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Stephan Herzig *Editor*

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Stephan Herzig
Editor

Metabolic Control

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Editor
Stephan Herzig
Institute for Diabetes and Cancer IDC
Helmholtz Center Munich
Neuherberg, Germany

Joint Heidelberg-IDC Translational Diabetes Program
Heidelberg University Hospital
Germany

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Preface

The proper control of energy homeostasis and metabolism is a prerequisite for the health of an organism. Indeed, metabolic dysfunction and the aberrant metabolic adaptation to changing environmental cues represent key features of severe disease conditions in humans, ranging from obesity, insulin resistance, and other components of the metabolic syndrome to end-stage complications in diabetes and even tumor diseases.

To acknowledge the increasing importance of both systemic and cellular metabolic hubs to serve as therapeutic targets in the aforementioned disease conditions, this edition of the "Handbook of Experimental Pharmacology" is dedicated to mechanisms and mediators of metabolic control. Highly distinguished leaders in the field of metabolism research have collected and summarized the current state of the art in the field. Individual chapters cover the importance of the central nervous system for energy balance, discuss both inter- and intra-organ communication routes in metabolic homeostasis and their molecular underpinnings, and highlight current treatment modalities in metabolic disorders.

I am convinced that this knowledge collection both spans a broad spectrum of conceptual aspects and at the same time provides an in-depth discussion of molecular principles in metabolic control, thereby allowing intriguing insights into this fascinating field of science.

Munich, Germany — Stephan Herzig

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A Genome-Wide Perspective on Metabolism

Alexander Rauch and Susanne Mandrup

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Abstract

Mammals have at least 210 histologically diverse cell types (Alberts, Molecular biology of the cell. Garland Science, New York, 2008) and the number would be even higher if functional differences are taken into account. The genome in each of these cell types is differentially programmed to express the specific set of genes needed to fulfill the phenotypical requirements of the cell. Furthermore, in each of these cell types, the gene program can be differentially modulated by exposure to external signals such as hormones or nutrients. The basis for the distinct gene programs relies on cell type-selective activation of transcriptional enhancers, which in turn are particularly sensitive to modulation. Until recently we had only fragmented insight into the regulation of a few of these enhancers;

A. Rauch • S. Mandrup (✉)
Department of Biochemistry and Molecular Biology, University of Southern Denmark, 5230 Odense M, Denmark
e-mail: s.mandrup@bmb.sdu.dk

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however, the recent advances in high-throughput sequencing technologies have enabled the development of a large number of technologies that can be used to obtain genome-wide insight into how genomes are reprogrammed during development and in response to specific external signals. By applying such technologies, we have begun to reveal the cross-talk between metabolism and the genome, i.e., how genomes are reprogrammed in response to metabolites, and how the regulation of metabolic networks is coordinated at the genomic level.

Keywords
Genomics · Gene regulation · Metabolism · Transcription factor action

1 Genome-Wide Technologies

One of the first technologies to be coupled with deep sequencing was chromatin immunoprecipitation (ChIP) (Jackson 1978). The so-called **ChIP-seq** technique relies on immunoprecipitation of a chromatin-associated protein of interest, e.g., transcription factors (TFs) (Robertson et al. 2007), or modified histone tails (Barski et al. 2007), followed by deep sequencing of the associated DNA (Fig. 1). By mapping these sequences to the genome, one obtains genome-wide and quantitative information regarding which regions are occupied by TFs and epigenomic marks. ChIP-seq has proven extremely powerful, and knowledge provided by this technology has greatly revolutionized our understanding of how transcription is regulated. The major limitations of this technique are the requirement for highly specific antibodies and the need for a priori knowledge regarding the selection of factors/histone marks of interest, i.e., there is a requirement to know what one is looking for. Furthermore, ChIP-seq requires relatively large amounts of cells, i.e., in the order of 10^5 to 10^7 depending on the antibody. Ongoing efforts aiming at scaling down include high-throughput ChIP (HT-ChIP) (Blecher-Gonen et al. 2013) and indexing first ChIP-seq (Lara-Astiaso et al. 2014) based on library preparation prior to the IP of chromatin. ChIP-seq works well for tissues, but ChIP-seq on isolated primary cells is complicated, due to (1) the requirement in terms of cell numbers, (2) the fact that cross-linking prior to cell sorting interferes with preparation of single cell suspensions, and (3) that the sorting procedure imposes stress that is likely to interfere with cellular signals and TF occupancy if cells are cross-linked after purification. To overcome this and more directly assess cell type-specific TF binding in vivo, transgenic approaches have been developed which enable the cell-type specific labeling of nuclei for IP (Bonn et al. 2012) or pull down (Deal and Henikoff 2011) procedures. In order to ensure a certain quality and reliability of the data sets, the ENCODE consortium recently published guidelines for ChIP-seq experiments (Landt et al. 2012).

Nucleosomes are actively depleted from promoter and enhancer regions by remodeling complexes (Felsenfeld and Groudine 2003; Gross and Garrard 1988), and determining the location of such nucleosome-depleted regions provides unbiased information about the position of these “action points” in the genome.

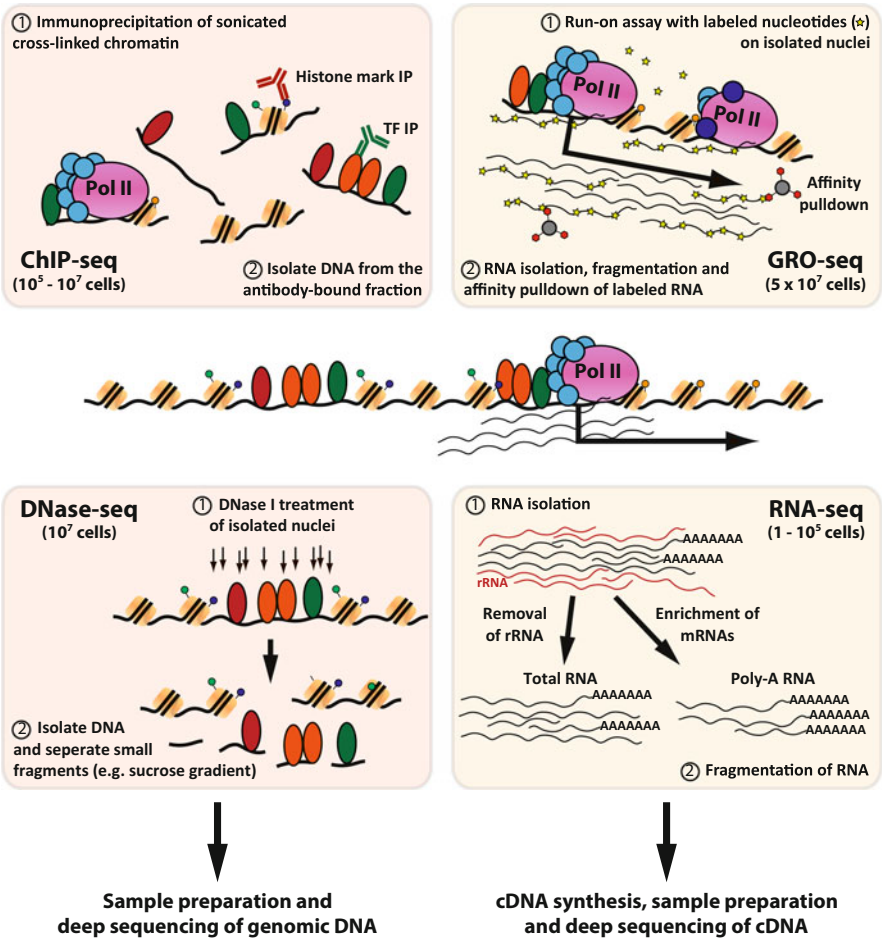

Fig. 1 Deep sequencing-based *technologies used to investigate transcriptional regulation*. Chromatin immunoprecipitation sequencing (**ChIP-seq**) is used to determine the genome-wide binding profile of TFs, cofactors, and histone marks to DNA. The procedure involves cross-linking of DNA and protein, fragmentation of chromatin, immunoprecipitation of the protein or mark of interest, de-cross-linking, and sequencing of the precipitated DNA. DNase I sequencing (**DNase-seq**) is used to determine nucleosome depleted (open) chromatin regions. The procedure involves isolation of nuclei, limited DNase I digestion, isolation of small DNA fragments, and sequencing. RNA sequencing (**RNA-seq**) is used to determine the level of total RNA or mRNA. **GRO-seq**, which combines purification of RNA species from in vitro global run-on transcription with sequencing, allows to measure acute transcriptional changes and transcription of very instable RNA species such as enhancer RNA. Whereas ChIP-seq and DNase-seq are characterized by sequencing enriched genomic DNA, RNA-seq and GRO-seq techniques are dependent on converting the RNA of interest into cDNA prior to library preparation

An accurate map of nucleosome-depleted regions (open chromatin) at a genome-wide level can be obtained by coupling DNase I digestion with high-throughput sequencing (**DNase-seq**) (Boyle et al. 2008; Crawford et al. 2006; Hesselberth et al. 2009). Here, limited DNase I digestion of isolated nuclei releases small fragments of DNA from accessible regions that can be sequenced and mapped to the genome to precisely locate their genomic position (Fig. 1). With the appropriate bioinformatic tools and sufficient sequencing depth, one can even determine the exact position of TF binding in open chromatin regions (footprints) (Hesselberth et al. 2009). While DNase-seq is a very powerful technology, it is time-consuming, and requires careful titration of DNase I concentration and large numbers of cells. In contrast, formaldehyde-assisted isolation of regulatory elements sequencing (**FAIRE-seq**), which relies on the enrichment of nucleosome-free DNA after consecutive rounds of phenol-chloroform extraction of cross-linked and sonicated chromatin (Giresi et al. 2007), constitutes an easy alternative albeit the signal-to-noise ratio provided is far lower than for DNase-seq. Another recently developed technology to map open chromatin regions is assay for transposase-accessible chromatin (ATAC-seq), which is based on the direct transposase-mediated integration of sequencing adaptors into accessible native chromatin (Buenrostro et al. 2013). These sequencing adapters can then be used for PCR amplification thereby circumventing the need for enrichment of open regions prior to library construction as for DNase-seq and FAIRE-seq. Thus, ATAC-seq can be performed with much lower numbers of cells but provides sequencing libraries with lower complexity due to PCR biases.

High-throughput sequencing is also widely used for the quantification of transcript levels by **RNA-seq** (Lister et al. 2008), which has now almost completely replaced microarrays due to higher reproducibility and higher dynamic range of detection (Wang et al. 2009). RNA-seq relies on sequencing of cDNA generated from rRNA-cleared total RNA (**total RNA-seq**), or mRNA-derived cDNA (**poly-A RNA-seq**) (Fig. 1). Due to the high sensitivity, RNA-seq can be applied to very small numbers of cells, including single cells (Tang et al. 2011). Further developments of RNA-seq such as 4sU-RNA-seq (Rabani et al. 2011) allow for specific detection of newly synthesized transcripts rather than quantification of the pool of transcripts present in a cell. Alternatively, using the newly developed iRNA-seq pipeline, one can determine newly synthesized transcripts and thereby estimate acute transcriptional changes by specifically quantifying reads in introns in total RNA-seq data sets (Madsen et al. 2015). Furthermore, transcription rate can be assessed by performing ChIP-seq on RNA polymerase II (**RNAPII-ChIP-seq**) (Nielsen et al. 2008) or by global run-on sequencing (**GRO-seq**) (Core et al. 2008). In the case of GRO-seq, RNA polymerase II is paused and allowed to continue transcription while incorporating labeled nucleotides in vitro. The newly synthesized RNA is then converted into cDNA and sequenced (Fig. 1). The transcripts detected are therefore not cellular transcripts per se but rather represents the activity of RNA polymerase II at the corresponding genomic positions. GRO-seq has been particularly useful for detection of active transcription at enhancers, which has been shown to correlate with enhancer activity (Kim et al. 2010; Wang et al. 2011).

There are several technologies that detect 3D chromatin interactions, and some of these have been combined with high-throughput sequencing to map such interactions at a genome-wide level (reviewed in de Wit and de Laat 2012). **HiC** is the most unbiased technology, as it determines interactions of all baits against all targets, but it is also the most demanding in terms of sequencing (Lieberman-Aiden et al. 2009). The resolution of the initial HiC analyses was poor because of the high complexity; however, recently the accumulation of 1.5–2 billion uniquely mapping paired-end reads revealed over one million long-range interactions with a resolution of 5–10 kb (Jin et al. 2013). HiC has recently been applied to single cells, thereby bridging the field of genomics and microscopy for chromosome studies (Nagano et al. 2013). The complexity of the interactions can be decreased by limiting the baits, e.g., by using arrays to capture specific baits in **capture HiC** (Dryden et al. 2014; Schoenfelder et al. 2015) or by using antibodies against a specific TF or cofactor such that only interactions involving this factor are detected as in "chromatin interaction analysis by paired-end tag sequencing" (**ChIA-PET**) (Fullwood et al. 2009). For instance, using RNA polymerase II as the target protein, one can detect how active promoter regions interact with enhancers (Li et al. 2012).

2 Predicting Key Regulatory Enhancers

Being able to identify the key regulatory enhancers involved in mediating a particular metabolic response is of great importance for understanding how gene expression is regulated. Genome-wide mapping of putative enhancers and their change of activity in response to a metabolic signal allows for prediction of which enhancers(s) are involved in the regulation of specific genes. Furthermore, bioinformatic analyses of the DNA sequence of all activated or repressed enhancers allows for prediction of which sequence-specific transcription factors might be involved in mediating the response. This is a powerful approach that has been used in a number of studies to predict the major regulators involved (Siersbaek et al. 2011; Carroll et al. 2005; Lin et al. 2010; Yanez-Cuna et al. 2012; Ling et al. 2010; Steger et al. 2010; Mikkelsen et al. 2010). Bioinformatic limitations for these predictions are the fact that large families of transcription factors bind to very similar DNA sequences and that only TFs for which there is a priori knowledge of the binding motif can be predicted. It is also important to bear in mind that the mitogenic environment of cell culture models may lead to activation of mitogenic transcription factors, such as AP-1, which will impact on the accessibility landscape and create a bias in such motif analyses (Zhu et al. 2013a).

2.1 Open Chromatin Regions Marks Putative Enhancers

Recent mapping of enhancer signatures in the genome of a large number of cell types has estimated the number of transcriptional enhancers to be in the order of 400,000 (Consortium 2012; Zhu et al. 2013a). Notably however, it is only a fraction of these enhancers that are active in a particular cell, and it remains incompletely

understood how enhancers are selectively activated in specific cell types. It is clear that cell type-selective expression and activation of TFs play an important role, but even highly active TFs only find a small percentage of their potential binding sites in the genome. A major determining factor for where a TF binds appears to be accessibility of the chromatin, i.e., TFs are more likely to bind to accessible chromatin regions. This "accessibility landscape" is cell type-specific (Degner et al. 2012; Thurman et al. 2012) as a result of the concerted binding of cellular TFs and their recruitment of remodeling complexes. Thus, open chromatin regions change dramatically during cellular differentiation (Siersbaek et al. 2011; Lara-Astiaso et al. 2014; Thomas et al. 2011) and more modestly in response to other metabolic signals (Ling et al. 2010; He et al. 2012; Lo et al. 2013; Leung et al. 2014; John et al. 2011). Of note, chromatin remodeling is ATP dependent and thus directly linked to the metabolic status of the cell (reviewed in Hargreaves and Crabtree 2011). Furthermore, genome-wide maps of DNase I hypersensitive sites (DHS) include a wealth of information about cell fate, lineage relationship, and dysfunction. The degree of overlap between open chromatin of embryonic stem cells and differentiating cells can be seen as a measure of cellular maturity, and unsupervised nearest-neighbor clustering of DNase I sites can reveal the developmental origin of cell types (Stergachis et al. 2013; Lara-Astiaso et al. 2014).

Traditional footprint assays use the ability of TFs to protect naked DNA from DNase digestion (Galas and Schmitz 1978). Similarly, footprints of TFs bound to chromatin can be determined by digital genomic footprinting that determines the precise DNase I cut events and creates maps of protected segments within the open chromatin regions (Hesselberth et al. 2009). One would expect that digital genomic footprinting would refine motif-based TF prediction in enhancers; however, current findings clearly demonstrate that not all TFs footprint equally well (He et al. 2014). Interestingly, a recent study showed that the degree of DNase I protection correlates with the occupancy time of the TF (Sung et al. 2014). Thus, digital genomic footprinting can provide insight into the architecture of enhancers (Siersbaek et al. 2014a), and the binding mode of TFs (Sherwood et al. 2014); however, it appears to add only limited power with respect to predicting TF binding in enhancers over analyzing motif occurrence in DHS sites.

2.2 Epigenomic Marks as Indicators of Enhancer Activity

Posttranslational modifications of the histone tails are dynamically placed by histone acetylases, methylases, and kinases and removed again by deacetylases, demethylases, and phosphatases. These histone-modifying enzymes are recruited to the chromatin by the sequence-specific TFs and are sensitive to fluctuations of energy substrates such as acetyl-CoA, UDP-glucose, NAD^+, α-ketoglutarate, and ATP and are thereby targeted by the intermediary metabolism of the cell (reviewed in Gut and Verdin 2013). ChIP-seq analyses have demonstrated that many histone modifications are associated with distinct genomic features, such as active enhancers (H3K4me1, H3K27ac), transcription (H3K36me3) and promoters (H3K4me3, H3K9ac), as well as repressed regions (H3K27me3, H3K9me3)

(Zentner et al. 2011; Kharchenko et al. 2011; Rada-Iglesias et al. 2011; Creyghton et al. 2010; Mikkelsen et al. 2007). Therefore, these marks hold predictive power in terms of defining promoters and enhancers and assessing their activity. Currently, H3K27ac appears to be the histone mark most dynamically marking active enhancers (Creyghton et al. 2010; Bogdanovic et al. 2012). H3K27ac distribution is more dynamic, and a better mark of enhancer activity compared to typical enhancer marks like H3K4me1 and DNase hypersensitivity, as they are also placed at poised enhancers (Zhang et al. 2013). It has been shown that H3K27ac is the best single predictor for enhancer activity, which can be excelled by analyzing the combination of H3K27ac, H3K27me3, H3K79me1, and H3K9ac. The combination of histone marks is highly predictive for enhancer activity and even enhancer RNA expression (Zhu et al. 2013b). Like DHS sites, histone marks have a cell type-specific distribution with an enrichment of cell type-specific TF motifs and show consecutive changes during cellular differentiation (Zhu et al. 2013a; Hnisz et al. 2013; Mikkelsen et al. 2010).

2.3 Cofactors and Enhancer RNAs as Indicators of Enhancer Activity

Enhancer activity can also be predicted by determining the association with co-activators such as the mediator subunit MED1, CREB binding protein (CBP), or bromodomain containing protein 4 BRD4, all of which have a more narrow window and appear to be more dynamic compared to histone acetylation marks (Brown et al. 2014). In addition, active enhancers are characterized by the expression of enhancer RNA (eRNAs), which are relatively short (50–2,000 nucleotides), non-polyadenylated transcripts (Kim et al. 2010; Wang et al. 2011). Enhancers with eRNA expression are more highly associated with active gene transcription than H3K27ac marked sites, indicating that eRNA is highly predictive for enhancer activity (Zhu et al. 2013b). Several reports have indicated that enhancer transcription is more than just transcriptional noise (Melo et al. 2013); however, it is currently unclear to what extent it is the transcription per se (Kaikkonen et al. 2013) or the actual transcripts that contribute to enhancer activity (Li et al. 2013; Lam et al. 2013; Mousavi et al. 2013).

2.4 Linking Enhancers to Promoters

Linking enhancers to the regulation of specific genes poses a major problem in all genome-wide analyses. The only correct way would seem to be the unfeasible task of systematically deleting putative enhancers and investigating the effect on gene expression (Kieffer-Kwon et al. 2013). Currently, most genome-wide analyses associate enhancers with promoters only based on proximity, sometimes taking into regard also promoter activity, i.e., linking active enhancers to nearest active promoter. Although this has proven to be a useful approximation (Sanyal

et al. 2012), it is clearly an oversimplification that fails to consider distal enhancers and fails to consider the fact that some enhancers may regulate multiple genes (Tsujimura et al. 2010; Link et al. 2013; Montavon et al. 2008). Assuming that physical interaction significantly increases the likelihood of regulation, a better approach for linking regulated genes with their enhancers is mapping of 3D chromatin interactions. The current technologies are all labor- and cost-intensive and pose major problems in terms of resolution (particularly in unbiased approached such as HiC) or in terms of bias (e.g., bias for a particular factor in ChIA-PET). However, despite these limitations, 3D technologies provide very useful insight into the specific interactions between enhancers and promoters as well as how these interactions are modulated. Results from such analyses have shown that about 27% of distal enhancers interact with the closest promoter, whereas almost 50% of distal enhancers interact with the closest active promoter (Sanyal et al. 2012). It is currently unclear to what extent these interactions are modulated by signal-dependent TFs as suggested by some studies (Li et al. 2013; Le Dily et al. 2014) or whether signal-dependent TFs act by modulating pre-established interactions as suggested by other studies (Hakim et al. 2011; Jin et al. 2013). However, in any case mapping these interactions will greatly help in defining which enhancers are involved (or has the potential to be involved) in the regulation of specific genes.

Knowing the binding sites of a TF and associating these with genes, either by proximity or by 3D technologies, provide valuable insight and hypotheses regarding the potential of the TF in controlling gene expression at a genome-wide level. By correlating TF binding with changes in epigenomic marks or cofactors in response to binding, one can predict whether the TF has an activating or repressing effect on the specific enhancer activity (Grontved et al. 2013; Uhlenhaut et al. 2013).

3 Cross-Talk Between Transcription Factors Is the Basis for Cell Type Specificity

It is becoming increasingly clear that there is considerable cross-talk between TFs at multiple levels and that this cross-talk is key to cell type-specific activation of enhancers and gene programs. First, transcription factors cooperate in gaining access to the DNA. This is important because the wrapping of DNA around nucleosomes in chromatin makes the DNA strand relatively inaccessible to TFs. There are numerous reports demonstrating that binding of one TF facilitates the recruitment of other TFs to chromatin. The ability of certain TFs to facilitate the binding of others to closed chromatin has been termed “pioneering” (reviewed in Iwafuchi-Doi and Zaret 2014). More recently the term “assisted loading” has been used to describe the interdependent binding of TFs to chromatin (Voss et al. 2011). FoxA1 has been shown to facilitate the recruitment of estrogen receptor (ER) in breast cancer cells (Hurtado et al. 2011; Carroll et al. 2005); AP1 facilitates the recruitment of glucocorticoid receptor (GR) in murine mammary cells (Biddie et al. 2011); C/EBPβ facilitates GR binding in mouse liver in vivo (Grontved

et al. 2013) and differentiating 3T3-L1 adipocytes (Steger et al. 2010; Siersbaek et al. 2011); and C/EBPα facilitates the recruitment of PPARγ and vice versa in adipocytes (Madsen et al. 2014). Furthermore, there is considerable cooperativity in TF binding to TF hotspots established early during 3T3-L1 adipogenesis (Siersbaek et al. 2011, 2014b). Interestingly, and consistent with this cooperativity, the binding of TFs to hotspots is less dependent on their cognate DNA motif compared to the binding to sites with fewer associated factors (Siersbaek et al. 2014a) (Fig. 2). This implies that genomic elements harboring a well-fitting consensus motif are likely to be bound by the corresponding TF more independently of other interacting factors. Accordingly, ER binding sites that are shared between different cell types typically contain high-affinity ER motifs, whereas cell type-specific ER sites are more

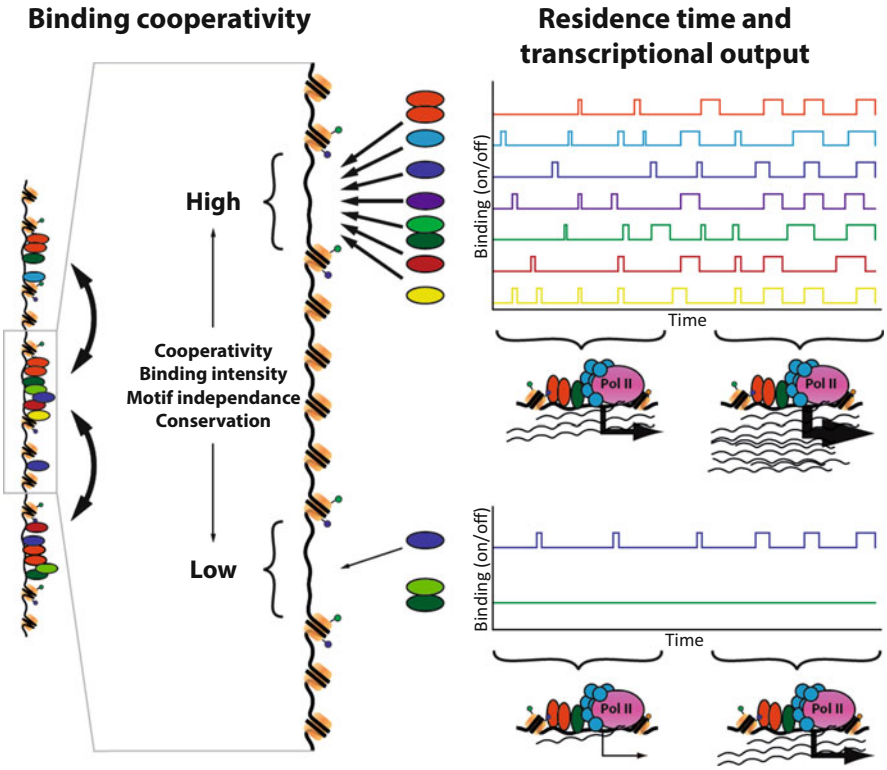

Fig. 2 *Transcription factors cooperate in binding and transcriptional output*. TFs only bind to a small subset of potential target sites in DNA. Most binding events are highly dependent on cooperative binding to TF hotspots and are therefore cell type-selective. There may also be cooperativity between hotspots as is seen in super-enhancers. Binding at such sites is less dependent on motif strength and is retained to a higher degree. Some binding events that are driven by strong TF binding motifs are less dependent on other TFs and tend to be bound in several different cell types. Transcriptional output is determined in part by association to hotspots (and super-enhancers) as well as the residence time of TF binding events, where mainly long binding events contribute to transcriptional rate

dependent on other ER cooperating factors (Gertz et al. 2013). Moreover, the ability of C/EBPα and PPARγ to act as the "leading factor" in priming the chromatin landscape for the other is dependent on the motif score of their corresponding binding sites (Madsen et al. 2014).

Importantly, and contrary to what has been the general dogma, TFs that bind to the same DNA sequence may cooperate rather than compete (Voss et al. 2011). This can be rationalized by the dynamic association of TFs with DNA, the limited occupancy times for TFs, and the ability of TFs to bring in different co-activator complexes. In line with this, photo-bleaching experiments (McNally et al. 2000; Mueller et al. 2008; Bosisio et al. 2006) and single molecule tracking studies (Chen et al. 2014; Gebhardt et al. 2013) showed that the average residence time of TFs on DNA is in the range of seconds to minutes. Interestingly, it has been shown that the residence time of a TF at a single site varies and that longer interactions correlate with higher transcriptional output (Lickwar et al. 2012; Karpova et al. 2008; Stavreva et al. 2004; Sharp et al. 2006) (Fig. 2). The dissociation of TFs from the chromatin is thought to be due to passive thermal processes as well as active cellular processes requiring chromatin remodeling (Nagaich et al. 2004; McKnight et al. 2011; Johnson et al. 2008; Karpova et al. 2004), chaperone (Agresti et al. 2005; Stavreva et al. 2004), and proteasomal activity (Stavreva et al. 2004; Stenoien et al. 2001). This turnover of transcriptional regulators at active sites may contribute positively to transcriptional activity. For example, it has been shown that proteasome inhibition leads to accumulation of ubiquitinated transcriptional regulators and reduced transcriptional activity of genes in the vicinity of ubiquitin-accumulating enhancers (Catic et al. 2013). The stochastic associations of TFs with DNA contribute to transcriptional noise and variability of transcriptional responses between different cells in a population (Raser and O'Shea 2004).

Interestingly, co-binding of TFs has been shown to be predictive for cross-species interdependent retention of binding sites. This was first shown for C/EBPα and PPARγ binding in human and mouse adipocytes (Schmidt et al. 2011), and later for three TFs in livers of six different rodent species (Stefflova et al. 2013). Loss of a single binding event leads to concomitant, systemic destabilization of the binding of the other TFs. Thus, it appears that co-bound TFs have a higher probability of binding conservation and that this binding is more robust to sequence variations in directly bound motifs. The ENCODE consortium reported 3,307 pairs of statistically co-associated factors involving 114 TFs (out of 117 analyzed) indicating a widespread genomic interaction between many but not all (analyzed) TFs (Gerstein et al. 2012). Furthermore, profiling of 15 TFs at 4 h following adipogenic stimulation of 3T3-L1 preadipocytes showed the presence of about 12,000 hotspots ($\geq$5 factors binding) and a remarkable overlap in binding of all TFs at 138 sites (Siersbaek et al. 2014b). Thus, although there might be pairs of TFs that are particularly good at facilitating the binding of each other, it is likely that cooperativity is a general phenomenon that extends to most TFs simply because they have capacity to make the chromatin more accessible through recruitment of remodeling complexes and cofactors.

Second, TFs also cooperate at the level of cofactor recruitment and chromatin remodeling (Siersbaek et al. 2014a), and hotspots are therefore highly associated with gene activation (He et al. 2011; Siersbaek et al. 2014b; Chen et al. 2008) (Fig. 2). Interestingly, analysis of C/EBPβ binding to hotspots showed that this TF contributed equally to the activity of the hotspot (as determined by recruitment of other TF and MED1) independent of whether it bound to DNA via a consensus C/EBP binding motif or via alternative mechanisms that could be both direct or indirect (Siersbaek et al. 2014a).

Third, recent results indicate that TFs also cooperate at the level of so-called super-enhancers (also termed stretch enhancers), which are clusters of highly active enhancers in the genome that typically span over several kb (Whyte et al. 2013; Parker et al. 2013). Super-enhancers are bound by cell type-specific TFs and high levels of co-activators such as the mediator complex. They are highly enriched in the vicinity of cell type-specific genes and are more sensitive to perturbations than normal enhancers (Whyte et al. 2013; Loven et al. 2013; Hnisz et al. 2013; Brown et al. 2014). Consistent with the importance of these enhancers, disease-associated sequence variations (SNPs) are enriched in super-enhancers compared to normal enhancers. SNPs associated with type I diabetes are enriched in super-enhancers of T-helper cells compared to, e.g., adipose tissue, brain, and heart (Hnisz et al. 2013). Indicative of cooperativity between the super-enhancer constituents, these have significantly higher levels of MED1 occupancy than regular enhancers with the same number of TFs associated (Siersbaek et al. 2014b). Furthermore, depletion of a TF that binds to constituents in a super-enhancer affects not only the activity of the associated constituents but also that of other not bound ones in the super-enhancer (Siersbaek et al. 2014b).

4 Genomic Approaches in Metabolism

The application of deep sequencing-based genome-wide approaches has transformed the way we study transcriptional regulation and has had major impact on our understanding of how gene programs are regulated. First, insight gained from these approaches has revolutionized our understanding of the molecular mechanisms and the complexity involved. Key findings include the fact that genes are typically regulated by multiple enhancers many of which are intragenic or far away from genes. Furthermore, enhancers are hotspots where multiple TF bind in a cooperative fashion, and this cooperativity appears to be the basis for the highly cell type- and context-dependent binding and action of TFs. It has also become clear that the majority of the genome is transcribed and that non-coding RNAs are heavily involved in the regulation of gene expression (Vollmers et al. 2012; Derrien et al. 2012; Howald et al. 2012; Banfai et al. 2012). Second, since we can now study all genes and all enhancers simultaneously rather than studying single gene-enhancers relations, we can obtain global insight into the role of a particular factor. Third, the genome-wide approaches have greatly facilitated the identification of novel regulators and made it possible to begin to define

transcriptional networks that interact in time and space (Fig. 3). Here TF networks can be thought of both as the cascades of factors that interact in time by regulating the expression of each other, as well as the functional cross-talk between TFs at a given time point.

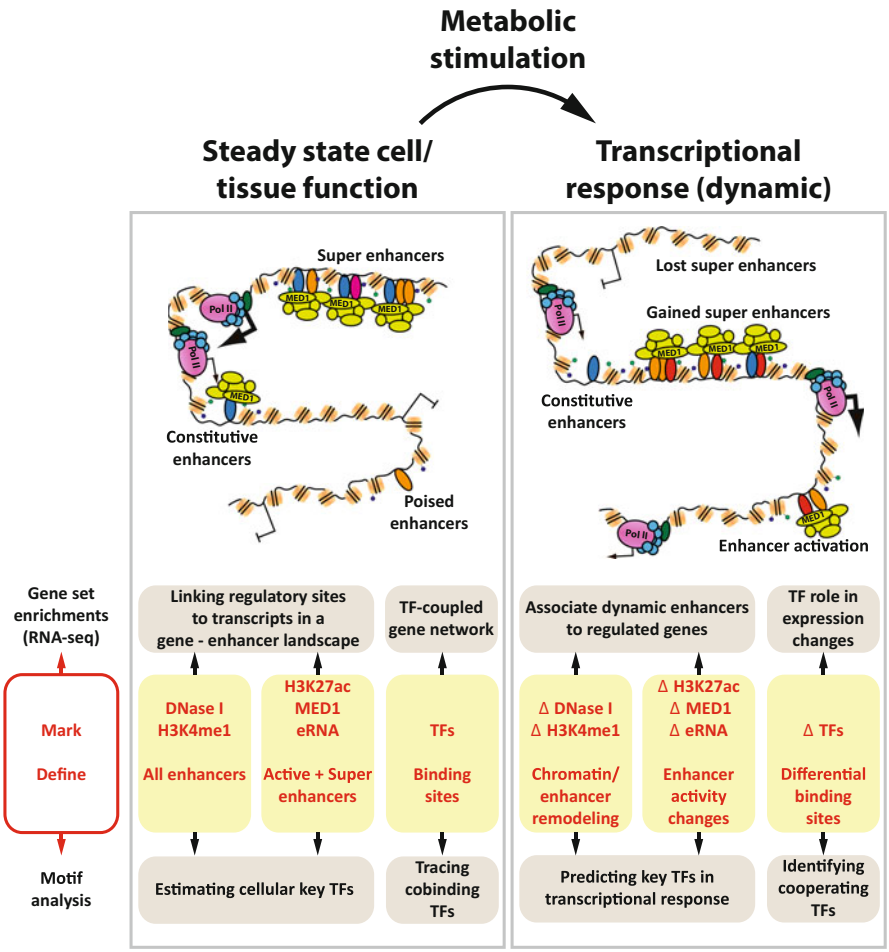

Fig. 3 *Genomic strategies for uncovering transcriptional networks*. Under steady-state conditions, profiling of (active) enhancer marks identifies the position of cell type-selective enhancers. Bioinformatic motif analysis of the underlying sequences can predict key cell type-specific TFs. Profiling of these TFs helps to validate their involvement in specific gene programs and to predict cooperating partners. Genome-wide profiling of changes in enhancer activity in response to specific stimuli is a powerful strategy for identifying key enhancers involved in transcriptional reprogramming and predicting the executing TFs based on motif analyses. Super-enhancers are particularly sensitive to regulatory signals, and super-enhancer-association can therefore be used to predict the most dramatically regulated/lineage-determining genes

4.1 Adipogenesis and Metabolic Regulation of Adipocytes

Genome-wide profiling of DHS sites during 3T3-L1 adipogenesis has revealed a dramatic remodeling of chromatin during the first few hours following stimulation with the adipogenic cocktail, and this remodeling is driven by the formation of early transcription factor hotspots (Siersbaek et al. 2011, 2014a). Some of the remodeled sites are only transiently remodeled and may be selectively required for the early gene program involved in, e.g., the early proliferative response (clonal expansion). Interestingly however, many remodeled sites remain open also in the mature adipocytes, and there is relatively little remodeling occurring at later time points during the differentiation. This demonstrates that the chromatin structure of these adipocytes is largely established early in differentiation long before the main transcriptional activators of the second wave, such as PPARγ and C/EBPα, are activated. However, once these factors become activated they occupy many of the early enhancers, indicating that the function of these important regulators in adipogenesis depends on the early remodeling process (reviewed in Siersbaek and Mandrup 2011; Lefterova et al. 2014).

Profiling of PPARγ and C/EBPα in mature mouse and human adipocytes has shown that PPARγ and C/EBPα are associated with most genes that are induced during adipogenesis and highly enriched in the vicinity of genes involved in glucose and lipid metabolism (Lefterova et al. 2008; Nielsen et al. 2008). This indicates that these key regulators are directly involved in the entire metabolic reprogramming during adipogenesis. Interestingly, PPARγ displays distinct binding patterns in adipocytes from different depots and is associated with lineage-selective genes, indicating that PPARγ is also involved in the activation of specialized adipocyte gene programs (Siersbaek et al. 2012). It is likely that lineage-selective factors cooperate with PPARγ to bind to these genes. Based on motif analyses EBF2 was discovered as a brown adipocyte factor that bind and cooperate with PPARγ at brown adipocyte-selective sites (Rajakumari et al. 2013). KLF11 was recently identified as another lineage-determining factor by analyzing de novo PPARγ super-enhancers during browning of human adipocytes (Loft et al. 2015). Furthermore, motif analyses of putative preadipocyte and adipocyte enhancers identified promyelocytic leukemia zinc finger protein (PLZF encoded by *Zbtb16*) and the serum response factor (SRF) as preadipocyte factors that inhibit differentiation (Mikkelsen et al. 2010) and nuclear factor 1 proteins NF1A and NF1B as factors that promote adipogenesis (Waki et al. 2011).

In addition, comparative transcriptome profiles have been used to identify common mechanisms in cell culture models and to determine to which extent these mechanisms resemble the transcriptional changes in vivo. For example, it was recently shown that in vitro insulin resistance of 3T3-L1 adipocytes through tumor necrosis factor α (TNF-α), hypoxia, or the combinatorial treatment compared to dexamethasone or high insulin exposure faithfully mimics the transcriptional perturbations of adipose tissue in diet-induced obesity (Lo et al. 2013).

4.2 Nutritional Reprogramming of the Genome in Hepatocytes

Liver metabolism is subject to marked nutritional and diurnal regulation. Genome-wide approaches have revealed that the genomic basis for that is a marked reprogramming of the chromatin landscape (Koike et al. 2012; Vollmers et al. 2009, 2012; Leung et al. 2014). By identification of the putative enhancers and the characterization of the mechanisms of individual factors involved, we have gained considerable insight into how this reprogramming occurs. One of the factors associated with the fasting response in hepatocytes is cAMP response element binding protein (CREB), which is activated by PKA phosphorylation of Ser133 during fasting (reviewed in Altarejos and Montminy 2011). Genome-wide profiling of CREB binding showed only minor differences in the binding of CREB and pSer133-CREB between different cell types cultured in vitro (Zhang et al. 2005). Furthermore, in the fasting liver CREB was found to be, associated with both activated and repressed genes (Everett et al. 2013). Bioinformatic analyses of liver ChIP-seq profiles demonstrated CREB binding sites near fasting-induced genes to be generally associated with co-occupancy of FOXA2, C/EBPβ, PPARα, and GR, whereas CREB binding sites near repressed genes were devoid of these factors and mostly enriched for CTCF binding (Everett et al. 2013). Thus, it appears that CREB generally marks enhancers of fasting-regulated genes, and whether it acts as a repressor or an activator depends on the cooperating TFs at the enhancer (Fig. 4).

Nuclear receptors (NRs) are a class of important signal-dependent TFs involved in the regulation a large number of cellular functions including differentiation and metabolism (reviewed in Aagaard et al. 2011; Hong et al. 2014; Poulsen et al. 2012). In mammals, members of the retinoid X receptors (RXRs) play a central role as the common heterodimerization partners of the class II receptors,

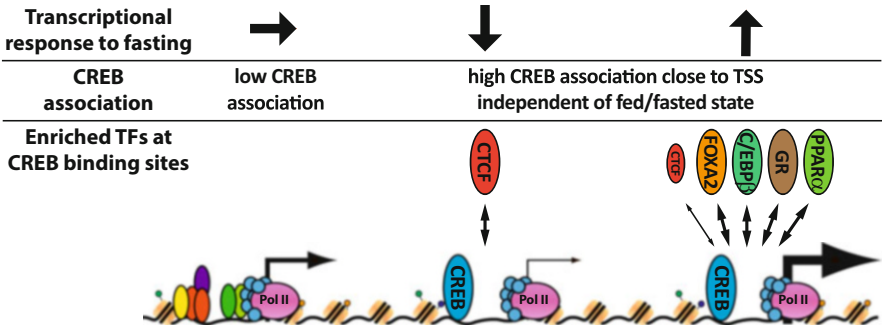

Fig. 4 *CREB marks fasting-responsive genes*. cAMP responsive element binding protein (CREB) is associated with genes that are differentially regulated upon fasting. The binding of CREB itself is independent of the nutritional state. However, near fasting-induced genes CREB binds to sites associated with *FOXA2* forkhead box protein A2, *C/EBPβ* CCAAT enhancer binding protein β, *GR* glucocorticoid receptor, and *PPARα* peroxisome proliferator-activated receptor α, whereas near fasting-repressed CREB binding sites are mainly associated with *CTCF* CCCTC-binding factor

which include a large number of metabolite-activated NRs such as PPARs, LXRs, and FXRs, as well as some hormone- and vitamin-activated receptors involved in the regulation of metabolism, such as TRs and RARs (reviewed in Evans and Mangelsdorf 2014). In vitro different types of RXR heterodimers bind to repeats of similar consensus sequences with distinct receptor-specific spacing, and these sequences have been found to be enriched at the chromatin binding sites of the corresponding receptors in many different studies (He et al. 2013; Boergesen et al. 2012; Cui et al. 2010; Chong et al. 2010; Thomas et al. 2010; Feng et al. 2011; Schmidt et al. 2010). The intriguing observation is that although nuclear receptor motifs are enriched at the binding sites, only a small fraction of the binding sites has a motif that fits the consensus reasonably well (Boergesen et al. 2012). It appears that nuclear receptors bind to many overlapping sites, i.e., "NR hotpots," in the genome and may do so by interacting with the same degenerate binding motifs in these hotspots (Boergesen et al. 2012) (Fig. 5). This finding challenges the concept of the DNA motif being the major contributor to defining NR specificity. It is likely that other TFs associated with enhancers play a key role not just in making the site accessible through chromatin remodeling, but also in facilitating the binding by protein-protein interaction (Siersbaek et al. 2014b) (reviewed in Voss and Hager 2014). It is intriguing that NRs that control opposing metabolic pathways occupy the same enhancers in mouse liver, as it was observed for PPARα and LXR, which activate fatty acid oxidation and fatty acid synthesis, respectively. This finding opens the possibility for an extensive and direct cross-talk between these different nuclear receptor pathways at the chromatin level; however, it remains to be seen how the individual NRs contribute to enhancer activity. It is likely that the level of importance of a particular NR for enhancer activity varies greatly between the different enhancers to which it binds.

4.3 Cross-Talk Between Metabolism and the Clock

There is considerable cross-talk between metabolism and the circadian clock. The clock controls metabolism in anticipation of a meal, and metabolites in turn regulate the clock (reviewed in Aguilar-Arnal and Sassone-Corsi 2013). Circadian genes are often tissue-specific and rate limiting steps of biological processes, e.g., glucose and lipid metabolism in liver (Kohsaka et al. 2007). The understanding of how this happens has recently made major leaps forwards based on discoveries generated by genome-wide studies (reviewed in Feng and Lazar 2012). Transcriptional reprogramming during the day is coordinated by changes in TF and cofactor recruitment that modulates chromatin structure and the epigenome (Feng et al. 2011; Vollmers et al. 2012; Koike et al. 2012; Menet et al. 2014; Stashi et al. 2014). The activity of cycling enhancers alternates between repressed, poised, and active state (Koike et al. 2012) which is dictated in part by the oscillating activities of the core clock TFs (Koike et al. 2012; Menet et al. 2014) as well as the accessory clock TFs such as REV-ERB proteins (Feng et al. 2011; Cho et al. 2012; Bugge et al. 2012). Core clock components are enriched at cycling promoters and

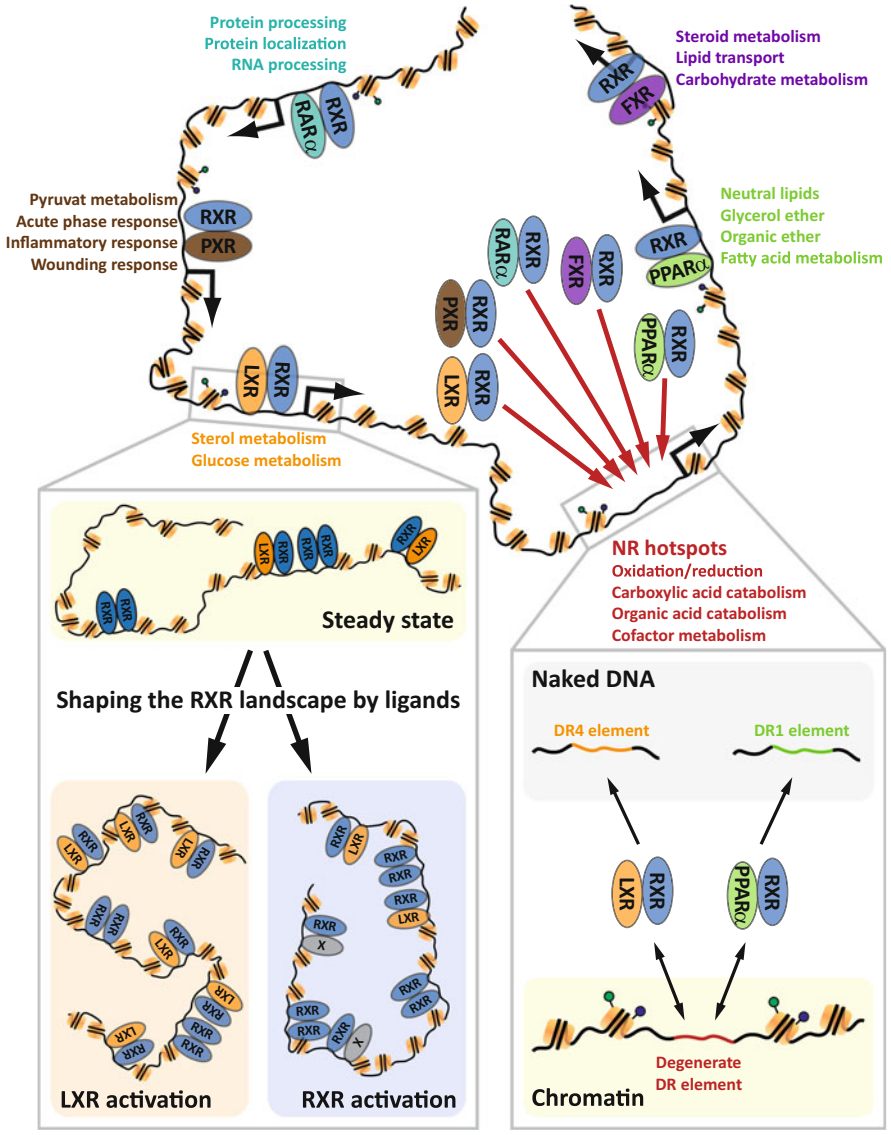

Fig. 5 *Nuclear receptors bind to many nuclear receptor hotspots*. Type II nuclear receptor dimers such as LXR-RXR, PXR-RXR, FXR-RXR, PPARα-RXR, and RARα-RXR bind to unique binding sites in the liver as well as to many nuclear receptor hotspots with degenerate motifs (*LXR* liver X receptor, *PXR* pregnane X receptor, *FXR* farnesoid X receptor, *PPARα* peroxisome proliferator-activated receptor α, *RARα* retinoic acid receptor α, *RXR* retinoid X receptor). *Lower panel left*: LXR agonist increases LXR as well as RXR occupancy and induces binding to many new sites. By contrast, RXR agonists only promote RXR occupancy and have little effect on LXR occupancy. *Lower panel right*: The binding of LXR-RXR dimers to naked DNA is limited to DR4 elements (direct repeat with four spacing nucleotides) and PPARα-RXR binding to DR1 elements. In contrast, both dimers are capable of binding to the same degenerate DR element in the (open) chromatin context

enhancers, and genes with a cycling activity of both components have an increased amplitude of transcripts (Vollmers et al. 2012). Loss of REV-ERBs leads to almost complete failure to recruit the co-repressor HDAC3 to genomic sites in liver, thereby resulting in derepression of many lipogenic genes and hepatic steatosis (Feng et al. 2011; Bugge et al. 2012). The activity of sterol-regulatory binding protein 1 (SREBP-1) is known to be activated by insulin and nutrients in the fed state (Green et al. 2009; Foretz et al. 1999). In addition, recent results showed that the binding profile of this central TF is regulated in a circadian manner, most likely through cooperative binding with TFs that are under control of the clock (Gilardi et al. 2014). This indicates that enhancers, such as those occupied by SREBP-1, integrate metabolic and circadian signals. One of the future challenges will be to dissect metabolic and clock-derived signals acting on regulatory enhancers.

5 Future Challenges

Genome-wide technologies have revolutionized our understanding of transcriptional regulation and had major impact on our ability to decode metabolic regulatory networks. However, these technologies have also revealed a hitherto unappreciated complexity that shows that there is a lot that remains to be understood. This includes the spatial and temporal integration of cellular signals on the chromatin and the driving forces of cell type-specific enhancer remodeling. Solving these questions will require overcoming some of the current technical limitations of genome-wide technologies.

One of the major limitations in genome-wide technologies is the requirement for large amount of sample material, and ongoing efforts therefore aim at scaling down. These include, e.g., new technologies that require much fewer cells to start with, such as ATAC-seq and HT-ChIP. The ultimate goal is to be able to determine molecular aspects of transcriptional regulation at a single cell level. Scaling down combined with cell sorting is important for the ability to analyze transcriptional regulation in primary cells. Current efforts include optimizing cell sorting by magnetic (MACS) or fluorescent (FACS) labeling and combining this with RNA-seq, DNase-seq, or ChIP-seq to get expression and chromatin profiles of pure cell populations (Thurman et al. 2012; Zhu et al. 2013a; Harzer et al. 2013). In order to overcome issues of cell sorting procedures and more directly assess genome-wide aspects of transcriptional regulation in vivo, it may be necessary to use transgenic approaches that allow for direct labeling and isolation of cell type-specific nuclei from tissues (Bonn et al. 2012; Deal and Henikoff 2011) or cell type-specific RNA species through "TU tagging," i.e., 4-thiouracil labeling and biotinylation of RNA from a particular cell type (Gay et al. 2014). Further development of these labeling technologies and their integration with genome-wide technologies will be an important step for the ability to investigate transcriptional regulation in vivo.

Another major challenge is the fact that the time resolution in the current genome-wide technologies is poor. Thus, these technologies provide averages not

just over many cells, but also averages over extended time periods compared to the highly dynamic behavior of transcriptional regulators. This means that fundamental insight into the dynamic actions of transcriptional regulators is lacking. Recent achievements, e.g., in single molecule tracking technologies, have provided a glimpse of this dynamic behavior and further developments in this area have great potential.

Finally, a major challenge is linking the complex insight gained through genome-wide technologies to a functional transcriptional readout. Here technologies that map the 3D structure of chromatin and record how this is changed in response to transcriptional signals have great potential. However, improving the time and spatial resolution of such techniques will be important for their general applicability and the information that can be obtained from such studies. Importantly, conclusions regarding causality require perturbation of the system, e.g., perturbation of specific enhancers, binding sites, factors, or processes. Here genome-editing holds great promise for establishing functional links that can validate and refine hypotheses for selected sites that can then be used as examples for prediction of functional links elsewhere in the genome. The major disadvantage of the current technologies for genome-editing is that they are labor-extensive, and moving beyond a few sites to eventually do genome-editing at a genome-wide level will therefore require development of new technologies.

References

Aagaard MM, Siersbaek R, Mandrup S (2011) Molecular basis for gene-specific transactivation by nuclear receptors. Biochim Biophys Acta 1812(8):824–835. doi:10.1016/j.bbadis.2010.12.018

Agresti A, Scaffidi P, Riva A, Caiolfa VR, Bianchi ME (2005) GR and HMGB1 interact only within chromatin and influence each other's residence time. Mol Cell 18(1):109–121. doi:10.1016/j.molcel.2005.03.005

Aguilar-Arnal L, Sassone-Corsi P (2013) The circadian epigenome: how metabolism talks to chromatin remodeling. Curr Opin Cell Biol 25(2):170–176. doi:10.1016/j.ceb.2013.01.003

Altarejos JY, Montminy M (2011) CREB and the CRTC co-activators: sensors for hormonal and metabolic signals. Nat Rev Mol Cell Biol 12(3):141–151. doi:10.1038/nrm3072

Banfai B, Jia H, Khatun J, Wood E, Risk B, Gundling WE Jr, Kundaje A, Gunawardena HP, Yu Y, Xie L, Krajewski K, Strahl BD, Chen X, Bickel P, Giddings MC, Brown JB, Lipovich L (2012) Long noncoding RNAs are rarely translated in two human cell lines. Genome Res 22 (9):1646–1657. doi:10.1101/gr.134767.111

Barski A, Cuddapah S, Cui K, Roh TY, Schones DE, Wang Z, Wei G, Chepelev I, Zhao K (2007) High-resolution profiling of histone methylations in the human genome. Cell 129(4):823–837. doi:10.1016/j.cell.2007.05.009

Biddie SC, John S, Sabo PJ, Thurman RE, Johnson TA, Schiltz RL, Miranda TB, Sung MH, Trump S, Lightman SL, Vinson C, Stamatoyannopoulos JA, Hager GL (2011) Transcription factor AP1 potentiates chromatin accessibility and glucocorticoid receptor binding. Mol Cell 43(1):145–155. doi:10.1016/j.molcel.2011.06.016

Blecher-Gonen R, Barnett-Itzhaki Z, Jaitin D, Amann-Zalcenstein D, Lara-Astiaso D, Amit I (2013) High-throughput chromatin immunoprecipitation for genome-wide mapping of in vivo protein-DNA interactions and epigenomic states. Nat Protoc 8(3):539–554. doi:10.1038/nprot.2013.023

Boergesen M, Pedersen TA, Gross B, van Heeringen SJ, Hagenbeek D, Bindesboll C, Caron S, Lalloyer F, Steffensen KR, Nebb HI, Gustafsson JA, Stunnenberg HG, Staels B, Mandrup S (2012) Genome-wide profiling of liver X receptor, retinoid X receptor, and peroxisome proliferator-activated receptor alpha in mouse liver reveals extensive sharing of binding sites. Mol Cell Biol 32(4):852–867. doi:10.1128/MCB. 06175-11

Bogdanovic O, Fernandez-Minan A, Tena JJ, de la Calle-Mustienes E, Hidalgo C, van Kruysbergen I, van Heeringen SJ, Veenstra GJ, Gomez-Skarmeta JL (2012) Dynamics of enhancer chromatin signatures mark the transition from pluripotency to cell specification during embryogenesis. Genome Res 22(10):2043–2053. doi:10.1101/gr.134833.111

Bonn S, Zinzen RP, Perez-Gonzalez A, Riddell A, Gavin AC, Furlong EE (2012) Cell type-specific chromatin immunoprecipitation from multicellular complex samples using BiTS-ChIP. Nat Protoc 7(5):978–994. doi:10.1038/nprot.2012.049

Bosisio D, Marazzi I, Agresti A, Shimizu N, Bianchi ME, Natoli G (2006) A hyper-dynamic equilibrium between promoter-bound and nucleoplasmic dimers controls NF-kappaB-dependent gene activity. EMBO J 25(4):798–810. doi:10.1038/sj.emboj.7600977

Boyle AP, Davis S, Shulha HP, Meltzer P, Margulies EH, Weng Z, Furey TS, Crawford GE (2008) High-resolution mapping and characterization of open chromatin across the genome. Cell 132 (2):311–322. doi:10.1016/j.cell.2007.12.014

Brown JD, Lin CY, Duan Q, Griffin G, Federation AJ, Paranal RM, Bair S, Newton G, Lichtman AH, Kung AL, Yang T, Wang H, Luscinskas FW, Croce KJ, Bradner JE, Plutzky J (2014) NF-kappaB directs dynamic super enhancer formation in inflammation and atherogenesis. Mol Cell. doi:10.1016/j.molcel.2014.08.024

Buenrostro JD, Giresi PG, Zaba LC, Chang HY, Greenleaf WJ (2013) Transposition of native chromatin for fast and sensitive epigenomic profiling of open chromatin, DNA-binding proteins and nucleosome position. Nat Methods 10(12):1213–1218. doi:10.1038/nmeth.2688

Bugge A, Feng D, Everett LJ, Briggs ER, Mullican SE, Wang F, Jager J, Lazar MA (2012) Rev-erbalpha and Rev-erbbeta coordinately protect the circadian clock and normal metabolic function. Genes Dev 26(7):657–667. doi:10.1101/gad.186858.112

Carroll JS, Liu XS, Brodsky AS, Li W, Meyer CA, Szary AJ, Eeckhoute J, Shao W, Hestermann EV, Geistlinger TR, Fox EA, Silver PA, Brown M (2005) Chromosome-wide mapping of estrogen receptor binding reveals long-range regulation requiring the forkhead protein FoxA1. Cell 122(1):33–43. doi:10.1016/j.cell.2005.05.008

Catic A, Suh CY, Hill CT, Daheron L, Henkel T, Orford KW, Dombkowski DM, Liu T, Liu XS, Scadden DT (2013) Genome-wide map of nuclear protein degradation shows NCoR1 turnover as a key to mitochondrial gene regulation. Cell 155(6):1380–1395. doi:10.1016/j.cell.2013.11.016

Chen X, Xu H, Yuan P, Fang F, Huss M, Vega VB, Wong E, Orlov YL, Zhang W, Jiang J, Loh YH, Yeo HC, Yeo ZX, Narang V, Govindarajan KR, Leong B, Shahab A, Ruan Y, Bourque G, Sung WK, Clarke ND, Wei CL, Ng HH (2008) Integration of external signaling pathways with the core transcriptional network in embryonic stem cells. Cell 133(6):1106–1117. doi:10.1016/j.cell.2008.04.043

Chen J, Zhang Z, Li L, Chen BC, Revyakin A, Hajj B, Legant W, Dahan M, Lionnet T, Betzig E, Tjian R, Liu Z (2014) Single-molecule dynamics of enhanceosome assembly in embryonic stem cells. Cell 156(6):1274–1285. doi:10.1016/j.cell.2014.01.062

Cho H, Zhao X, Hatori M, Yu RT, Barish GD, Lam MT, Chong LW, DiTacchio L, Atkins AR, Glass CK, Liddle C, Auwerx J, Downes M, Panda S, Evans RM (2012) Regulation of circadian behaviour and metabolism by REV-ERB-alpha and REV-ERB-beta. Nature 485 (7396):123–127. doi:10.1038/nature11048

Chong HK, Infante AM, Seo YK, Jeon TI, Zhang Y, Edwards PA, Xie X, Osborne TF (2010) Genome-wide interrogation of hepatic FXR reveals an asymmetric IR-1 motif and synergy with LRH-1. Nucleic Acids Res 38(18):6007–6017. doi:10.1093/nar/gkq397

Consortium EP (2012) An integrated encyclopedia of DNA elements in the human genome. Nature 489(7414):57–74. doi:10.1038/nature11247

Core LJ, Waterfall JJ, Lis JT (2008) Nascent RNA sequencing reveals widespread pausing and divergent initiation at human promoters. Science 322(5909):1845–1848. doi:10.1126/science.1162228

Crawford GE, Holt IE, Whittle J, Webb BD, Tai D, Davis S, Margulies EH, Chen Y, Bernat JA, Ginsburg D, Zhou D, Luo S, Vasicek TJ, Daly MJ, Wolfsberg TG, Collins FS (2006) Genome-wide mapping of DNase hypersensitive sites using massively parallel signature sequencing (MPSS). Genome Res 16(1):123–131. doi:10.1101/gr.4074106

Creyghton MP, Cheng AW, Welstead GG, Kooistra T, Carey BW, Steine EJ, Hanna J, Lodato MA, Frampton GM, Sharp PA, Boyer LA, Young RA, Jaenisch R (2010) Histone H3K27ac separates active from poised enhancers and predicts developmental state. Proc Natl Acad Sci U S A 107(50):21931–21936. doi:10.1073/pnas.1016071107

Cui JY, Gunewardena SS, Rockwell CE, Klaassen CD (2010) ChIPing the cistrome of PXR in mouse liver. Nucleic Acids Res 38(22):7943–7963. doi:10.1093/nar/gkq654

de Wit E, de Laat W (2012) A decade of 3C technologies: insights into nuclear organization. Genes Dev 26(1):11–24. doi:10.1101/gad.179804.111

Deal RB, Henikoff S (2011) The INTACT method for cell type-specific gene expression and chromatin profiling in *Arabidopsis thaliana*. Nat Protoc 6(1):56–68. doi:10.1038/nprot.2010.175

Degner JF, Pai AA, Pique-Regi R, Veyrieras JB, Gaffney DJ, Pickrell JK, De Leon S, Michelini K, Lewellen N, Crawford GE, Stephens M, Gilad Y, Pritchard JK (2012) DNase I sensitivity QTLs are a major determinant of human expression variation. Nature 482(7385):390–394. doi:10.1038/nature10808

Derrien T, Johnson R, Bussotti G, Tanzer A, Djebali S, Tilgner H, Guernec G, Martin D, Merkel A, Knowles DG, Lagarde J, Veeravalli L, Ruan X, Ruan Y, Lassmann T, Carninci P, Brown JB, Lipovich L, Gonzalez JM, Thomas M, Davis CA, Shiekhattar R, Gingeras TR, Hubbard TJ, Notredame C, Harrow J, Guigo R (2012) The GENCODE v7 catalog of human long noncoding RNAs: analysis of their gene structure, evolution, and expression. Genome Res 22(9):1775–1789. doi:10.1101/gr.132159.111

Dryden NH, Broome LR, Dudbridge F, Johnson N, Orr N, Schoenfelder S, Nagano T, Andrews S, Wingett S, Kozarewa I, Assiotis I, Fenwick K, Maguire SL, Campbell J, Natrajan R, Lambros M, Perrakis E, Ashworth A, Fraser P, Fletcher O (2014) Unbiased analysis of potential targets of breast cancer susceptibility loci by Capture Hi-C. Genome Res. doi:10.1101/gr.175034.114

Evans RM, Mangelsdorf DJ (2014) Nuclear receptors, RXR, and the Big Bang. Cell 157(1):255–266. doi:10.1016/j.cell.2014.03.012

Everett LJ, Le Lay J, Lukovac S, Bernstein D, Steger DJ, Lazar MA, Kaestner KH (2013) Integrative genomic analysis of CREB defines a critical role for transcription factor networks in mediating the fed/fasted switch in liver. BMC Genomics 14:337. doi:10.1186/1471-2164-14-337

Felsenfeld G, Groudine M (2003) Controlling the double helix. Nature 421(6921):448–453. doi:10.1038/nature01411

Feng D, Lazar MA (2012) Clocks, metabolism, and the epigenome. Mol Cell 47(2):158–167. doi:10.1016/j.molcel.2012.06.026

Feng D, Liu T, Sun Z, Bugge A, Mullican SE, Alenghat T, Liu XS, Lazar MA (2011) A circadian rhythm orchestrated by histone deacetylase 3 controls hepatic lipid metabolism. Science 331(6022):1315–1319. doi:10.1126/science.1198125

Foretz M, Guichard C, Ferre P, Foufelle F (1999) Sterol regulatory element binding protein-1c is a major mediator of insulin action on the hepatic expression of glucokinase and lipogenesis-related genes. Proc Natl Acad Sci U S A 96(22):12737–12742

Fullwood MJ, Liu MH, Pan YF, Liu J, Xu H, Mohamed YB, Orlov YL, Velkov S, Ho A, Mei PH, Chew EG, Huang PY, Welboren WJ, Han Y, Ooi HS, Ariyaratne PN, Vega VB, Luo Y, Tan PY, Choy PY, Wansa KD, Zhao B, Lim KS, Leow SC, Yow JS, Joseph R, Li H, Desai KV, Thomsen JS, Lee YK, Karuturi RK, Herve T, Bourque G, Stunnenberg HG, Ruan X, Cacheux-

Rataboul V, Sung WK, Liu ET, Wei CL, Cheung E, Ruan Y (2009) An oestrogen-receptor-alpha-bound human chromatin interactome. Nature 462(7269):58–64. doi:10.1038/nature08497

Galas DJ, Schmitz A (1978) DNAse footprinting: a simple method for the detection of protein-DNA binding specificity. Nucleic Acids Res 5(9):3157–3170

Gay L, Karfilis KV, Miller MR, Doe CQ, Stankunas K (2014) Applying thiouracil tagging to mouse transcriptome analysis. Nat Protoc 9(2):410–420. doi:10.1038/nprot.2014.023

Gebhardt JC, Suter DM, Roy R, Zhao ZW, Chapman AR, Basu S, Maniatis T, Xie XS (2013) Single-molecule imaging of transcription factor binding to DNA in live mammalian cells. Nat Methods 10(5):421–426. doi:10.1038/nmeth.2411

Gerstein MB, Kundaje A, Hariharan M, Landt SG, Yan KK, Cheng C, Mu XJ, Khurana E, Rozowsky J, Alexander R, Min R, Alves P, Abyzov A, Addleman N, Bhardwaj N, Boyle AP, Cayting P, Charos A, Chen DZ, Cheng Y, Clarke D, Eastman C, Euskirchen G, Frietze S, Fu Y, Gertz J, Grubert F, Harmanci A, Jain P, Kasowski M, Lacroute P, Leng J, Lian J, Monahan H, O'Geen H, Ouyang Z, Partridge EC, Patacsil D, Pauli F, Raha D, Ramirez L, Reddy TE, Reed B, Shi M, Slifer T, Wang J, Wu L, Yang X, Yip KY, Zilberman-Schapira G, Batzoglou S, Sidow A, Farnham PJ, Myers RM, Weissman SM, Snyder M (2012) Architecture of the human regulatory network derived from ENCODE data. Nature 489(7414):91–100. doi:10.1038/nature11245

Gertz J, Savic D, Varley KE, Partridge EC, Safi A, Jain P, Cooper GM, Reddy TE, Crawford GE, Myers RM (2013) Distinct properties of cell-type-specific and shared transcription factor binding sites. Mol Cell 52(1):25–36. doi:10.1016/j.molcel.2013.08.037

Gilardi F, Migliavacca E, Naldi A, Baruchet M, Canella D, Le Martelot G, Guex N, Desvergne B, Cycli XC (2014) Genome-wide analysis of SREBP1 activity around the clock reveals its combined dependency on nutrient and circadian signals. PLoS Genet 10(3):e1004155. doi:10.1371/journal.pgen.1004155

Giresi PG, Kim J, McDaniell RM, Iyer VR, Lieb JD (2007) FAIRE (Formaldehyde-Assisted Isolation of Regulatory Elements) isolates active regulatory elements from human chromatin. Genome Res 17(6):877–885. doi:10.1101/gr.5533506

Green CD, Jump DB, Olson LK (2009) Elevated insulin secretion from liver X receptor-activated pancreatic beta-cells involves increased de novo lipid synthesis and triacylglyceride turnover. Endocrinology 150(6):2637–2645. doi:10.1210/en.2008-1039

Grontved L, John S, Baek S, Liu Y, Buckley JR, Vinson C, Aguilera G, Hager GL (2013) C/EBP maintains chromatin accessibility in liver and facilitates glucocorticoid receptor recruitment to steroid response elements. EMBO J 32(11):1568–1583. doi:10.1038/emboj.2013.106

Gross DS, Garrard WT (1988) Nuclease hypersensitive sites in chromatin. Annu Rev Biochem 57:159–197. doi:10.1146/annurev.bi.57.070188.001111

Gut P, Verdin E (2013) The nexus of chromatin regulation and intermediary metabolism. Nature 502(7472):489–498. doi:10.1038/nature12752

Hakim O, Sung MH, Voss TC, Splinter E, John S, Sabo PJ, Thurman RE, Stamatoyannopoulos JA, de Laat W, Hager GL (2011) Diverse gene reprogramming events occur in the same spatial clusters of distal regulatory elements. Genome Res 21(5):697–706. doi:10.1101/gr.111153.110

Hargreaves DC, Crabtree GR (2011) ATP-dependent chromatin remodeling: genetics, genomics and mechanisms. Cell Res 21(3):396–420. doi:10.1038/cr.2011.32

Harzer H, Berger C, Conder R, Schmauss G, Knoblich JA (2013) FACS purification of Drosophila larval neuroblasts for next-generation sequencing. Nat Protoc 8(6):1088–1099. doi:10.1038/nprot.2013.062

He A, Kong SW, Ma Q, Pu WT (2011) Co-occupancy by multiple cardiac transcription factors identifies transcriptional enhancers active in heart. Proc Natl Acad Sci U S A 108 (14):5632–5637. doi:10.1073/pnas.1016959108

He HH, Meyer CA, Chen MW, Jordan VC, Brown M, Liu XS (2012) Differential DNase I hypersensitivity reveals factor-dependent chromatin dynamics. Genome Res 22 (6):1015–1025. doi:10.1101/gr.133280.111

He Y, Gong L, Fang Y, Zhan Q, Liu HX, Lu Y, Guo GL, Lehman-McKeeman L, Fang J, Wan YJ (2013) The role of retinoic acid in hepatic lipid homeostasis defined by genomic binding and transcriptome profiling. BMC Genomics 14:575. doi:10.1186/1471-2164-14-575

He HH, Meyer CA, Hu SS, Chen MW, Zang C, Liu Y, Rao PK, Fei T, Xu H, Long H, Liu XS, Brown M (2014) Refined DNase-seq protocol and data analysis reveals intrinsic bias in transcription factor footprint identification. Nat Methods 11(1):73–78. doi:10.1038/nmeth.2762

Hesselberth JR, Chen X, Zhang Z, Sabo PJ, Sandstrom R, Reynolds AP, Thurman RE, Neph S, Kuehn MS, Noble WS, Fields S, Stamatoyannopoulos JA (2009) Global mapping of protein-DNA interactions in vivo by digital genomic footprinting. Nat Methods 6(4):283–289. doi:10.1038/nmeth.1313

Hnisz D, Abraham BJ, Lee TI, Lau A, Saint-Andre V, Sigova AA, Hoke HA, Young RA (2013) Super-enhancers in the control of cell identity and disease. Cell 155(4):934–947. doi:10.1016/j.cell.2013.09.053

Hong SH, Ahmadian M, Yu RT, Atkins AR, Downes M, Evans RM (2014) Nuclear receptors and metabolism: from feast to famine. Diabetologia 57(5):860–867. doi:10.1007/s00125-014-3209-9

Howald C, Tanzer A, Chrast J, Kokocinski F, Derrien T, Walters N, Gonzalez JM, Frankish A, Aken BL, Hourlier T, Vogel JH, White S, Searle S, Harrow J, Hubbard TJ, Guigo R, Reymond A (2012) Combining RT-PCR-seq and RNA-seq to catalog all genic elements encoded in the human genome. Genome Res 22(9):1698–1710. doi:10.1101/gr.134478.111

Hurtado A, Holmes KA, Ross-Innes CS, Schmidt D, Carroll JS (2011) FOXA1 is a key determinant of estrogen receptor function and endocrine response. Nat Genet 43(1):27–33. doi:10.1038/ng.730

Iwafuchi-Doi M, Zaret KS (2014) Pioneer transcription factors in cell reprogramming. Genes Dev 28(24):2679–2692. doi:10.1101/gad.253443.114

Jackson V (1978) Studies on histone organization in the nucleosome using formaldehyde as a reversible cross-linking agent. Cell 15(3):945–954

Jin F, Li Y, Dixon JR, Selvaraj S, Ye Z, Lee AY, Yen CA, Schmitt AD, Espinoza CA, Ren B (2013) A high-resolution map of the three-dimensional chromatin interactome in human cells. Nature 503(7475):290–294. doi:10.1038/nature12644

John S, Sabo PJ, Thurman RE, Sung MH, Biddie SC, Johnson TA, Hager GL, Stamatoyannopoulos JA (2011) Chromatin accessibility pre-determines glucocorticoid receptor binding patterns. Nat Genet 43(3):264–268. doi:10.1038/ng.759

Johnson TA, Elbi C, Parekh BS, Hager GL, John S (2008) Chromatin remodeling complexes interact dynamically with a glucocorticoid receptor-regulated promoter. Mol Biol Cell 19(8):3308–3322. doi:10.1091/mbc.E08-02-0123

Kaikkonen MU, Spann NJ, Heinz S, Romanoski CE, Allison KA, Stender JD, Chun HB, Tough DF, Prinjha RK, Benner C, Glass CK (2013) Remodeling of the enhancer landscape during macrophage activation is coupled to enhancer transcription. Mol Cell 51(3):310–325. doi:10.1016/j.molcel.2013.07.010

Karpova TS, Chen TY, Sprague BL, McNally JG (2004) Dynamic interactions of a transcription factor with DNA are accelerated by a chromatin remodeller. EMBO Rep 5(11):1064–1070. doi:10.1038/sj.embor.7400281

Karpova TS, Kim MJ, Spriet C, Nalley K, Stasevich TJ, Kherrouche Z, Heliot L, McNally JG (2008) Concurrent fast and slow cycling of a transcriptional activator at an endogenous promoter. Science 319(5862):466–469. doi:10.1126/science.1150559

Kharchenko PV, Alekseyenko AA, Schwartz YB, Minoda A, Riddle NC, Ernst J, Sabo PJ, Larschan E, Gorchakov AA, Gu T, Linder-Basso D, Plachetka A, Shanower G, Tolstorukov MY, Luquette LJ, Xi R, Jung YL, Park RW, Bishop EP, Canfield TK, Sandstrom R, Thurman RE, MacAlpine DM, Stamatoyannopoulos JA, Kellis M, Elgin SC, Kuroda MI, Pirrotta V, Karpen GH, Park PJ (2011) Comprehensive analysis of the chromatin landscape in *Drosophila melanogaster*. Nature 471(7339):480–485. doi:10.1038/nature09725

Kieffer-Kwon KR, Tang Z, Mathe E, Qian J, Sung MH, Li G, Resch W, Baek S, Pruett N, Grontved L, Vian L, Nelson S, Zare H, Hakim O, Reyon D, Yamane A, Nakahashi H, Kovalchuk AL, Zou J, Joung JK, Sartorelli V, Wei CL, Ruan X, Hager GL, Ruan Y, Casellas R (2013) Interactome maps of mouse gene regulatory domains reveal basic principles of transcriptional regulation. Cell 155(7):1507–1520. doi:10.1016/j.cell.2013.11.039

Kim TK, Hemberg M, Gray JM, Costa AM, Bear DM, Wu J, Harmin DA, Laptewicz M, Barbara-Haley K, Kuersten S, Markenscoff-Papadimitriou E, Kuhl D, Bito H, Worley PF, Kreiman G, Greenberg ME (2010) Widespread transcription at neuronal activity-regulated enhancers. Nature 465(7295):182–187. doi:10.1038/nature09033

Kohsaka A, Laposky AD, Ramsey KM, Estrada C, Joshu C, Kobayashi Y, Turek FW, Bass J (2007) High-fat diet disrupts behavioral and molecular circadian rhythms in mice. Cell Metab 6 (5):414–421. doi:10.1016/j.cmet.2007.09.006

Koike N, Yoo SH, Huang HC, Kumar V, Lee C, Kim TK, Takahashi JS (2012) Transcriptional architecture and chromatin landscape of the core circadian clock in mammals. Science 338 (6105):349–354. doi:10.1126/science.1226339

Lam MT, Cho H, Lesch HP, Gosselin D, Heinz S, Tanaka-Oishi Y, Benner C, Kaikkonen MU, Kim AS, Kosaka M, Lee CY, Watt A, Grossman TR, Rosenfeld MG, Evans RM, Glass CK (2013) Rev-Erbs repress macrophage gene expression by inhibiting enhancer-directed transcription. Nature 498(7455):511–515. doi:10.1038/nature12209

Landt SG, Marinov GK, Kundaje A, Kheradpour P, Pauli F, Batzoglou S, Bernstein BE, Bickel P, Brown JB, Cayting P, Chen Y, DeSalvo G, Epstein C, Fisher-Aylor KI, Euskirchen G, Gerstein M, Gertz J, Hartemink AJ, Hoffman MM, Iyer VR, Jung YL, Karmakar S, Kellis M, Kharchenko PV, Li Q, Liu T, Liu XS, Ma L, Milosavljevic A, Myers RM, Park PJ, Pazin MJ, Perry MD, Raha D, Reddy TE, Rozowsky J, Shoresh N, Sidow A, Slattery M, Stamatoyannopoulos JA, Tolstorukov MY, White KP, Xi S, Farnham PJ, Lieb JD, Wold BJ, Snyder M (2012) ChIP-seq guidelines and practices of the ENCODE and modENCODE consortia. Genome Res 22(9):1813–1831. doi:10.1101/gr.136184.111

Lara-Astiaso D, Weiner A, Lorenzo-Vivas E, Zaretsky I, Jaitin DA, David E, Keren-Shaul H, Mildner A, Winter D, Jung S, Friedman N, Amit I (2014) Immunogenetics. Chromatin state dynamics during blood formation. Science 345(6199):943–949. doi:10.1126/science.1256271

Le Dily F, Bau D, Pohl A, Vicent GP, Serra F, Soronellas D, Castellano G, Wright RH, Ballare C, Filion G, Marti-Renom MA, Beato M (2014) Distinct structural transitions of chromatin topological domains correlate with coordinated hormone-induced gene regulation. Genes Dev 28(19):2151–2162. doi:10.1101/gad.241422.114

Lefterova MI, Zhang Y, Steger DJ, Schupp M, Schug J, Cristancho A, Feng D, Zhuo D, Stoeckert CJ Jr, Liu XS, Lazar MA (2008) PPARgamma and C/EBP factors orchestrate adipocyte biology via adjacent binding on a genome-wide scale. Genes Dev 22(21):2941–2952. doi:10.1101/gad.1709008

Lefterova MI, Haakonsson AK, Lazar MA, Mandrup S (2014) PPARgamma and the global map of adipogenesis and beyond. Trends Endocrinol Metab 25(6):293–302. doi:10.1016/j.tem.2014.04.001

Leung A, Parks BW, Du J, Trac C, Setten R, Chen Y, Brown K, Lusis AJ, Natarajan R, Schones DE (2014) Open chromatin profiling in mice livers reveals unique chromatin variations induced by high fat diet. J Biol Chem 289(34):23557–23567. doi:10.1074/jbc.M114.581439

Li G, Ruan X, Auerbach RK, Sandhu KS, Zheng M, Wang P, Poh HM, Goh Y, Lim J, Zhang J, Sim HS, Peh SQ, Mulawadi FH, Ong CT, Orlov YL, Hong S, Zhang Z, Landt S, Raha D, Euskirchen G, Wei CL, Ge W, Wang H, Davis C, Fisher-Aylor KI, Mortazavi A, Gerstein M, Gingeras T, Wold B, Sun Y, Fullwood MJ, Cheung E, Liu E, Sung WK, Snyder M, Ruan Y (2012) Extensive promoter-centered chromatin interactions provide a topological basis for transcription regulation. Cell 148(1–2):84–98. doi:10.1016/j.cell.2011.12.014

Li W, Notani D, Ma Q, Tanasa B, Nunez E, Chen AY, Merkurjev D, Zhang J, Ohgi K, Song X, Oh S, Kim HS, Glass CK, Rosenfeld MG (2013) Functional roles of enhancer RNAs for

oestrogen-dependent transcriptional activation. Nature 498(7455):516–520. doi:10.1038/nature12210

Lickwar CR, Mueller F, Hanlon SE, McNally JG, Lieb JD (2012) Genome-wide protein-DNA binding dynamics suggest a molecular clutch for transcription factor function. Nature 484 (7393):251–255. doi:10.1038/nature10985

Lieberman-Aiden E, van Berkum NL, Williams L, Imakaev M, Ragoczy T, Telling A, Amit I, Lajoie BR, Sabo PJ, Dorschner MO, Sandstrom R, Bernstein B, Bender MA, Groudine M, Gnirke A, Stamatoyannopoulos J, Mirny LA, Lander ES, Dekker J (2009) Comprehensive mapping of long-range interactions reveals folding principles of the human genome. Science 326(5950):289–293. doi:10.1126/science.1181369

Lin YC, Jhunjhunwala S, Benner C, Heinz S, Welinder E, Mansson R, Sigvardsson M, Hagman J, Espinoza CA, Dutkowski J, Ideker T, Glass CK, Murre C (2010) A global network of transcription factors, involving E2A, EBF1 and Foxo1, that orchestrates B cell fate. Nat Immunol 11(7):635–643. doi:10.1038/ni.1891

Ling G, Sugathan A, Mazor T, Fraenkel E, Waxman DJ (2010) Unbiased, genome-wide in vivo mapping of transcriptional regulatory elements reveals sex differences in chromatin structure associated with sex-specific liver gene expression. Mol Cell Biol 30(23):5531–5544. doi:10.1128/MCB. 00601-10

Link N, Kurtz P, O'Neal M, Garcia-Hughes G, Abrams JM (2013) A p53 enhancer region regulates target genes through chromatin conformations in cis and in trans. Genes Dev 27 (22):2433–2438. doi:10.1101/gad.225565.113

Lister R, O'Malley RC, Tonti-Filippini J, Gregory BD, Berry CC, Millar AH, Ecker JR (2008) Highly integrated single-base resolution maps of the epigenome in Arabidopsis. Cell 133 (3):523–536. doi:10.1016/j.cell.2008.03.029

Lo KA, Labadorf A, Kennedy NJ, Han MS, Yap YS, Matthews B, Xin X, Sun L, Davis RJ, Lodish HF, Fraenkel E (2013) Analysis of in vitro insulin-resistance models and their physiological relevance to in vivo diet-induced adipose insulin resistance. Cell Rep 5(1):259–270. doi:10.1016/j.celrep.2013.08.039

Loft A, Forss I, Siersbaek MS, Schmidt SF, Larsen AS, Madsen JG, Pisani DF, Nielsen R, Aagaard MM, Mathison A, Neville MJ, Urrutia R, Karpe F, Amri EZ, Mandrup S (2015) Browning of human adipocytes requires KLF11 and reprogramming of PPARgamma superenhancers. Genes Dev 29(1):7–22. doi:10.1101/gad.250829.114

Loven J, Hoke HA, Lin CY, Lau A, Orlando DA, Vakoc CR, Bradner JE, Lee TI, Young RA (2013) Selective inhibition of tumor oncogenes by disruption of super-enhancers. Cell 153 (2):320–334. doi:10.1016/j.cell.2013.03.036

Madsen MS, Siersbaek R, Boergesen M, Nielsen R, Mandrup S (2014) Peroxisome proliferator-activated receptor gamma and C/EBPalpha synergistically activate key metabolic adipocyte genes by assisted loading. Mol Cell Biol 34(6):939–954. doi:10.1128/MCB. 01344-13

Madsen JG, Schmidt SF, Larsen BD, Loft A, Nielsen R, Mandrup S (2015) iRNA-seq: computational method for genome-wide assessment of acute transcriptional regulation from total RNA-seq data. Nucl Acids Res. doi:10.1093/nar/gku1365

McKnight JN, Jenkins KR, Nodelman IM, Escobar T, Bowman GD (2011) Extranucleosomal DNA binding directs nucleosome sliding by Chd1. Mol Cell Biol 31(23):4746–4759. doi:10.1128/MCB. 05735-11

McNally JG, Muller WG, Walker D, Wolford R, Hager GL (2000) The glucocorticoid receptor: rapid exchange with regulatory sites in living cells. Science 287(5456):1262–1265

Melo CA, Drost J, Wijchers PJ, van de Werken H, de Wit E, Oude Vrielink JA, Elkon R, Melo SA, Leveille N, Kalluri R, de Laat W, Agami R (2013) eRNAs are required for p53-dependent enhancer activity and gene transcription. Mol Cell 49(3):524–535. doi:10.1016/j.molcel.2012.11.021

Menet JS, Pescatore S, Rosbash M (2014) CLOCK:BMAL1 is a pioneer-like transcription factor. Genes Dev 28(1):8–13. doi:10.1101/gad.228536.113

Mikkelsen TS, Ku M, Jaffe DB, Issac B, Lieberman E, Giannoukos G, Alvarez P, Brockman W, Kim TK, Koche RP, Lee W, Mendenhall E, O'Donovan A, Presser A, Russ C, Xie X, Meissner A, Wernig M, Jaenisch R, Nusbaum C, Lander ES, Bernstein BE (2007) Genome-wide maps of chromatin state in pluripotent and lineage-committed cells. Nature 448 (7153):553–560. doi:10.1038/nature06008

Mikkelsen TS, Xu Z, Zhang X, Wang L, Gimble JM, Lander ES, Rosen ED (2010) Comparative epigenomic analysis of murine and human adipogenesis. Cell 143(1):156–169. doi:10.1016/j.cell.2010.09.006

Montavon T, Le Garrec JF, Kerszberg M, Duboule D (2008) Modeling Hox gene regulation in digits: reverse collinearity and the molecular origin of thumbness. Genes Dev 22(3):346–359. doi:10.1101/gad.1631708

Mousavi K, Zare H, Dell'orso S, Grontved L, Gutierrez-Cruz G, Derfoul A, Hager GL, Sartorelli V (2013) eRNAs promote transcription by establishing chromatin accessibility at defined genomic loci. Mol Cell 51(5):606–617. doi:10.1016/j.molcel.2013.07.022

Mueller F, Wach P, McNally JG (2008) Evidence for a common mode of transcription factor interaction with chromatin as revealed by improved quantitative fluorescence recovery after photobleaching. Biophys J 94(8):3323–3339. doi:10.1529/biophysj.107.123182

Nagaich AK, Walker DA, Wolford R, Hager GL (2004) Rapid periodic binding and displacement of the glucocorticoid receptor during chromatin remodeling. Mol Cell 14(2):163–174

Nagano T, Lubling Y, Stevens TJ, Schoenfelder S, Yaffe E, Dean W, Laue ED, Tanay A, Fraser P (2013) Single-cell Hi-C reveals cell-to-cell variability in chromosome structure. Nature 502 (7469):59–64. doi:10.1038/nature12593

Nielsen R, Pedersen TA, Hagenbeek D, Moulos P, Siersbaek R, Megens E, Denissov S, Borgesen M, Francoijs KJ, Mandrup S, Stunnenberg HG (2008) Genome-wide profiling of PPARgamma:RXR and RNA polymerase II occupancy reveals temporal activation of distinct metabolic pathways and changes in RXR dimer composition during adipogenesis. Genes Dev 22(21):2953–2967. doi:10.1101/gad.501108

Parker SC, Stitzel ML, Taylor DL, Orozco JM, Erdos MR, Akiyama JA, van Bueren KL, Chines PS, Narisu N, Program NCS, Black BL, Visel A, Pennacchio LA, Collins FS, National Institutes of Health Intramural Sequencing Center Comparative Sequencing Program A, Authors NCSP (2013) Chromatin stretch enhancer states drive cell-specific gene regulation and harbor human disease risk variants. Proc Natl Acad Sci U S A 110(44):17921–17926. doi:10.1073/pnas.1317023110

Poulsen L, Siersbaek M, Mandrup S (2012) PPARs: fatty acid sensors controlling metabolism. Semin Cell Dev Biol 23(6):631–639. doi:10.1016/j.semcdb.2012.01.003

Rabani M, Levin JZ, Fan L, Adiconis X, Raychowdhury R, Garber M, Gnirke A, Nusbaum C, Hacohen N, Friedman N, Amit I, Regev A (2011) Metabolic labeling of RNA uncovers principles of RNA production and degradation dynamics in mammalian cells. Nat Biotechnol 29(5):436–442. doi:10.1038/nbt.1861

Rada-Iglesias A, Bajpai R, Swigut T, Brugmann SA, Flynn RA, Wysocka J (2011) A unique chromatin signature uncovers early developmental enhancers in humans. Nature 470 (7333):279–283. doi:10.1038/nature09692

Rajakumari S, Wu J, Ishibashi J, Lim HW, Giang AH, Won KJ, Reed RR, Seale P (2013) EBF2 determines and maintains brown adipocyte identity. Cell Metab 17(4):562–574. doi:10.1016/j.cmet.2013.01.015

Raser JM, O'Shea EK (2004) Control of stochasticity in eukaryotic gene expression. Science 304 (5678):1811–1814. doi:10.1126/science.1098641

Robertson G, Hirst M, Bainbridge M, Bilenky M, Zhao Y, Zeng T, Euskirchen G, Bernier B, Varhol R, Delaney A, Thiessen N, Griffith OL, He A, Marra M, Snyder M, Jones S (2007) Genome-wide profiles of STAT1 DNA association using chromatin immunoprecipitation and massively parallel sequencing. Nat Methods 4(8):651–657. doi:10.1038/nmeth1068

Sanyal A, Lajoie BR, Jain G, Dekker J (2012) The long-range interaction landscape of gene promoters. Nature 489(7414):109–113. doi:10.1038/nature11279

Schmidt D, Wilson MD, Ballester B, Schwalie PC, Brown GD, Marshall A, Kutter C, Watt S, Martinez-Jimenez CP, Mackay S, Talianidis I, Flicek P, Odom DT (2010) Five-vertebrate ChIP-seq reveals the evolutionary dynamics of transcription factor binding. Science 328 (5981):1036–1040. doi:10.1126/science.1186176

Schmidt SF, Jorgensen M, Chen Y, Nielsen R, Sandelin A, Mandrup S (2011) Cross species comparison of C/EBPalpha and PPARgamma profiles in mouse and human adipocytes reveals interdependent retention of binding sites. BMC Genomics 12:152. doi:10.1186/1471-2164-12-152

Schoenfelder S, Furlan-Magaril M, Mifsud B, Tavares-Cadete F, Sugar R, Javierre BM, Nagano T, Katsman Y, Sakthidevi M, Wingett SW, Dimitrova E, Dimond A, Edelman LB, Elderkin S, Tabbada K, Darbo E, Andrews S, Herman B, Higgs A, LeProust E, Osborne CS, Mitchell JA, Luscombe NM, Fraser P (2015) The pluripotent regulatory circuitry connecting promoters to their long-range interacting elements. Genome Res 25(4):582–597. doi:10.1101/gr.185272.114

Sharp ZD, Mancini MG, Hinojos CA, Dai F, Berno V, Szafran AT, Smith KP, Lele TP, Ingber DE, Mancini MA (2006) Estrogen-receptor-alpha exchange and chromatin dynamics are ligand- and domain-dependent. J Cell Sci 119(Pt 19):4101–4116. doi:10.1242/jcs.03161

Sherwood RI, Hashimoto T, O'Donnell CW, Lewis S, Barkal AA, van Hoff JP, Karun V, Jaakkola T, Gifford DK (2014) Discovery of directional and nondirectional pioneer transcription factors by modeling DNase profile magnitude and shape. Nat Biotechnol 32(2):171–178. doi:10.1038/nbt.2798

Siersbaek R, Mandrup S (2011) Transcriptional networks controlling adipocyte differentiation. Cold Spring Harb Symp Quant Biol 76:247–255. doi:10.1101/sqb.2011.76.010512

Siersbaek R, Nielsen R, John S, Sung MH, Baek S, Loft A, Hager GL, Mandrup S (2011) Extensive chromatin remodelling and establishment of transcription factor 'hotspots' during early adipogenesis. EMBO J 30(8):1459–1472. doi:10.1038/emboj.2011.65

Siersbaek MS, Loft A, Aagaard MM, Nielsen R, Schmidt SF, Petrovic N, Nedergaard J, Mandrup S (2012) Genome-wide profiling of peroxisome proliferator-activated receptor gamma in primary epididymal, inguinal, and brown adipocytes reveals depot-selective binding correlated with gene expression. Mol Cell Biol 32(17):3452–3463. doi:10.1128/MCB. 00526-12

Siersbaek R, Baek S, Rabiee A, Nielsen R, Traynor S, Clark N, Sandelin A, Jensen ON, Sung MH, Hager GL, Mandrup S (2014a) Molecular architecture of transcription factor hotspots in early adipogenesis. Cell Rep 7(5):1434–1442. doi:10.1016/j.celrep.2014.04.043

Siersbaek R, Rabiee A, Nielsen R, Sidoli S, Traynor S, Loft A, La Cour Poulsen L, Rogowska-Wrzesinska A, Jensen ON, Mandrup S (2014b) Transcription factor cooperativity in early adipogenic hotspots and super-enhancers. Cell Rep 7(5):1443–1455. doi:10.1016/j.celrep.2014.04.042

Stashi E, Lanz RB, Mao J, Michailidis G, Zhu B, Kettner NM, Putluri N, Reineke EL, Reineke LC, Dasgupta S, Dean A, Stevenson CR, Sivasubramanian N, Sreekumar A, Demayo F, York B, Fu L, O'Malley BW (2014) SRC-2 is an essential coactivator for orchestrating metabolism and circadian rhythm. Cell Rep 6(4):633–645. doi:10.1016/j.celrep.2014.01.027

Stavreva DA, Muller WG, Hager GL, Smith CL, McNally JG (2004) Rapid glucocorticoid receptor exchange at a promoter is coupled to transcription and regulated by chaperones and proteasomes. Mol Cell Biol 24(7):2682–2697

Stefflova K, Thybert D, Wilson MD, Streeter I, Aleksic J, Karagianni P, Brazma A, Adams DJ, Talianidis I, Marioni JC, Flicek P, Odom DT (2013) Cooperativity and rapid evolution of cobound transcription factors in closely related mammals. Cell 154(3):530–540. doi:10.1016/j.cell.2013.07.007

Steger DJ, Grant GR, Schupp M, Tomaru T, Lefterova MI, Schug J, Manduchi E, Stoeckert CJ Jr, Lazar MA (2010) Propagation of adipogenic signals through an epigenomic transition state. Genes Dev 24(10):1035–1044. doi:10.1101/gad.1907110

Stenoien DL, Patel K, Mancini MG, Dutertre M, Smith CL, O'Malley BW, Mancini MA (2001) FRAP reveals that mobility of oestrogen receptor-alpha is ligand- and proteasome-dependent. Nat Cell Biol 3(1):15–23. doi:10.1038/35050515

Stergachis AB, Neph S, Reynolds A, Humbert R, Miller B, Paige SL, Vernot B, Cheng JB, Thurman RE, Sandstrom R, Haugen E, Heimfeld S, Murry CE, Akey JM, Stamatoyannopoulos JA (2013) Developmental fate and cellular maturity encoded in human regulatory DNA landscapes. Cell 154(4):888–903. doi:10.1016/j.cell.2013.07.020

Sung MH, Guertin MJ, Baek S, Hager GL (2014) DNase footprint signatures are dictated by factor dynamics and DNA sequence. Mol Cell. doi:10.1016/j.molcel.2014.08.016

Tang F, Lao K, Surani MA (2011) Development and applications of single-cell transcriptome analysis. Nat Methods 8(4 Suppl):S6–S11. doi:10.1038/nmeth.1557

Thomas AM, Hart SN, Kong B, Fang J, Zhong XB, Guo GL (2010) Genome-wide tissue-specific farnesoid X receptor binding in mouse liver and intestine. Hepatology 51(4):1410–1419. doi:10.1002/hep.23450

Thomas S, Li XY, Sabo PJ, Sandstrom R, Thurman RE, Canfield TK, Giste E, Fisher W, Hammonds A, Celniker SE, Biggin MD, Stamatoyannopoulos JA (2011) Dynamic reprogramming of chromatin accessibility during Drosophila embryo development. Genome Biol 12(5):R43. doi:10.1186/gb-2011-12-5-r43

Thurman RE, Rynes E, Humbert R, Vierstra J, Maurano MT, Haugen E, Sheffield NC, Stergachis AB, Wang H, Vernot B, Garg K, John S, Sandstrom R, Bates D, Boatman L, Canfield TK, Diegel M, Dunn D, Ebersol AK, Frum T, Giste E, Johnson AK, Johnson EM, Kutyavin T, Lajoie B, Lee BK, Lee K, London D, Lotakis D, Neph S, Neri F, Nguyen ED, Qu H, Reynolds AP, Roach V, Safi A, Sanchez ME, Sanyal A, Shafer A, Simon JM, Song L, Vong S, Weaver M, Yan Y, Zhang Z, Zhang Z, Lenhard B, Tewari M, Dorschner MO, Hansen RS, Navas PA, Stamatoyannopoulos G, Iyer VR, Lieb JD, Sunyaev SR, Akey JM, Sabo PJ, Kaul R, Furey TS, Dekker J, Crawford GE, Stamatoyannopoulos JA (2012) The accessible chromatin landscape of the human genome. Nature 489(7414):75–82. doi:10.1038/nature11232

Tsujimura T, Hosoya T, Kawamura S (2010) A single enhancer regulating the differential expression of duplicated red-sensitive opsin genes in zebrafish. PLoS Genet 6(12):e1001245. doi:10.1371/journal.pgen.1001245

Uhlenhaut NH, Barish GD, Yu RT, Downes M, Karunasiri M, Liddle C, Schwalie P, Hubner N, Evans RM (2013) Insights into negative regulation by the glucocorticoid receptor from genome-wide profiling of inflammatory cistromes. Mol Cell 49(1):158–171. doi:10.1016/j.molcel.2012.10.013

Vollmers C, Gill S, DiTacchio L, Pulivarthy SR, Le HD, Panda S (2009) Time of feeding and the intrinsic circadian clock drive rhythms in hepatic gene expression. Proc Natl Acad Sci U S A 106(50):21453–21458. doi:10.1073/pnas.0909591106

Vollmers C, Schmitz RJ, Nathanson J, Yeo G, Ecker JR, Panda S (2012) Circadian oscillations of protein-coding and regulatory RNAs in a highly dynamic mammalian liver epigenome. Cell Metab 16(6):833–845. doi:10.1016/j.cmet.2012.11.004

Voss TC, Hager GL (2014) Dynamic regulation of transcriptional states by chromatin and transcription factors. Nat Rev Genet 15(2):69–81. doi:10.1038/nrg3623

Voss TC, Schiltz RL, Sung MH, Yen PM, Stamatoyannopoulos JA, Biddie SC, Johnson TA, Miranda TB, John S, Hager GL (2011) Dynamic exchange at regulatory elements during chromatin remodeling underlies assisted loading mechanism. Cell 146(4):544–554. doi:10.1016/j.cell.2011.07.006

Waki H, Nakamura M, Yamauchi T, Wakabayashi K, Yu J, Hirose-Yotsuya L, Take K, Sun W, Iwabu M, Okada-Iwabu M, Fujita T, Aoyama T, Tsutsumi S, Ueki K, Kodama T, Sakai J, Aburatani H, Kadowaki T (2011) Global mapping of cell type-specific open chromatin by FAIRE-seq reveals the regulatory role of the NFI family in adipocyte differentiation. PLoS Genet 7(10):e1002311. doi:10.1371/journal.pgen.1002311

Wang Z, Gerstein M, Snyder M (2009) RNA-Seq: a revolutionary tool for transcriptomics. Nat Rev Genet 10(1):57–63. doi:10.1038/nrg2484

Wang D, Garcia-Bassets I, Benner C, Li W, Su X, Zhou Y, Qiu J, Liu W, Kaikkonen MU, Ohgi KA, Glass CK, Rosenfeld MG, Fu XD (2011) Reprogramming transcription by distinct classes

of enhancers functionally defined by eRNA. Nature 474(7351):390–394. doi:10.1038/nature10006

Whyte WA, Orlando DA, Hnisz D, Abraham BJ, Lin CY, Kagey MH, Rahl PB, Lee TI, Young RA (2013) Master transcription factors and mediator establish super-enhancers at key cell identity genes. Cell 153(2):307–319. doi:10.1016/j.cell.2013.03.035

Yanez-Cuna JO, Dinh HQ, Kvon EZ, Shlyueva D, Stark A (2012) Uncovering cis-regulatory sequence requirements for context-specific transcription factor binding. Genome Res 22(10):2018–2030. doi:10.1101/gr.132811.111

Zentner GE, Tesar PJ, Scacheri PC (2011) Epigenetic signatures distinguish multiple classes of enhancers with distinct cellular functions. Genome Res 21(8):1273–1283. doi:10.1101/gr.122382.111

Zhang X, Odom DT, Koo SH, Conkright MD, Canettieri G, Best J, Chen H, Jenner R, Herbolsheimer E, Jacobsen E, Kadam S, Ecker JR, Emerson B, Hogenesch JB, Unterman T, Young RA, Montminy M (2005) Genome-wide analysis of cAMP-response element binding protein occupancy, phosphorylation, and target gene activation in human tissues. Proc Natl Acad Sci U S A 102(12):4459–4464. doi:10.1073/pnas.0501076102

Zhang B, Day DS, Ho JW, Song L, Cao J, Christodoulou D, Seidman JG, Crawford GE, Park PJ, Pu WT (2013) A dynamic H3K27ac signature identifies VEGFA-stimulated endothelial enhancers and requires EP300 activity. Genome Res 23(6):917–927. doi:10.1101/gr.149674.112

Zhu J, Adli M, Zou JY, Verstappen G, Coyne M, Zhang X, Durham T, Miri M, Deshpande V, De Jager PL, Bennett DA, Houmard JA, Muoio DM, Onder TT, Camahort R, Cowan CA, Meissner A, Epstein CB, Shoresh N, Bernstein BE (2013a) Genome-wide chromatin state transitions associated with developmental and environmental cues. Cell 152(3):642–654. doi:10.1016/j.cell.2012.12.033

Zhu Y, Sun L, Chen Z, Whitaker JW, Wang T, Wang W (2013b) Predicting enhancer transcription and activity from chromatin modifications. Nucleic Acids Res 41(22):10032–10043. doi:10.1093/nar/gkt826

Role of the cAMP Pathway in Glucose and Lipid Metabolism

Kim Ravnskjaer, Anila Madiraju, and Marc Montminy

Contents

Abstract

3′–5′-Cyclic adenosine monophosphate (cyclic AMP or cAMP) was first described in 1957 as an intracellular second messenger mediating the effects of glucagon and epinephrine on hepatic glycogenolysis (Berthet et al., J Biol Chem 224(1):463–475, 1957). Since this initial characterization, cAMP has been firmly established as a versatile molecular signal involved in both central and peripheral regulation of energy homeostasis and nutrient partitioning. Many of these effects appear to be mediated at the transcriptional level, in part through the activation of the transcription factor CREB and its coactivators. Here we review current understanding of the mechanisms by which the cAMP signaling pathway triggers metabolic programs in insulin-responsive tissues.

Keywords

CBP (CREB Binding Protein) • CREB (cAMP Response Element Binding protein) • CRTC (cAMP Regulated Transcriptional Coactivator)

K. Ravnskjaer • A. Madiraju • M. Montminy (✉)
Salk Institute, La Jolla, CA 92037, USA
e-mail: montminy@salk.edu

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1 Introduction

In mammals, cAMP is produced from ATP by a family of enzymes called adenylate cyclases (ACs). There are nine transmembrane members of this family (tmACs Type I–IX) and one *soluble* isoform (AC10 or sAC) (Kamenetsky et al. 2006). The soluble AC is conserved across species, from prokaryotes and fungi, to humans (Buck et al. 1999). This enzyme is localized to the cytosol, the nucleus, and the mitochondria where it is activated by increases in bicarbonate from cellular respiration (Zippin et al. 2004; Acin-Perez et al. 2009). The nine transmembrane ACs are expressed at different levels in various cell types (Defer et al. 2000) where they are confined to discrete functional domains together with upstream regulators and downstream targets of cAMP signaling. The most extensively characterized regulators of tmAC activity are heterotrimeric G proteins composed of β and γ subunits (Chen et al. 1995) and either stimulatory ($G_{\alpha s}$) or inhibitory ($G_{\alpha i}$) subunits that convert extracellular stimuli engaging G-protein-coupled receptors (GPCRs) into intracellular signals through modulation of tmAC activity. Other signal transducers that regulate tmAC activity include calmodulin (Valverde et al. 1979), protein kinase A (PKA) (Iwami et al. 1995), protein kinase C (PKC) (Yoshimasa et al. 1987), as well as regulator of G-protein signaling 2 (RGS2) (Sinnarajah et al. 2001). These regulators affect specific tmAC types, allowing versatile feedback loops that are cell-type specific and that integrate cAMP signaling with calcium, $G_{\alpha q}$, and growth factor signals.

Precise regulation of cAMP turnover, clustering of ACs with downstream targets in microdomains, and inhibitory feedback mechanisms all serve the purpose of compartmentalizing the cAMP signal both spatially and temporally. Separation of discrete cAMP signals is the functional basis for the coexistence and fidelity of multiple signaling pathways using cAMP as a second messenger in the same cell (Hayes et al. 1980; Di Benedetto et al. 2008; Zaccolo 2011; Houslay 2010). It is important to note that under physiological conditions, diffusion of cAMP beyond these defined microdomains is insignificant (Zaccolo and Pozzan 2002). Indeed, protein microdomains are instrumental in maintaining distinct cAMP signaling compartments, and the scaffolding proteins known as A-kinase anchor proteins (AKAPs) play a key role in the formation of these microdomains (Dessauer 2009; Smith et al. 2013). Through direct physical interactions, AKAPs station cAMP-effector proteins optimally relative to ACs and phosphodiesterases (PDEs) allowing exposure to cAMP concentrations within their dynamic range.

The physiological outcome of signals eliciting a cellular cAMP response depends on the subcellular localization of the effectors relative to the source of cAMP and to potential downstream targets. Three classes of cAMP-effector proteins have been established to date: PKA (Walsh et al. 1968), exchange proteins directly activated by cAMP (EPACs) (de Rooij et al. 1998), and hyperpolarization-activated cyclic nucleotide-gated (HCN) ion channels (DiFrancesco and Tortora 1991). These effectors bind cAMP allosterically, leading to direct effects on downstream targets or permitting them to serve as integrators of the cAMP signal with other secondary messenger systems and signaling pathways. In addition to the

three established cAMP-effector mechanisms, PDE10A isozymes (Handa et al. 2008) and Popeye domain-containing (Popdc) proteins (Froese et al. 2012) have been identified as cAMP-binding proteins. The importance of PDE10A and Popdc proteins as cAMP effectors is yet to be determined.

While cAMP synthesis is determined by the activities of ACs, the superfamily of PDEs is the predominant cAMP-lowering mechanism in mammalian cells. These cyclic nucleotide-hydrolyzing enzymes are classified into multiple families (PDE1–11) with distinct expression patterns and specificities. Eight of these families can hydrolyze cAMP to AMP (PDEs 4, 7, and 8 are specific to cAMP), while the others are selective for 3′–5′-cyclic guanosine monophosphate (cGMP). Each family consists of several isozymes with distinct subcellular distributions and means of regulation (for a comprehensive review, see Francis et al. 2011). Dynamic changes in PDE expression, localization, oligomerization, and relative cGMP levels in the case of dual-specificity PDEs are all crucial determinants of total cAMP hydrolytic activity. The magnitude of the cAMP signal and fine-tuning of its kinetics by the PDEs is also coordinated by interactions with regulatory proteins and posttranslational modifications of the PDEs, such as phosphorylation by PKA itself (Francis et al. 2011; Keravis and Lugnier 2010). The amplitude, propagation, and duration of the cAMP signal are not only restricted by localization of PDE activities but are also regulated by a negative feedback mechanism in which cAMP itself activates PDE3 and PDE4 isozymes (Sette et al. 1994; Gettys et al. 1987). As will be discussed later in this chapter, PDEs are widely targeted by pharmacological agents to correct cAMP signaling in human disease.

2 cAMP in Metabolic Control

Cyclic AMP signaling has enormous impact on metabolic pathways both at the cellular and systemic levels. In the following sections, we will focus on inducers, regulators, and effectors of cAMP signals in important metabolic tissues. Mechanisms will be discussed in the context of nutrient homeostasis and the metabolic syndrome, a state of severe metabolic imbalance signified by perturbations in cAMP signaling in multiple tissues.

Most tissues are under the control of the sympathetic nervous system (SNS). Through the coordinated release of epinephrine from the adrenal glands into the circulation and the release of synaptic norepinephrine, the SNS activates membrane-bound adrenergic receptors on target cells. Of these, α2-adrenergic receptors inhibit and β-adrenergic receptors (β1, β2, and β3) stimulate cAMP production through G_i and $G_{\alpha s}$ activation, respectively (Insel 1996). The SNS has classically been associated with stress responses (Seematter et al. 2004), but synaptic tone is also involved in regulating basal metabolic rate, glucose disposal, and lipid partitioning (Monroe et al. 2001; Boyda et al. 2013; Arner et al. 1990). In this light, it is interesting to note that a growing body of literature links metabolic disorders like obesity and diabetes to dysregulation of adrenergic receptor signaling (Boyda et al. 2013; Yasuda et al. 2006; Ziegler et al. 2012).

2.1 Liver

Cyclic AMP signaling was first discovered in the liver as a critical mediator of glucose metabolism. Mammals use highly interconnected hormonal signaling mechanisms that include the opposing actions of glucagon and insulin to maintain glucose homeostasis. Decreases in circulating glucose concentrations during fasting trigger the release of pancreatic glucagon, which stimulates gluconeogenesis and provides substrate supply to glucose-dependent tissues like the brain and red blood cell compartments. During feeding, increases in circulating insulin downregulate hepatic gluconeogenesis in part through activation of the Ser/Thr kinase Akt. Increases in insulin resistance promote hyperglycemia, in part due to a failure of insulin to suppress hepatic glucose production. Indeed, lowering hepatic glucose production represents a major objective for treatment of type II diabetic individuals. A potential regulatory target in this process is the transcription factor CREB (Altarejos and Montminy 2011a) and its associated coactivators the CREB-binding protein (CBP) and cAMP-regulated transcriptional coactivators (CRTCs).

Triggering of the glucagon receptor on hepatocytes activates adenylate cyclase, leading to production of cAMP and increases in PKA activity. Following its PKA-mediated phosphorylation, CREB interacts with CREB-binding protein (CBP) (Chrivia et al. 1993a; Eckner et al. 1994) and initiates transcription of key gluconeogenic enzymes such as pyruvate carboxylase (PC), phosphoenolpyruvate carboxykinase (PEPCK-C) (Herzig et al. 2001; Quinn and Granner 1990; Wynshaw-Boris et al. 1986), and glucose-6-phosphatase (Lin et al. 1997). The induction of gluconeogenic genes by CREB is further augmented by the CREB-regulated transcription coactivator 2 (CRTC2), which is dephosphorylated in response to glucagon, where it migrates to the nucleus and associates with CREB over relevant promoters (Koo et al. 2005; Chrivia et al. 1993b). Under feeding conditions, CRTC2 is phosphorylated by salt-inducible kinases (SIKs) and sequestered in the cytoplasm via an association with 14-3-3 proteins. Exposure to glucagon promotes the PKA-mediated phosphorylation and inhibition of SIKs, leading to the dephosphorylation of CRTC2.

The CREB/CRTC2 pathway appears to be active primarily during short-term fasting; with prolonged fasting, forkhead box protein O1 (FOXO1) activity appears critical in maintaining expression of gluconeogenic genes (Liu et al. 2008). The physiological relevance of CREB and CRTC2 in hepatic glucose production is demonstrated in vivo with a mouse model expressing dominant-negative CREB inhibitor A-CREB (Ahn et al. 1998) and in cultured hepatocytes with a knockout of CRTC2 (Wang et al. 2010).

In addition to these transcriptional effects, PKA modulates gluconeogenesis by altering substrate flux. PKA phosphorylates the bifunctional enzyme fructose-2,6-bisphosphatase/phosphofructokinase-2, favoring the phosphatase activity and therefore decreasing production of the metabolic intermediate fructose 2,6-bisphosphate. This metabolite is a powerful allosteric activator of phosphofructokinase-1 (PFK-1), the first rate-limiting enzyme in glycolysis. By depleting the activator, PKA inhibits glycolytic flux through PFK-1, enhancing

glucose production and liver glucose output (El-Maghrabi et al. 1982; Richards et al. 1982).

Liver glycogenolysis is another means by which glucose homeostasis is maintained during fasting and exercise or in response to stress as manifested by increased catecholamine signaling. Following its activation by glucagon and epinephrine, PKA phosphorylates and activates phosphorylase kinase, which in turn stimulates glycogen phosphorylase, allowing for glucose release from liver glycogen stores (Studer and Borle 1982; Studer et al. 1984).

In addition to its regulation by fasting and feeding signals, hepatic glucose production is also modulated by the circadian clock. Like other tissues, the liver clock is governed by E-box transcription factors called brain and muscle ARNT-like (BMAL) and circadian locomotor output cycles kaput (CLOCK), which trigger expression of cryptochrome (CRY) and period (PER) proteins. In turn, CRY and PER repress transcription of CLOCK and BMAL, providing feedback regulation. Although they are regarded primarily as transcriptional repressors, CRY1 and CRY2 appear to inhibit expression of the gluconeogenic program during the night-to-day transition by binding to the cytoplasmic $G_s\alpha$ subunit of the heterotrimeric G protein. Conversely, decreases in CRY1 and CRY2 levels during the day-to-night transition enhance hepatic glucose production due to increases in cAMP signaling (Zhang et al. 2010). CREB and CRTC2 have been reported to promote the expression of BMAL1, suggesting that this pathway may modulate the core clock (Sun et al. 2015).

2.2 White Adipose Tissue

White adipose tissue (WAT) in mammals serves as an insulation, a storage depot of energy in the form of triglycerides, and an endocrine organ. The characteristics of fat depots throughout the body are largely dictated by their location; they are under complex endocrine, nutritional, neuronal, and immunological control (Rosen and Spiegelman 2014). Many of these regulatory signals are mediated by cAMP, which is known to have a significant role in both adipogenesis and lipid partitioning in white adipose tissue. Mature adipocytes arise from fibroblastic precursors through a dynamic differentiation process that requires extensive chromatin remodeling (Park et al. 2012; Siersbaek et al. 2014; Tang and Lane 2012). The cAMP signaling pathways are among the most well-characterized mechanisms controlling adipocyte differentiation. PDE inhibitors and synthetic cAMP analogs are commonly employed to switch on the adipogenic program in vitro (Russell and Ho 1976). An early increase in preadipocyte cAMP levels stimulates PKA, which in turn phosphorylates and activates the nuclear basic leucine zipper transcription factor cAMP-response element-binding (CREB) protein and members of the ATF family (Zhang et al. 2004; Fox et al. 2006a). These transcriptional activators have been linked to the induction of critical regulators of adipogenesis, including peroxisome proliferator-activated receptor-γ (PPAR-γ) and CCAAT/enhancer-binding protein (C/EBP) α and β (Fox et al. 2006a; Niehof et al. 1997; Birsoy et al. 2008).

Expression of constitutively active CREB is sufficient to promote adipogenesis in 3T3-L1 cells (Fox et al. 2006b), highlighting the importance of the cAMP pathway in adipocyte differentiation. Activation of EPAC1 also appears important for a subset of cAMP effects (Petersen et al. 2008) possibly in synergy with the effects of PKA. Adipogenesis is impaired in mice lacking $G_{\alpha s}$ expression in adipose tissue (Chen et al. 2010), providing strong evidence that cAMP is crucial to adipogenesis in vivo. Embryonic fibroblasts isolated from these mice have significantly reduced adipogenic potential in vitro even when PDE activity is inhibited. The nature of the signals stimulating cAMP production during adipogenesis in vivo remains to be elucidated, however.

In addition to its effects on differentiation, cAMP signaling also regulates lipid metabolism in WAT. During instances of high energy demand, such as fasting, triglycerides stored in adipocyte lipid droplets are hydrolyzed to fatty acids and glycerol in a process known as lipolysis (Frayn 2002). For decades, it has been suggested that adipokinetic factors released from the pituitary (e.g., growth hormone), adrenal (e.g., glucocorticoids), and pancreas (e.g., glucagon) can stimulate lipolysis from WAT (Hollenberg et al. 1961; White and Engel 1958). Although some of these factors have been shown to induce lipid breakdown from adipocytes in vitro, the significance of their lipolytic actions in vivo is likely to be of limited importance (Coppack et al. 1994). Rather, lipolytic signals come primarily from sympathetic innervation of the adipose depots (Adler et al. 2012; Dodt et al. 1999; Nishizawa and Bray 1978). In humans, catecholamines are the primary hormones involved in triggering lipolysis in WAT. β-Adrenergic stimuli increase cAMP levels and promote lipid breakdown. However, stimulation of receptors coupled to inhibitory G_i proteins, such as EP3 receptor by prostaglandin E_2 (PGE_2), will lead to inhibition of adenylate cyclase activity, hindering cAMP synthesis and decreasing lipolysis (Kolditz and Langin 2010; Richelsen and Pedersen 1985; Cummings et al. 1996). By releasing PGE2, adipose tissue macrophages may contribute to catecholamine resistance in certain depots.

Studies of several genetically modified animal models point to an important role for cAMP in lipolysis in vivo. A direct lipolytic function for PKA and cAMP signaling in WAT is further supported by numerous studies documenting PKA phosphorylation and activation of several key regulators of lipolysis in response to elevated cAMP levels. These include the hormone-sensitive lipase (HSL) (Anthonsen et al. 1998), adipocyte-specific triglyceride lipase (ATGL) (Pagnon et al. 2012), and lipid droplet-associated protein perilipin (Brasaemle et al. 2009).

Another genetic mouse model showing increased cAMP-induced lipolysis is the PDE3B knockout mouse. In addition to an increased lipolytic response to adrenergic stimuli in vivo, the well-known anti-lipolytic effect of insulin (Olefsky 1977) is not observed in isolated PDE3B null adipocytes (Choi et al. 2006). This dual phenotype can be explained by the distinct localization of PDE3B in separate microdomains. In wild-type adipocytes, specific pools of PDE3B are phosphorylated and activated by PKA in response to β-adrenergic stimulation, whereas insulin receptor signaling will promote AKT-mediated phosphorylation and activation of other PDE3B pools (Ahmad et al. 2009). PKA maintains temporal

autoregulation of the cAMP signal while promoting lipolysis by allowing PDE3B fine-tuning of the cAMP signal to a concentration range that is ideal to sustain activity, whereas AKT blocks lipolysis by dissociating it from lipolytic signals at least in part by activating discrete pools of PDE3B. Significantly, PDE3B mutant mice show hepatic lipid accumulation and insulin resistance, suggesting that the increase in lipolysis is not accompanied by an increase in fatty acid oxidation and may reflect the redundancy between PDE family members or the diminished importance of PDE3B in the regulation of fatty acid oxidation in non-adipose tissues such as the liver.

2.3 Brown Adipose Tissue

Brown adipose tissue (BAT) is an oxidative tissue specialized in dissipating energy as heat, crucial for maintaining optimal body temperature when exposed to changes in the environment (Rosen and Spiegelman 2014). BAT was initially characterized as an interscapular fat pad in newborn rodents but has since been identified in both infants (Lidell et al. 2013) and adult humans (Virtanen et al. 2009; Cypess et al. 2009; van Marken Lichtenbelt et al. 2009; Huttunen et al. 1981). Unlike WAT, BAT expresses uncoupling protein 1 (UCP1, also known as thermogenin) allowing uncoupling of mitochondrial oxidative phosphorylation and thermogenesis (Cannon et al. 1982; Heaton et al. 1978; Enerback et al. 1997). Cold exposure induces the oxidative and thermogenic capacity of BAT (Cameron and Smith 1964) through SNS activation and adrenergic stimulation of cAMP production (Thomas and Palmiter 1997). Cyclic AMP immediately activates PKA (Skala and Knight 1977) leading to increased lipolysis and activation of UCP1 (Fedorenko et al. 2012). In an adaptive response to prolonged cold exposure, cAMP also contributes to increased UCP1 levels (Mattsson et al. 2011), mitochondrial biogenesis (Bogacka et al. 2005), and expanded BAT mass. These adaptive effects are believed to require transcriptional changes although the exact mechanisms, and the relative importance of these pathways, are still debated. Transcriptional activators that have been found to play prominent roles in BAT adaptation to cold exposure include CREB (Rim and Kozak 2002), PPAR gamma-coactivator 1-alpha (PGC1a), IRF4 (Kong et al. 2014), and PRDM16 (Kajimura et al. 2009; Seale et al. 2007). In particular, PGC1a, which is itself induced by cAMP, is required for commitment of preadipocytes to differentiate into brown adipocytes (Puigserver et al. 1998) and appears to be decreased in the adipose tissue of obese patients (Semple et al. 2004).

In order to sustain thermogenesis, the BAT must fuel ATP synthesis by oxidizing substrate. Cyclic AMP signaling, via adrenergic stimuli from sympathetic innervation, will lead to the increased synthesis of both lipoprotein lipase and GLUT1 (Shimizu et al. 1998). This allows increased release of fatty acids and uptake of glucose for oxidation. Importantly, the enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which plays a significant role in glycolysis and lipid metabolism, is also upregulated by norepinephrine and cAMP analogs (Barroso et al. 1999). Bone morphogenic protein 8 (BMP8) was found to enhance

thermogenesis in a manner dependent upon on the CREB pathway, and loss of BMP8 was associated with decreased metabolic rate and thermogenesis (Whittle et al. 2012).

Importantly, the thyroid hormone triiodothyronine (T3) (Silva and Larsen 1983; de Jesus et al. 2001) also has a significant effect on obligatory thermogenesis and BAT function. T3 is required for maintaining BAT lipolysis and for sustaining the basic metabolic rate by promoting uncoupling of oxidative phosphorylation (Mullur et al. 2014). T3 levels in the BAT are carefully regulated by conversion of T4 to T3 by deiodinases. In the BAT, adrenergic signaling as well as activation of the bile acid receptor TGR5 leads to cAMP increases, which promote 5′-deiodinase type 2 activity, increasing local T3 levels (Silva 2006; Arrojo et al. 2013).

Induction of cAMP signaling can also trigger "browning" of white adipose tissue to a more oxidative tissue phenotype (Dempersmier et al. 2015). The possibility of inducing BAT characteristics in WAT by modulating cAMP signaling points to cAMP agonists as potential targets for drug development for the treatment of obesity. Indeed, administration of β3-adrenergic agonist to mice with a knockout of phosphodiesterase 3B (PDE3B) increases cAMP accumulation in epididymal WAT, leading to browning of these WAT depots (Guirguis et al. 2013). Furthermore, bone morphogenic protein 7 (BMP7), which is upregulated by cAMP (Ishibashi et al. 1993), also promotes brown adipose characteristics in human adipogenic stem cells (Okla et al. 2015).

2.4 Pancreas

The cAMP pathway contributes importantly to pancreatic β-cell growth and insulin secretion (Furman et al. 2010; Altarejos and Montminy 2011b; Dalle et al. 2011). The incretin hormone glucagon-like peptide-1 (GLP-1) increases cAMP levels by acting through its GPCR, leading to CREB activation, increased glucose-stimulated insulin secretion and β-cell expansion (Wang et al. 1997; Tourrel et al. 2001). Another incretin, glucose-dependent insulinotropic peptide (GIP), also increases cAMP signaling via interaction with its GPCR (Yabe and Seino 2011). However, receptors for this hormone are downregulated in response to high glucose, making it a less attractive target for the treatment of diabetes compared to GLP-1 signaling (Puddu et al. 2015). Insulin-like growth factor 1 (IGF-1), which is produced in the liver, has been shown to be important for proper development, growth, and proliferation of β-cells. Its actions are mediated through insulin response substrate 2 (IRS2), which is known to play a role in the protection of β-cells against apoptosis and in promoting their growth by activating the pro-growth kinase Akt (George et al. 2002; Withers et al. 1999). Cyclic AMP signaling via CREB also activates IRS2 and in this manner will enhance IGF-1 signaling (Jhala et al. 2003). Notably, activation of the IGF-1 pathway also increases the activity of PDE3B, which degrades cAMP (Zhao et al. 1997); cAMP itself activates PDE3B via PKA (Heimann et al. 2010), creating a negative feedback loop and also modulating

cAMP levels to ensure proper cAMP signaling and to prevent uncontrolled insulin secretion (Härndahl et al. 2002).

Other signals in addition to GLP-1 appear capable of stimulating islet function. Cyclic AMP levels are also elevated in response to γ-aminobutyric acid (GABA), for example, which prevents apoptosis and increases β-cell mass (Purwana et al. 2014). Indeed, acetylcholine, like glucose, induces membrane depolarization and calcium influx, this stimulating insulin secretion. This effect is dependent on protein kinase C (PKC) and phospholipase C coupled to muscarinic receptors (Love et al. 1998; Niwa et al. 1998). Acetylcholine was also found to activate adenylate cyclase activity and to increase cAMP production in diabetic rat islets, leading to CREB activation and enhanced cell viability (Screaton et al. 2004; Eckert et al. 1996). Whether this effect occurs via increases in Ca^{2+} or PKC activity or is due to signaling variations in the diabetic islets remains to be fully elucidated.

In addition to PDE3B, several additional mechanisms limit accumulation of cAMP in β-cells. Increases in intracellular Ca^{2+} in response to glucose elevations activate PDE1, increasing degradation of cAMP. Neuropeptide Y (NPY) (Morgan et al. 1998), PGE_2 (Kimple et al. 2013), and adrenaline (Metz 1988) all signal through GPCRs coupled to G_i, inhibiting adenylate cyclase activity and negatively regulating cAMP accumulation.

Insulin secretion from β-cells is tightly regulated by increases in extracellular glucose concentrations. Glucose transport and phosphorylation allow for its subsequent oxidation; the production of ATP stimulates insulin granule release. The primary transporter involved in facilitated diffusion of glucose in human β-cells is GLUT1. This transporter is not hormonally regulated, suggesting that cellular amounts of this protein have a predominant effect on the efficiency of glucose transport (Thorens et al. 2000). The GLUT1 promoter region has been reported to contain a CREB-binding site that mediates induction of this gene by cAMP (Murakami et al. 1992). However, increased GLUT1 expression may not necessarily affect insulin response (Ishihara et al. 1994); increases in hexokinase I enhance insulin secretion in response to glucose. Cyclic AMP has been shown to increase hexokinase I expression and may in this manner contribute to heightened insulin detection (Yokomori et al. 1992; Borboni et al. 1999).

CREB has been demonstrated to protect β-cells from cytokine-mediated apoptosis and glucotoxicity (Jhala et al. 2003; Jambal et al. 2003; Costes et al. 2009). Overexpression of A-CREB led to diminished replication and increased apoptosis of β-cells, causing hyperglycemia in mice (Jhala et al. 2003). Additionally, it has been shown that the CREB coactivator CRTC2 is necessary for glucose-mediated insulin release (Eberhard et al. 2013; Blanchet et al. 2015). These data collectively underscore the centrality of cAMP signaling through CREB in β-cell survival and endurance. Cyclic AMP is also thought to augment insulin secretion by promoting the PKA-mediated phosphorylation of the SUR1 subunit of the ATP-sensitive potassium channel, thereby inhibiting channel activity and enhancing depolarization (Light et al. 2002); by PKA-dependent phosphorylation of voltage-sensitive calcium channels, increasing Ca^{2+} influx (Leiser and Fleischer 1996; Gerhardstein

et al. 1999); and by directly regulating exocytosis of insulin-containing vesicles (Ammälä et al. 1993; Ding and Gromada 1997).

Though most effects of cAMP in β-cells appear to proceed via a PKA-dependent mechanism, a subset of these effects may be mediated by EPACs (Kang et al. 2006; Henquin and Nenquin 2014; Yoshida et al. 2014). The pancreatic β-cell expresses both EPAC1 and EPAC2, which are directly activated by cAMP. Knockdown of EPAC expression in vivo led to abrogation of cAMP-mediated stimulation of glucose-induced insulin secretion from the pancreas and development of metabolic syndrome in vivo (Kashima et al. 2001; Kai et al. 2013), highlighting the importance of alternative cAMP-dependant pathways to β-cell function.

Incretin hormones have been found to promote β-cell replication at least in rodent cells by increasing the expression of the cell cycle regulators cyclins D1 (Kim et al. 2006) and A2 (Song et al. 2008). cAMP also stimulates the expression of Bcl-2 and Bcl-xL (Hui et al. 2003; Kim et al. 2008), which protect β-cells from apoptosis. cAMP may also terminate cell death cascades by inhibiting caspases (Ehses et al. 2003; Welters et al. 2006).

In addition to its effects on insulin secretion, β-cell growth and proliferation, cAMP may also enhance differentiation. GLP-1 appears to increase the expression of pancreatic duodenal homeobox – 1 (PDX-1) (Perfetti et al. 2000), a transcription factor central to the differentiation of pancreatic endocrine, exocrine, and ductal cell populations from endoderm during fetal development (Zhou et al. 2002; Offield et al. 1996). PDX-1 is also necessary for maturation of β-cells and allows selective differentiation of pancreatic endocrine progenitors to form insulin-producing β-cells while suppressing formation of glucagon-producing α-cells (McKinnon and Docherty 2001; Ber et al. 2003; Gao et al. 2014). It has been shown that cAMP, via PKA, is required to maintain GLP-1-mediated PDX-1 increases and PDX-1 translocation to the nucleus. PDX-1 also promotes expression of the GLP-1 receptor, enhancing cAMP production (Wang et al. 2005).

In contrast to PDX-1 expression, the transcription factor MafA is upregulated during the later development of the endocrine pancreas, where it promotes full maturation of β-cells and stimulates insulin biosynthesis (Kaneto et al. 2008; Artner et al. 2010; Hang and Stein 2011). GLP-1 and cAMP agonists were found to increase MafA expression via induction of the CREB pathway, further implicating cAMP in pancreatic development and differentiation (Blanchet et al. 2015).

The role of cAMP in other pancreatic cell types within the islet is not as well understood. GLP-1 has been demonstrated to increase glucagon release from α-cells via the cAMP/PKA pathway (Ding et al. 1997). Inhibiting EPAC-2 activity in α-cells was also found to decrease glucagon gene transcription, suggesting that both pathways (PKA and EPAC) modulate glucagon production (Islam et al. 2009). Though GLP-1 appears to increase glucagon secretion from α-cells in vitro, this effect may be largely inhibited in vivo due to effects of GLP-1 on somatostatin release from pancreatic δ-cells. Indeed, cAMP stimulates somatostatin secretion from δ-cells, thereby inhibiting adenylate cyclase and cAMP synthesis in α-cells via G_i-coupled GPCR (Elliott et al. 2015; Hauge-Evans et al. 2009). Finally,

adrenergic stimulation (e.g., adrenaline action) can increase both glucagon (Dai et al. 2014) and somatostatin (Gromada et al. 1997) release via cAMP elevation.

2.5 Muscle

Skeletal muscle has a specialized architecture that directly relates to its function in movement and to its regulation by neuronal inputs. Muscle contraction is dependent upon proper calcium signaling, and cAMP plays a significant role in regulating this process. Beta-adrenergic signaling elevates muscle cAMP and thereby modulates Ca^{2+}-induced Ca^{2+} release from the sarcoplasmic reticulum, acutely increasing contractile force (Saida and Van Breemen 1984; Cairns and Dulhunty 1993). This effect has been postulated to reflect cAMP-regulated phosphorylation of L-type voltage-dependent Ca^{2+} channels (Sculptoreanu et al. 1993). Cyclic AMP is also necessary for PKA-mediated activation of the Na^{+}–K^{+} pump, which is important in membrane hyperpolarization and in the restoration of muscle excitability (Clausen 2003).

Glycogenolysis, which is critical for meeting energy demands of the muscle, is also promoted by cAMP. Acute cAMP effects are especially important during exercise, when energy consumption by the muscle is at its peak and when epinephrine levels are high, leading to increases in muscular cAMP (Ezrailson et al. 1983; Soderling et al. 1970). Significantly, chronic activation of cAMP pathways in the muscle promotes adaptive responses that include increased myofiber size as well as metabolic transition to a more glycolytic fiber type (Chen et al. 2009; Maltin et al. 1989). Activation of β-adrenergic receptors, which stimulate the cAMP pathway, may prove to be an effective mechanism to enhance muscle function and slow atrophy in disease states such as Duchenne's muscular dystrophy (Harcourt et al. 2007; Hinkle et al. 2007; Ryall et al. 2008).

3 Conclusion

Cyclic AMP mediates the effects of glucagon and beta-adrenergic signals in regulating glucose and lipid metabolism. A key feature of type II diabetes (T2D) is the failure of insulin to trigger glucose uptake into the muscle and to suppress glucose production from the liver (Centers for Disease Control and Prevention 2015; Brown and Goldstein 2008; Basit et al. 2004). Suppressing liver gluconeogenesis and glycogenolysis via inhibition of cAMP signaling or CREB-dependent transcription may have salutary effects on blood glucose concentrations.

While lowering cAMP signaling in the liver may prove beneficial, upregulating cAMP in other tissues may also improve glucose and lipid homeostasis. The chronic hyperglycemia associated with insulin resistance is accompanied initially by compensatory increases in pancreatic islet mass and in insulin secretion. However, unremitting insulin resistance eventually causes β-cell failure and apoptosis, ultimately leading to T2D (Poitout and Robertson 2008; Kahn 2003). In some cases,

an innate susceptibility of the β-cell may magnify the risk of developing T2D; a number of studies have concluded that prediabetes and youth-onset T2D in certain ethnic groups are more strongly associated with β-cell dysfunction (Gujral et al. 2014; Staimez et al. 2013; Dowse et al. 1990). Developing therapeutic strategies to protect β-cells from glucolipotoxicity may provide effective treatment for T2D in this setting. The cAMP signaling pathway is important for β-cell viability, proliferation, and glucose responsiveness. Increasing cAMP signaling or CREB activity may further potentiate β-cell function and provide therapeutic benefit to insulin-resistant individuals.

Upregulating the cAMP pathway in brown and white adipose tissues may also have positive effects on fat burning and thereby decrease inflammatory infiltrates that contribute to insulin resistance in obesity. In view of the pleiotropic effects of the cAMP pathway on glucose and lipid metabolism, a major challenge will be to target relevant modulators in specific tissues. Based on the considerable number of phosphodiesterases with distinct pharmacological properties and tissue localization, these regulators may prove particularly useful in this regard. Future studies on region-specific cAMP signaling in different organelles may also provide further insight into the regulatory properties of this second messenger.

References

Acin-Perez R et al (2009) Cyclic AMP produced inside mitochondria regulates oxidative phosphorylation. Cell Metab 9(3):265–276

Adler ES et al (2012) Neurochemical characterization and sexual dimorphism of projections from the brain to abdominal and subcutaneous white adipose tissue in the rat. J Neurosci 32 (45):15913–15921

Ahmad F et al (2009) Differential regulation of adipocyte PDE3B in distinct membrane compartments by insulin and the beta3-adrenergic receptor agonist CL316243: effects of caveolin-1 knockdown on formation/maintenance of macromolecular signalling complexes. Biochem J 424(3):399–410

Ahn S et al (1998) A dominant-negative inhibitor of CREB reveals that it is a general mediator stimulus-dependent transcription of c-fos. Mol Cell Biol 18:967–977

Altarejos JY, Montminy M (2011) CREB and the CRTC co-activators: sensors for hormonal and metabolic signals. Nat Rev Mol Cell Biol 12(3):141–151

Ammälä C, Ashcroft F, Rorsman P (1993) Calcium-independent potentiation of insulin release by cyclic AMP in single beta-cells. Nature 363(6427):356–358

Anthonsen MW et al (1998) Identification of novel phosphorylation sites in hormone-sensitive lipase that are phosphorylated in response to isoproterenol and govern activation properties in vitro. J Biol Chem 273(1):215–221

Arner P et al (1990) Adrenergic regulation of lipolysis in situ at rest and during exercise. J Clin Invest 85(3):893–898

Arrojo E, Drigo R, Fonseca TL, Werneck-de-Castro JP, Bianco AC (2013) Role of the type 2 iodothyronine deiodinase (D2) in the control of thyroid hormone signaling. Biochim Biophys Acta 1830(7):3956–3964

Artner I, Hang Y, Mazur M, Yamamoto T, Guo M, Lindner J, Magnuson MA, Stein R (2010) MafA and MafB regulate genes critical to beta-cells in a unique temporal manner. Diabetes 59 (10):2530–2539

Barroso I, Benito B, Garcí-Jiménez C, Hernández A, Obregón MJ, Santisteban P (1999) Norepinephrine, tri-iodothyronine and insulin upregulate glyceraldehyde-3-phosphate dehydrogenase mRNA during Brown adipocyte differentiation. Eur J Endocrinol 141(2):169–179

Basit A, Hydrie M, Hakeem R, Ahmedani MY, Masood Q (2004) Frequency of chronic complications of type II diabetes. J Coll Physicians Surg Pak 14:79–83

Ber I, Shternhall K, Perl S, Ohanuna Z, Goldberg I, Barshack I, Benvenisti-Zarum L, Meivar-Levy I, Ferber S (2003) Functional, persistent, and extended liver to pancreas transdifferentiation. J Biol Chem 278(34):31950–31957

Berthet J, Rall TW, Sutherland EW (1957) The relationship of epinephrine and glucagon to liver phosphorylase. IV. Effect of epinephrine and glucagon on the reactivation of phosphorylase in liver homogenates. J Biol Chem 224(1):463–475

Birsoy K, Chen Z, Friedman J (2008) Transcriptional regulation of adipogenesis by KLF4. Cell Metab 7(4):339–347

Blanchet E, Van de Velde S, Matsumura S, Hao E, LeLay J, Kaestner K, Montminy M (2015) Feedback inhibition of CREB signaling promotes beta cell dysfunction in insulin resistance. Cell Rep 10:1149–1157

Bogacka I, Ukropcova B, McNeil M, Gimble JM, Smith SR (2005) Structural and functional consequences of mitochondrial biogenesis in human adipocytes in vitro. J Clin Endocrinol Metab 90(12):6650–6656

Borboni P, Porzio O, Pierucci D, Cicconi S, Magnaterra R, Federici M, Sesti G, Lauro D, D'Agata V, Cavallaro S, Marlier LN (1999) Molecular and functional characterization of pituitary adenylate cyclase-activating polypeptide (PACAP-38)/vasoactive intestinal polypeptide receptors in pancreatic beta-cells and effects of PACAP-38 on components of the insulin secretory system. Endocrinology 140(12):5530–5537

Boyda HN et al (2013) Peripheral adrenoceptors: the impetus behind glucose dysregulation and insulin resistance. J Neuroendocrinol 25(3):217–228

Brasaemle DL et al (2009) Perilipin A and the control of triacylglycerol metabolism. Mol Cell Biochem 326(1–2):15–21

Brown MS, Goldstein J (2008) Selective versus total insulin resistance: a pathogenic paradox. Cell Metab 7:95–96

Buck J et al (1999) Cytosolic adenylyl cyclase defines a unique signaling molecule in mammals. Proc Natl Acad Sci U S A 96(1):79–84

Cairns SP, Dulhunty A (1993) Beta-adrenergic potentiation of E-C coupling increases force in rat skeletal muscle. Muscle Nerve 16:1317–1325

Cameron IL, Smith RE (1964) Cytological responses of brown fat tissue in cold-exposed rats. J Cell Biol 23:89–100

Cannon B, Hedin A, Nedergaard J (1982) Exclusive occurrence of thermogenin antigen in brown adipose tissue. FEBS Lett 150(1):129–132

Centers for Disease Control and Prevention (2015) Diabetes public health resource. http://www.cdc.gov/diabetes/. Accessed June 2015

Chen J et al (1995) A region of adenylyl cyclase 2 critical for regulation by G protein beta gamma subunits. Science 268(5214):1166–1169

Chen M, Feng H, Gupta D, Kelleher J, Dickerson KE, Wang J, Hunt D, Jou W, Gavrilova O, Jin JP, Weinstein LS (2009) G(s)alpha deficiency in skeletal muscle leads to reduced muscle mass, fiber-type switching, and glucose intolerance without insulin resistance or deficiency. Am J Physiol Cell Physiol 296(4):C930–C940

Chen M et al (2010) G(s)alpha deficiency in adipose tissue leads to a lean phenotype with divergent effects on cold tolerance and diet-induced thermogenesis. Cell Metab 11(4):320–330

Choi YH et al (2006) Alterations in regulation of energy homeostasis in cyclic nucleotide phosphodiesterase 3B-null mice. J Clin Invest 116(12):3240–3251

Chrivia JC et al (1993) Phosphorylated CREB binds specifically to the nuclear protein CBP. Nature 365(6449):855–859

Clausen T (2003) Na + −K+ pump regulation and skeletal muscle contractility. Physiol Rev 83 (4):1269–1324
Coppack SW, Jensen M, Miles JM (1994) In vivo regulation of lipolysis in humans. J Lipid Res 35 (2):177–193
Costes S, Vandewalle B, Tourrel-Cuzin C, Broca C, Linck N, Bertrand G, Kerr-Conte J, Portha B, Pattou F, Bockaert J, Dalle S (2009) Degradation of cAMP-responsive element-binding protein by the ubiquitin-proteasome pathway contributes to glucotoxicity in beta-cells and human pancreatic islets. Diabetes 58:1105–1115
Cummings DE et al (1996) Genetically lean mice result from targeted disruption of the RII beta subunit of protein kinase A. Nature 382(6592):622–626
Cypess AM et al (2009) Identification and importance of brown adipose tissue in adult humans. N Engl J Med 360(15):1509–1517
Dai XQ, Spigelman A, Khan S, Braun M, Manning Fox JE, MacDonald PE (2014) SUMO1 enhances cAMP-dependent exocytosis and glucagon secretion from pancreatic α-cells. J Physiol 592(Pt 17):3715–3726
Dalle S, Quoyer J, Varin E, Costes S (2011) Roles and regulation of the transcription factor CREB in pancreatic β-cells. Curr Mol Pharmacol 4:187–195
de Jesus LA et al (2001) The type 2 iodothyronine deiodinase is essential for adaptive thermogenesis in brown adipose tissue. J Clin Invest 108(9):1379–1385
de Rooij J et al (1998) Epac is a Rap1 guanine-nucleotide-exchange factor directly activated by cyclic AMP. Nature 396(6710):474–477
Defer N, Best-Belpomme M, Hanoune J (2000) Tissue specificity and physiological relevance of various isoforms of adenylyl cyclase. Am J Physiol Renal Physiol 279(3):F400–F416
Dempersmier J, Sambeat A, Gulyaeva O, Paul SM, Hudak CS, Raposo HF, Kwan HY, Kang C, Wong RH, Sul HS (2015) Cold-inducible Zfp516 activates UCP1 transcription to promote browning of white fat and development of brown fat. Mol Cell 57(2):235–246
Dessauer CW (2009) Adenylyl cyclase--A-kinase anchoring protein complexes: the next dimension in cAMP signaling. Mol Pharmacol 76(5):935–941
Di Benedetto G et al (2008) Protein kinase A type I and type II define distinct intracellular signaling compartments. Circ Res 103(8):836–844
DiFrancesco D, Tortora P (1991) Direct activation of cardiac pacemaker channels by intracellular cyclic AMP. Nature 351(6322):145–147
Ding WG, Gromada J (1997) Protein kinase A-dependent stimulation of exocytosis in mouse pancreatic beta-cells by glucose-dependent insulinotropic polypeptide. Diabetes 46 (4):615–621
Ding WG, Renström E, Rorsman P, Buschard K, Gromada J (1997) Glucagon-like peptide I and glucose-dependent insulinotropic polypeptide stimulate Ca2 + −induced secretion in rat alpha-cells by a protein kinase A-mediated mechanism. Diabetes 46(5):792–800
Dodt C et al (1999) Intraneural stimulation elicits an increase in subcutaneous interstitial glycerol levels in humans. J Physiol 521(Pt 2):545–552
Dowse GK, Qin H, Collins VR, Zimmet PZ, Alberti KG, Gareeboo H (1990) Determinants of estimated insulin resistance and beta-cell function in Indian, Creole and Chinese Mauritians. The Mauritius NCD Study Group. Diabetes Res Clin Pract 10:265–279
Eberhard CE, Fu A, Reeks C, Screaton RA (2013) CRTC2 is required for β-cell function and proliferation. Endocrinology 154:2308–2317
Eckert B, Schwaninger M, Knepel W (1996) Calcium-mobilizing insulin secretagogues stimulate transcription that is directed by the cyclic adenosine 3′,5′-monophosphate/calcium response element in a pancreatic islet beta-cell line. Endocrinology 137:225–233
Eckner R et al (1994) Molecular cloning and functional analysis of the adenovirus E1A-associated 300-kD protein (p300) reveals a protein with properties of a transcriptional adaptor. Genes Dev 8(8):869–884
Ehses JA, Casilla V, Doty T, Pospisilik JA, Winter KD, Demuth HU, Pederson RA, McIntosh CH (2003) Glucose-dependent insulinotropic polypeptide promotes beta-(INS-1) cell survival via

cyclic adenosine monophosphate-mediated caspase-3 inhibition and regulation of p38 mitogen-activated protein kinase. Endocrinology 144(10):4433–4445
Elliott AD, Ustione A, Piston DW (2015) Somatostatin and insulin mediate glucose-inhibited glucagon secretion in the pancreatic α-cell by lowering cAMP. Am J Physiol Endocrinol Metab 308(2):E130–E143
El-Maghrabi MR, Claus T, Pilkis J, Pilkis SJ (1982) Regulation of 6-phosphfructo-2-kinase activity by cyclic AMP-dependent phosphorylation. Proc Natl Acad Sci U S A 79:315–319
Enerback S et al (1997) Mice lacking mitochondrial uncoupling protein are cold-sensitive but not obese. Nature 387(6628):90–94
Ezrailson EG, Entman M, Garber AJ (1983) Adrenergic and serotonergic regulation of skeletal muscle metabolism in rat. I. The effects of adrenergic and serotonergic antagonists on the regulation of muscle amino acid release, glycogenolysis, and cyclic nucleotide levels. J Biol Chem 258(20):12494–12498
Fedorenko A, Lishko PV, Kirichok Y (2012) Mechanism of fatty-acid-dependent UCP1 uncoupling in brown fat mitochondria. Cell 151(2):400–413
Fox KE et al (2006) Depletion of cAMP-response element-binding protein/ATF1 inhibits adipogenic conversion of 3T3-L1 cells ectopically expressing CCAAT/enhancer-binding protein (C/EBP) alpha, C/EBP beta, or PPAR gamma 2. J Biol Chem 281(52):40341–40353
Francis SH, Blount MA, Corbin JD (2011) Mammalian cyclic nucleotide phosphodiesterases: molecular mechanisms and physiological functions. Physiol Rev 91(2):651–690
Frayn KN (2002) Adipose tissue as a buffer for daily lipid flux. Diabetologia 45(9):1201–1210
Froese A et al (2012) Popeye domain containing proteins are essential for stress-mediated modulation of cardiac pacemaking in mice. J Clin Invest 122(3):1119–1130
Furman B, Ong W, Pyne NJ (2010) Cyclic AMP signaling in pancreatic islets. Adv Exp Med Biol 654:281–304
Gao T, McKenna B, Li C, Reichert M, Nguyen J, Singh T, Yang C, Pannikar A, Doliba N, Zhang T, Stoffers DA, Edlund H, Matschinsky F, Stein R, Stanger BZ (2014) Pdx1 maintains β cell identity and function by repressing an α cell program. Cell Metab 19(2):259–271
George M, Ayuso E, Casellas A, Costa C, Devedjian JC, Bosch F (2002) Beta cell expression of IGF-I leads to recovery from type 1 diabetes. J Clin Invest 109:1153–1163
Gerhardstein BL, Puri T, Chien AJ, Hosey MM (1999) Identification of the sites phosphorylated by cyclic AMP-dependent protein kinase on the beta 2 subunit of L-type voltage-dependent calcium channels. Biochemistry 38(32):10361–10370
Gettys TW et al (1987) Short-term feedback regulation of cAMP by accelerated degradation in rat tissues. J Biol Chem 262(1):333–339
Gromada J, Bokvist K, Ding WG, Barg S, Buschard K, Renström E, Rorsman P (1997) Adrenaline stimulates glucagon secretion in pancreatic A-cells by increasing the Ca2+ current and the number of granules close to the L-type Ca2+ channels. J Gen Physiol 110(3):217–228
Guirguis E, Hockman S, Chung YW, Ahmad F, Gavrilova O, Raghavachari N, Yang Y, Niu G, Chen X, Yu ZX, Liu S, Degerman E, Manganiello V (2013) A role for phosphodiesterase 3B in acquisition of brown fat characteristics by white adipose tissue in male mice. Endocrinology 154(9):3152–3167
Gujral UP, Narayan K, Kahn SE, Kanaya AM (2014) The relative associations of β-cell function and insulin sensitivity with glycemic status and incident glycemic progression in migrant Asian Indians in the United States: the MASALA study. J Diabetes Complications 28:45–50
Handa N et al (2008) Crystal structure of the GAF-B domain from human phosphodiesterase 10A complexed with its ligand, cAMP. J Biol Chem 283(28):19657–19664
Hang Y, Stein R (2011) MafA and MafB activity in pancreatic β cells. Trends Endocrinol Metab 22(9):364–373
Harcourt LJ, Schertzer J, Ryall JG, Lynch GS (2007) Low dose formoterol administration improves muscle function in dystrophic mdx mice without increasing fatigue. Neuromuscul Disord 17:47–55

Härndahl L, Jing X, Ivarsson R, Degerman E, Ahrén B, Manganiello VC, Renström E, Holst LS (2002) Important role of phosphodiesterase 3B for the stimulatory action of cAMP on pancreatic beta-cell exocytosis and release of insulin. J Biol Chem 277(40):37446–37455

Hauge-Evans AC, King A, Carmignac D, Richardson CC, Robinson IC, Low MJ, Christie MR, Persaud SJ, Jones PM (2009) Somatostatin secreted by islet delta-cells fulfills multiple roles as a paracrine regulator of islet function. Diabetes 58(2):403–411

Hayes JS, Brunton LL, Mayer SE (1980) Selective activation of particulate cAMP-dependent protein kinase by isoproterenol and prostaglandin E1. J Biol Chem 255(11):5113–5119

Heaton GM et al (1978) Brown-adipose-tissue mitochondria: photoaffinity labelling of the regulatory site of energy dissipation. Eur J Biochem 82(2):515–521

Heimann E, Jones H, Resjö S, Manganiello VC, Stenson L, Degerman E (2010) Expression and regulation of cyclic nucleotide phosphodiesterases in human and rat pancreatic islets. PLoS One 5(12):e14191

Henquin JC, Nenquin M (2014) Activators of PKA and Epac distinctly influence insulin secretion and cytosolic Ca2+ in female mouse islets stimulated by glucose and tolbutamide. Endocrinology 155(9):3274–3287

Herzig S et al (2001) CREB regulates hepatic gluconeogenesis via the co-activator PGC-1. Nature 413:179–183

Hinkle RT, Lefever F, Dolan ET, Reichart DL, Dietrich JA, Gropp KE, Thacker RI, Demuth JP, Stevens PJ, Qu XA, Varbanov AR, Wang F, Isfort RJ (2007) Corticortophin releasing factor 2 receptor agonist treatment significantly slows disease progression in mdx mice. BMC Med 5:18

Hollenberg CH, Raben MS, Astwood EB (1961) The lipolytic response to corticotropin. Endocrinology 68:589–598

Houslay MD (2010) Underpinning compartmentalised cAMP signalling through targeted cAMP breakdown. Trends Biochem Sci 35(2):91–100

Hui H, Nourparvar A, Zhao X, Perfetti R (2003) Glucagon-like peptide-1 inhibits apoptosis of insulin-secreting cells via a cyclic 5′-adenosine monophosphate-dependent protein kinase A- and a phosphatidylinositol 3-kinase-dependent pathway. Endocrinology 144(4):1444–1455

Huttunen P, Hirvonen J, Kinnula V (1981) The occurrence of brown adipose tissue in outdoor workers. Eur J Appl Physiol Occup Physiol 46(4):339–345

Insel PA (1996) Seminars in medicine of the Beth Israel Hospital, Boston. Adrenergic receptors – evolving concepts and clinical implications. N Engl J Med 334(9):580–585

Ishibashi K, Sasaki S, Akiba T, Marumo F (1993) Expression of bone morphogenic protein 7 mRNA in MDCK cells. Biochem Biophys Res Commun 193(1):235–239

Ishihara H, Asano T, Tsukuda K, Katagiri H, Inukai K, Anai M, Kikuchi M, Yazaki Y, Miyazaki J, Oka Y (1994) Overexpression of hexokinase I but not GLUT1 glucose transporter alters concentration dependence of glucose-stimulated insulin secretion in pancreatic beta-cell line MIN6. J Biol Chem 269(4):3081–3087

Islam D, Zhang N, Wang P, Li H, Brubaker PL, Gaisano HY, Wang Q, Jin T (2009) Epac is involved in cAMP-stimulated proglucagon expression and hormone production but not hormone secretion in pancreatic alpha- and intestinal L-cell lines. Am J Physiol Endocrinol Metab 296(1):E174–E181

Iwami G et al (1995) Regulation of adenylyl cyclase by protein kinase A. J Biol Chem 270 (21):12481–12484

Jambal P, Masterson S, Nesterova A, Bouchard R, Bergman B, Hutton JC, Boxer LM, Reusch JE, Pugazhenthi S (2003) Cytokine-mediated down-regulation of the transcription factor cAMP-response element-binding protein in pancreatic beta-cells. J Biol Chem 278:23055–23065

Jhala US, Canettieri G, Screaton RA, Kulkarni RN, Krajewski S, Reed J, Walker J, Lin X, White M, Montminy M (2003) cAMP promotes pancreatic beta-cell survival via CREB-mediated induction of IRS2. Genes Dev 17:1575–1580

Kahn SE (2003) The relative contributions of insulin resistance and beta-cell dysfunction to the pathophysiology of type 2 diabetes. Diabetologia 46:3–19

Kai AK, Lam A, Chen Y, Tai AC, Zhang X, Lai AK, Yeung PK, Tam S, Wang J, Lam KS, Vanhoutte PM, Bos JL, Chung SS, Xu A, Chung SK (2013) Exchange protein activated by cAMP 1 (Epac1)-deficient mice develop β-cell dysfunction and metabolic syndrome. FASEB J 27(10):4122–4135

Kajimura S, Seale P, Kubota K, Lunsford E, Frangioni JV, Gygi SP, Spiegelman BM (2009) Initiation of myoblast to brown fat switch by a PRDM16-C/EBP-beta transcriptional complex. Nature 460(7259):1154–1158

Kamenetsky M et al (2006) Molecular details of cAMP generation in mammalian cells: a tale of two systems. J Mol Biol 362(4):623–639

Kaneto H, Miyatsuka T, Kawamori D, Yamamoto K, Kato K, Shiraiwa T, Katakami N, Yamasaki Y, Matsuhisa M, Matsuoka TA (2008) PDX-1 and MafA play a crucial role in pancreatic beta-cell differentiation and maintenance of mature beta-cell function. Endocr J 55 (2):235–252

Kang G, Chepurny O, Malester B, Rindler MJ, Rehmann H, Bos JL, Schwede F, Coetzee WA, Holz GG (2006) cAMP sensor Epac as a determinant of ATP-sensitive potassium channel activity in human pancreatic beta cells and rat INS-1 cells. J Physiol 573(Pt 3):595–609

Kashima Y, Miki T, Shibasaki T, Ozaki N, Miyazaki M, Yano H, Seino S (2001) Critical role of cAMP-GEFII—Rim2 complex in incretin-potentiated insulin secretion. J Biol Chem 276:46046–46053

Keravis T, Lugnier C (2010) Cyclic nucleotide phosphodiesterases (PDE) and peptide motifs. Curr Pharm Des 16(9):1114–1125

Kim MJ, Kang J, Park YG, Ryu GR, Ko SH, Jeong IK, Koh KH, Rhie DJ, Yoon SH, Hahn SJ, Kim MS, Jo YH (2006) Exendin-4 induction of cyclin D1 expression in INS-1 beta-cells: involvement of cAMP-responsive element. J Endocrinol 188(3):623–633

Kim SJ, Nian C, Widenmaier S, McIntosh CH (2008) Glucose-dependent insulinotropic polypeptide-mediated up-regulation of beta-cell antiapoptotic Bcl-2 gene expression is coordinated by cyclic AMP (cAMP) response element binding protein (CREB) and cAMP-responsive CREB coactivator 2. Mol Cell Biol 28(5):1644–1656

Kimple ME, Keller M, Rabaglia MR, Pasker RL, Neuman JC, Truchan NA, Brar HK, Attie AD (2013) Prostaglandin E2 receptor, EP3, is induced in diabetic islets and negatively regulates glucose- and hormone-stimulated insulin secretion. Diabetes 62(6):1904–1912

Kolditz CI, Langin D (2010) Adipose tissue lipolysis. Curr Opin Clin Nutr Metab Care 13 (4):377–381

Kong X, Banks A, Liu T, Kazak L, Rao RR, Cohen P, Wang X, Yu S, Lo JC, Tseng YH, Cypess AM, Xue R, Kleiner S, Kang S, Spiegelman BM, Rosen ED (2014) IRF4 is a key thermogenic transcriptional partner of PGC-1α. Cell 158(1):69–83

Koo SH, Flechner L, Qi L, Zhang X, Screaton RA, Jeffries S, Hedrick S, Xu W, Boussouar F, Brindle P, Takemori H, Montminy M (2005) The CREB coactivator TORC2 is a key regulator of fasting glucose metabolism. Nature 437(7062):1109–1111

Leiser M, Fleischer N (1996) cAMP-dependent phosphorylation of the cardiac-type alpha 1 subunit of the voltage-dependent Ca2+ channel in a murine pancreatic beta-cell line. Diabetes 45 (10):1412–1418

Lidell ME et al (2013) Evidence for two types of brown adipose tissue in humans. Nat Med 19 (5):631–634

Light PE, Manning Fox J, Riedel MJ, Wheeler MB (2002) Glucagon-like peptide-1 inhibits pancreatic ATP-sensitive potassium channels via a protein kinase A- and ADP-dependent mechanism. Mol Endocrinol 16(9):2135–2144

Lin B, Morris D, Chou JY (1997) The role of HNF1alpha, HNF3gamma, and cyclic AMP in glucose-6-phosphatase gene activation. Biochemistry 36(46):14096–14106

Liu Y, Dentin R, Chen D, Hedrick S, Ravnskjaer K, Schenk S, Milne J, Meyers DJ, Cole P, Yates J 3rd, Olefsky J, Guarente L, Montminy M (2008) A fasting inducible switch modulates gluconeogenesis via activator/coactivator exchange. Nature 456(7219):269–273

Love JA, Richards N, Owyang C, Dawson DC (1998) Muscarinic modulation of voltage-dependent Ca2+ channels in insulin-secreting HIT-T15 cells. Am J Physiol 274(2 Pt 1): G397–G405

Maltin CA, Hay S, Delday MI, Lobley GE, Reeds PJ (1989) The action of the beta-agonist clenbuterol on protein metabolism in innervated and denervated phasic muscles. Biochem J 261:965–971

Mattsson CL, Csikasz R, Chernogubova E, Yamamoto DL, Hogberg HT, Amri EZ, Hutchinson DS, Bengtsson T (2011) β_1-Adrenergic receptors increase UCP1 in human MADS brown adipocytes and rescue cold-acclimated β_3-adrenergic receptor-knockout mice via nonshivering thermogenesis. Am J Physiol Endocrinol Metab 301(6):E1108–E1118

McKinnon CM, Docherty K (2001) Pancreatic duodenal homeobox-1, PDX-1, a major regulator of beta cell identity and function. Diabetologia 44(10):1203–1214

Metz SA (1988) Epinephrine impairs insulin release by a mechanism distal to calcium mobilization. Similarity to lipoxygenase inhibitors. Diabetes 37(1):65–73

Monroe MB et al (2001) Direct evidence for tonic sympathetic support of resting metabolic rate in healthy adult humans. Am J Physiol Endocrinol Metab 280(5):E740–E744

Morgan DG, Kulkarni R, Hurley JD, Wang ZL, Wang RM, Ghatei MA, Karlsen AE, Bloom SR, Smith DM (1998) Inhibition of glucose stimulated insulin secretion by neuropeptide Y is mediated via the Y1 receptor and inhibition of adenylyl cyclase in RIN 5AH rat insulinoma cells. Diabetologia 41(12):1482–1491

Mullur R, Liu Y, Brent GA (2014) Thyroid hormone regulation of metabolism. Physiol Rev 94 (2):355–382

Murakami T, Nishiyama T, Shirotani T, Shinohara Y, Kan M, Ishii K, Kanai F, Nakazuru S, Ebina Y (1992) Identification of two enhancer elements in the gene encoding the type 1 glucose transporter from the mouse which are responsive to serum, growth factor, and oncogenes. J Biol Chem 267:9300–9306

Niehof M, Manns MP, Trautwein C (1997) CREB controls LAP/C/EBP beta transcription. Mol Cell Biol 17(7):3600–3613

Nishizawa Y, Bray GA (1978) Ventromedial hypothalamic lesions and the mobilization of fatty acids. J Clin Invest 61(3):714–721

Niwa T, Matsukawa Y, Senda T, Nimura Y, Hidaka H, Niki I (1998) Acetylcholine activates intracellular movement of insulin granules in pancreatic beta-cells via inositol trisphosphate-dependent [correction of triphosphate-dependent] mobilization of intracellular Ca2+. Diabetes 47(11):1699–1706

Offield MF, Jetton T, Labosky PA, Ray M, Stein RW, Magnuson MA, Hogan BL, Wright CV (1996) PDX-1 is required for pancreatic outgrowth and differentiation of the rostral duodenum. Development 122(3):983–995

Okla M, Ha J, Temel RE, Chung S (2015) BMP7 drives human adipogenic stem cells into metabolically active beige adipocytes. Lipids 50(2):111–120

Olefsky JM (1977) Insensitivity of large rat adipocytes to the antilipolytic effects of insulin. J Lipid Res 18(4):459–464

Pagnon J et al (2012) Identification and functional characterization of protein kinase A phosphorylation sites in the major lipolytic protein, adipose triglyceride lipase. Endocrinology 153 (9):4278–4289

Park BO, Ahrends R, Teruel MN (2012) Consecutive positive feedback loops create a bistable switch that controls preadipocyte-to-adipocyte conversion. Cell Rep 2(4):976–990

Perfetti R, Zhou J, Doyle ME, Egan JM (2000) Glucagon-like peptide-1 induces cell proliferation and pancreatic-duodenum homeobox-1 expression and increases endocrine cell mass in the pancreas of old, glucose-intolerant rats. Endocrinology 141(12):4600–4605

Petersen RK et al (2008) Cyclic AMP (cAMP)-mediated stimulation of adipocyte differentiation requires the synergistic action of Epac- and cAMP-dependent protein kinase-dependent processes. Mol Cell Biol 28(11):3804–3816

Poitout V, Robertson R (2008) Glucolipotoxicity: fuel excess and beta-cell dysfunction. Endocr Rev 29:351–366

Puddu A, Sanguineti R, Montecucco F, Viviani GL (2015) Effects of high glucose levels and glycated serum on GIP responsiveness in the pancreatic beta cell line HIT-T15. J Diabetes Res 2015:326359

Puigserver P, Wu Z, Park CW, Graves R, Wright M, Spiegelman BM (1998) A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. Cell 92(6):829–839

Purwana I, Zheng J, Li X, Deurloo M, Son DO, Zhang Z, Liang C, Shen E, Tadkase A, Feng ZP, Li Y, Hasilo C, Paraskevas S, Bortell R, Greiner DL, Atkinson M, Prud'homme GJ, Wang Q (2014) GABA promotes human β-cell proliferation and modulates glucose homeostasis. Diabetes 63:4197–4205

Quinn PG, Granner DK (1990) Cyclic AMP-dependent protein kinase regulates transcription of the phosphoenolpyruvate carboxykinase gene but not binding of nuclear factors to the cyclic AMP regulatory element. Mol Cell Biol 10:3357–3364

Richards CS, Yokoyama M, Furuya E, Uyeda K (1982) Reciprocal changes in fructose-2,6-bisphosphate, 2-kinase and fructose-2,6-bisphosphatase activity in response to glucagon and epinephrine. Biochem Biophys Res Commun 104:1073–1079

Richelsen B, Pedersen O (1985) Beta-adrenergic regulation of prostaglandin E2 receptors in human and rat adipocytes. Endocrinology 116(3):1182–1188

Rim JS, Kozak L (2002) Regulatory motifs for CREB-binding protein and Nfe2l2 transcription factors in the upstream enhancer of the mitochondrial uncoupling protein 1 gene. J Biol Chem 277(37):34589–34600

Rosen ED, Spiegelman BM (2014) What we talk about when we talk about fat. Cell 156 (1–2):20–44

Russell TR, Ho R (1976) Conversion of 3T3 fibroblasts into adipose cells: triggering of differentiation by prostaglandin F2alpha and 1-methyl-3-isobutyl xanthine. Proc Natl Acad Sci U S A 73 (12):4516–4520

Ryall JG, Schertzer J, Alabakis TM, Gehrig SM, Plant DR, Lynch GS (2008) Intramuscular beta2-agonist administration enhances early regeneration and functional repair in rat skeletal muscle after myotoxic injury. J Appl Physiol 105:165–172

Saida K, Van Breemen C (1984) Cyclic AMP modulation of adrenoreceptor-mediated arterial smooth muscle contraction. J Gen Physiol 84(2):307–318

Screaton RA, Conkright M, Katoh Y, Best JL, Canettieri G, Jeffries S, Guzman E, Niessen S, Yates JR 3rd, Takemori H, Okamoto M, Montminy M (2004) The CREB coactivator TORC2 functions as a calcium- and cAMP-sensitive coincidence detector. Cell 119:61–74

Sculptoreanu A, Scheuer T, Catterall WA (1993) Voltage-dependent potentiation of L-type Ca2+ channels due to phosphorylation by cAMP-dependent protein kinase. Nature 364 (6434):240–243

Seale P, Kajimura S, Yang W, Chin S, Rohas LM, Uldry M, Tavernier G, Langin D, Spiegelman BM (2007) Transcriptional control of brown fat determination by PRDM16. Cell Metab 6 (1):38–54

Seematter G et al (2004) Relationship between stress, inflammation and metabolism. Curr Opin Clin Nutr Metab Care 7(2):169–173

Semple RK, Crowley V, Sewter CP, Laudes M, Christodoulides C, Considine RV, Vidal-Puig A, O'Rahilly S (2004) Expression of the thermogenic nuclear hormone receptor coactivator PGC-1alpha is reduced in the adipose tissue of morbidly obese subjects. Int J Obes Relat Metab Disord 28(1):176–179

Sette C, Iona S, Conti M (1994) The short-term activation of a rolipram-sensitive, cAMP-specific phosphodiesterase by thyroid-stimulating hormone in thyroid FRTL-5 cells is mediated by a cAMP-dependent phosphorylation. J Biol Chem 269(12):9245–9252

Shimizu Y, Satoh S, Yano H, Minokoshi Y, Cushman SW, Shimazu T (1998) Effects of noradrenaline on the cell-surface glucose transporters in cultured brown adipocytes: novel

mechanism for selective activation of GLUT1 glucose transporters. Biochem J 330 (Pt 1):397–403
Siersbaek R et al (2014) Molecular architecture of transcription factor hotspots in early adipogenesis. Cell Rep 7(5):1434–1442
Silva JE (2006) Thermogenic mechanisms and their hormonal regulation. Physiol Rev 86 (2):435–464
Silva JE, Larsen PR (1983) Adrenergic activation of triiodothyronine production in brown adipose tissue. Nature 305(5936):712–713
Sinnarajah S et al (2001) RGS2 regulates signal transduction in olfactory neurons by attenuating activation of adenylyl cyclase III. Nature 409(6823):1051–1055
Skala JP, Knight BL (1977) Protein kinases in brown adipose tissue of developing rats. State of activation of protein kinase during development and cold exposure and its relationship to adenosine 3′:5′-monophosphate, lipolysis, and heat production. J Biol Chem 252 (3):1064–1070
Smith FD et al (2013) Intrinsic disorder within an AKAP-protein kinase A complex guides local substrate phosphorylation. Elife 2:e01319
Soderling TR, Hickenbottom J, Reimann EM, Hunkeler FL, Walsh DA, Krebs EG (1970) Inactivation of glycogen synthetase and activation of phosphorylase kinase by muscle adenosine 3′,5′-monophosphate-dependent protein kinases. J Biol Chem 245:6317–6328
Song WJ, Schreiber W, Zhong E, Liu FF, Kornfeld BD, Wondisford FE, Hussain MA (2008) Exendin-4 stimulation of cyclin A2 in beta-cell proliferation. Diabetes 57(9):2371–2381
Staimez LR, Weber M, Ranjani H, Ali MK, Echouffo-Tcheugui JB, Phillips LS, Mohan V, Narayan KM (2013) Evidence of reduced β-cell function in Asian Indians with mild dysglycemia. Diabetes Care 36:2772–2778
Studer RK, Borle AB (1982) Differences between male and female rats in the regulation of hepatic glycogenolysis. The relative role of calcium and cAMP in phosphorylase activation by catecholamines. J Biol Chem 257(14):7987–7993
Studer RK, Snowdowne KW, Borle AB (1984) Regulation of hepatic glycogenolysis by glucagon in male and female rats. Role of cAMP and Ca2+ and interactions between epinephrine and glucagon. J Biol Chem 259(6):3596–3604
Sun X, Dang F, Zhang D, Yuan Y, Zhang C, Wu Y, Wang Y, Liu Y (2015) Glucagon-CREB/CRTC2 signaling cascade regulates hepatic BMAL1 protein. J Biol Chem 290(4):2189–2197
Tang QQ, Lane MD (2012) Adipogenesis: from stem cell to adipocyte. Annu Rev Biochem 81:715–736
Thomas SA, Palmiter RD (1997) Thermoregulatory and metabolic phenotypes of mice lacking noradrenaline and adrenaline. Nature 387(6628):94–97
Thorens B, Guillam M, Beermann F, Burcelin R, Jaquet M (2000) Transgenic reexpression of GLUT1 or GLUT2 in pancreatic beta cells rescues GLUT2-null mice from early death and restores normal glucose-stimulated insulin secretion. J Biol Chem 275(31):23751–23758
Tourrel C, Bailbé D, Meile MJ, Kergoat M, Portha B (2001) Glucagon-like peptide-1 and exendin-4 stimulate beta-cell neogenesis in streptozotocin-treated newborn rats resulting in persistently improved glucose homeostasis at adult age. Diabetes 50:1562–1570
Valverde I et al (1979) Calmodulin activation of adenylate cyclase in pancreatic islets. Science 206 (4415):225–227
van Marken Lichtenbelt WD et al (2009) Cold-activated brown adipose tissue in healthy men. N Engl J Med 360(15):1500–1508
Virtanen KA et al (2009) Functional brown adipose tissue in healthy adults. N Engl J Med 360 (15):1518–1525
Walsh DA, Perkins JP, Krebs EG (1968) An adenosine 3′,5′-monophosphate-dependant protein kinase from rabbit skeletal muscle. J Biol Chem 243(13):3763–3765
Wang Y, Perfetti R, Greig NH, Holloway HW, DeOre KA, Montrose-Rafizadeh C, Elahi D, Egan JM (1997) Glucagon-like peptide-1 can reverse the age-related decline in glucose tolerance in rats. J Clin Invest 99:2883–2889

Wang H, Iezzi M, Theander S, Antinozzi PA, Gauthier BR, Halban PA, Wollheim CB (2005) Suppression of Pdx-1 perturbs proinsulin processing, insulin secretion and GLP-1 signalling in INS-1 cells. Diabetologia 48(4):720–731

Wang Y, Inoue H, Ravnskjaer K, Viste K, Miller N, Liu Y, Hedrick S, Vera L, Montminy M (2010) Targeted disruption of the CREB coactivator Crtc2 increases insulin sensitivity. Proc Natl Acad Sci U S A 107(7):3087–3092

Welters HJ, Diakogiannaki E, Mordue JM, Tadayyon M, Smith SA, Morgan NG (2006) Differential protective effects of palmitoleic acid and cAMP on caspase activation and cell viability in pancreatic beta-cells exposed to palmitate. Apoptosis 11(7):1231–1238

White JE, Engel FL (1958) Lipolytic action of corticotropin on rat adipose tissue in vitro. J Clin Invest 37(11):1556–1563

Whittle AJ, Carobbio S, Martins L, Slawik M, Hondares E, Vázquez MJ, Morgan D, Csikasz RI, Gallego R, Rodriguez-Cuenca S, Dale M, Virtue S, Villarroya F, Cannon B, Rahmouni K, López M, Vidal-Puig A (2012) BMP8B increases brown adipose tissue thermogenesis through both central and peripheral actions. Cell 149(4):871–885

Withers DJ, Burks D, Towery HH, Altamuro SL, Flint CL, White MF (1999) Irs-2 coordinates Igf-1 receptor-mediated beta-cell development and peripheral insulin signalling. Nat Genet 23:32–40

Wynshaw-Boris A, Short J, Loose DS, Hanson RW (1986) Characterization of the phosphoenolpyruvate carboxykinase (GTP) promoter-regulatory region. I. Multiple hormone regulatory elements and the effects of enhancers. J Biol Chem 261:9714–9720

Yabe D, Seino Y (2011) Two incretin hormones GLP-1 and GIP: comparison of their actions in insulin secretion and β cell preservation. Prog Biophys Mol Biol 107(2):248–256

Yasuda K et al (2006) Adrenergic receptor polymorphisms and autonomic nervous system function in human obesity. Trends Endocrinol Metab 17(7):269–275

Yokomori N, Tawata M, Hosaka Y, Onaya T (1992) Transcriptional regulation of hexokinase I mRNA levels by TSH in cultured rat thyroid FRTL5 cells. Life Sci 51(20):1613–1619

Yoshimasa T et al (1987) Cross-talk between cellular signalling pathways suggested by phorbol-ester-induced adenylate cyclase phosphorylation. Nature 327(6117):67–70

Yosida M, Dezaki K, Uchida K, Kodera S, Lam NV, Ito K, Rita RS, Yamada H, Shimomura K, Ishikawa SE, Sugawara H, Kawakami M, Tominaga M, Yada T, Kakei M (2014) Involvement of cAMP/EPAC/TRPM2 activation in glucose- and incretin-induced insulin secretion. Diabetes 63(10):3394–3403

Zaccolo M (2011) Spatial control of cAMP signalling in health and disease. Curr Opin Pharmacol 11(6):649–655

Zaccolo M, Pozzan T (2002) Discrete microdomains with high concentration of cAMP in stimulated rat neonatal cardiac myocytes. Science 295(5560):1711–1715

Zhang JW et al (2004) Role of CREB in transcriptional regulation of CCAAT/enhancer-binding protein beta gene during adipogenesis. J Biol Chem 279(6):4471–4478

Zhang EE, Liu Y, Dentin R, Pongsawakul PY, Liu AC, Hirota T, Nusinow DA, Sun X, Landais S, Kodama Y, Brenner DA, Montminy M, Kay SA (2010) Cryptochrome mediates circadian regulation of cAMP signaling and hepatic gluconeogenesis. Nat Med 16(10):1152–1156

Zhao AZ, Zhao H, Teague J, Fujimoto W, Beavo JA (1997) Attenuation of insulin secretion by insulin-like growth factor 1 is mediated through activation of phosphodiesterase 3B. Proc Natl Acad Sci U S A 94(7):3223–3228

Zhou J, Pineyro M, Wang X, Doyle ME, Egan JM (2002) Exendin-4 differentiation of a human pancreatic duct cell line into endocrine cells: involvement of PDX-1 and HNF3beta transcription factors. J Cell Physiol 192(3):304–314

Ziegler MG et al (2012) Epinephrine and the metabolic syndrome. Curr Hypertens Rep 14(1):1–7

Zippin JH et al (2004) Bicarbonate-responsive "soluble" adenylyl cyclase defines a nuclear cAMP microdomain. J Cell Biol 164(4):527–534

Insulin Signaling in the Control of Glucose and Lipid Homeostasis

Alan R. Saltiel

Contents

Abstract

A continuous supply of glucose is necessary to ensure proper function and survival of all organs. Plasma glucose levels are thus maintained in a narrow range around 5 mM, which is considered the physiological set point. Glucose homeostasis is controlled primarily by the liver, fat, and skeletal muscle. Following a meal, most glucose disposals occur in the skeletal muscle, whereas fasting plasma glucose levels are determined primarily by glucose output from the liver.

The balance between the utilization and production of glucose is primarily maintained at equilibrium by two opposing hormones, insulin and glucagon. In response to an elevation in plasma glucose and amino acids (after consumption of a meal), insulin is released from the beta cells of the islets of Langerhans in

A.R. Saltiel (✉)
Life Sciences Institute, University of Michigan, AnnArbor, MI, USA
e-mail: saltiel@umich.edu

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the pancreas. When plasma glucose falls (during fasting or exercise), glucagon is secreted by α cells, which surround the beta cells in the pancreas. Both cell types are extremely sensitive to glucose concentrations, can regulate hormone synthesis, and are released in response to small changes in plasma glucose levels. At the same time, insulin serves as the major physiological anabolic agent, promoting the synthesis and storage of glucose, lipids, and proteins and inhibiting their degradation and release back into the circulation.

This chapter will focus mainly on signal transduction mechanisms by which insulin exerts its plethora of effects in liver, muscle, and fat cells, focusing on those pathways that are crucial in the control of glucose and lipid homeostasis.

Keywords
Glucose, Glycogen, Lipid, Phosphatase, Protein kinase, Receptor

1 Early Signaling Pathways

1.1 The Insulin Receptor and Its Substrates

The insulin receptor is a tetrameric protein consisting of two α-subunits and two β-subunits that belongs to a subfamily of receptor tyrosine kinases also including the insulin-like growth factor 1 (IGF-1) receptor and the insulin receptor-related receptor (IRR) (Hedo et al. 1983) (Fig. 1). For each receptor, the two subunits are derived from a single-chain precursor or proreceptor that is processed by a furin-like enzyme to give a single α–β subunit complex, in which the α-subunits bind to insulin and the β-subunits act as tyrosine kinases (Lane et al. 1985). Two of the α–β dimers undergo disulfide linkage to form the functional tetramer. The α-subunit attenuates the β-subunit tyrosine kinase activity; insulin binding to the α-subunit relieves this inhibition. Activation results in transphosphorylation of the β-subunits, leading to a conformational change and a further increase in activity of the kinase domain. The α–β heterodimers of the insulin and IGF-1 receptors and the IRR form functional hybrids in which binding to one receptor leads to transphosphorylation of the other. Likewise, heterodimers between a dominant-negative form of one receptor can inhibit the activity of the other, perhaps explaining why individuals with mutations in the insulin receptor exhibit both insulin resistance and growth retardation (Ablooglu and Kohanski 2001).

At least nine intracellular substrates of the insulin and IGF-1 receptor tyrosine kinases have been identified (Fig. 2). Four of these belong to the family of insulin/IGF-1 receptor substrate (IRS) proteins (Sun et al. 1991, 1995; Lavan et al. 1997). These IRS proteins are characterized by the presence of both pleckstrin homology (PH) and phosphotyrosine binding (PTB) domains near the N-terminus that account for the high affinity of these substrates for the insulin receptor, and up to 20 potential tyrosine phosphorylation sites spread throughout the molecule. IRS-1 and IRS-2 are

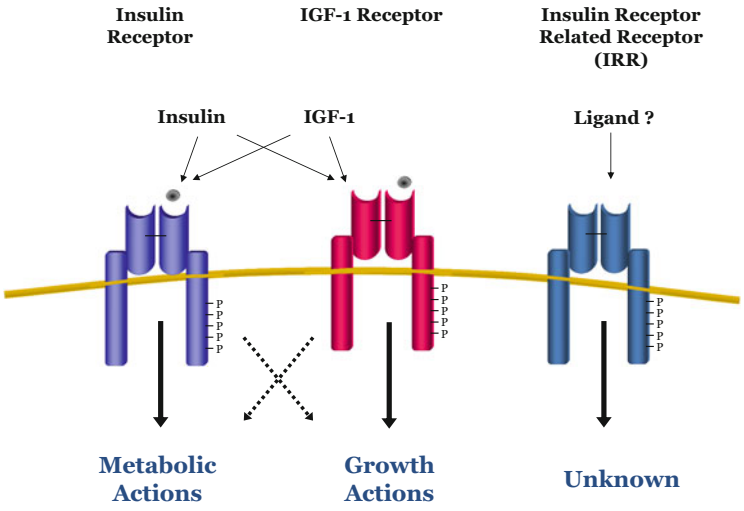

Fig. 1 Subunit structures of the insulin and insulin-like receptors

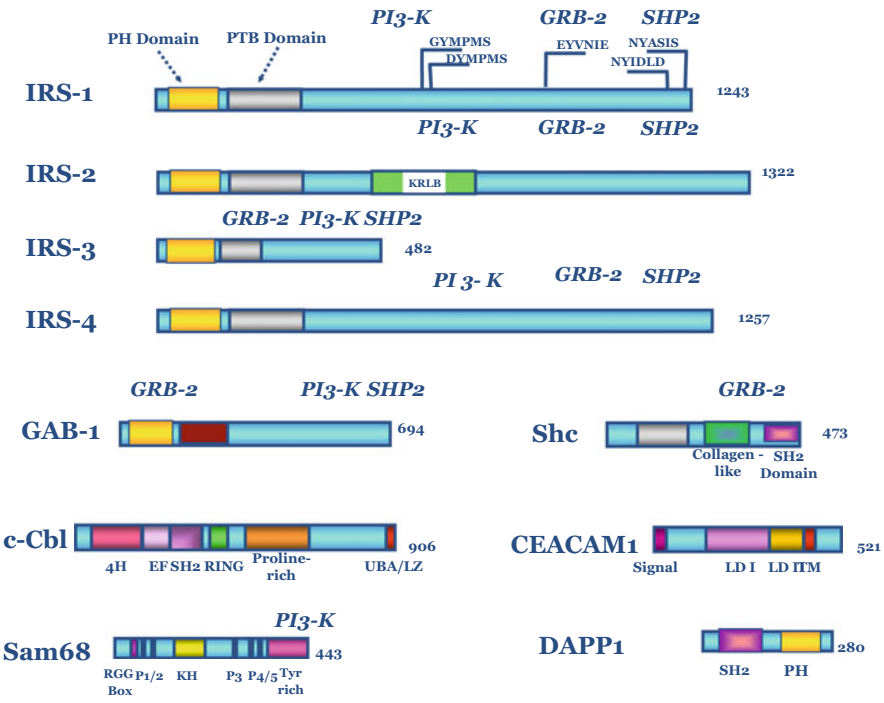

Fig. 2 Substrates of the insulin and IGF-1 receptors

widely distributed, whereas IRS-3 and IRS-4 have more limited distributions. IRS-3 is most abundant in adipocytes, and its mRNA is also detected in the liver, heart, lung, brain, and kidney (Myers and White 1995), while IRS-4 is present at very low levels in the fibroblasts, embryonic tissues, skeletal muscle, liver, heart, hypothalamus, and kidney (Fantin et al. 1999). Other direct substrates of the insulin/IGF-1 receptor kinases include Gab-1 (Lehr et al. 2000), $p62^{dok}$ (Wick et al. 2001), Cbl (Baumann et al. 2000), APS (Liu et al. 2002), and the various isoforms of Shc (Gustafson et al. 1995), each of which initiates a separate signaling pathway. Following insulin stimulation, the receptor directly phosphorylates most of these substrates on multiple tyrosine residues. The phosphorylated tyrosines in each of these substrates occur in specific sequence motifs; once phosphorylated, they serve as docking sites for intracellular molecules that contain SH2 (Src-homology2) domains (Saltiel and Kahn 2001).

The SH2 proteins that bind to phosphorylated substrate proteins fall into two major categories. The best studied are adaptor molecules, such as the regulatory subunit of PI 3-kinase, or Grb2, which associates with SOS to activate the Ras–ERK pathway (Saltiel and Kahn 2001; Jhun et al. 1994). The other major category of proteins that bind to IRS proteins are enzymes, such as the phosphotyrosine phosphatase SHP2 (Milarski and Saltiel 1994), and cytoplasmic tyrosine kinases, such as Fyn.

Although the IRS proteins are highly homologous and possess similar tyrosine phosphorylation motifs, recent studies in knockout (KO) mice and knockout cell lines suggest that the various IRS proteins serve complementary rather than redundant roles in insulin and IGF-1 signaling. IRS-1 knockout mice exhibit IGF-1 resistance as manifested by prenatal and postnatal growth retardation, as well as insulin resistance, primarily in muscle and fat, resulting in impaired glucose tolerance (Araki et al. 1994; Yamauchi et al. 1996). IRS-2 knockout mice also exhibit insulin resistance, but primarily in the liver, and have defects in growth in only selected tissues of the body, including certain regions of the brain, β-cells, and retinal cells (Withers et al. 1998). Likewise at the cellular level, IRS-1 knockout preadipocytes exhibit defective differentiation (Miki et al. 2001), while IRS-2 knockout preadipocytes differentiate normally but fail to respond to insulin (Fasshauer et al. 2000).

There is also a difference in β-cell compensatory response in KO mice. Although there is some element of β-cell dysfunction, there is sufficient islet hyperplasia such that IRS-1 knockout mice only develop mildly impaired glucose tolerance. In contrast, IRS-2 knockout mice exhibit decreased islet mass due to altered β-cell development. The combination of insulin resistance and decreased β-cell mass leads the IRS-$2^{-/-}$ mice to develop early-onset type 2 diabetes (Withers et al. 1998). In contrast, IRS-3 knockout mice have normal growth and metabolism, while IRS-4 knockout mice exhibit only minimal abnormalities in glucose tolerance (Fantin et al. 2000).

2 Turning Off the Insulin Receptor

The action of insulin on glucose homeostasis demands a rapid on-and-off response to avoid the dangers of hypoglycemia. Upon its dissociation from its receptor, insulin can be degraded. The insulin signal may also be terminated by internalization and degradation of its receptor (Carpentier et al. 1992). Following dissociation of the ligand, phosphorylation of the insulin receptor and its substrates is rapidly reversed by the action of protein tyrosine phosphatases (PTPases). Several PTPases have been identified that are capable of catalyzing dephosphorylation of the insulin receptor in vitro or in vivo, and some are even upregulated in insulin-resistant states (Goldstein et al. 2000; Sugimoto et al. 1994). Most attention has focused on the cytoplasmic phosphatase PTP-1b. Disruption of the gene encoding this enzyme in mice produces increased insulin-dependent tyrosine phosphorylation of the insulin receptor and IRS proteins in muscle and leads to improved insulin sensitivity (Zinker et al. 2002). PTP-1b knockout mice are also resistant to diet-induced obesity, suggesting that PTP-1b deletion in the brain may influence energy uptake and expenditure.

IRS proteins also undergo serine phosphorylation in response to insulin and other stimuli to reduce insulin action. In general, serine phosphorylation appears to act as a negative regulator of insulin signaling by decreasing tyrosine phosphorylation of IRS proteins, as well as by promoting interaction with 14-3-3 proteins (Craparo et al. 1997). A number of different intracellular enzymes have been implicated in this serine phosphorylation, including some in the insulin signaling pathway, such as Akt (Li et al. 1999), JNK (Hirosumi et al. 2002), ERK (De Fea and Roth 1997), and PI 3-kinase (which also has serine kinase activity) (Tanti et al. 1994), all of which have the potential to provide feedback inhibition.

3 Phosphatidylinositol 3-Kinase and Downstream Targets

The first SH2 domain protein identified to interact with IRS-1 was the enzyme PI 3-kinase, specifically members of the class Ia form. This enzyme plays a pivotal role in the metabolic and mitogenic actions of insulin and IGF-1 (Cheatham et al. 1994) (Fig. 3). Inhibitors of PI 3-kinase or transfection with dominant-negative constructs of the enzyme block virtually all of the metabolic actions of insulin, including stimulation of glucose transport, glycogen synthesis, and lipid synthesis. The enzyme itself consists of a regulatory and a catalytic subunit. Activation of the catalytic subunit depends on the interaction of the two SH2 domains in the regulatory subunit with specific tyrosine-phosphorylated motifs in the IRS proteins of the sequence pYMXM and pYXXM (Backer et al. 1992; Myers et al. 1994a).

At least eight isoforms of the regulatory subunits of PI 3-kinase have been identified. These are derived from three genes which undergo alternative splicing (Carpenter and Cantley 1996). p85α and p85β represent the “full-length” versions of the regulatory subunits and contain an SH3 domain, a bcr homology domain

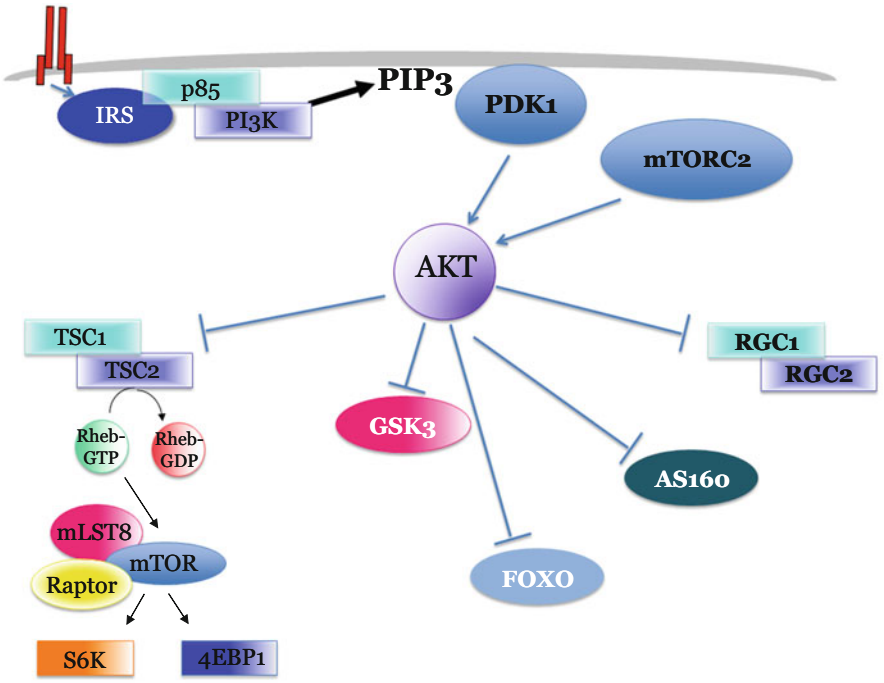

Fig. 3 The PI 3-kinase pathway in insulin action

flanked by two proline-rich domains, two SH2 domains, and an inter-SH2 (iSH2) domain containing the p110 binding region (Carpenter and Cantley 1996). The shorter versions of regulatory subunits, p55α and p50α, are splicing variants derived from the same gene encoding p85α (*Pik3r1*) (Inukai et al. 1997). They share the common nSH2-iSH2-cSH2 with p85α but lack the N-terminal half. p85α is ubiquitously expressed; however, the splice variants, p55α/AS53 and p50α appear to play specific roles in some selected tissues or in particular states of insulin resistance (Kerouz et al. 1997). The exact role of the different regulatory subunits of PI 3-kinase in insulin action is unclear. Knockout mice with a disruption of all three isoforms of *Pik3r1* gene die within a few weeks of birth, indicating the importance of p85α and its spliced variants in normal growth and normal metabolism (Fruman et al. 2000). One explanation for the increased sensitivity in both cases is the improved stoichiometry. Under normal conditions, the concentration of regulatory subunits is in excess of that of the catalytic subunits and phosphorylated IRS proteins. This leads to the binding of free monomeric forms of regulatory subunit to phosphorylated IRS proteins, thus blocking the binding of the active heterodimer. Mice with a heterozygous knockout *Pik3r1* gene also have improved stoichiometry of interaction between the regulatory and catalytic subunits, resulting in improved sensitivity to insulin and IGF-1 and protection of mice with genetic insulin resistance from developing diabetes. Likewise, cell lines derived from heterozygous *Pik3r1* gene knockout embryos exhibit increased insulin/IGF-1 signaling (Ueki et al. 2002; Mauvais-Jarvis et al. 2002).

PI 3-kinase itself catalyzes the phosphorylation of phosphoinositides on the 3′-position to PI-(3)P, PI-(3,4)P_2, and PI-(3,4,5)P_3 (also known as PIP_3). These lipids bind to the pleckstrin homology (PH) domains of a variety of signaling molecules and alter their activity or subcellular localization. Three major classes of signaling molecules are regulated by PI 3-phosphates: the AGC superfamily of serine/threonine protein kinases, guanine nucleotide exchange proteins of the Rho family of GTPase, and the TEC family of tyrosine kinases, including BTK and ITK.

The best-characterized pathway involves the AGC kinase known as PDK1. This enzyme, along with the serine kinase mTORC2, phosphorylates and activates the serine/threonine kinase Akt. Akt is thought to play an important role in transmission of insulin's metabolic pathways by phosphorylating glycogen synthase kinase-3 (Rommel et al. 2001), the forkhead (FOXO) transcription factors, the cyclic AMP regulatory element-binding protein CREB (Burgering and Coffer 1995; Downward 1998; Lawlor and Alessi 2001), and the GAP proteins TSC2, AS160, and RGC2 also known as RalGAPA (Leto and Saltiel 2012). However, studies using inhibitors and activators of Akt have not uniformly inhibited or mimicked insulin actions (Lawlor and Alessi 2001). Part of the variability may relate to the fact that there are three isoforms of Akt/PKB (Datta et al. 1999). Although the major form, Akt1, is clearly important for cell survival and growth, Akt2 appears to be more important in mediating insulin action in the liver (Cho et al. 2001). Other AGC kinases that are downstream of PI 3-kinase are the atypical forms of protein kinase C (PKC), including PKCζ and PKC. Stable expression of a constitutively active, membrane-bound form of Akt in 3T3L1 adipocytes resulted in increased glucose transport and persistent localization of GLUT4 to the plasma membrane (Kohn et al. 1996), but does not fully reproduce insulin action. Conversely, expression of a dominant-interfering Akt mutant inhibited insulin-stimulated GLUT4 translocation. Likewise, overexpression of PKCζ or PKCλ results in GLUT4 translocation (Bandyopadhyay et al. 1997; Standaert et al. 1997), whereas expression of a dominant-interfering PKCλ blocks the action of insulin (Kotani et al. 1998).

The insulin signal can also be terminated by reducing the level of PIP_3 in the cell (Lazar and Saltiel 2006). This is achieved through the activity of PIP_3 phosphatases, such as PTEN (Nakashima et al. 2000) and SHIP2 (Clement et al. 2001). PTEN dephosphorylates phosphoinositides on the 3′-position, thus lowering the level of the second messengers. SHIP2 is a 5′-phosphoinositide phosphatase. Disruption of the gene encoding this enzyme also yields mice with increased insulin sensitivity (Clement et al. 2001).

4 The APS/Cbl-Associated Protein/Cbl Pathway

Although PI 3-kinase activity is clearly necessary for insulin-stimulated glucose uptake, several lines of evidence suggest that additional signals may also be required. Indeed, other hormones or growth factors that activate PI 3-kinase, such as platelet-derived growth factor (PDGF) and interleukin-4, do not stimulate glucose transport (Wiese et al. 1995). Likewise, addition of a PIP_3 analogue alone had

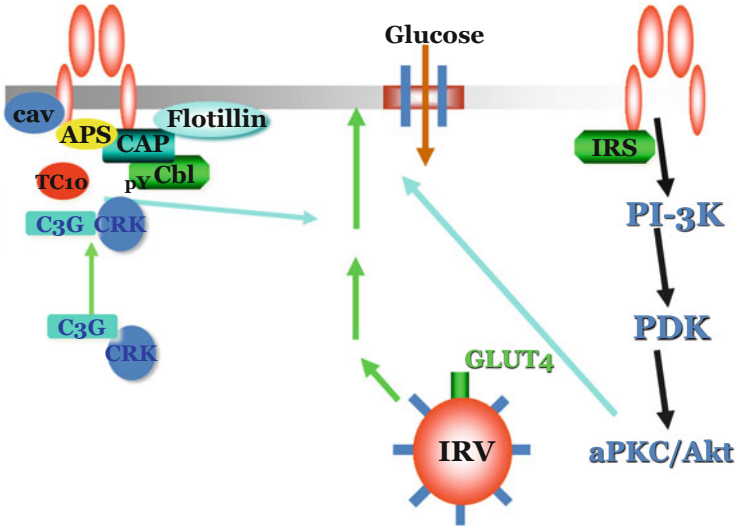

Fig. 4 The APS/CAP/Cbl pathway in insulin action

no effect on glucose transport (Jiang et al. 1998). In addition, two naturally occurring insulin-receptor mutants that appear to be fully capable of activating PI 3-kinase are unable to mediate full insulin action (Krook et al. 1996).

As mentioned above, insulin also initiates a PI-3-kinase-independent signaling pathway by recruiting the adaptor protein APS, which binds with high affinity to the activated insulin receptor (Hu et al. 2003) (Fig. 4). Upon its phosphorylation, APS recruits a complex of two proteins, CAP and c-Cbl (Liu et al. 2002; Ribon et al. 1998a). This triggers insulin receptor-catalyzed tyrosine phosphorylation of c-Cbl, which then interacts with the adaptor protein Crk, which is in complex with the GEF C3G (Ribon et al. 1996). C3G in turn catalyses activation of TC10, a member of the Rho family of small GTPases (Knudsen et al. 1994; Chiang et al. 2001) that is localized in lipid rafts in the plasma membrane, and it is discussed below. CAP expression correlates well with insulin responsiveness, and its expression is increased by treatment of cells with insulin-sensitizing thiazolidinediones (Ribon et al. 1998b). CAP belongs to a family of adapter proteins that contain a sorbin homology (SoHo) domain. This allows CAP to interact with one of the components of the lipid raft domain of the plasma membrane, a protein called flotillin. Expression of CAP mutants that cannot bind to Cbl or flotillin inhibits Cbl translocation and insulin-stimulated glucose uptake (Baumann et al. 2000). The importance of this pathway remains uncertain, since mice with a targeted knockout of CAP display increased insulin sensitivity, due mainly to reduced inflammation (Lesniewski et al. 2007). However, there are other members of the SoHo domain family that can compensate (Zhang et al. 2007).

5 The Ras–Mitogen-Activated Protein Kinase Cascade and mTOR

Insulin also activates the Ras–mitogen-activated protein (MAP) kinase (ERK) cascade. Following the tyrosine phosphorylation of one of the IRS proteins or Shc, there is binding of the adaptor protein Grb2, which in turn recruits the guanyl nucleotide exchange protein SOS to the plasma membrane, thus activating Ras (Skolnik et al. 1993; Myers et al. 1994b). Full activation of Ras by insulin also requires stimulation of the tyrosine phosphatase SHP2, which also interacts with insulin-receptor substrates such as Gab-1 and IRS1/2 (Milarski and Saltiel 1994; Yamauchi et al. 1995). Once activated, Ras operates as a molecular switch, converting upstream tyrosine phosphorylations into a second serine kinase cascade, via the stepwise activation of Raf, the MAP kinase kinase MEK, and the MAP kinases themselves, ERK1 and ERK2. The MAP kinases can phosphorylate substrates in the cytoplasm or translocate into the nucleus and catalyze the phosphorylation of transcription factors, such as $p62^{TCF}$, initiating a transcriptional program that leads the cell to commit to a proliferative or differentiative cycle. Blockade of the Ras–MAP kinase pathway with dominant-negative mutants or pharmacologic inhibitors can prevent the stimulation of cell growth by insulin but has no effect on any of the anabolic or metabolic actions of the hormone (Lazar et al. 1995).

Yet another component of insulin signaling, particularly effects on protein synthesis/degradation and interaction with nutrient sensing, involves the protein kinase mTOR (mammalian target of rapamycin). mTOR is a member of the PI 3-kinase family but appears to serve primarily as a protein kinase. Stimulation of mTOR appears to involve PI 3-kinase as well as another signal (Ozes et al. 2001; Laplante and Sabatini 2009; Inoki et al. 2003; Alessi et al. 2009; Mori et al. 2009; Li et al. 2010). mTOR itself helps regulate mRNA translation via phosphorylation and activation of the p70 ribosomal S6 kinase (p70 S6 kinase), as well as the phosphorylation of the eIF-4E inhibitor, PHAS-1 or 4E-BP1. p70 S6 kinase phosphorylates ribosomal S6 protein, thus activating ribosome biosynthesis and increasing translation of mRNAs with a 5′-terminal oligopyrimidine tract. Phosphorylation of PHAS-1 by mTOR results in its dissociation from eIF-2, allowing cap-dependent translation of mRNAs with a highly structured 5′-untranslated region.

6 The Regulation of Glucose Transport

The classical effect of insulin on glucose homeostasis is its ability to stimulate glucose transport in fat and muscle. This occurs via a translocation of GLUT4 glucose transporters from intracellular sites to the plasma membrane, and many of these steps are controlled by the regulation of small G proteins (Fig. 5). The GLUT4 protein consists of 12 transmembrane helices with a characteristic C-terminal tail containing two adjacent leucine residues commonly found in proteins that undergo regulated trafficking. In the basal state, GLUT4 continuously recycles between the

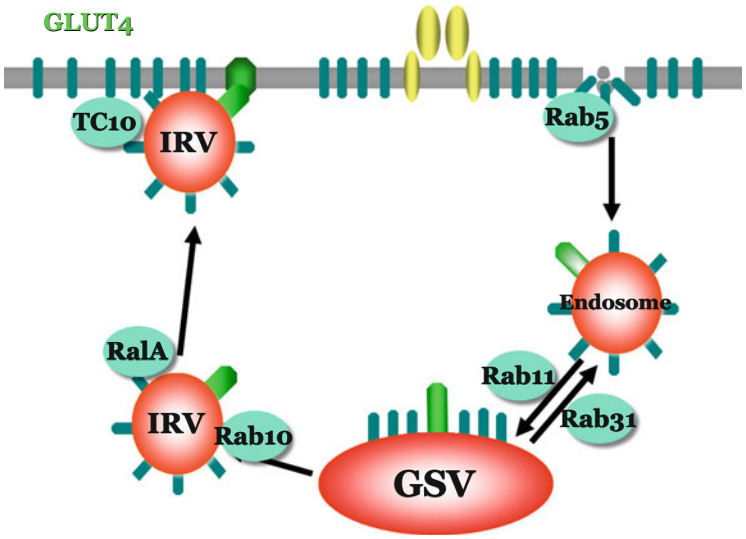

Fig. 5 The role of G proteins in the regulation of GLUT4 trafficking

cell surface and various intracellular compartments. The GLUT4 vesicle is highly specialized and derived from a sorting endosomal population. Insulin markedly increases the rate of GLUT4 vesicle exocytosis and appears to slightly decrease the rate of internalization of the GLUT4 protein, although this latter effect is controversial. While the exact domains of the protein involved in localization and trafficking remain controversial, the C- and N-terminal tails of the protein, both of which are oriented on the cytoplasmic side of the vesicle, appear to be required (Haney et al. 1995).

The trafficking itinerary of GLUT4 is controlled by insulin (Leto and Saltiel 2012). After endocytosis, recycled membrane proteins can return quickly to the plasma membrane from sorting endosomes or they can sort through intracellular compartments, including recycling endosomes, late endosomes, and the TGN, before returning to the plasma membrane (Maxfield and McGraw 2004). A single GLUT4 molecule undergoes multiple cycles of exocytosis and endocytosis. In the basal state, at least half of the GLUT4 population is found in a specialized vesicle compartment, and stimulation with insulin depletes a proportion of these GLUT4-enriched vesicles (GSVs). Although insulin causes rapid translocation of GSVs to the plasma membrane, prolonged stimulation with the hormone triggers GLUT4 movement to the plasma membrane in endosomes, suggesting that once preformed GSVs have been depleted, GLUT4 recycling from endosomes is favored rather than reforming new GSVs (Muretta et al. 2008; Xu et al. 2011). Thus, insulin may increase surface GLUT4 levels by acting on at least two processes in GLUT4 trafficking: exocytosis of GSVs and recycling via endosomes (Fig. 5).

GLUT4 sorting and delivery into GSVs relies on the actions of small GTPases, which assemble effectors that mediate vesicle budding, transport, and fusion. In adipocytes and muscle cells, Rabs 4, 5, 8, 10, 11, 14, and 31 have been implicated in regulating different steps in GLUT4 sorting, although additional small GTPases (including Arf6 and RalA) have been found associated with GLUT4-containing vesicles and some of these also affect GSV formation (Miinea et al. 2005). Rab5, which drives homotypic and heterotypic early endosomal fusion, is activated at the plasma membrane by insulin through TC10, which recruits the Rab5 GEF Gapex-5 to the surface of the cell. Rab5 may regulate mobility and sorting of GLUT4-containing vesicles after endocytosis (Lodhi et al. 2008; Huang et al. 2001; Zerial and McBride 2001).

The Akt substrate AS160 is a RabGAP that targets Rabs 8 and 14 in muscle cells (Sun et al. 2010) and Rab10 in adipocytes (Miinea et al. 2005; Sano et al. 2007). These Rabs have a positive role in GLUT4 translocation, suggesting that they may regulate GSV formation and/or intracellular retention (Sun et al. 2010; Sano et al. 2007, 2008; Ishikura and Klip 2008). As insulin stimulates phosphorylation of AS160 via Akt, blocking the function of the protein, which relieves its inhibitory effect on its target Rabs (Kane et al. 2002; Sano et al. 2003) (Fig. 1). Whereas activation of Rabs 8 and 14 by insulin has been demonstrated in muscle cells (Sun et al. 2010), so far activation of Rab10 in adipocytes has not been detected (Sano et al. 2008). Nevertheless, Rab10 is necessary for maximal GLUT4 exocytosis in response to insulin, and several lines of evidence indicate that cycling of this small GTPase may increase glucose uptake (Sano et al. 2007, 2011).

Incoming vesicles are targeted through a tethering/docking step to regions of the plasma membrane that contain the fusion machinery. In adipocytes, GSV targeting to lipids rafts is important for efficient insertion of GLUT4 into the plasma membrane (Inoue et al. 2006). A critical component of the GLUT4 tethering machinery is the exocyst, an evolutionarily conserved octameric complex that assembles at sites of exocytosis and tethers exocytic vesicles on the plasma membrane (He and Guo 2009). The exocyst is thought to flexibly mediate the initial contact between exocytic vesicles and the plasma membrane from a relatively long distance and can thus concentrate GSVs before the final membrane fusion step. Inhibition of exocyst assembly in adipocytes disrupts fusion of GSVs without affecting their translocation, demonstrating that this complex is necessary for vesicle targeting at the plasma membrane (Inoue et al. 2003).

Insulin regulates exocyst-mediated targeting through three steps: exocyst assembly, engagement of the exocyst by GSVs, and GSV disengagement from the exocyst to enable fusion (Inoue et al. 2003, 2006; Chen et al. 2007, 2011a). First, insulin directs assembly of the exocyst at the plasma membrane by promoting an interaction between active TC10 and the exocyst scaffolding subunit Exo70 (Inoue et al. 2003). Exo70 is constitutively associated with other exocyst subunits and thereby assembles the complex at the plasma membrane (Inoue et al. 2006). Other interactions between exocyst subunits and the plasma membrane facilitate complex assembly at discrete locations; multiple interactions between plasma membrane

constituents and exocyst subunits coordinate exocyst assembly in lipid rafts and localized GLUT4 targeting.

The exocyst is recognized by GSVs via the small GTPase RalA, which is present in GLUT4-containing vesicles. Insulin controls RalA activity by inhibiting a complex of proteins with RalGAP activity. The RalGAP complex is composed of a regulatory subunit (RGC1 or RalGAPA) and a catalytic subunit (RGC2 or RalGAPB) that contains a GAP domain with specific activity toward Ral GTPases. Akt-catalyzed phosphorylation of RGC2 on at least three residues inhibits the complex and allows for GTP loading on RalA (Chen et al. 2011b). SiRNA-mediated knockdown of RGC1 or RGC2 increases RalA activity and insulin-stimulated glucose uptake, demonstrating the regulatory role of this complex (De Fea and Roth 1997). SiRNA-mediated knockdown or overexpression of a dominant-negative RalA mutant blocks insulin-stimulated glucose uptake and GLUT4 insertion into the plasma membrane; by contrast, constitutively active RalA mutants increase the effect of insulin, indicating that activation of this small GTPase is required for insulin-regulated GLUT4 exocytosis (Chen et al. 2007). Once activated, RalA interacts with exocyst subunits Sec5 and Exo84 (Chen et al. 2007; Moskalenko et al. 2002, 2003). Although the precise role of these two RalA-binding proteins remains uncertain, both are required for insulin-stimulated glucose uptake (Chen et al. 2007).

Large tethering complexes such as the exocyst are thought to disengage from vesicles and/or disassemble before fusion occurs (Munson and Novick 2006). Disengagement may allow for fusion of opposing membranes and recycling of tethering machinery for additional rounds of vesicle targeting. Indeed, RalA dissociates from the exocyst through insulin-dependent, PKC-catalyzed phosphorylation of Ser89 in the RalA-binding domain of Sec5, which triggers Sec5 release (Chen et al. 2011a). Mutation of Ser89 to either alanine (to block phosphorylation) or aspartic acid (to mimic it) blocks GLUT4 insertion into the plasma membrane, suggesting that both engagement and disengagement from the targeting machinery are necessary steps preceding fusion (Chen et al. 2011a). This phosphorylation-dependent release of GSVs from the exocyst raises the possibility that the exocyst also serves a "gatekeeper" function in controlling GSV fusion.

7 Regulation of Glucose and Lipid Synthesis, Utilization, and Storage

7.1 Glucose Oxidation and Storage

Upon entering the muscle cell, glucose is rapidly phosphorylated by hexokinase and either stored as glycogen via the activity of glycogen synthase or oxidized to generate ATP synthesis via enzymes such as pyruvate kinase. In the liver and adipose tissue, glucose can also be stored as fat. Some of the enzymes involved in glycolysis, as well as in glycogen and lipid synthesis, are regulated by insulin via changes in their phosphorylation state due to a combination of protein kinase

inhibition and phosphatase activation. In addition, some of these enzymes are also regulated at the transcriptional level.

Insulin stimulates glycogen accumulation through a coordinated increase in glucose transport and glycogen synthesis. Activation of glycogen synthase involves promoting its dephosphorylation via both the inhibition of kinases that can phosphorylate glycogen synthase, such as PKA or GSK3 (Newgard et al. 2000; Brady et al. 1999), and the activation of phosphatases that dephosphorylate glycogen synthase, such as protein phosphatase 1 (PP1) (Brady et al. 1997). This process is downstream of PI 3-kinase and involves Akt phosphorylation of GSK-3. This inactivates GSK-3, resulting in a decrease in the phosphorylation of glycogen synthase and an increase in its activity state. However, the inhibition of GSK-3 is not sufficient for full activation of glycogen, since GSK-3 does not phosphorylate all of the residues of glycogen synthase that are dephosphorylated in response to insulin (Lawrence and Roach 1997).

Activation of PP1 correlates well with changes in glycogen synthase activity (Brady et al. 1998). However, insulin does not appear to globally activate PP1 but rather to activate specific pools of the phosphatase localized on the glycogen particle. The compartmentalized activation of PP1 by insulin is due to glycogen-targeting subunits that serve as "molecular scaffolds," bringing together the enzyme with its substrates glycogen synthase and glycogen phosphorylase in a macromolecular complex (Newgard et al. 2000). Four different proteins (G_M, G_L, PTG, and R_6) have been reported to target PP1 to the glycogen particle. Overexpression of these scaffolding proteins in cells or in vivo by adenovirus-mediated gene transfer results in a dramatic increase in basal cellular glycogen levels (Berman et al. 1998). Furthermore, glycogen stores in cells overexpressing PTG are refractory to breakdown by agents that raise intracellular cAMP levels, suggesting that PTG locks the cell into a glycogenic mode. The mechanism by which insulin activates glycogen-associated PP1 remains unknown. Although it had been proposed that activation of MAP kinase leads to the phosphorylation of the targeting protein G_M and the subsequent release of inhibition of the enzyme by insulin, blockade of the pathway had no effect of the activation of glycogen synthase by insulin, and mutation of the identified phosphorylation sites did not impair insulin action (Lazar et al. 1995). However, inhibitors of PI 3-kinase can block activation of PP1 by insulin (Shepherd 2005), indicating that PIP_3-dependent protein kinases are involved.

8 Regulation of Gluconeogenesis

Insulin inhibits the production and release of glucose by the liver and kidney by blockade of gluconeogenesis and glycogenolysis (Pilkis and Granner 1992). Insulin achieves these effects by directly controlling the activities of a subset of metabolic enzymes via the process of phosphorylation and dephosphorylation described above, as well as by regulation of the expression of a number of genes encoding hepatic enzymes. Insulin dramatically inhibits the transcription of the gene encoding phosphoenolpyruvate carboxylase (PEPCK), the rate-limiting step in

gluconeogenesis. The hormone also decreases transcription of the genes encoding fructose 1,6-bisphosphatase and glucose 6-phosphatase and increases transcription of those encoding glycolytic enzymes such as glucokinase and pyruvate kinase and lipogenic enzymes such as fatty acid synthase and acetyl CoA carboxylase (Lin and Accili 2011).

Several transcription factors play a role in this insulin-mediated regulation. Hepatic nuclear factor-3 (NF3) and HNF4 both appear to be involved in regulation of the PEPCK gene, which is the rate-limiting enzyme of gluconeogenesis (Lin and Accili 2011). Sterol regulatory element-binding protein-1c (SREBP-1c) is regulated by insulin and also influences the negative effect of insulin on PEPCK gene transcription (Chakravarty et al. 2001). The forkhead transcription factor FOXO1 also appears to be involved in the regulation of PEPCK and glucose 6-phosphatase (G6Pase), since both PEPCK and glucose 6-phosphatase contain putative FKHR binding sites in their promoter sequences, and overexpression of FKHR in hepatoma cells markedly increases the expression of the catalytic subunit of glucose 6-phosphatase. Phosphorylation of FOXO1 by Akt inhibits its activity by retaining the transcription factor in the cytoplasm. Both HNF4 and FOXO1 may be modified in their activity by the co-activator PGC-1. PGC-1 levels are increased in insulin-deficient and insulin-resistant diabetes. Thus, this creates an attractive hypothesis by bringing together multiple regulators under one common master regulator (Yoon et al. 2001).

While there is no doubt that insulin plays a key role in the regulation of the enzymes of gluconeogenesis, insulin can also indirectly influence glucose metabolism. This occurs via changes in the availability of substrates for gluconeogenesis that are released from muscle and fat (Bradley et al. 1993). Thus, when insulin levels are low, there is a breakdown of muscle protein and adipocyte triglycerides, leading to increased levels of gluconeogenic substrates such as alanine and free fatty acids. Physiologic experiments performed in dogs suggested that, under some circumstances, this indirect pathway may be the major pathway of insulin regulation of gluconeogenesis (Bradley et al. 1993). However, recent experiments with mice with a genetic knockout of the insulin receptor in liver indicate that the direct pathway is more important in that species (Fisher and Kahn 2003). In any case, in humans, the indirect pathway may contribute to the pathogenesis of diabetes, especially in individuals with central obesity, since visceral fat is less sensitive than subcutaneous fat to insulin inhibition of lipolysis, resulting in direct flux of fatty acids derived from these fat cells through the portal vein to the liver.

9 Regulation of Lipogenesis and Lipolysis

As is the case with carbohydrate metabolism, insulin also promotes the synthesis of lipids and inhibits their degradation. Recent studies suggest that many of these changes also might require an increase in levels of the transcription factor SREBP1-c (Kim et al. 1998; Foretz et al. 1999a, b). Dominant-negative forms of SREBP1 can block expression of these gluconeogenic and lipogenic genes (Foretz

et al. 1999a, b), while overexpression can increase their expression (Shimomura et al. 1999). Interestingly, hepatic SREBP levels are increased in rodent models of lipodystrophy, and this is associated with coordinated increases in fatty acid synthesis and gluconeogenesis, mimicking the phenotype observed in genetic models of obesity-induced diabetes. These observations led Shimomura et al. (1999) to speculate that increased expression of SREBP-1c might lead to the mixed insulin resistance observed in the diabetic liver, with increased rates of both gluconeogenesis and lipogenesis. The pathways that account for the changes in SREBP1-c expression lie downstream of the IRS/PI 3-kinase pathway.

In adipocytes, glucose is stored primarily as lipid. This is the result of increased uptake of glucose and activation of lipid synthetic enzymes, including pyruvate dehydrogenase, fatty acid synthase, and acetyl CoA carboxylase. Insulin also profoundly inhibits lipolysis in adipocytes, primarily through inhibition of the enzyme hormone-sensitive lipase. This enzyme is acutely regulated by control of its phosphorylation state, activated by PKA-dependent phosphorylation, and inhibited due to a combination of kinase inhibition and phosphatase activation. Insulin inhibits the activity of the lipase primarily via reductions in cAMP levels due to the activation of a cAMP-specific phosphodiesterase in fat cells (Bjorgell et al. 1984; Stralfors et al. 1984).

References

Ablooglu AJ, Kohanski RA (2001) Activation of the insulin receptor's kinase domain changes the rate-determining step of substrate phosphorylation. Biochemistry 40:504–513

Alessi DR, Pearce LR, Garcia-Martinez JM (2009) New insights into mTOR signaling: mTORC2 and beyond. Sci Signal 2:pe27. doi:10.1126/scisignal.267pe27

Araki E et al (1994) Alternative pathway of insulin signalling in mice with targeted disruption of the IRS-1 gene. Nature 372:186–190. doi:10.1038/372186a0

Backer JM et al (1992) Phosphatidylinositol 3′-kinase is activated by association with IRS-1 during insulin stimulation. EMBO J 11:3469–3479

Bandyopadhyay G et al (1997) Activation of protein kinase C (alpha, beta, and zeta) by insulin in 3T3/L1 cells. Transfection studies suggest a role for PKC-zeta in glucose transport. J Biol Chem 272:2551–2558

Baumann CA et al (2000) CAP defines a second signalling pathway required for insulin-stimulated glucose transport. Nature 407:202–207. doi:10.1038/35025089

Berman HK, O'Doherty RM, Anderson P, Newgard CB (1998) Overexpression of protein targeting to glycogen (PTG) in rat hepatocytes causes profound activation of glycogen synthesis independent of normal hormone- and substrate-mediated regulatory mechanisms. J Biol Chem 273:26421–26425

Bjorgell P, Rosberg S, Isaksson O, Belfrage P (1984) The antilipolytic, insulin-like effect of growth hormone is caused by a net decrease of hormone-sensitive lipase phosphorylation. Endocrinology 115:1151–1156. doi:10.1210/endo-115-3-1151

Bradley DC, Poulin RA, Bergman RN (1993) Dynamics of hepatic and peripheral insulin effects suggest common rate-limiting step in vivo. Diabetes 42:296–306

Brady MJ, Printen JA, Mastick CC, Saltiel AR (1997) Role of protein targeting to glycogen (PTG) in the regulation of protein phosphatase-1 activity. J Biol Chem 272:20198–20204

Brady MJ, Bourbonais FJ, Saltiel AR (1998) The activation of glycogen synthase by insulin switches from kinase inhibition to phosphatase activation during adipogenesis in 3T3-L1 cells. J Biol Chem 273:14063–14066

Brady MJ, Pessin JE, Saltiel AR (1999) Spatial compartmentalization in the regulation of glucose metabolism by insulin. Trends Endocrinol Metab 10:408–413. doi:10.1016/S1043-2760(99)00201-5

Burgering BM, Coffer PJ (1995) Protein kinase B (c-Akt) in phosphatidylinositol-3-OH kinase signal transduction. Nature 376:599–602. doi:10.1038/376599a0

Carpenter CL, Cantley LC (1996) Phosphoinositide kinases. Curr Opin Cell Biol 8:153–158

Carpentier JL, Paccaud JP, Gorden P, Rutter WJ, Orci L (1992) Insulin-induced surface redistribution regulates internalization of the insulin receptor and requires its autophosphorylation. Proc Natl Acad Sci U S A 89:162–166

Chakravarty K et al (2001) Sterol regulatory element-binding protein-1c mimics the negative effect of insulin on phosphoenolpyruvate carboxykinase (GTP) gene transcription. J Biol Chem 276:34816–34823. doi:10.1074/jbc.M103310200

Cheatham B et al (1994) Phosphatidylinositol 3-kinase activation is required for insulin stimulation of pp 70 S6 kinase, DNA synthesis, and glucose transporter translocation. Mol Cell Biol 14:4902–4911

Chen XW, Leto D, Chiang SH, Wang Q, Saltiel AR (2007) Activation of RalA is required for insulin-stimulated Glut4 trafficking to the plasma membrane via the exocyst and the motor protein Myo1c. Dev Cell 13:391–404. doi:10.1016/j.devcel.2007.07.007

Chen XW et al (2011a) Exocyst function is regulated by effector phosphorylation. Nat Cell Biol 13:580–588. doi:10.1038/ncb2226

Chen XW et al (2011b) A Ral GAP complex links PI 3-kinase/Akt signaling to RalA activation in insulin action. Mol Biol Cell 22:141–152. doi:10.1091/mbc.E10-08-0665

Chiang SH et al (2001) Insulin-stimulated GLUT4 translocation requires the CAP-dependent activation of TC10. Nature 410:944–948

Cho H et al (2001) Insulin resistance and a diabetes mellitus-like syndrome in mice lacking the protein kinase Akt2 (PKB beta). Science 292:1728–1731

Clement S et al (2001) The lipid phosphatase SHIP2 controls insulin sensitivity. Nature 409:92–97. doi:10.1038/35051094

Craparo A, Freund R, Gustafson TA (1997) 14-3-3 (epsilon) interacts with the insulin-like growth factor I receptor and insulin receptor substrate I in a phosphoserine-dependent manner. J Biol Chem 272:11663–11669

Datta SR, Brunet A, Greenberg ME (1999) Cellular survival: a play in three Akts. Genes Dev 13:2905–2927

De Fea K, Roth RA (1997) Modulation of insulin receptor substrate-1 tyrosine phosphorylation and function by mitogen-activated protein kinase. J Biol Chem 272:31400–31406

Downward J (1998) Mechanisms and consequences of activation of protein kinase B/Akt. Curr Opin Cell Biol 10:262–267

Fantin VR et al (1999) Cloning, tissue expression, and chromosomal location of the mouse insulin receptor substrate 4 gene. Endocrinology 140:1329–1337. doi:10.1210/endo.140.3.6578

Fantin VR, Wang Q, Lienhard GE, Keller SR (2000) Mice lacking insulin receptor substrate 4 exhibit mild defects in growth, reproduction, and glucose homeostasis. Am J Physiol Endocrinol Metab 278:E127–E133

Fasshauer M et al (2000) Essential role of insulin receptor substrate-2 in insulin stimulation of Glut4 translocation and glucose uptake in brown adipocytes. J Biol Chem 275:25494–25501. doi:10.1074/jbc.M004046200

Fisher SJ, Kahn CR (2003) Insulin signaling is required for insulin's direct and indirect action on hepatic glucose production. J Clin Invest 111:463–468. doi:10.1172/JCI16426

Foretz M, Guichard C, Ferre P, Foufelle F (1999a) Sterol regulatory element binding protein-1c is a major mediator of insulin action on the hepatic expression of glucokinase and lipogenesis-related genes. Proc Natl Acad Sci U S A 96:12737–12742

Foretz M et al (1999b) ADD1/SREBP-1c is required in the activation of hepatic lipogenic gene expression by glucose. Mol Cell Biol 19:3760–3768

Fruman DA et al (2000) Hypoglycaemia, liver necrosis and perinatal death in mice lacking all isoforms of phosphoinositide 3-kinase p85 alpha. Nat Genet 26:379–382. doi:10.1038/81715

Goldstein BJ, Bittner-Kowalczyk A, White MF, Harbeck M (2000) Tyrosine dephosphorylation and deactivation of insulin receptor substrate-1 by protein-tyrosine phosphatase 1B. Possible facilitation by the formation of a ternary complex with the Grb2 adaptor protein. J Biol Chem 275:4283–4289

Gustafson TA, He W, Craparo A, Schaub CD, O'Neill TJ (1995) Phosphotyrosine-dependent interaction of SHC and insulin receptor substrate 1 with the NPEY motif of the insulin receptor via a novel non-SH2 domain. Mol Cell Biol 15:2500–2508

Haney PM, Levy MA, Strube MS, Mueckler M (1995) Insulin-sensitive targeting of the GLUT4 glucose transporter in L6 myoblasts is conferred by its COOH-terminal cytoplasmic tail. J Cell Biol 129:641–658

He B, Guo W (2009) The exocyst complex in polarized exocytosis. Curr Opin Cell Biol 21:537–542. doi:10.1016/j.ceb.2009.04.007

Hedo JA, Kahn CR, Hayashi M, Yamada KM, Kasuga M (1983) Biosynthesis and glycosylation of the insulin receptor. Evidence for a single polypeptide precursor of the two major subunits. J Biol Chem 258:10020–10026

Hirosumi J et al (2002) A central role for JNK in obesity and insulin resistance. Nature 420:333–336. doi:10.1038/nature01137

Hu J, Liu J, Ghirlando R, Saltiel AR, Hubbard SR (2003) Structural basis for recruitment of the adaptor protein APS to the activated insulin receptor. Mol Cell 12:1379–1389

Huang J, Imamura T, Olefsky JM (2001) Insulin can regulate GLUT4 internalization by signaling to Rab5 and the motor protein dynein. Proc Natl Acad Sci U S A 98:13084–13089. doi:10.1073/pnas.241368698

Inoki K, Li Y, Xu T, Guan KL (2003) Rheb GTPase is a direct target of TSC2 GAP activity and regulates mTOR signaling. Genes Dev 17:1829–1834. doi:10.1101/gad.1110003

Inoue M, Chang L, Hwang J, Chiang SH, Saltiel AR (2003) The exocyst complex is required for targeting of Glut4 to the plasma membrane by insulin. Nature 422:629–633. doi:10.1038/nature01533

Inoue M, Chiang SH, Chang L, Chen XW, Saltiel AR (2006) Compartmentalization of the exocyst complex in lipid rafts controls Glut4 vesicle tethering. Mol Biol Cell 17:2303–2311. doi:10.1091/mbc.E06-01-0030

Inukai K et al (1997) p85alpha gene generates three isoforms of regulatory subunit for phosphatidylinositol 3-kinase (PI 3-Kinase), p50alpha, p55alpha, and p85alpha, with different PI 3-kinase activity elevating responses to insulin. J Biol Chem 272:7873–7882

Ishikura S, Klip A (2008) Muscle cells engage Rab8A and myosin Vb in insulin-dependent GLUT4 translocation. Am J Physiol Cell Physiol 295:C1016–C1025. doi:10.1152/ajpcell.00277.2008

Jhun BH et al (1994) Microinjection of the SH2 domain of the 85-kilodalton subunit of phosphatidylinositol 3-kinase inhibits insulin-induced DNA synthesis and c-fos expression. Mol Cell Biol 14:7466–7475

Jiang T et al (1998) Membrane-permeant esters of phosphatidylinositol 3,4,5-trisphosphate. J Biol Chem 273:11017–11024

Kane S et al (2002) A method to identify serine kinase substrates. Akt phosphorylates a novel adipocyte protein with a Rab GTPase-activating protein (GAP) domain. J Biol Chem 277:22115–22118. doi:10.1074/jbc.C200198200

Kerouz NJ, Horsch D, Pons S, Kahn CR (1997) Differential regulation of insulin receptor substrates-1 and -2 (IRS-1 and IRS-2) and phosphatidylinositol 3-kinase isoforms in liver and muscle of the obese diabetic (ob/ob) mouse. J Clin Invest 100:3164–3172. doi:10.1172/JCI119872

Kim JB et al (1998) Nutritional and insulin regulation of fatty acid synthetase and leptin gene expression through ADD1/SREBP1. J Clin Invest 101:1–9. doi:10.1172/JCI1411
Knudsen BS, Feller SM, Hanafusa H (1994) Four proline-rich sequences of the guanine-nucleotide exchange factor C3G bind with unique specificity to the first Src homology 3 domain of Crk. J Biol Chem 269:32781–32787
Kohn AD, Summers SA, Birnbaum MJ, Roth RA (1996) Expression of a constitutively active Akt Ser/Thr kinase in 3T3-L1 adipocytes stimulates glucose uptake and glucose transporter 4 translocation. J Biol Chem 271:31372–31378
Kotani K et al (1998) Requirement of atypical protein kinase clambda for insulin stimulation of glucose uptake but not for Akt activation in 3T3-L1 adipocytes. Mol Cell Biol 18:6971–6982
Krook A, Moller DE, Dib K, O'Rahilly S (1996) Two naturally occurring mutant insulin receptors phosphorylate insulin receptor substrate-1 (IRS-1) but fail to mediate the biological effects of insulin. Evidence that IRS-1 phosphorylation is not sufficient for normal insulin action. J Biol Chem 271:7134–7140
Lane MD, Ronnett G, Slieker LJ, Kohanski RA, Olson TL (1985) Post-translational processing and activation of insulin and EGF proreceptors. Biochimie 67:1069–1080
Laplante M, Sabatini DM (2009) mTOR signaling at a glance. J Cell Sci 122:3589–3594. doi:10.1242/jcs.051011
Lavan BE, Lane WS, Lienhard GE (1997) The 60-kDa phosphotyrosine protein in insulin-treated adipocytes is a new member of the insulin receptor substrate family. J Biol Chem 272:11439–11443
Lawlor MA, Alessi DR (2001) PKB/Akt: a key mediator of cell proliferation, survival and insulin responses? J Cell Sci 114:2903–2910
Lawrence JC Jr, Roach PJ (1997) New insights into the role and mechanism of glycogen synthase activation by insulin. Diabetes 46:541–547
Lazar DF, Saltiel AR (2006) Lipid phosphatases as drug discovery targets for type 2 diabetes. Nat Rev Drug Discov 5:333–342. doi:10.1038/nrd2007
Lazar DF et al (1995) Mitogen-activated protein kinase kinase inhibition does not block the stimulation of glucose utilization by insulin. J Biol Chem 270:20801–20807
Lehr S et al (2000) Identification of major tyrosine phosphorylation sites in the human insulin receptor substrate Gab-1 by insulin receptor kinase in vitro. Biochemistry 39:10898–10907
Lesniewski LA et al (2007) Bone marrow-specific Cap gene deletion protects against high-fat diet-induced insulin resistance. Nat Med 13:455–462. doi:10.1038/nm1550
Leto D, Saltiel AR (2012) Regulation of glucose transport by insulin: traffic control of GLUT4. Nat Rev Mol Cell Biol 13:383–396. doi:10.1038/nrm3351
Li J, DeFea K, Roth RA (1999) Modulation of insulin receptor substrate-1 tyrosine phosphorylation by an Akt/phosphatidylinositol 3-kinase pathway. J Biol Chem 274:9351–9356
Li S, Brown MS, Goldstein JL (2010) Bifurcation of insulin signaling pathway in rat liver: mTORC1 required for stimulation of lipogenesis, but not inhibition of gluconeogenesis. Proc Natl Acad Sci U S A 107:3441–3446. doi:10.1073/pnas.0914798107
Lin HV, Accili D (2011) Hormonal regulation of hepatic glucose production in health and disease. Cell Metab 14:9–19. doi:10.1016/j.cmet.2011.06.003
Liu J, Kimura A, Baumann CA, Saltiel AR (2002) APS facilitates c-Cbl tyrosine phosphorylation and GLUT4 translocation in response to insulin in 3T3-L1 adipocytes. Mol Cell Biol 22:3599–3609
Lodhi IJ et al (2008) Insulin stimulates phosphatidylinositol 3-phosphate production via the activation of Rab5. Mol Biol Cell 19:2718–2728. doi:10.1091/mbc.E08-01-0105
Mauvais-Jarvis F et al (2002) Reduced expression of the murine p85alpha subunit of phosphoinositide 3-kinase improves insulin signaling and ameliorates diabetes. J Clin Invest 109:141–149
Maxfield FR, McGraw TE (2004) Endocytic recycling. Nat Rev Mol Cell Biol 5:121–132. doi:10.1038/nrm1315

Miinea CP et al (2005) AS160, the Akt substrate regulating GLUT4 translocation, has a functional Rab GTPase-activating protein domain. Biochem J 391:87–93. doi:10.1042/BJ20050887

Miki H et al (2001) Essential role of insulin receptor substrate 1 (IRS-1) and IRS-2 in adipocyte differentiation. Mol Cell Biol 21:2521–2532. doi:10.1128/MCB.21.7.2521-2532.2001

Milarski KL, Saltiel AR (1994) Expression of catalytically inactive Syp phosphatase in 3T3 cells blocks stimulation of mitogen-activated protein kinase by insulin. J Biol Chem 269:21239–21243

Mori H et al (2009) Critical role for hypothalamic mTOR activity in energy balance. Cell Metab 9:362–374. doi:10.1016/j.cmet.2009.03.005

Moskalenko S et al (2002) The exocyst is a Ral effector complex. Nat Cell Biol 4:66–72. doi:10.1038/ncb728

Moskalenko S et al (2003) Ral GTPases regulate exocyst assembly through dual subunit interactions. J Biol Chem 278:51743–51748. doi:10.1074/jbc.M308702200

Munson M, Novick P (2006) The exocyst defrocked, a framework of rods revealed. Nat Struct Mol Biol 13:577–581. doi:10.1038/nsmb1097

Muretta JM, Romenskaia I, Mastick CC (2008) Insulin releases Glut4 from static storage compartments into cycling endosomes and increases the rate constant for Glut4 exocytosis. J Biol Chem 283:311–323. doi:10.1074/jbc.M705756200

Myers MG Jr, White MF (1995) New frontiers in insulin receptor substrate signaling. Trends Endocrinol Metab 6:209–215

Myers MG Jr et al (1994a) Insulin receptor substrate-1 mediates phosphatidylinositol 3′-kinase and p70S6k signaling during insulin, insulin-like growth factor-1, and interleukin-4 stimulation. J Biol Chem 269:28783–28789

Myers MG Jr et al (1994b) Role of IRS-1-GRB-2 complexes in insulin signaling. Mol Cell Biol 14:3577–3587

Nakashima N, Sharma PM, Imamura T, Bookstein R, Olefsky JM (2000) The tumor suppressor PTEN negatively regulates insulin signaling in 3T3-L1 adipocytes. J Biol Chem 275:12889–12895

Newgard CB, Brady MJ, O'Doherty RM, Saltiel AR (2000) Organizing glucose disposal: emerging roles of the glycogen targeting subunits of protein phosphatase-1. Diabetes 49:1967–1977

Ozes ON et al (2001) A phosphatidylinositol 3-kinase/Akt/mTOR pathway mediates and PTEN antagonizes tumor necrosis factor inhibition of insulin signaling through insulin receptor substrate-1. Proc Natl Acad Sci U S A 98:4640–4645. doi:10.1073/pnas.051042298

Pilkis SJ, Granner DK (1992) Molecular physiology of the regulation of hepatic gluconeogenesis and glycolysis. Annu Rev Physiol 54:885–909. doi:10.1146/annurev.ph.54.030192.004321

Ribon V, Hubbell S, Herrera R, Saltiel AR (1996) The product of the cbl oncogene forms stable complexes in vivo with endogenous Crk in a tyrosine phosphorylation-dependent manner. Mol Cell Biol 16:45–52

Ribon V, Printen JA, Hoffman NG, Kay BK, Saltiel AR (1998a) A novel, multifunctional c-Cbl binding protein in insulin receptor signaling in 3T3-L1 adipocytes. Mol Cell Biol 18:872–879

Ribon V, Johnson JH, Camp HS, Saltiel AR (1998b) Thiazolidinediones and insulin resistance: peroxisome proliferatoractivated receptor gamma activation stimulates expression of the CAP gene. Proc Natl Acad Sci U S A 95:14751–14756

Rommel C et al (2001) Mediation of IGF-1-induced skeletal myotube hypertrophy by PI(3)K/Akt/mTOR and PI(3)K/Akt/GSK3 pathways. Nat Cell Biol 3:1009–1013. doi:10.1038/ncb1101-1009

Saltiel AR, Kahn CR (2001) Insulin signalling and the regulation of glucose and lipid metabolism. Nature 414:799–806

Sano H et al (2003) Insulin-stimulated phosphorylation of a Rab GTPase-activating protein regulates GLUT4 translocation. J Biol Chem 278:14599–14602

Sano H et al (2007) Rab10, a target of the AS160 Rab GAP, is required for insulin-stimulated translocation of GLUT4 to the adipocyte plasma membrane. Cell Metab 5:293–303. doi:10.1016/j.cmet.2007.03.001

Sano H, Roach WG, Peck GR, Fukuda M, Lienhard GE (2008) Rab10 in insulin-stimulated GLUT4 translocation. Biochem J 411:89–95. doi:10.1042/BJ20071318

Sano H, Peck GR, Kettenbach AN, Gerber SA, Lienhard GE (2011) Insulin-stimulated GLUT4 protein translocation in adipocytes requires the Rab10 guanine nucleotide exchange factor Dennd4C. J Biol Chem 286:16541–16545. doi:10.1074/jbc.C111.228908

Shepherd PR (2005) Mechanisms regulating phosphoinositide 3-kinase signalling in insulin-sensitive tissues. Acta Physiol Scand 183:3–12

Shimomura I et al (1999) Insulin selectively increases SREBP-1c mRNA in the livers of rats with streptozotocin-induced diabetes. Proc Natl Acad Sci U S A 96:13656–13661

Skolnik EY et al (1993) The SH2/SH3 domain-containing protein GRB2 interacts with tyrosine-phosphorylated IRS1 and Shc: implications for insulin control of ras signalling. EMBO J 12:1929–1936

Standaert ML et al (1997) Protein kinase C-zeta as a downstream effector of phosphatidylinositol 3-kinase during insulin stimulation in rat adipocytes. Potential role in glucose transport. J Biol Chem 272:30075–30082

Stralfors P, Bjorgell P, Belfrage P (1984) Hormonal regulation of hormone-sensitive lipase in intact adipocytes: identification of phosphorylated sites and effects on the phosphorylation by lipolytic hormones and insulin. Proc Natl Acad Sci U S A 81:3317–3321

Sugimoto S, Wandless TJ, Shoelson SE, Neel BG, Walsh CT (1994) Activation of the SH2-containing protein tyrosine phosphatase, SH-PTP2, by phosphotyrosine-containing peptides derived from insulin receptor substrate-1. J Biol Chem 269:13614–13622

Sun XJ et al (1991) Structure of the insulin receptor substrate IRS-1 defines a unique signal transduction protein. Nature 352:73–77. doi:10.1038/352073a0

Sun XJ et al (1995) Role of IRS-2 in insulin and cytokine signalling. Nature 377:173–177. doi:10.1038/377173a0

Sun Y, Bilan PJ, Liu Z, Klip A (2010) Rab8A and Rab13 are activated by insulin and regulate GLUT4 translocation in muscle cells. Proc Natl Acad Sci U S A 107:19909–19914. doi:10.1073/pnas.1009523107

Tanti JF, Gremeaux T, Van Obberghen E, Le Marchand-Brustel Y (1994) Insulin receptor substrate 1 is phosphorylated by the serine kinase activity of phosphatidylinositol 3-kinase. Biochem J 304(Pt 1):17–21

Ueki K et al (2002) Molecular balance between the regulatory and catalytic subunits of phosphoinositide 3-kinase regulates cell signaling and survival. Mol Cell Biol 22:965–977

Wick MJ, Dong LQ, Hu D, Langlais P, Liu F (2001) Insulin receptor-mediated p62dok tyrosine phosphorylation at residues 362 and 398 plays distinct roles for binding GTPase-activating protein and Nck and is essential for inhibiting insulin-stimulated activation of Ras and Akt. J Biol Chem 276:42843–42850. doi:10.1074/jbc.M102116200

Wiese RJ, Mastick CC, Lazar DF, Saltiel AR (1995) Activation of mitogen-activated protein kinase and phosphatidylinositol 3′-kinase is not sufficient for the hormonal stimulation of glucose uptake, lipogenesis, or glycogen synthesis in 3T3-L1 adipocytes. J Biol Chem 270:3442–3446

Withers DJ et al (1998) Disruption of IRS-2 causes type 2 diabetes in mice. Nature 391:900–904. doi:10.1038/36116

Xu Y et al (2011) Dual-mode of insulin action controls GLUT4 vesicle exocytosis. J Cell Biol 193:643–653. doi:10.1083/jcb.201008135

Yamauchi K, Milarski KL, Saltiel AR, Pessin JE (1995) Protein-tyrosine-phosphatase SHPTP2 is a required positive effector for insulin downstream signaling. Proc Natl Acad Sci U S A 92:664–668

Yamauchi T et al (1996) Insulin signalling and insulin actions in the muscles and livers of insulin-resistant, insulin receptor substrate 1-deficient mice. Mol Cell Biol 16:3074–3084

Yoon JC et al (2001) Control of hepatic gluconeogenesis through the transcriptional coactivator PGC-1. Nature 413:131–138. doi:10.1038/35093050

Zerial M, McBride H (2001) Rab proteins as membrane organizers. Nat Rev Mol Cell Biol 2:107–117. doi:10.1038/35052055

Zhang M, Liu J, Cheng A, Deyoung SM, Saltiel AR (2007) Identification of CAP as a costameric protein that interacts with filamin C. Mol Biol Cell 18:4731–4740. doi:10.1091/mbc.E07-06-0628

Zinker BA et al (2002) PTP1B antisense oligonucleotide lowers PTP1B protein, normalizes blood glucose, and improves insulin sensitivity in diabetic mice. Proc Natl Acad Sci U S A 99:11357–11362. doi:10.1073/pnas.142298199

Glucocorticoids and Metabolic Control

Lilia Magomedova and Carolyn L. Cummins

Contents

Abstract

In response to stress, the central nervous system initiates a signaling cascade, which leads to the production of glucocorticoids (GCs). GCs act through the glucocorticoid receptor (GR) to coordinate the appropriate cellular response with the primary goal of mobilizing the storage forms of carbon precursors to generate a continuous glucose supply for the brain. Although GCs are critical for maintaining energy homeostasis, excessive GC stimulation leads to a number of undesirable side effects, including hyperglycemia, insulin resistance, fatty liver, obesity, and muscle wasting leading to severe metabolic dysfunction. Summarized below are the diverse metabolic roles of glucocorticoids in energy

L. Magomedova • C.L. Cummins (✉)
Department of Pharmaceutical Sciences, University of Toronto, Toronto, ON M5S 3M2, Canada
e-mail: Lilia.magomedova@utoronto.ca; Carolyn.cummins@utoronto.ca

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homeostasis and dysregulation, focusing specifically on glucose, lipid, and protein metabolism.

Keywords
Adipose tissue · Glucocorticoid hormones · Glucocorticoid receptor · Gluconeogenesis · Lipid metabolism · Liver · Muscle · Protein metabolism

1 Introduction

Glucocorticoids (GCs) are steroid hormones, essential for survival under stress. The physiologic stress response is mediated by the release of ACTH from the pituitary that acts on the adrenal gland to increase the production and release of cortisol (in humans) or corticosterone (in rodents) into the circulation. The GC hormone then acts through the GC receptor (GR) to coordinate the appropriate cellular response to stress with the primary outcome of increasing blood glucose levels. The mechanisms by which GCs achieve this effect involve the interplay primarily between liver, muscle, and adipose tissue. This adaptive response to stress, however, is meant to be of short duration and is regulated by negative feedback at the level of the hypothalamus and pituitary gland. Prolonged, elevated GC exposure, as observed with therapeutic use of GCs or in Cushing's syndrome, leads to increased insulin secretion eventually resulting in severe metabolic dysfunction and insulin resistance.

2 Glucose Metabolism

Under stressful stimuli, GCs coordinate a number of physiological processes with the end goal of generating a sustained glucose supply for the brain. GCs affect whole-body glucose metabolism by decreasing peripheral glucose uptake and inducing hepatic gluconeogenesis by mechanisms described below (Fig. 1).

2.1 Liver

The most well-studied effects of GCs are by far those related to hepatic gluconeogenesis. Glucose is the primary energy source for the brain, renal medulla, and erythrocytes, and the liver is the main organ responsible for de novo glucose production under fasting conditions. Not surprisingly, therefore, hepatic gluconeogenesis is under very tight hormonal regulation. In the fed state, insulin facilitates glucose uptake and utilization, whereas in the fasted state, glucagon, catecholamines, and GCs stimulate glucose production and release. In fact, mice

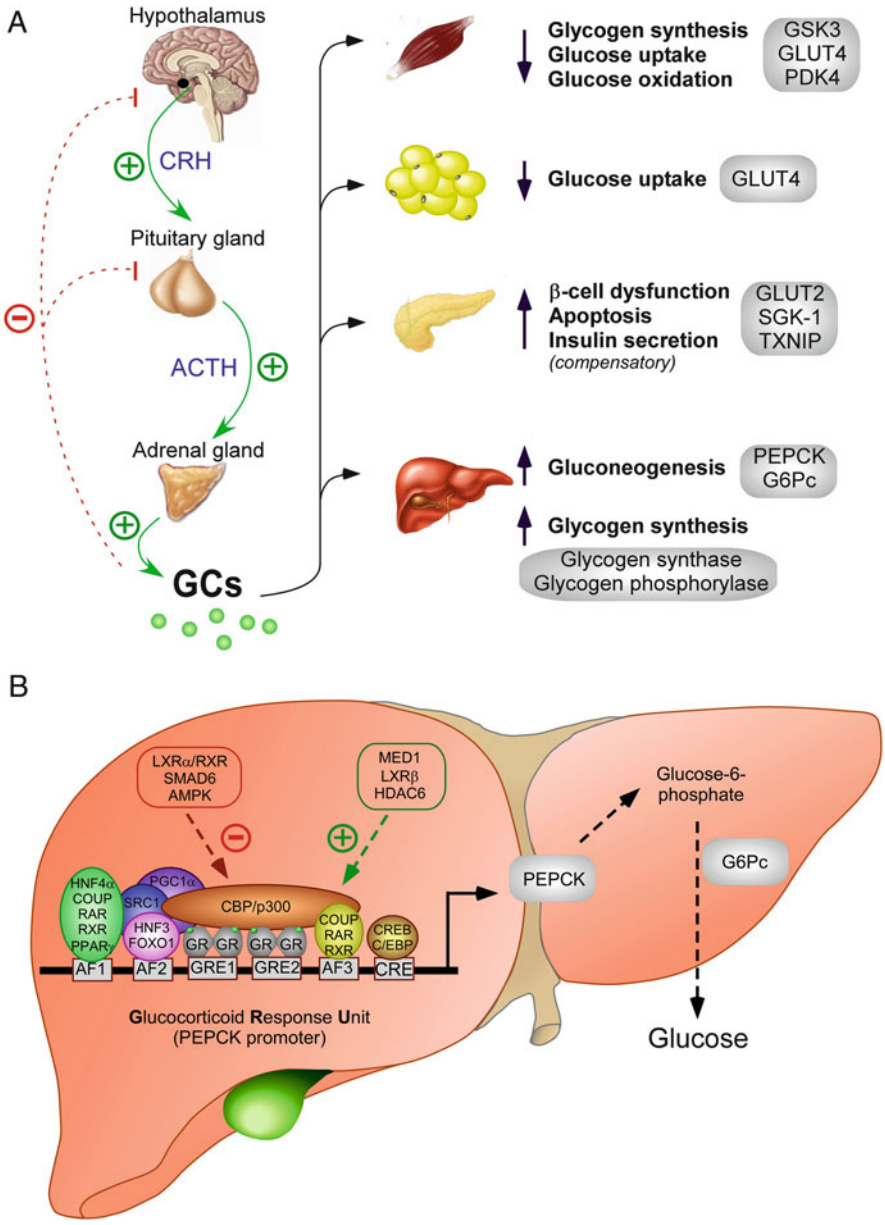

Fig. 1 Mechanisms by which GCs regulate whole-body glucose homeostasis. (**a**) Schematic representation of the HPA axis and the effects of GCs/GR on glucose metabolism in the liver, adipose tissue, muscle, and pancreas. Genes/proteins that are involved (either directly or indirectly) in the mentioned events are in *shaded boxes*. (**b**) Representation of the PEPCK glucocorticoid response unit in the liver, together with the location of some of the accessory factors necessary to initiate transcription. Depicted in the *square boxes* are some of the known positive

lacking GR in the hepatocytes fail to appropriately respond to prolonged fasting, resulting in severe hypoglycemia (Opherk et al. 2004).

Ligand-bound GR directly activates the transcription of two key enzymes involved in gluconeogenesis: phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pc). PEPCK is the rate-limiting enzyme required to generate glucose-6-phosphate, whereas G6Pc is the enzyme that cleaves the phosphate allowing for glucose release into the circulation. PEPCK regulation is complex and requires a myriad of accessory proteins and transcription factors to ensure a maximal gluconeogenic response. Through extensive promoter mapping, it was found that the *Pepck* promoter harbors a GR response unit (GRU), which has two GR response elements (GREs) as well as binding sites for forkhead transcription factor (FOXO1), retinoid X receptor (RXR), chicken ovalbumin upstream promoter-transcription factor (COUP-TF), CCAAT/enhancer-binding protein β (C/EBPβ), hepatocyte nuclear factors 3 and 4 (HNF-3 and HNF-4), peroxisome proliferator-activated receptor γ (PPARγ2), and retinoic acid receptor (RAR) [reviewed in Chakravarty et al. (2005)]. Similarly, three functional GREs have been identified in the proximal *G6Pc* promoter, and similar to *Pepck* regulation, GCs act in cooperation with HNF-1, HNF-4, and FOXO1 to fully induce *G6Pc* transcription (Lin et al. 1998; Nakae et al. 2001; Vander Kooi et al. 2005).

Interestingly, cholesterol-sensing liver X receptors (LXRα and LXRβ) can also influence the recruitment of GR to gluconeogenic gene promoters (Nader et al. 2012; Patel et al. 2011). Specifically, rats treated with GW3965 (a dual LXRα/β agonist) were found to be refractory to the GC-induced hyperglycemia (Nader et al. 2012). This is believed to be due to direct competition for DNA binding, where the LXRα/RXRα dimer was found to displace GR from its GRE on the *G6Pc* promoter. Making matters more complex, it was found that LXR's effects on GC-mediated induction of gluconeogenesis are isoform specific. In fact, LXRβ is necessary for GR binding to the *Pepck* promoter, and LXRβ knockout mice are protected from dexamethasone (Dex)-induced hyperglycemia (Patel et al. 2011).

Adding another layer of control to the systemic regulation of energy homeostasis, the transcriptional activity of GR can also be modified through the recruitment of various coactivator and corepressor complexes. Coactivators including SRC1,

Fig. 1 (continued) (+) and negative (−) regulators of GC signaling. Also shown are the steps leading to the release of glucose into circulation. *ACTH* adrenocorticotropin hormone, *AMPK* AMP kinase, *CBP* CREB-binding protein, *C/EBP* CCAAT/enhancer-binding protein, *COUP* chicken ovalbumin upstream promoter-transcription factor, *CREB* cAMP-response element binding protein, *CRH* corticotropin-releasing hormone, *GLUT2* glucose transporter 2, *GLUT4* glucose transporter 4, *FOXO1* foxhead box protein O1, *G6Pc* glucose-6-phosphatase, *GR* glucocorticoid receptor, *GRE* glucocorticoid response element, *GSK3* glycogen synthase kinase 3, *HDAC6* histone deacetylase 6, *HNF* hepatic nuclear factor, *LXR* liver X receptor, *MED1* mediator complex subunit 1, *PDK4* pyruvate dehydrogenase kinase 4, *PEPCK* PEP carboxykinase, *PGC1α* PPAR-γ coactivator-1, *PPARγ* peroxisome proliferator-activated receptor γ, *RAR* retinoic acid receptor, *RXR* retinoid X receptor, *SGK-1* serum- and glucocorticoid-regulated kinase 1, *SMAD6* SMAD family member 6, *SRC-1* steroid receptor coactivator 1, *TXNIP* thioredoxin-interacting protein

CBP/p300, and PGC1α have all been shown to be involved in *Pepck* transactivation (Sommerfeld et al. 2011). Under fasting conditions, the expression of *Pgc1α* is induced synergistically by glucagon and GCs (Yoon et al. 2001). PGC1α then binds and coactivates GR as well as HNF-4 and FOXO1 to induce a coordinated gluconeogenic response on both *Pepck* and *G6pc* promoters (Puigserver et al. 2003; Rhee et al. 2003).

GCs also recruit chromatin-modifying enzymes, p300 and CBP, to the *Pepck* promoter in order to maintain the surrounding chromatin in an open conformation, whereas insulin opposes these actions partly by displacing p300/CBP, leading to chromatin condensation (Hall et al. 2007; Wang et al. 2004). In addition, AMPK, which acts as a "low-energy sensor" within the cells, also counteracts GC-induced expression of *Pepck* by phosphorylating GR at serine 211 leading to the release of p300 and the SWF/SNF chromatin remodeling complex from the promoters of *Pepck* and *G6pc* (Nader et al. 2010). In fact, rats treated with the AMPK activator, AICAR, were refractory to Dex-induced hepatic gluconeogenesis. Moreover, SMAD6, a member of the transforming growth factor β family, was identified as a GR corepressor protein, which recruits histone deacetylase 3 (HDAC3) and opposes histone H3 and H4 acetylation mediated by the coactivator SRC1 (Ichijo et al. 2005). Finally, HDAC6 was found to affect GC signaling by deacetylating the heat shock protein 90 (HSP90) (Kovacs et al. 2005). Inhibition of HDAC6 activity results in hyper-acetylation of HSP90 leading to an impaired GR nuclear translocation and activation (Kovacs et al. 2005). In agreement, HDAC6 knockout animals were protected from GC-induced hyperglycemia and insulin intolerance (Rhee et al. 2003).

Another mechanism by which GCs can affect liver glucose homeostasis is by directly antagonizing the actions of insulin. For example, the expression of a pseudo kinase, *Trb3*, is increased by GC treatment leading to the inhibition of AKT phosphorylation and development of hyperglycemia and insulin resistance (Du et al. 2003). Similarly, ceramides, which are lipid-derived signaling molecules, can also mediate GC-induced hepatic insulin resistance by blocking AKT activation (Holland et al. 2007). This mechanism will be discussed in further detail below (see: lipid metabolism/liver).

Paradoxically, GC-treatment results in an increase in glycogen synthesis. This represents one of the few anabolic actions of this otherwise catabolic hormone. Our understanding of the mechanism by which GCs increase glycogen synthesis is derived largely from long-standing biochemical studies. Regulation of glycogen synthesis requires the reciprocal action of two key enzymes: glycogen synthase and glycogen phosphorylase. Both enzymes exist in active and inactive states regulated by phosphorylation and dephosphorylation events. Interestingly, studies found that GCs lead to inactivation of glycogen phosphorylase (glycogen-mobilizing enzyme) and a concomitant activation of glycogen synthase, resulting in an overall increase in hepatic glycogen content (de Wulf and Hers 1968; Laloux et al. 1983).

2.2 Muscle and Adipose Tissue

Muscle is the organ that makes the largest contribution to glucose utilization in the body, with more than 80% of circulating glucose being taken up by muscle in an insulin-dependent fashion. Insulin is an anabolic hormone, whose actions in the muscle are to stimulate glucose uptake, utilization, and storage. Most of the catabolic actions of GCs in muscle arise through antagonizing the actions of insulin. The main mechanism by which GCs decrease muscle glucose uptake is by inhibiting the translocation of the glucose transporter, GLUT4, to the plasma membrane (Haber and Weinstein 1992; Weinstein et al. 1995, 1998). Suppression of insulin-stimulated glycogen synthesis by GCs is mediated by decreasing the phosphorylation of GSK3, leading to the repression of glycogen synthase (Ruzzin et al. 2005). Both GLUT4 and GSK3 are downstream targets of AKT in the insulin-signaling cascade, highlighting the antagonistic interaction between insulin and GCs. The mechanism of this crosstalk between GCs and insulin has been extensively studied. The ability of GCs to inhibit AKT phosphorylation has been observed in vitro (C2C12 myotubes) and in vivo (rat skeletal muscle) (Long et al. 2003; Sandri et al. 2004). In rat skeletal muscle, GC excess decreases insulin receptor tyrosine phosphorylation (Giorgino et al. 1993). Dex treatment in rats has also been shown to reduce muscle PI3 kinase activity (Saad et al. 1993).

Inhibition of glucose oxidation is another mechanism by which GCs decrease glucose utilization in the muscle. GCs strongly upregulate the expression of the pyruvate dehydrogenase kinase 4 (*Pdk4*) (Sugden and Holness 2003). PDK4 inhibits the activity of the pyruvate dehydrogenase complex, thus inhibiting glucose oxidation to acetyl-CoA, resulting in decreased glucose utilization. *Pdk4* is a direct target gene of GR. Interestingly, the *Pdk4* GRE overlaps with the FOXO binding site, which is in turn required for insulin-mediated suppression of *Pdk4* expression (Connaughton et al. 2010; Kwon et al. 2004).

Similar to their effects in muscle, GCs also antagonize insulin signaling in adipose tissue, leading to decreased localization of GLUT4 transporters to the plasma membrane (Sakoda et al. 2000). Moreover, Dex treatment in rats was shown to decrease insulin-induced IRS-1 and IRS-2 phosphorylation with a concomitant decrease in AKT phosphorylation (Caperuto et al. 2006).

3 Lipid Metabolism

GCs are important regulators of whole-body lipid homeostasis. When fasting, or under starvation conditions, elevated systemic GC levels stimulate adipose tissue lipolysis, resulting in the generation of free fatty acids and glycerol. Muscle and liver both utilize the energy (ATP) derived from the oxidation of FFAs, whereas glycerol is used primarily by the liver as a precursor for gluconeogenesis. Given these effects, it is not surprising that elevated GC levels can lead to central obesity, dyslipidemia, and fatty liver. Summarized below are some of the complex effects of GCs on adipose tissue and liver lipid metabolism (Fig. 2).

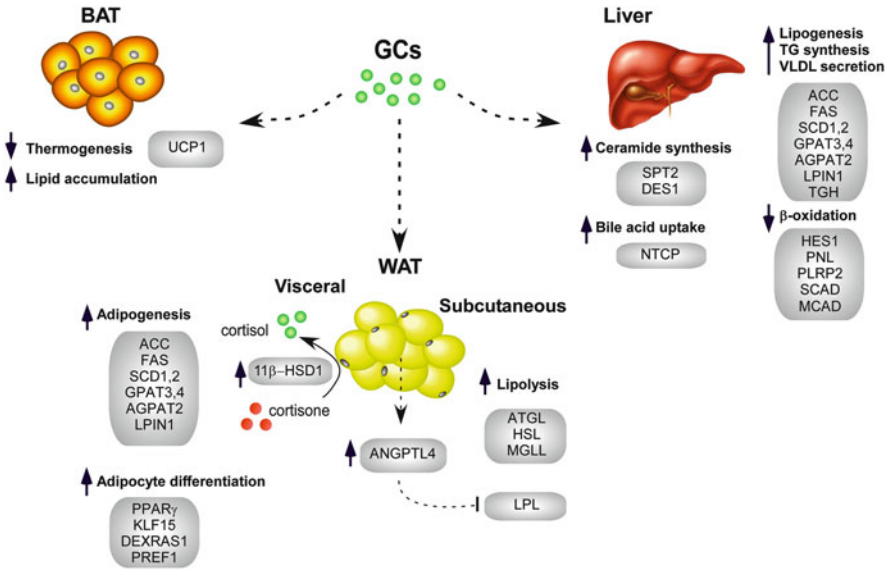

Fig. 2 Schematic view of the role of GCs in lipid metabolism in the liver, white adipose tissue (WAT) and brown adipose tissue (BAT). Proteins that are involved (either directly or indirectly) in the depicted metabolic processes are in *shaded boxes*. *11β-HSD* 11β-hydroxysteroid dehydrogenase, *ACC* acyl-CoA carboxylase, *AGPAT2* acylglycerolphosphate acyltransferase 2, *ANGPTL4* angiopoietin-like 4, *ATGL* adipose triglyceride lipase, *DES1* dihydroceramide synthase, *DEXRAS1* dexamethasone-induced Ras 1, *FAS* fatty acid synthase, *GPAT* glycerophosphate acyltransferase, *HES1* hairy and enhancer of split-1, *HSL* hormone-sensitive lipase, *KLF15* Kruppel-like factor 15, *LPIN1* lipin 1, *LPL* lipoprotein lipase, *MCAD* medium-chain acyl-CoA dehydrogenase, *MGLL* monoacyl glycerol lipase (MGLL), *NTCP* Na^+-taurocholate cotransporting polypeptide, *PPARγ* peroxisome proliferator-activated receptor γ, *PREF1* pre-adipogenic factor 1, *PNL* pancreatic lipase, *PLRP2* pancreatic lipase-related protein-2, *SCAD* short-chain acyl-CoA dehydrogenase, *SCD1* stearoyl-CoA desaturase, *SPT2* serine palmitoyltransferase 2, *TGH* triacylglycerol hydrolase, *UCP1* uncoupling protein 1

3.1 Adipose Tissue

GCs exhibit pleiotropic effects on lipid metabolism by causing both increased lipolysis and increased adipogenesis [reviewed recently by Peckett et al. (2011)]. Under fasting conditions, when GC levels are elevated, increased adipose tissue lipolysis occurs due to increased expression of adipose triglyceride lipase (*Atgl*) and hormone-sensitive lipase (*Hsl* or *Lipe*) (Slavin et al. 1994; Villena et al. 2004; Xu et al. 2009). Monoacyl glycerol lipase (MGLL), which converts monoacyl glycerol to glycerol, is also known to be induced by GCs (Yu et al. 2010). GC regulation of *Hsl* and *Mgll* appears to be direct through a functional GR binding site, whereas no GRE has been identified to date in *Atgl* (Yu et al. 2010).

Recently, GCs were found to directly upregulate the expression of angiopoietin-like 4 (*Angptl4*), a secreted protein synthesized in WAT and liver in response to

fasting. ANGPTL4 inhibits the activity of extracellular lipoprotein lipase (LPL) (Shan et al. 2009), important for FFA uptake, and, at the same time, induces intracellular adipocyte lipolysis (Gray et al. 2012), resulting in an overall increase in plasma triglyceride (TG) levels. In vitro and in vivo studies have shown that GCs regulate *Angptl4* expression though a GRE located in the 3′ untranslated region of the gene (Koliwad et al. 2009). *Angptl4*-/- mice were protected from Dex-induced hypertriglyceridemia and hepatosteatosis (Koliwad et al. 2009). In agreement, treatment of mice with a synthetic GC antagonist, RU486, also attenuated fasting-induced expression of *Angptl4* (Gray et al. 2012). It should be noted that although GCs are believed to be in general "lipolytic," there is mounting evidence suggesting that they also have anti-lipolytic actions (Peckett et al. 2011). In fact, studies in 3T3-L1 adipocytes showed that both dose and duration of GC stimulation dictate the net outcome of increased or decreased lipolysis (Campbell et al. 2011).

In the fed state, when insulin levels are elevated, GCs may act synergistically with insulin to promote de novo lipogenesis by directly upregulating (via a functional GRE) the expression of two key enzymes involved in fatty acid synthesis: acyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) (Diamant and Shafrir 1975; Volpe and Marasa 1975). Studies in cultured adipocytes showed that corticosterone in combination with insulin was able to increase lipogenesis by 66% when compared to insulin alone (Minshull and Strong 1985). Involvement of GCs in TG synthesis was also demonstrated in a genome-wide analysis of 3T3-L1 adipocytes and in vivo studies of mice treated with Dex, where a large number of GR target genes were identified in the TG synthetic pathway: *Scd1*, *Scd2*, *Gpat3*, *Gpat4*, *Agpat2*, and *Lpin1* (Yu et al. 2010). Most of these genes, with the exception of *Agpat2*, have at least one functional GR binding site (Yu et al. 2010).

Excessive GC stimulation has been shown to be instrumental for the development of central obesity and its associated metabolic disorders. Although there is some controversy surrounding the correlation of plasma GCs with obesity (Abraham et al. 2013; Hautanen et al. 1997; Kjolhede et al. 2014; Praveen et al. 2011), positive correlations between elevated GC activity and the development of metabolic syndrome have been observed in humans (Phillips et al. 1998; Reynolds et al. 2001; Stolk et al. 1996; Walker et al. 1998). HPA axis hyperactivity has similarly been linked to the development of insulin resistance and hypertension. Studies in Zucker rats showed that both adrenalectomy and GR antagonist treatment were able to improve the metabolic phenotype in these animals, directly implicating GCs in the development of obesity (Langley and York 1990; Yukimura et al. 1978). In fact, patients with Cushing's syndrome exhibit a characteristic redistribution of adipose tissue from the periphery to the abdominal depots. This fat-mass redistribution is believed to arise from the differential activity of GCs in various fat depots. In the periphery, GCs induce the activity of HSL and ATGL leading to increased lipolysis (Slavin et al. 1994), whereas, in central fat depots, GCs promote lipogenesis (Chimin et al. 2014; Rebuffe-Scrive et al. 1988; Seckl et al. 2004).

Pre-receptor metabolism has also been implicated in the depot-specific actions of GCs. 11β-Hydroxysteroid dehydrogenase (11β-HSD1) is an enzyme that

catalyzes the conversion of inactive cortisone to cortisol (in humans), thus increasing the intra-tissue levels of active GCs (Seckl and Walker 2001). Interestingly, the activity of 11β-HSD1 in omental adipocytes was found to be higher than that in subcutaneous depots, suggesting that GCs might have a greater impact in the abdominal depots (Bujalska et al. 1997). Indeed, mice overexpressing 11β-HSD1 have higher intra-abdominal GC levels and exhibit central adipocyte hypertrophy (Masuzaki et al. 2001).

Adding more complexity to our understanding of GC-regulated lipid metabolism is a recent study using a stable isotope (heavy water) labeling technique which showed that GCs can, in fact, simultaneously increase TG synthesis and lipolysis in inguinal fat pads of wild-type mice treated with Dex and in subcutaneous and visceral depots of CRH-Tg mice (Yu et al. 2010). It was found that 4-day Dex treatment of wild-type mice was able to induce the expression of genes involved in TG synthesis (*Scd2*, *Gpat3*, *Gpat4*, *Agpat2*, and *Lpin1*), lipolysis (*Lipe* and *Mgll*), lipid storage (*S3-12*), and lipid transport (*Cd36*, *Lrp1*, *Slc27a2*, *Vldlr*) (Yu et al. 2010). Most of these genes had at least one functional GR binding site, hinting at the direct regulation by GCs. Several unanswered questions remain: (1) why do GCs stimulate lipolysis and lipogenesis simultaneously resulting in futile cycling, and (2) what dictates the fat redistribution in Cushing's patients or in patients following chronic GC treatment? One possibility is that other hormones participate in the regulation of lipid metabolism by tipping the scale from TG synthesis to lipolysis or vice versa leading to a depot-specific adiposity.

Another mechanism by which GCs can increase adipose tissue mass is by stimulating pre-adipocyte differentiation. In vitro, GCs are required to fully induce adipocyte differentiation and as such they represent a key component of the adipogenic differentiation cocktail (Steger et al. 2010). In 3T3-L1 cells, activated GR transiently induces the expression of a key adipogenic transcription factor *Pparγ* (a master regulator of adipogenesis) and suppresses the expression of pre-adipogenic factor 1 (*Pref1*) (Steger et al. 2010). Interestingly, two direct target genes of GR, *Klf15* and *Dexras1*, have been recently implicated in GC-induced adipogenesis. MEFs and 3T3-L1 cells lacking KLF15 or DEXRAS1, respectively, were unable to stimulate adipocyte differentiation in vitro and animals lacking DEXRAS1 were protected against Dex-induced obesity. In vivo, depot-specific actions of GCs on adipocyte differentiation have also been observed, where treatment of rats for 10 days with corticosterone was able to increase adipocyte differentiation in visceral adipose tissue but not in subcutaneous depots (Campbell et al. 2011). However, the relative contribution of adipocyte hypertrophy vs. hyperplasia in the development of central obesity still needs to be examined.

Interestingly, GCs are also reported to induce the differentiation of brown preadipocytes (Shima et al. 1994) while inhibiting uncoupling protein 1 (*Ucp1*) expression and activity (Soumano et al. 2000). In fact, GC treatment in rats resulted in decreased thermogenesis and increased lipid accumulation in both BAT and WAT (Strack et al. 1995). In rodents, BAT plays an important role in regulating insulin sensitivity and glucose homeostasis by regulating thermogenesis (Stanford et al. 2013). With the recent discovery of metabolically active BAT in adult humans, it will be

exciting to investigate the role of GCs at this site to determine the relative contribution of BAT to GC-mediated glucose and lipid dysregulation (Cypess et al. 2014).

3.2 Liver

GC excess can lead to the ectopic accumulation of fat in the liver, causing the formation of "fatty liver" also known as hepatic steatosis, which is implicated in the development of insulin resistance and metabolic syndrome. Indeed, increased liver fat content has been observed in patients with Cushing's syndrome (Shibli-Rahhal et al. 2006) and in patients undergoing chronic GC treatment (Schacke et al. 2002). Unlike the extensive literature describing the role of GCs in adipose tissue lipid metabolism, the role of GR signaling in hepatic lipid metabolism is not well defined. A number of in vitro and in vivo studies have shown that GCs act in the liver to increase fatty acid synthesis (Diamant and Shafrir 1975; Altman et al. 1951), decrease fatty acid oxidation (Letteron et al. 1997), and increase VLDL secretion (Cole et al. 1982), although the latter is controversial (Dolinsky et al. 2004). Similar to adipose tissue, GCs in the liver can regulate de novo lipogenesis by directly upregulating the expression of *Fas* and *Acc*, and these effects are synergistic with insulin (Diamant and Shafrir 1975; Altman et al. 1951). In addition, acyl-CoA dehydrogenase enzymes involved in fatty acid β-oxidation are decreased by GC treatment in mice (Letteron et al. 1997). Similar observations have been made in primary hepatocytes suggesting that these effects are at least partially cell autonomous (Amatruda et al. 1983; Mangiapane and Brindley 1986). Moreover, downstream genes encoding enzymes in TG synthetic pathways, such as DGAT1 and DGAT2, were found to be upregulated by GCs, but whether this regulation is direct requires further investigation (Dolinsky et al. 2004). The combined effect of increasing lipogenesis and decreasing β-oxidation is thought to contribute to the observed hepatic steatosis. The effects of GCs on VLDL secretion are not well defined. Studies looking at patients with Cushing's syndrome are inconclusive, showing either elevated (Taskinen et al. 1983) or normal (Tiryakioglu et al. 2010) plasma VLDL levels. Numerous in vitro studies in both mouse and rat primary hepatocytes and isolated livers found an increase in VLDL secretion following Dex treatment; however, Dolinsky et al. found that VLDL secretion rates were not affected in vivo or in primary hepatocytes (Dolinsky et al. 2004). Interestingly, the stability of triacylglycerol hydrolase (TGH/Ces3), a lipase involved in intracellular TG hydrolyses prior to incorporation into VLDL, was found to be decreased by Dex treatment (Dolinsky et al. 2004).

A recent study performed by de Guia et al. 2015 has implicated microRNAs in the regulation of hepatic triglyceride metabolism by GCs (de Guia et al. 2015). miR-379/410 cluster was found to be a direct target of GR in the liver, and miR-379 levels were shown to be positively correlated with serum GCs and triglyceride levels in humans (de Guia et al. 2015). Moreover, knockdown of miR-379 in wild-type mice as well as obese animals decreased plasma TG and VLDL levels (de Guia et al. 2015). It was discovered that miR-379 acts by decreasing the levels of LDLR

and the lipolysis stimulated lipoprotein receptor (LSR), leading to decreased hepatic TG uptake and increased circulating lipids (de Guia et al. 2015).

The ability of GR to orchestrate these complex events relies on its interaction with a number of accessory proteins. For example, LXRβ was recently identified as a critical player in GC-induced hepatosteatosis (Patel et al. 2011). Mice lacking LXRβ were refractory to developing fatty liver following Dex treatment, although the exact molecular mechanism of GR-LXRβ interaction is not known. Furthermore, liver-specific knockouts of MED1, a GR coactivator, are protected from Dex-induced TG accumulation (Jia et al. 2009). In MED1-null livers, Dex fails to inhibit fatty acid β-oxidation leading to reduced TG accumulation.

GR can also elicit its control over hepatic dyslipidemia via the repression of *Hes1* gene expression (Lemke et al. 2008). GCs were found to reduce *Hes1* mRNA and protein levels in vitro (U2OS-GR cells and rat primary hepatocytes) and in livers of adrenalectomized mice (Revollo et al. 2013). In accordance, shRNA-mediated knockdown of GR in the liver of *db*/*db* mice was found to induce the expression of *Hes1* with a concomitant reduction in hepatosteatosis, suggesting a direct role of GR in the regulation of *Hes1* expression. Overexpression of HES1 in the liver of *db*/*db* mice was shown to be protective against GC-induced hepatosteatosis. Beneficial effects of HES1 overexpression are believed to be due to its ability to upregulate the expression of pancreatic lipases, *Pnl* and *Pnlrp2*, both of which contribute to TG hydrolysis. Chromatin immunoprecipitation analyses and luciferase-reporter assays revealed that *Hes1* is a direct target gene of GR in vivo (Lemke et al. 2008; Revollo et al. 2013). However, the exact molecular mechanism of*Hes1* regulation by GR is controversial, with studies hinting at the involvement of HDAC and NFκB proteins (Lemke et al. 2008; Revollo et al. 2013). In conclusion, GCs were found to stimulate hepatic TG accumulation via the repression of *Hes1*, thus blocking the induction of pancreatic lipase gene expression.

GCs can also regulate the production and accumulation of ceramides in the liver by stimulating the expression of genes involved in ceramide synthesis (serine palmitoyltransferase 2, SPT2, and dihydroceramide synthase, DES1) (Holland et al. 2007). Ceramides are sphingolipids composed of a fatty acid and sphingosine moiety (Hannun 1994), which act as important signaling molecules that generally promote catabolic processes. Ceramide levels are markedly elevated in rodent models of insulin resistance induced by GC excess, whereas mice heterozygous for *Des1* are protected from Dex-induced insulin resistance (Holland et al. 2007). This represents a mechanism by which GCs can indirectly antagonize insulin signaling.

With respect to regulation of cholesterol metabolism, studies have revealed that liver-specific GR deficiency results in dysregulation of cholesterol and bile acid homeostasis (Lemke et al. 2008; Rose et al. 2011). Hepatocyte-specific GR knockout mice exhibit reduced serum cholesterol levels, increased cholesterol accumulation in the liver, and elevated fasting bile acid levels. Moreover, mice lacking liver-specific GR had lower gallbladder bile acid concentrations and were more prone to developing cholesterol gallstones when placed on a cholesterol-rich diet (Rose et al. 2011). It was then found that liver GR deficiency impaired hepatic bile acid

uptake due to decreased expression of the basolateral bile acid transporter, *Ntcp* (*Slc10a1*) (Rose et al. 2011).

4 Protein Metabolism

4.1 Muscle

It is known that GCs both increase skeletal muscle catabolism and decrease muscle synthesis. The result of these combined processes is an increased rate of muscle breakdown, which is observed in patients with Cushing's disease. In vitro studies have shown that GCs can elicit their catabolic actions in a cell autonomous manner. For example, Dex treatment resulted in decreased cell diameters in C2C12 and L6 myotubes compared to vehicle treatment (Menconi et al. 2008). In vivo, animals treated with GCs exhibit a decrease in skeletal muscle size (Baehr et al. 2011), whereas muscle-specific GR knockout animals are resistant to Dex-induced muscle atrophy (Watson et al. 2012). GC control of muscle breakdown comes from its ability to upregulate two muscle-specific E3 ubiquitin ligases: muscle RING finger 1 (MuRF1) and muscle atrophy F-box (MAFbx) (Bodine et al. 2001). MuRF1 and MAFbx are induced in many catabolic states including starvation, diabetes, and GC treatment. Through ubiquitination, MAFbx and MuRF1 mark distinct protein targets for proteosomal degradation. MuRF1 has been shown to target primarily myofibrillar proteins such as myosin heavy chain (MYHC), whereas MAFbx was found to interact with regulatory proteins including MyoD and eIF3-f (Clarke et al. 2007; Csibi et al. 2009; Lagirand-Cantaloube et al. 2009). Interestingly, mice lacking MuRF1 were spared from Dex-induced muscle wasting, while *Mafbx-/-* animals were not (Baehr et al. 2011). Even more surprising is the fact that sparing of the *Murf1-/-* muscle mass was found to be primarily due to maintenance of protein synthesis rather than changes in proteolytic pathways (Baehr et al. 2011). These findings suggest that MuRF1 can regulate muscle atrophy through yet unknown non-proteolytic pathways, and this regulation is distinct from that of MAFbx. It should be noted than unlike skeletal muscle, cardiac muscle responds to GCs by cardiomyocyte hypertrophy suggesting that the catabolic actions of GCs on protein turnover are also tissue specific (Ren et al. 2012).

GCs can also directly increase the expression of myostatin, which in turn negatively regulates muscle growth (Ma et al. 2003). Mice lacking myostatin are resistant to developing Dex-induced muscle atrophy (Gilson et al. 2007). The expression levels of *Murf1* and *Mafbx* are also decreased in myostatin-null mice, implicating myostatin as an important mediator of GC-induced muscle atrophy (Ma et al. 2003). More recently, it was found that Dex was able to suppress muscle satellite cell function through the upregulation of myostatin and a resultant suppression of *Akirin1* (promyogenic gene) (Dong et al. 2013).

In addition to increased proteolysis, GCs can induce muscle atrophy by decreasing protein synthesis. GCs achieve this via the inhibition of mTOR, a kinase that phosphorylates S6K1 and 4E-BP1, two proteins involved in mRNA translation

initiation (Schakman et al. 2008). Recent studies identified *Klf15* and *Ddit4* (*Redd1*) as two direct target genes of GCs involved in mTOR inhibition (Shimizu et al. 2011). KLF15 has been shown to induce the expression of *Bcat2*, a mitochondrial enzyme that decreases mTOR activity (Shimizu et al. 2011). DDIT4, on the other hand, was found to increase the activity of the regulatory TSC1/TSC2 protein complex leading to mTOR inhibition (Shimizu et al. 2011; Wang et al. 2006). Interestingly, KLF15 was also found to regulate the atrophy genes, *Murf1* and *Mafbx*, and is also regulated by GCs in adipose tissue to promote adipocyte differentiation. Several other GR target genes, *Sesn1*, *Depdc6*, and *Mknk2*, have also been shown to interact and inhibit mTOR activity or signaling (Kuo et al. 2012, 2013). Finally, GR was found to upregulate the expression of *p85*α through a GRE (Kuo et al. 2012). Studies utilizing shRNA to knockdown p85α in C2C12 myotubes found that Dex failed to inhibit AKT activity and atrophy gene expression. Interestingly, studies by Hu et al. found that activated GR is able to directly bind p85α (regulatory subunit of PI3 kinase) and prevent its association with IRS-1, thus inhibiting insulin signaling (Hu et al. 2009). Overall, these data suggest that GCs may suppress insulin signaling via p85α through genomic (direct DNA binding) and non-genomic mechanisms.

5 Glucocorticoids and Other Target Organs

5.1 Pancreas

The endocrine pancreas is a major sensor of circulating glucose levels. Pancreatic β-cells respond to elevated blood glucose by secreting insulin to promote glucose uptake and utilization in peripheral tissues. The role of GCs on insulin secretion is complex and a detailed review was published recently (Rafacho et al. 2012). GCs impact pancreatic β-cell function early during embryonic development. Studies in $Gr^{lox/lox}$ and Gr^{PdxCre} mice have shown that maternal food restriction during late pregnancy (which causes elevated fetal corticosterone levels) irreversibly decreases the β-cell mass of newborn mice (Valtat et al. 2011). Moreover, there is evidence suggesting that GCs may shift the fate of pancreatic progenitor cells from an endocrine to an exocrine lineage, thus compromising β-cell expansion later in life (Valtat et al. 2011). Interestingly, excessive GC signaling in mature β-cells does not affect cell numbers but instead leads to impaired insulin secretion (Blondeau et al. 2012). Studies performed in vitro on isolated islets and cultured β-cells also showed that GCs inhibit insulin secretion and promote apoptosis (Lambillotte et al. 1997; Ranta et al. 2006; Reich et al. 2012). Mechanistically, GCs impair pancreatic cell function via several distinct mechanisms. Dex treatment of isolated pancreatic β-cells decreases the stability and protein levels of the GLUT2 glucose transporter leading to impaired insulin secretion (Gremlich et al. 1997). Moreover, GC-mediated induction of serum-/glucocorticoid-regulated kinase 1 (*Sgk-1*) in INS-1 cells led to increased activity of voltage-gated K^+ channels, leading to reduced insulin release (Ullrich et al. 2005). Furthermore, recent studies found

that Dex can induce the expression of *Txnip*, a negative regulator of the antioxidant thioredoxin in β-cells of mice and human islets, resulting in apoptosis (Reich et al. 2012). Lastly, the unfolded protein response was also recently implicated in β-cell dysfunction, where prednisolone administration to β-cells resulted in the activation of ATF6 and IRE1/XBP1 pathways and increased caspase-3 activity leading to apoptosis (Linssen et al. 2011).

Intriguingly, oral glucose tolerance tests performed in normal subjects immediately after receiving a single i.v. bolus of hydrocortisone showed an increase in insulin secretion compared to vehicle treatment (Vila et al. 2010). Similarly, Dex administration in healthy individuals was shown to cause hyperinsulinemia (Nicod et al. 2003). Higher insulin levels were able to compensate for Dex-mediated insulin resistance in skeletal muscle and adipose but not in the liver since hepatic glucose production remained elevated during the clamp (Nicod et al. 2003). It is believed that hyperinsulinemia, which arises following acute GC treatment, is a result of compensatory actions by pancreatic β-cells to respond to hyperglycemia. Chronic GC stimulation, on the other hand, leads to a decrease in insulin signaling due to β-cell dysfunction and apoptosis.

5.2 CNS

A well-known role of GCs in the brain is the classical negative feedback of the HPA axis, where circulating GCs inhibit the expression of the hormones CRH (hypothalamus) and ACTH (pituitary gland) leading to inhibition of GC synthesis from the adrenal cortex. A number of recent studies have shown that GC signaling in the brain can also regulate peripheral metabolic responses. GR is highly expressed in the paraventricular (PVN) and arcuate (ARC) nuclei in the brain where it was discovered to regulate feeding behavior and glucose homeostasis by regulating the expression of the orexigenic peptide neuropeptide Y (NPY). Local administration of Dex (via retrodialysis) into the ARC, but not the PVN, was able to induce hepatic insulin resistance during a hyperinsulinemic-euglycemic clamp (Yi et al. 2012). In agreement, intracerebroventricular coadministration of the NPY1 receptor antagonist BIBP3226, or hepatic sympathetic denervation, was able to block this effect (Yi et al. 2012). In summary, GCs seem to be able to regulate peripheral insulin responsiveness via hypothalamic signaling and the sympathetic nervous system.

Interestingly, hepatic vagal innervation is also required for GC-induced insulin resistance, hyperglycemia, and hypertension. Studies by Bernal-Mizrachi et al. revealed that selective hepatic afferent vagotomy, as well as central afferent vagal nerve sectioning, decrease Dex-induced *Ppar*α and *Pepck* expression and reverse insulin resistance in wild-type mice (Bernal-Mizrachi et al. 2007). PPARα's involvement in GC-induced insulin resistance and hyperglycemia has been previously established, and animals lacking hepatic PPARα are protected against Dex-mediated effects (Bernal-Mizrachi et al. 2003; Lemberger et al. 1994). Intriguingly, adenoviral reconstitution of hepatic PPARα in normoglycemic Dex-treated *Pparα*-/- animals increased PEPCK activity, blood glucose, and blood pressure in

sham-operated mice but not after vagotomy, suggesting that both hepatic vagal innervation and intact PPARα signaling are necessary for GC-induced metabolic effects (Bernal-Mizrachi et al. 2007).

6 Concluding Remarks

The metabolic actions of glucocorticoids are highly coordinated between multiple tissues, facilitating the rapid catabolic actions of GCs that have the overall effect of increasing circulating glucose levels. While many of the biochemical processes mediating these effects are now understood, the individual genes responsible for these effects and the molecular mechanisms regulating their expression are still being elucidated. Further understanding the complex feedback responses mediated by hormones and the sympathetic nervous system will provide new insight into possible mechanisms of inhibiting the detrimental metabolic consequences of chronic GC exposure.

References

Abraham SB, Rubino D, Sinaii N, Ramsey S, Nieman LK (2013) Cortisol, obesity, and the metabolic syndrome: a cross-sectional study of obese subjects and review of the literature. Obesity 21:E105–E117

Altman KI, Miller LL, Bly CG (1951) The synergistic effect of cortisone and insulin on lipogenesis in the perfused rat liver as studied with alpha-C14-acetate. Arch Biochem Biophys 31:329–331

Amatruda JM, Danahy SA, Chang CL (1983) The effects of glucocorticoids on insulin-stimulated lipogenesis in primary cultures of rat hepatocytes. Biochem J 212:135–141

Baehr LM, Furlow JD, Bodine SC (2011) Muscle sparing in muscle RING finger 1 null mice: response to synthetic glucocorticoids. J Physiol 589:4759–4776

Bernal-Mizrachi C, Weng S, Feng C, Finck BN, Knutsen RH, Leone TC, Coleman T, Mecham RP, Kelly DP, Semenkovich CF (2003) Dexamethasone induction of hypertension and diabetes is PPAR-alpha dependent in LDL receptor-null mice. Nat Med 9:1069–1075

Bernal-Mizrachi C, Xiaozhong L, Yin L, Knutsen RH, Howard MJ, Arends JJ, Desantis P, Coleman T, Semenkovich CF (2007) An afferent vagal nerve pathway links hepatic PPARalpha activation to glucocorticoid-induced insulin resistance and hypertension. Cell Metab 5:91–102

Blondeau B, Sahly I, Massourides E, Singh-Estivalet A, Valtat B, Dorchene D, Jaisser F, Breant B, Tronche F (2012) Novel transgenic mice for inducible gene overexpression in pancreatic cells define glucocorticoid receptor-mediated regulations of beta cells. PLoS One 7:e30210

Bodine SC, Latres E, Baumhueter S, Lai VK, Nunez L, Clarke BA, Poueymirou WT, Panaro FJ, Na E, Dharmarajan K, Pan ZQ, Valenzuela DM, DeChiara TM, Stitt TN, Yancopoulos GD, Glass DJ (2001) Identification of ubiquitin ligases required for skeletal muscle atrophy. Science 294:1704–1708

Bujalska IJ, Kumar S, Stewart PM (1997) Does central obesity reflect "Cushing's disease of the omentum"? Lancet 349:1210–1213

Campbell JE, Peckett AJ, D'Souza AM, Hawke TJ, Riddell MC (2011) Adipogenic and lipolytic effects of chronic glucocorticoid exposure. Am J Physiol Cell Physiol 300:C198–C209

Caperuto LC, Anhe GF, Amanso AM, Ribeiro LM, Medina MC, Souza LC, Carvalho OM, Bordin S, Saad MJ, Carvalho CR (2006) Distinct regulation of IRS proteins in adipose tissue from obese aged and dexamethasone-treated rats. Endocrine 29:391–398

Chakravarty K, Cassuto H, Reshef L, Hanson RW (2005) Factors that control the tissue-specific transcription of the gene for phosphoenolpyruvate carboxykinase-C. Crit Rev Biochem Mol Biol 40:129–154

Chimin P, Farias Tda S, Torres-Leal FL, Bolsoni-Lopes A, Campana AB, Andreotti S, Lima FB (2014) Chronic glucocorticoid treatment enhances lipogenic activity in visceral adipocytes of male Wistar rats. Acta Physiol 211:409–420

Clarke BA, Drujan D, Willis MS, Murphy LO, Corpina RA, Burova E, Rakhilin SV, Stitt TN, Patterson C, Latres E, Glass DJ (2007) The E3 Ligase MuRF1 degrades myosin heavy chain protein in dexamethasone-treated skeletal muscle. Cell Metab 6:376–385

Cole TG, Wilcox HG, Heimberg M (1982) Effects of adrenalectomy and dexamethasone on hepatic lipid metabolism. J Lipid Res 23:81–91

Connaughton S, Chowdhury F, Attia RR, Song S, Zhang Y, Elam MB, Cook GA, Park EA (2010) Regulation of pyruvate dehydrogenase kinase isoform 4 (PDK4) gene expression by glucocorticoids and insulin. Mol Cell Endocrinol 315:159–167

Csibi A, Leibovitch MP, Cornille K, Tintignac LA, Leibovitch SA (2009) MAFbx/Atrogin-1 controls the activity of the initiation factor eIF3-f in skeletal muscle atrophy by targeting multiple C-terminal lysines. J Biol Chem 284:4413–4421

Cypess AM, Haft CR, Laughlin MR, Hu HH (2014) Brown fat in humans: consensus points and experimental guidelines. Cell Metab 20:408–415

de Guia RM, Rose AJ, Sommerfeld A, Seibert O, Strzoda D, Zota A, Feuchter Y, Krones-Herzig A, Sijmonsma T, Kirilov M, Sticht C, Gretz N, Dallinga-Thie G, Diederichs S, Kloting N, Bluher M, Berriel Diaz M, Herzig S (2015) microRNA-379 couples glucocorticoid hormones to dysfunctional lipid homeostasis. EMBO J 34:344–360

de Wulf H, Hers HG (1968) The role of glucose, glucagon and glucocorticoids in the regulation of liver glycogen synthesis. Eur J Biochem 6:558–564

Diamant S, Shafrir E (1975) Modulation of the activity of insulin-dependent enzymes of lipogenesis by glucocorticoids. Eur J Biochem 53:541–546

Dolinsky VW, Douglas DN, Lehner R, Vance DE (2004) Regulation of the enzymes of hepatic microsomal triacylglycerol lipolysis and re-esterification by the glucocorticoid dexamethasone. Biochem J 378:967–974

Dong Y, Pan JS, Zhang L (2013) Myostatin suppression of Akirin1 mediates glucocorticoid-induced satellite cell dysfunction. PLoS One 8:e58554

Du K, Herzig S, Kulkarni RN, Montminy M (2003) TRB3: a tribbles homolog that inhibits Akt/PKB activation by insulin in liver. Science 300:1574–1577

Gilson H, Schakman O, Combaret L, Lause P, Grobet L, Attaix D, Ketelslegers JM, Thissen JP (2007) Myostatin gene deletion prevents glucocorticoid-induced muscle atrophy. Endocrinology 148:452–460

Giorgino F, Almahfouz A, Goodyear LJ, Smith RJ (1993) Glucocorticoid regulation of insulin receptor and substrate IRS-1 tyrosine phosphorylation in rat skeletal muscle in vivo. J Clin Invest 91:2020–2030

Gray NE, Lam LN, Yang K, Zhou AY, Koliwad S, Wang JC (2012) Angiopoietin-like 4 (Angptl4) protein is a physiological mediator of intracellular lipolysis in murine adipocytes. J Biol Chem 287:8444–8456

Gremlich S, Roduit R, Thorens B (1997) Dexamethasone induces posttranslational degradation of GLUT2 and inhibition of insulin secretion in isolated pancreatic beta cells. Comparison with the effects of fatty acids. J Biol Chem 272:3216–3222

Haber RS, Weinstein SP (1992) Role of glucose transporters in glucocorticoid-induced insulin resistance. GLUT4 isoform in rat skeletal muscle is not decreased by dexamethasone. Diabetes 41:728–735

Hall RK, Wang XL, George L, Koch SR, Granner DK (2007) Insulin represses phosphoenolpyruvate carboxykinase gene transcription by causing the rapid disruption of an active transcription complex: a potential epigenetic effect. Mol Endocrinol 21:550–563

Hannun YA (1994) The sphingomyelin cycle and the second messenger function of ceramide. J Biol Chem 269:3125–3128

Hautanen A, Raikkonen K, Adlercreutz H (1997) Associations between pituitary-adrenocortical function and abdominal obesity, hyperinsulinaemia and dyslipidaemia in normotensive males. J Intern Med 241:451–461

Holland WL, Brozinick JT, Wang LP, Hawkins ED, Sargent KM, Liu Y, Narra K, Hoehn KL, Knotts TA, Siesky A, Nelson DH, Karathanasis SK, Fontenot GK, Birnbaum MJ, Summers SA (2007) Inhibition of ceramide synthesis ameliorates glucocorticoid-, saturated-fat-, and obesity-induced insulin resistance. Cell Metab 5:167–179

Hu Z, Wang H, Lee IH, Du J, Mitch WE (2009) Endogenous glucocorticoids and impaired insulin signaling are both required to stimulate muscle wasting under pathophysiological conditions in mice. J Clin Invest 119:3059–3069

Ichijo T, Voutetakis A, Cotrim AP, Bhattachryya N, Fujii M, Chrousos GP, Kino T (2005) The Smad6-histone deacetylase 3 complex silences the transcriptional activity of the glucocorticoid receptor: potential clinical implications. J Biol Chem 280:42067–42077

Jia Y, Viswakarma N, Fu T, Yu S, Rao MS, Borensztajn J, Reddy JK (2009) Conditional ablation of mediator subunit MED1 (MED1/PPARBP) gene in mouse liver attenuates glucocorticoid receptor agonist dexamethasone-induced hepatic steatosis. Gene Expr 14:291–306

Kjolhede EA, Gustafsson PE, Gustafsson PA, Nelson N (2014) Overweight and obese children have lower cortisol levels than normal weight children. Acta Paediatr 103:295–299

Koliwad SK, Kuo T, Shipp LE, Gray NE, Backhed F, So AY, Farese RV Jr, Wang JC (2009) Angiopoietin-like 4 (ANGPTL4, fasting-induced adipose factor) is a direct glucocorticoid receptor target and participates in glucocorticoid-regulated triglyceride metabolism. J Biol Chem 284:25593–25601

Kovacs JJ, Murphy PJ, Gaillard S, Zhao X, Wu JT, Nicchitta CV, Yoshida M, Toft DO, Pratt WB, Yao TP (2005) HDAC6 regulates Hsp90 acetylation and chaperone-dependent activation of glucocorticoid receptor. Mol Cell 18:601–607

Kuo T, Lew MJ, Mayba O, Harris CA, Speed TP, Wang JC (2012) Genome-wide analysis of glucocorticoid receptor-binding sites in myotubes identifies gene networks modulating insulin signaling. Proc Natl Acad Sci U S A 109:11160–11165

Kuo T, Harris CA, Wang JC (2013) Metabolic functions of glucocorticoid receptor in skeletal muscle. Mol Cell Endocrinol 380:79–88

Kwon HS, Huang B, Unterman TG, Harris RA (2004) Protein kinase B-alpha inhibits human pyruvate dehydrogenase kinase-4 gene induction by dexamethasone through inactivation of FOXO transcription factors. Diabetes 53:899–910

Lagirand-Cantaloube J, Cornille K, Csibi A, Batonnet-Pichon S, Leibovitch MP, Leibovitch SA (2009) Inhibition of atrogin-1/MAFbx mediated MyoD proteolysis prevents skeletal muscle atrophy in vivo. PLoS One 4:e4973

Laloux M, Stalmans W, Hers HG (1983) On the mechanism by which glucocorticoids cause the activation of glycogen synthase in mouse and rat livers. Eur J Biochem 136:175–181

Lambillotte C, Gilon P, Henquin JC (1997) Direct glucocorticoid inhibition of insulin secretion. An in vitro study of dexamethasone effects in mouse islets. J Clin Invest 99:414–423

Langley SC, York DA (1990) Effects of antiglucocorticoid RU 486 on development of obesity in obese fa/fa Zucker rats. Am J Physiol 259:R539–R544

Lemberger T, Staels B, Saladin R, Desvergne B, Auwerx J, Wahli W (1994) Regulation of the peroxisome proliferator-activated receptor alpha gene by glucocorticoids. J Biol Chem 269:24527–24530

Lemke U, Krones-Herzig A, Berriel Diaz M, Narvekar P, Ziegler A, Vegiopoulos A, Cato AC, Bohl S, Klingmuller U, Screaton RA, Muller-Decker K, Kersten S, Herzig S (2008) The glucocorticoid receptor controls hepatic dyslipidemia through Hes1. Cell Metab 8:212–223

Letteron P, Brahimi-Bourouina N, Robin MA, Moreau A, Feldmann G, Pessayre D (1997) Glucocorticoids inhibit mitochondrial matrix acyl-CoA dehydrogenases and fatty acid beta-oxidation. Am J Physiol 272:G1141–G1150

Lin B, Morris DW, Chou JY (1998) Hepatocyte nuclear factor 1alpha is an accessory factor required for activation of glucose-6-phosphatase gene transcription by glucocorticoids. DNA Cell Biol 17:967–974

Linssen MM, van Raalte DH, Toonen EJ, Alkema W, van der Zon GC, Dokter WH, Diamant M, Guigas B, Ouwens DM (2011) Prednisolone-induced beta cell dysfunction is associated with impaired endoplasmic reticulum homeostasis in INS-1E cells. Cell Signal 23:1708–1715

Long W, Barrett EJ, Wei L, Liu Z (2003) Adrenalectomy enhances the insulin sensitivity of muscle protein synthesis. Am J Physiol Endocrinol Metab 284:E102–E109

Ma K, Mallidis C, Bhasin S, Mahabadi V, Artaza J, Gonzalez-Cadavid N, Arias J, Salehian B (2003) Glucocorticoid-induced skeletal muscle atrophy is associated with upregulation of myostatin gene expression. Am J Physiol Endocrinol Metab 285:E363–E371

Mangiapane EH, Brindley DN (1986) Effects of dexamethasone and insulin on the synthesis of triacylglycerols and phosphatidylcholine and the secretion of very-low-density lipoproteins and lysophosphatidylcholine by monolayer cultures of rat hepatocytes. Biochem J 233:151–160

Masuzaki H, Paterson J, Shinyama H, Morton NM, Mullins JJ, Seckl JR, Flier JS (2001) A transgenic model of visceral obesity and the metabolic syndrome. Science 294:2166–2170

Menconi M, Gonnella P, Petkova V, Lecker S, Hasselgren PO (2008) Dexamethasone and corticosterone induce similar, but not identical, muscle wasting responses in cultured L6 and C2C12 myotubes. J Cell Biochem 105:353–364

Minshull M, Strong CR (1985) The stimulation of lipogenesis in white adipose tissue from fed rats by corticosterone. Int J Biochem 17:529–532

Nader N, Ng SS, Lambrou GI, Pervanidou P, Wang Y, Chrousos GP, Kino T (2010) AMPK regulates metabolic actions of glucocorticoids by phosphorylating the glucocorticoid receptor through p38 MAPK. Mol Endocrinol 24:1748–1764

Nader N, Ng SS, Wang Y, Abel BS, Chrousos GP, Kino T (2012) Liver x receptors regulate the transcriptional activity of the glucocorticoid receptor: implications for the carbohydrate metabolism. PLoS One 7:e26751

Nakae J, Kitamura T, Silver DL, Accili D (2001) The forkhead transcription factor Foxo1 (Fkhr) confers insulin sensitivity onto glucose-6-phosphatase expression. J Clin Invest 108:1359–1367

Nicod N, Giusti V, Besse C, Tappy L (2003) Metabolic adaptations to dexamethasone-induced insulin resistance in healthy volunteers. Obes Res 11:625–631

Opherk C, Tronche F, Kellendonk C, Kohlmuller D, Schulze A, Schmid W, Schutz G (2004) Inactivation of the glucocorticoid receptor in hepatocytes leads to fasting hypoglycemia and ameliorates hyperglycemia in streptozotocin-induced diabetes mellitus. Mol Endocrinol 18:1346–1353

Patel R, Patel M, Tsai R, Lin V, Bookout AL, Zhang Y, Magomedova L, Li T, Chan JF, Budd C, Mangelsdorf DJ, Cummins CL (2011) LXRbeta is required for glucocorticoid-induced hyperglycemia and hepatosteatosis in mice. J Clin Invest 121:431–441

Peckett AJ, Wright DC, Riddell MC (2011) The effects of glucocorticoids on adipose tissue lipid metabolism. Metabolism 60:1500–1510

Phillips DI, Barker DJ, Fall CH, Seckl JR, Whorwood CB, Wood PJ, Walker BR (1998) Elevated plasma cortisol concentrations: a link between low birth weight and the insulin resistance syndrome? J Clin Endocrinol Metab 83:757–760

Praveen EP, Sahoo JP, Kulshreshtha B, Khurana ML, Gupta N, Dwivedi SN, Kumar G, Ammini AC (2011) Morning cortisol is lower in obese individuals with normal glucose tolerance. Diabetes Metab Syndr Obes 4:347–352

Puigserver P, Rhee J, Donovan J, Walkey CJ, Yoon JC, Oriente F, Kitamura Y, Altomonte J, Dong H, Accili D, Spiegelman BM (2003) Insulin-regulated hepatic gluconeogenesis through FOXO1-PGC-1alpha interaction. Nature 423:550–555

Rafacho A, Boschero AC, Ortsäter H (2012) Functional and molecular aspects of glucocorticoids in the endocrine pancreas and glucose homeostasis. In: Magdeldin S (ed) State of the art of therapeutic endocrinology. InTech, Rijeka

Ranta F, Avram D, Berchtold S, Dufer M, Drews G, Lang F, Ullrich S (2006) Dexamethasone induces cell death in insulin-secreting cells, an effect reversed by exendin-4. Diabetes 55:1380–1390

Rebuffe-Scrive M, Krotkiewski M, Elfverson J, Bjorntorp P (1988) Muscle and adipose tissue morphology and metabolism in Cushing's syndrome. J Clin Endocrinol Metab 67:1122–1128

Reich E, Tamary A, Sionov RV, Melloul D (2012) Involvement of thioredoxin-interacting protein (TXNIP) in glucocorticoid-mediated beta cell death. Diabetologia 55:1048–1057

Ren R, Oakley RH, Cruz-Topete D, Cidlowski JA (2012) Dual role for glucocorticoids in cardiomyocyte hypertrophy and apoptosis. Endocrinology 153:5346–5360

Revollo JR, Oakley RH, Lu NZ, Kadmiel M, Gandhavadi M, Cidlowski JA (2013) HES1 is a master regulator of glucocorticoid receptor-dependent gene expression. Sci Signal 6:ra103

Reynolds RM, Walker BR, Syddall HE, Andrew R, Wood PJ, Whorwood CB, Phillips DI (2001) Altered control of cortisol secretion in adult men with low birth weight and cardiovascular risk factors. J Clin Endocrinol Metab 86:245–250

Rhee J, Inoue Y, Yoon JC, Puigserver P, Fan M, Gonzalez FJ, Spiegelman BM (2003) Regulation of hepatic fasting response by PPARgamma coactivator-1alpha (PGC-1): requirement for hepatocyte nuclear factor 4alpha in gluconeogenesis. Proc Natl Acad Sci U S A 100:4012–4017

Rose AJ, Berriel Diaz M, Reimann A, Klement J, Walcher T, Krones-Herzig A, Strobel O, Werner J, Peters A, Kleyman A, Tuckermann JP, Vegiopoulos A, Herzig S (2011) Molecular control of systemic bile acid homeostasis by the liver glucocorticoid receptor. Cell Metab 14:123–130

Ruzzin J, Wagman AS, Jensen J (2005) Glucocorticoid-induced insulin resistance in skeletal muscles: defects in insulin signalling and the effects of a selective glycogen synthase kinase-3 inhibitor. Diabetologia 48:2119–2130

Saad MJ, Folli F, Kahn JA, Kahn CR (1993) Modulation of insulin receptor, insulin receptor substrate-1, and phosphatidylinositol 3-kinase in liver and muscle of dexamethasone-treated rats. J Clin Invest 92:2065–2072

Sakoda H, Ogihara T, Anai M, Funaki M, Inukai K, Katagiri H, Fukushima Y, Onishi Y, Ono H, Fujishiro M, Kikuchi M, Oka Y, Asano T (2000) Dexamethasone-induced insulin resistance in 3T3-L1 adipocytes is due to inhibition of glucose transport rather than insulin signal transduction. Diabetes 49:1700–1708

Sandri M, Sandri C, Gilbert A, Skurk C, Calabria E, Picard A, Walsh K, Schiaffino S, Lecker SH, Goldberg AL (2004) Foxo transcription factors induce the atrophy-related ubiquitin ligase atrogin-1 and cause skeletal muscle atrophy. Cell 117:399–412

Schacke H, Docke WD, Asadullah K (2002) Mechanisms involved in the side effects of glucocorticoids. Pharmacol Ther 96:23–43

Schakman O, Gilson H, Thissen JP (2008) Mechanisms of glucocorticoid-induced myopathy. J Endocrinol 197:1–10

Seckl JR, Walker BR (2001) Minireview: 11beta-hydroxysteroid dehydrogenase type 1 – a tissue-specific amplifier of glucocorticoid action. Endocrinology 142:1371–1376

Seckl JR, Morton NM, Chapman KE, Walker BR (2004) Glucocorticoids and 11beta-hydroxysteroid dehydrogenase in adipose tissue. Recent Prog Horm Res 59:359–393

Shan L, Yu XC, Liu Z, Hu Y, Sturgis LT, Miranda ML, Liu Q (2009) The angiopoietin-like proteins ANGPTL3 and ANGPTL4 inhibit lipoprotein lipase activity through distinct mechanisms. J Biol Chem 284:1419–1424

Shibli-Rahhal A, Van Beek M, Schlechte JA (2006) Cushing's syndrome. Clin Dermatol 24:260–265

Shima A, Shinohara Y, Doi K, Terada H (1994) Normal differentiation of rat brown adipocytes in primary culture judged by their expressions of uncoupling protein and the physiological isoform of glucose-transporter. Biochim Biophys Acta-Mol Cell Res 1223:1–8

Shimizu N, Yoshikawa N, Ito N, Maruyama T, Suzuki Y, Takeda S, Nakae J, Tagata Y, Nishitani S, Takehana K, Sano M, Fukuda K, Suematsu M, Morimoto C, Tanaka H (2011) Crosstalk between glucocorticoid receptor and nutritional sensor mTOR in skeletal muscle. Cell Metab 13:170–182

Slavin BG, Ong JM, Kern PA (1994) Hormonal regulation of hormone-sensitive lipase activity and mRNA levels in isolated rat adipocytes. J Lipid Res 35:1535–1541

Sommerfeld A, Krones-Herzig A, Herzig S (2011) Transcriptional co-factors and hepatic energy metabolism. Mol Cell Endocrinol 332:21–31

Soumano K, Desbiens S, Rabelo R, Bakopanos E, Camirand A, Silva JE (2000) Glucocorticoids inhibit the transcriptional response of the uncoupling protein-1 gene to adrenergic stimulation in a brown adipose cell line. Mol Cell Endocrinol 165:7–15

Stanford KI, Middelbeek RJ, Townsend KL, An D, Nygaard EB, Hitchcox KM, Markan KR, Nakano K, Hirshman MF, Tseng YH, Goodyear LJ (2013) Brown adipose tissue regulates glucose homeostasis and insulin sensitivity. J Clin Invest 123:215–223

Steger DJ, Grant GR, Schupp M, Tomaru T, Lefterova MI, Schug J, Manduchi E, Stoeckert CJ Jr, Lazar MA (2010) Propagation of adipogenic signals through an epigenomic transition state. Genes Dev 24:1035–1044

Stolk RP, Lamberts SW, de Jong FH, Pols HA, Grobbee DE (1996) Gender differences in the associations between cortisol and insulin in healthy subjects. J Endocrinol 149:313–318

Strack AM, Bradbury MJ, Dallman MF (1995) Corticosterone decreases nonshivering thermogenesis and increases lipid storage in brown adipose tissue. Am J Physiol 268:R183–R191

Sugden MC, Holness MJ (2003) Recent advances in mechanisms regulating glucose oxidation at the level of the pyruvate dehydrogenase complex by PDKs. Am J Physiol Endocrinol Metab 284:E855–E862

Taskinen MR, Nikkila EA, Pelkonen R, Sane T (1983) Plasma lipoproteins, lipolytic enzymes, and very low density lipoprotein triglyceride turnover in Cushing's syndrome. J Clin Endocrinol Metab 57:619–626

Tiryakioglu O, Ugurlu S, Yalin S, Yirmibescik S, Caglar E, Yetkin DO, Kadioglu P (2010) Screening for Cushing's syndrome in obese patients. Clinics 65:9–13

Ullrich S, Berchtold S, Ranta F, Seebohm G, Henke G, Lupescu A, Mack AF, Chao CM, Su J, Nitschke R, Alexander D, Friedrich B, Wulff P, Kuhl D, Lang F (2005) Serum- and glucocorticoid-inducible kinase 1 (SGK1) mediates glucocorticoid-induced inhibition of insulin secretion. Diabetes 54:1090–1099

Valtat B, Dupuis C, Zenaty D, Singh-Estivalet A, Tronche F, Breant B, Blondeau B (2011) Genetic evidence of the programming of beta cell mass and function by glucocorticoids in mice. Diabetologia 54:350–359

Vander Kooi BT, Onuma H, Oeser JK, Svitek CA, Allen SR, Vander Kooi CW, Chazin WJ, O'Brien RM (2005) The glucose-6-phosphatase catalytic subunit gene promoter contains both positive and negative glucocorticoid response elements. Mol Endocrinol 19:3001–3022

Vila G, Krebs M, Riedl M, Baumgartner-Parzer SM, Clodi M, Maier C, Pacini G, Luger A (2010) Acute effects of hydrocortisone on the metabolic response to a glucose load: increase in the first-phase insulin secretion. Eur J Endocrinol/European Federation of Endocrine Societies 163:225–231

Villena JA, Roy S, Sarkadi-Nagy E, Kim KH, Sul HS (2004) Desnutrin, an adipocyte gene encoding a novel patatin domain-containing protein, is induced by fasting and glucocorticoids: ectopic expression of desnutrin increases triglyceride hydrolysis. J Biol Chem 279:47066–47075

Volpe JJ, Marasa JC (1975) Hormonal regulation of fatty acid synthetase, acetyl-CoA carboxylase and fatty acid synthesis in mammalian adipose tissue and liver. Biochim Biophys Acta 380:454–472

Walker BR, Phillips DI, Noon JP, Panarelli M, Andrew R, Edwards HV, Holton DW, Seckl JR, Webb DJ, Watt GC (1998) Increased glucocorticoid activity in men with cardiovascular risk factors. Hypertension 31:891–895

Wang XL, Herzog B, Waltner-Law M, Hall RK, Shiota M, Granner DK (2004) The synergistic effect of dexamethasone and all-trans-retinoic acid on hepatic phosphoenolpyruvate carboxykinase gene expression involves the coactivator p300. J Biol Chem 279:34191–34200

Wang H, Kubica N, Ellisen LW, Jefferson LS, Kimball SR (2006) Dexamethasone represses signaling through the mammalian target of rapamycin in muscle cells by enhancing expression of REDD1. J Biol Chem 281:39128–39134

Watson ML, Baehr LM, Reichardt HM, Tuckermann JP, Bodine SC, Furlow JD (2012) A cell-autonomous role for the glucocorticoid receptor in skeletal muscle atrophy induced by systemic glucocorticoid exposure. Am J Physiol Endocrinol Metab 302:E1210–E1220

Weinstein SP, Paquin T, Pritsker A, Haber RS (1995) Glucocorticoid-induced insulin resistance: dexamethasone inhibits the activation of glucose transport in rat skeletal muscle by both insulin- and non-insulin-related stimuli. Diabetes 44:441–445

Weinstein SP, Wilson CM, Pritsker A, Cushman SW (1998) Dexamethasone inhibits insulin-stimulated recruitment of GLUT4 to the cell surface in rat skeletal muscle. Metabolism 47:3–6

Xu C, He J, Jiang H, Zu L, Zhai W, Pu S, Xu G (2009) Direct effect of glucocorticoids on lipolysis in adipocytes. Mol Endocrinol 23:1161–1170

Yi CX, Foppen E, Abplanalp W, Gao Y, Alkemade A, la Fleur SE, Serlie MJ, Fliers E, Buijs RM, Tschop MH, Kalsbeek A (2012) Glucocorticoid signaling in the arcuate nucleus modulates hepatic insulin sensitivity. Diabetes 61:339–345

Yoon JC, Puigserver P, Chen G, Donovan J, Wu Z, Rhee J, Adelmant G, Stafford J, Kahn CR, Granner DK, Newgard CB, Spiegelman BM (2001) Control of hepatic gluconeogenesis through the transcriptional coactivator PGC-1. Nature 413:131–138

Yu CY, Mayba O, Lee JV, Tran J, Harris C, Speed TP, Wang JC (2010) Genome-wide analysis of glucocorticoid receptor binding regions in adipocytes reveal gene network involved in triglyceride homeostasis. PLoS One 5:e15188

Yukimura Y, Bray GA, Wolfsen AR (1978) Some effects of adrenalectomy in the fatty rat. Endocrinology 103:1924–1928

Nuclear Receptor Coregulators in Metabolism and Disease

Marco Giudici, Saioa Goni, Rongrong Fan, and Eckardt Treuter

Contents

Abstract

Within the past two decades, coregulators have emerged as essential chromatin components of metabolic signaling by nuclear receptors and additional metabolite-sensing transcription factors. Intriguingly, coregulators themselves are efficient sensors and effectors of metabolic stimuli that modulate gene expression at different levels, often via post-translational modifications of

M. Giudici • S. Goni • R. Fan • E. Treuter (✉)
Department of Biosciences and Nutrition, Karolinska Institutet, Huddinge S-14157, Sweden
e-mail: eckardt.treuter@ki.se

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histones or other factors. There is already evidence that alterations of expression or function of coregulators contributes to metabolic disease by propagating disease-specific epigenomes linked to the dysregulation of transcription and downstream pathways. In this chapter we review the current progress made in understanding the role of coregulators in metabolic pathways, with a particular emphasis on their study in vivo and in the context of metabolic disease.

Keywords
Coregulators • Inflammation • Metabolic disease • Metabolism • Nuclear receptors

Abbreviations

aP2 (FABP4)	Fatty acid binding protein 4, adipocyte
ABCA1	ATP-binding cassette, subfamily A (ABC1), member 1
ABCB11	ATP-binding cassette, subfamily B (MDR/TAP), member 11
ABCG1	ATP-binding cassette, subfamily G (WHITE), member 1
AMPK	AMP-activated kinase
AP-1	Activator protein 1
APR	Acute phase response
ATGL	Adipose triglyceride lipase
BAT	Brown adipose tissue
cAMP	Cyclic adenosine monophosphate
C/EBPα	CCAAT/enhancer binding protein (C/EBP), alpha
CACT	Solute carrier family 25 (mitochondrial carnitine-acylcarnitine translocase), member 20
CARM1/PRMT4	Coactivator-associated arginine methyltransferase 1
CARTPT	CART prepropeptide
CBP/p300	CREB binding protein
CDK	Cyclin-dependent kinase
CIDEA	Cell death-inducing DNA fragmentation factor, alpha subunit-like effector A
CLK2	CDC-like kinase 2
CREB	cAMP responsive element binding protein
CRTC	CREB regulated transcription coactivator
CYP27A1	Cytochrome P450, family 27, subfamily a, polypeptide 1
CYP3A11	Cytochrome P450, family 3, subfamily a, polypeptide 11
CYP7A1	Cytochrome P450, family 7, subfamily a, polypeptide 1
CYP7B1	Cytochrome P450, family 7, subfamily b, polypeptide 1
CYP8B1	Cytochrome P450, family 8, subfamily b, polypeptide 1
EHMT1	Euchromatic histone methyltransferase 1

ERK1/2	Mitogen-activated protein kinase 3
ERR	Estrogen receptor-related receptor
FGF	Fibroblast growth factor
FOXO1	Forkhead box O1
FXR	NR subfamily 1, group H, member 4
GABPα	GA repeat binding protein, alpha
GCN5/PCAF	Lysine acetyltransferase 2A
GLUT2	Solute carrier family 2 (facilitated glucose transporter)
GPS2	G-protein pathway suppressor 2
GYS1	Glycogen synthase 1
H3K18/27	Histone H3 Lysine 18/27
H3K9	Histone H3 Lysine 9
HDAC	Histone deacetylase
HFD	High-fat diet
HIF1α	Hypoxia-inducible factor 1, alpha subunit
HMGCS2	3-Hydroxy-3-methylglutaryl-coenzyme A synthase 2
HNF4α	Hepatic nuclear factor 4, alpha
HSL	Lipase, hormone sensitive
IGF	Insulin-like growth factor
IRS2	Insulin receptor substrate 2
JNK (MAPK8)	Mitogen-activated protein kinase 8
KCNQ1	Potassium voltage-gated channel, subfamily Q, member 1
KDM4A/JMJD2	Lysine-specific demethylase 4A
LRH-1	NR subfamily 5, group A, member 2
LXR	Liver X receptor
MED1	Mediator complex subunit 1
MTP	Microsomal triglyceride transfer protein
NCOR/NCOR1	NR corepressor 1
NRF-1	Nuclear respiratory factor 1
OTC	Ornithine transcarbamylase
p/CIP (NCOA3)	NR coactivator 3
PDK1	Pyruvate dehydrogenase kinase, isoenzyme 1
PGAM2	Phosphoglycerate mutase 2
PGC1	Peroxisome proliferator-activated receptor gamma, coactivator 1
PI3K	Phosphatidylinositol 3-kinase
POMC	Proopiomelanocorticoid
PPAR	Peroxisome proliferator-activated receptor
PRDM16	PR domain containing 16
PROX1	Prospero homeobox 1
PYGM	Muscle glycogen phosphorylase
RALBP1	ralA binding protein 1
RGS2	Regulator of G-protein signaling 2
RIP140/NRIP1	NR interacting protein 1
RNF8	Ring finger protein 8
RXR	Retinoid X receptor
SHP	Small heterodimer partner

SIRT	Sirtuin
SMRT/NCOR2	Silencing mediator of retinoid and thyroid receptors/NR corepressor 2
SRC1/NCOA1	Steroid receptor coactivator 1/NR coactivator 1
SREBP1	Sterol regulatory element binding transcription factor 1
T2D	Type 2 diabetes; T3, T4 = thyroid hormone 3, 4
TBL1	Transducin (beta)-like 1 X-linked
TBLR1	Transducin (beta)-like 1 X-linked receptor 1
TCF7L2	Transcription factor 7-like 2, T cell specific, HMG box
THRAP3	Thyroid hormone receptor-associated protein 3
TIF2/NCOA2	Transcription intermediary factor 2/NR coactivator 2
TLE3	Transducin-like enhancer of split 3
TNFα	Tumor necrosis factor
TR	Thyroid receptor
TRAF2	TNF receptor-associated factor 2
TZD	Thiazolidinedione
UBC13	Ubiquitin-conjugating enzyme E2N
UCP	Uncoupling protein (mitochondrial, proton carrier)
WAT	White adipose tissue
WD	Western diet

1 Introduction: Discovery and Classification of Coregulators

Since their discovery more than three decades ago, members of the nuclear receptor (NR) family have emerged as principal sensor, signal transducers, and transcriptional regulators of metabolic pathways. Their place among the more than 1,500 human transcription factors is unique because NR activity can directly be regulated by small molecule compounds (endocrine hormones, lipid metabolites, xenobiotics, pharmaceutics), which bind to them as ligands. Efforts to characterize the principal components of NR signaling led in 1994 to the identification of the first NR-associated proteins, many of which are today established NR coregulators (for the purpose of our review just termed *coregulators*). Over the past two decades, a large number of coregulators have been identified, currently estimated to include more than 200 different proteins (Rosenfeld et al. 2006; McKenna and O'Malley 2010; Dasgupta et al. 2014). Two complementary experimental approaches turned out to be essential for the identification of coregulators, first the genetic two-hybrid protein-protein interaction screening and second, though in fewer cases, the biochemical purification and mass spectrometry analysis of multi-protein complexes. While two-hybrid screenings (Vidal and Fields 2014), today also commercially available, remain a simple and effective method of choice to identify coregulators,

advances in the sensitivity and capacity of mass spectrometry methods have already yielded exciting snapshots of cell-type- or ligand-selective coregulator complexes at the proteome-wide scale, referred to as the "coregulator complexome" or simply "coregulatorsome" (Malovannaya et al. 2011; O'Malley et al. 2012). Without doubt, the future integration of genomic, proteomic, and metabolomic high-throughput data promises to reveal a substantially improved understanding of metabolism from individual pathways to cells, tissues, and ultimately to organisms.

NR coregulators are commonly categorized into coactivators or corepressors that facilitate NR-dependent transcriptional activation or repression, respectively. Although coregulators do not require direct DNA binding to execute their function, many have a high affinity to chromatin making the distinction less useful in vivo. Due to the growing number of coregulators identified, and the many distinct mechanisms of action characterized, today's perception of the coregulator concept has to become probably broader. Thus, NR coregulators can be best described as proteins that associate (directly or indirectly, e.g., within a multi-protein complex) with NRs and regulate their activity (which is mainly but not exclusively transcriptional). This broader view takes into account that some crucial aspects of NR activity control include also non-genomic mechanisms *off* chromatin, for example, the regulation of NR protein stability or of the nuclear/cytoplasmic distribution. Otherwise, since most coregulators are structurally and functionally diverse proteins, no unifying classification of these proteins is possible. In line with that, it is likely and already shown in many cases that NR coregulators are seldom specific to one or a few NRs but even target other classes of transcription factors. This at first sight promiscuous feature turns out to have important physiological consequences, as exemplified by the role of coregulators in transcriptional crosstalk between NRs and other transcription factors. Table 1 summarizes key members and features of today's established NR coregulators, with a particular emphasis on those that already are known for their involvement in metabolism.

Despite substantial progress in characterizing individual coregulators, we are just beginning to understand the multifaceted control of transcription and metabolism by coregulators in vivo, in particular in the context of metabolic disease. Unlike in the cancer field, mutations in genes encoding coregulators have not yet been linked to metabolic disease states. However, evidence is emerging that alterations in function or expression of coregulators can cause metabolic disease by propagating disease-specific epigenomes linked to dysregulation of transcription and downstream pathways. Coregulators are regulated by metabolic signals but also mediate metabolic signaling, thus making them efficient metabolic sensors and effectors at the same time. They act in different ways to modulate NR functions. Besides the direct interaction, post-translational modifications, both at the chromatin and NR level, appear to be their most important modulating tools. Moreover, coregulators influence NRs by altering their stability and intracellular localization. All of these influences lead to the proper response of the whole body to metabolic changes that appear either physiologically or under different stresses.

Table 1 Nuclear receptor coregulators and key functions in relation to metabolism

Coregulator category	Gene symbol[a]	Official full name	Aliases[b]	Established molecular functions	Suspected functions in metabolism and Metabolic disease[c]	Genetically engineered mouse models[d]
Prototypical CoA (histone acetyltransferase complex)	NCOA1	NR coactivator 1	SRC1	– Association with NRs via NR-Box LxxLL motifs, tightly associated with CBP/p300 and CARM1/PRMTs – Regulation by signal-dependent PTMs – NR-independent functions, e.g., growth factor pathways linked to cancer – GRIP1: specific role in anti-inflammatory transpression by the glucocortoicoid receptor GR	– Implicated in CACT deficiency syndrome	SRC1 Liver KO (Qi et al. 1999; Louet et al. 2010)
	NCOA2	NR coactivator 2	SRC2, TIF2, GRIP1			SRC2 Liver KO (Chopra et al. 2008, 2011)
	NCOA3	NR coactivator 3	SRC3, AIB1, ACTR			
Mediator complex	MED1	Mediator Subunit 1	TRAP220	– Association with NRs via LxxLL motifs, established interaction with POL2 and the transcription initiation complex	– Required for adipogenesis by cooperating with PPARγ and C/EBPs	Liver KO (Bai et al. 2011)
						Muscle KO (Chen et al. 2010)
Histone acetyltransferase	CREBBP P300	CREB Binding Protein	CBP, KAT3A	– Association with NRs via LxxLL motifs and/or via SRCs/MED1, tightly link to the Mediator complex and RNAP2 – Acetyltransferase towards histones (e.g., H3K27Ac) and NRs, epigenetic enhancer mark	– No specific metabolic or inflammatory functions have been characterized	–
Histone methyltransferase	CARM1	CoA-associated Arginine Methylase	PRMT4, PRMT5	– Association with NRs directly and via SRCs – Arginine methyltransferase towards histones and NRs	– Fundamental for glycogen metabolism and dysregulated in glycogen storage diseases	–

Inducible, tissue-specific CoA	PPARGC1A	PPAR Gamma Coactivator 1α	PGC-1α	– Signal-inducible CoA, association with NRs via LxxLL-like motifs, particular affinity for PPARγ and ERRs, association with NRF1 to regulate mitochondrial gene expression and biogenesis	– Induced by multiple signals in metabolic tissues, such as cAMP (liver), cold (BAT), exercise (muscle) – Key regulator of metabolic NR pathways and mitochondrial biogenesis in many cell types – Involved in adipogenesis, WAT browing, BAT thermogenesis, crosstalk with inflammation – Gene variants associated with hypertension and T2D	Full KO (Lin et al. 2004) MHC – PGC-1α TG (Lehman et al. 2000) cs-tet-on – PGC-1α TG (Russell et al. 2004)
	PPARGC1B	PPAR Gamma Coactivator 1β	PGC-1β	– Stimulation of the activity of ERα, NRF1, GR	– Downregulated in prediabetic and T2D patients – Allelic variations increase the risk of the development of obesity	Full KO (Lelliott et al. 2006)
	PRDM16	PR domain containing 16	CMD1LL, LVNC8, MEL1, PFM13	– Zn-finger transcription factor, chromatin modifier, coregulator that cooperates with PPARγ, C/EBPs and MED1 in adipocytes	– Master coregulator for the development of BAT and WAT browning – Human SNPs associated with higher lean body mass	aP2-PRDM16 KO, TG (Seale et al. 2007, 2011)
Prototypical CoR (HDAC3 complex)	GPS2	G-protein pathway suppressor 2	AMF1	– Association with NRs directly and/or via NCOR/SMRT Gene-selective CoR, atypical CoA function that regulates chromatin-binding of metabolic NTs (LXRs, PPARs) Mediator of NR transrepression, which involves binding to SUMOylated NRs	– Key role in NR metaflammation: crosstalk between metabolism and inflammation, in hepatocytes, adipocytes and macrophages – Positive regulator of PPARγ-dependent lipolysis in adipocytes – CoR of inflammatory gene expression in many cell types (hepatocytes – NCOR, adipocytes – with SMRT)	Adipose tissue TG, KD (Cardamone et al. 2012; Toubal et al. 2013)

(continued)

Table 1 (continued)

Coregulator category	Gene symbol[a]	Official full name	Aliases[b]	Established molecular functions	Suspected functions in metabolism and Metabolic disease[c]	Genetically engineered mouse models[d]
	NCOR1	NR corepressor 1	N-CoR	– Association with NRs via CoR-NR-type peptide motifs, docking sites for many other TFs and coregulators, signal integrators via PTMs	– Repressor of NR metabolic pathways – Unexpected pro-inflammatory action: repression of PPARγ phosphorylation by CDK5 in adipocytes; repression of LXR-mediated synthesis of anti-inflammatory ω3 fatty acid in macrophages – Linked to oxidative phosphorylation in muscle – Cooperation with HDAC3 in hepatic circadian gene expression	Liver KI (NCOR-binding deficient mutant) (Astapova et al. 2014) Liver KO (Sun et al. 2013)
						Monocyte/macrophage KO (Li et al. 2013)
						Adipose tissue KO (Li et al. 2011)
						Muscle KO (Yamamoto et al. 2011; Pérez-Schindler et al. 2012)
	NCOR2	NR corepressor 2	SMRT		– Adipocyte regulator of oxidative phosphorylation and inflammation (along with GPS2) – Several polymorphisms associated with T2D and adiponectin levels	Adipose tissue KI (NR-binding-deficient mutant) (Reilly et al. 2010; Nofsinger et al. 2008; Fang et al. 2011)
	TBL1XR1	Transducin β-like 1 X-linked receptor 1	TBLR1	– Indirect association with NRs via NCOR – SMRT – GPS2 – Histone-binding domains, implicated in docking of the CoR complex to chromatin – Function as CoA/CoR exchange factors for many NRs/TFs	– Key regulator of adipocyte lipolysis, increased in lipolytic conditions in WAT, correlating with serum FFAs – Cooperates with PPARs in liver, altered expression in human liver disease (steatosis, NAFLD)	Adipose tissue KO (Rohm et al. 2013)
	TBL1X	Transducin β-like 1 X-linked	TBL1			Liver KO (Kulozik et al. 2011)

	HDAC3	Histone deacetylase 3	HD3, RPD3, RPD3-2	– Class I histone deacetylase: major HDAC associated with NCOR/SMRT – HDAC activity-independent function in NR transcription	– Key regulator of hepatic circadian transcription (partly via REV-ERB) – Implicated in alternative macrophage M2 activation, atherogenesis, intestinal inflammatory disease (IBD, colitis)	Liver KO (Sun et al. 2012, 2013) Macrophage KO (Chen et al. 2012) Intestine KO (Alenghat et al. 2013) Muscle KO (Sun et al. 2011)
Atypical CoR	NRIP1	NR/receptor interacting protein 1	RIP140	– Association with NRs via LxxLL motifs, regulated by multiple PTMs – Context-dependent CoR/CoA (NR3C1, NR3C2, ESR1)	– Regulator of lipid and glucose metabolism in liver, adipocytes, muscle – RIP140 KO mice are lean with increased oxygen consumption, resistant to high-fat diet-induced obesity, improved insulin sensitivity – WAT KO induces browning – Skeletal muscle KO enriches oxidative fibers	Full KO (Leonardsson et al. 2004)
	PROX1	Prospero-related homeobox 1	–	– Corepressor of LRH-1/NRs in the liver	– Represses NR-dependent hepatic metabolism	–
	TLE3	Transducin-like enhancer of split 3	ESG, ESG3, GRG3, HsT18976	– Corepressor by blocking the interaction of Prdm16 with PPARγ	– Modulates brown fat programs and lipid storage in adipose tissue	aP2-TLE3 TG (Villanueva Claudio et al. 2011)

(continued)

Table 1 (continued)

Coregulator category	Gene symbol[a]	Official full name	Aliases[b]	Established molecular functions	Suspected functions in metabolism and Metabolic disease[c]	Genetically engineered mouse models[d]
Histone deacetylases (others than HDAC3)	HDAC 4	Histone deacetylase 4	HDAC-4	– Class II histone deacetylases: deacetylation of lysine residues of histone tails	– HDAC4 participates in second messenger cAMP signaling, which in macrophages controls pro-inflammatory activation	Full KO (HDAC5) (Chang et al. 2004)
	HDAC 5	Histone deacetylase 5	HD5			Heart KO (HDAC4) (Hohl et al. 2013)
	HDAC 7	Histone deacetylase 7	HD7A, HDAC7A			
	SIRT1	Sirtuin 1	SIR2L1	– Class III histone deacetylases: activity dependent on NAD^+/NADH ratio, relying upon NAD^+ hydrolysis	– SIRT6 decreases in fatty liver and failing hearts	Full KO (SIRT1-SIRT3) (Li et al. 2007; Hirschey et al. 2010; Shimazu et al. 2010)
	SIRT3	Sirtuin 3	SIR2L3			Liver KO (SIRT1-SIRT6) (Purushotham et al. 2009; Kim et al. 2010)
	SIRT5	Sirtuin 5	SIR2L5			Myeloid KO (SIRT1) (Schug et al. 2010)
	SIRT6	Sirtuin 6	SIR2L6			Brain/Neuron KO (SIRT1) (Cohen et al. 2009; Ramadori et al. 2010)
						Full TG (SIRT1) (Banks et al. 2008)
						Heart TG (SIRT6) (Sundaresan et al. 2012)
						Pancreas TG (SIRT1) (Moynihan et al. 2005)
						Brain/Adipose tissue TG (SIRT1) (Bordone et al. 2007)

Atypical orphan NRs that function as CoR	NR0B2	Short heterodimer partner	SHP	– Inducible CoR for metabolic NRs, association with NRs via LxxLL motifs, high affinity for NR5A2 (LRH-1), NR5A1 (SF-1) – Regulated by mono-ubiquitination	– Feedback control of hepatic bile acid synthesis, inducible by oxysterols, bile acids, cytokines – Mediator of crosstalk with inflammation	Full KO (Kerr et al. 2002)
	NR0B1	Nuclear receptor subfamily 0, group B, member 1	DAX-1		– Feedback control of steroidogenesis – Mutated in humans with DSS/AHC syndrome	–

BAT brown adipose tissue, *CACT* carnitine-acylcarnitine translocase, *CoA* coactivator, *CoR* corepressor, *DIO* diet-induced obesity (in mice), *DSS/AHC* dosage sensitive sex reversal/adrenal hypoplasia congenita, *FFA* free fatty acid, *HAT* histone acetyltransferase, *HDAC* histone deacetylase, *IBD* inflammatory bowel disease, *KO* knockout (Cre-mediated), *KD* knockdown (RNAi-mediated), *KI* knock-in (mutants), *NAFLD* nonalcoholic fatty liver disease, *NR* nuclear receptor, *PTM* post-translational modification, *SNP* single nucleotide polymorphism, *T2D* type 2 diabetes, *TF* transcription factor, *TG* transgenic overexpression (WT, mutants), *WAT* white adipose tissue

[a]NCBI Gene Symbol: human reference genes (RefSeq gene ID); mostly corresponds to the common protein name

[b]Aliases: alternative names, also known as, often linked to the history of initial discovery, e.g., *SRC1* steroid receptor coactivator 1

[c]Functions in development or cancer are not listed here

[d]Knockout (KO), knock-in (KI), transgenic overexpression (TG): the examples refer to tissue-specific models that yielded a metabolic phenotype; many coregulator KO mice have early developmental defects or are related to cancer and thus are not listed here

2 Molecular Mechanisms of Coregulator Action and Regulation

2.1 Coregulators: Key Components of Genomic NR Signaling

Coregulators (chromatin modifiers, coactivators, corepressors, etc.) are crucial components of NR signaling that include NRs itself, ligands (hormones, metabolites, synthetic compounds, endocrine disrupters), post-translational modifications (PTMs, e.g., phosphorylation, SUMOylation), cistromes (genomic NR-binding sites), transcriptomes (NR-regulated genes), and epigenomes (chromatin/DNA and histone modifications) (Fig. 1). Due to the presence or absence of individual key components, many NR signaling pathways are highly cell type specific, and some differ significantly between species such as rodents and humans.

In vitro evidence including three-dimensional structure data suggests that coregulators recognize distinct ligand-dependent NR conformations and establish interactions with the transcriptional machinery and chromatin at regulatory regions

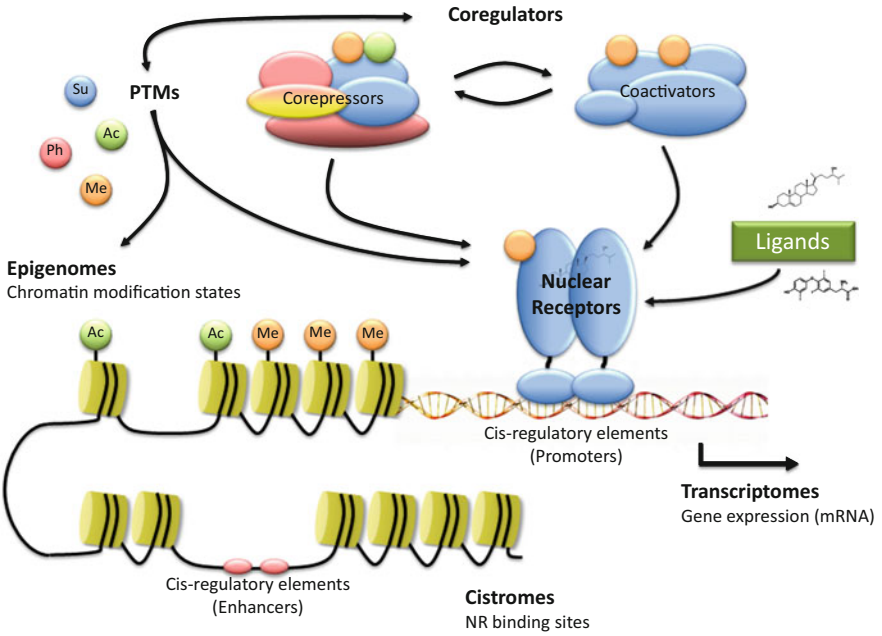

Fig. 1 Coregulators are molecular key components of NR signaling. NR signaling has to be understood as the complex interplay of various key components which determine genomic responses linked to gene expression and physiological outcomes in a highly cell-type-selective manner. Ligands, PTMs, and coregulators are major regulatory components that directly control NR activity, while cistromes and epigenomes specify the genome-wide target gene range (transcriptomes). Coregulators often function as "writers," "erasers," or "readers" of specific PTMs towards NRs and chromatin, and some of these activities are uniquely responsible to trigger metabolic and (anti-)inflammatory NR pathways. *PTMs*, post-translational modifications, such as Su (SUMOylation), Ac (acetylation), Me (methylation), and Ph (phosphorylation)

(promoters, enhancers) of target genes, thereby acting at the genomic level. Primary docking site for many coregulators is the conserved NR ligand-binding domain (LBD), a multifunctional domain responsible for ligand-binding, dimerization, and coregulator recruitment. Structure determination revealed a conserved NR LBD fold consisting of 12 α-helices. Helices 3–5 and 12 form the common coactivator/LxxLL/NR-box-interaction surface called AF-2, which usually is exposed upon binding of activating ligands (agonists) but blocked by inhibitory ligands (antagonists). Thereby, they function either as coactivators, by recognizing the active NR state and promoting transcription, or as corepressors, by recognizing the inactive NR state and repressing NR-dependent transcription.

Coregulators often function in larger multi-protein complexes to establish cell-type- and ligand-dependent epigenomes by "writing" or "reading" reversible epigenetic chromatin modifications linked to transcription (e.g., acetylation or methylation of histone tails). Individual coregulators have been identified as modulators of different post-translational modifications (PTMs) of chromatin, in particular of histone tails. Some of these histone PTMs have emerged as powerful analytic marks to characterize the epigenomic chromatin landscapes including promoters, enhancers, transcriptional activity states, and alterations thereof in different cellular and signaling contexts.

As a first example, GCN5/PCAF and CBP/p300 enhance, respectively, H3K9 and H3K18/27 acetylation, and their deletion in cells reduces their specific effects. The action of CBP/p300 correlates well with the activation of NR target genes and with the propagation of the H3K27 promoter/enhancer mark, while in the case of GCN5/PCAF this seems to be dispensable (Jin et al. 2011). As a second example, while the general function of HDACs as histone deacetylases is well established, the role of HDAC activity in NR repression/deactivation of target genes remains uncertain. In fact, the enzymatic activity of HDAC3 seems to be dispensable for NR repression and circadian gene expression in the liver, while the integration of HDAC3 into the NCOR complex was an absolute requirement (Sun et al. 2013). This example suggests that certain chromatin modifiers have evolved to accommodate more specialized coregulator functions, some of which do not require the original enzymatic activity towards histones. Overall, with the recent progress of second-generation sequencing approaches including ChIP-Sequencing (Siersbæk et al. 2012), the genome-wide analysis of key histone marks along with NRs, other transcription factors, and coregulators rose as an important tool for the further understanding of the cell-type-specific changes that occur in the genome as both cause and consequence of metabolic signaling.

2.2 Exception from the Rule: Non-genomic Action of Coregulators

Certain coregulators are multitasking by carrying out additional functions *off* chromatin or even out of the nucleus, leading to the contribution of non-genomic actions (Fig. 2). For example, HDACs can be found in the cytoplasm where they control cell division and modify cytoplasmic substrates (Luan et al. 2014).

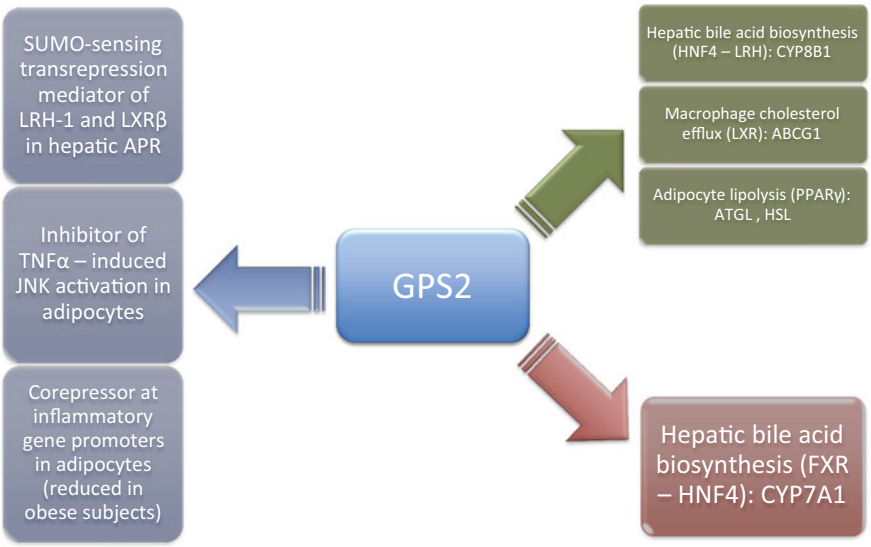

Fig. 2 One coregulator, multiple functions: GPS2. Depending on specific metabolic pathways and cellular and (epi-)genomic context, many coregulators show the ability of both positively or negatively modulating gene transcription. GPS2, a subunit of the HDAC3 corepressor complex, has been characterized as an atypical coactivator in bile acid biosynthesis, cholesterol efflux from macrophages, and lipogenesis in adipocytes (*green*). GPS2 acts as a corepressor in hepatic bile acid biosynthesis (*red*) and anti-inflammatory pathways in liver and adipose tissue (*blue*)

Intriguing reports suggest that corepressors such as SHP and GPS2 modulate inflammatory NFkB activation pathways in the cytoplasm (Yuk et al. 2011; Cardamone et al. 2012). Cytoplasmic RIP140 regulates a variety of cellular pathways linked to metabolism, including lipolysis and adipocyte GLUT4 trafficking (Ho et al. 2009). Finally, there are many NR-binding proteins that modify NRs by PTMs (thus referred to as PTM modifiers). Some of these actions may occur in the cytoplasm and/or result in nuclear export or cytoplasmic retention. However, to which extent these different non-genomic actions contribute to the identified metabolic actions of coregulators is in the most cases currently unclear.

2.3 Beyond Histone Modifications: Coregulators are PTM Modifiers of Many Targets

Many coregulators are known as PTM modifiers of the histone tails, which is linked to their fundamental role in transcriptional and epigenomic reprogramming. More recently, evidence is emerging that many non-histone proteins, including NRs and

other transcription factors, can also become post-translationally modified by these coregulators. There are additional examples that coregulators themselves can be the targets for multiple PTMs, some of which may directly be regulated by metabolic signals and/or have direct consequences on regulated metabolic pathways. As a matter of concern it has to be considered that many PTMs are transient and dynamic in vivo, thus difficult to catch. Further, PTMs crosstalk and thereby influence each other at many substrates, making the study of metabolic cause and consequence quite challenging. As with histones, different PTMs may modify the same surface-exposed amino acid residue, often lysine residues, which are target for acetylation, ubiquitination, SUMOylation, and methylation.

Acetylation and Deacetylation Acetylation of non-histone substrates often inhibits transcriptional activity of proteins, while deacetylation has the opposite effect. The action of acetyltransferase GCN5 on PGC1α is one example of this mechanism, causing PGC1α to re-localize from promoter regions to nuclear foci (Lerin et al. 2006). GCN5 is itself phosphorylated and activated by insulin-CDK4, exerting its activity on PGC1α and suppressing hepatic glucose production (Lee et al. 2014). Similarly, CBP/p300 is implicated in the acetylation of a number of NRs. The deacetylases sirtuins (also referred to as class III HDACs) have been demonstrated to be key regulators of NR acetylation states. As an example, acetylation is fundamental for FXR stability, and it inhibits FXR heterodimerization with RXR and consequently affects its DNA binding and transcriptional activity. SIRT1 deacetylates FXR, and lack of hepatic SIRT1 results in increased FXR acetylation leading to metabolic dysfunction (Kemper et al. 2009). SIRT1 also deacetylates CREB, preventing its cAMP-dependent phosphorylation, leading to reduced expression of glucogenic genes and promoting hepatic lipid accumulation and secretion (Qiang et al. 2011). Moreover, SIRT1 interacts and deacetylates LXRs (at K432 in LXRα and K433 in LXRβ), positively regulating them and leading to their ubiquitination. Thus, lack of SIRT1 has profound effects also on lipid metabolism, reducing the expression of different LXR target genes, including ABCA1 (Li et al. 2007). Finally, SIRT1 deacetylates IRS-2 and upregulates the phosphorylation level of IRS-2 and ERK1/2 in striatum, altering the neurotransmitter signaling in striatum and the expression of endocrine hormones in hypothalamus and serum T3 and T4 levels (Wu et al. 2011).

SUMOylation SUMOylation plays important modulating roles in different metabolic and inflammatory processes (Treuter and Venteclef 2011). Cholestasis is influenced by the SUMOylation status of PPARα; SUMOylated PPARα interacts with GABPα at the CYP7B1 promoter, resulting in repression of the latter and leading to protection against estrogen-induced intrahepatic cholestasis in female mice (Leuenberger et al. 2009). Using an in vivo knock-in mouse model, it was shown that non-SUMOylated LRH-1 (mutated at K289R) is unable to interact with the corepressor PROX1, leading to increased reverse cholesterol transport and

atheroprotection (Stein et al. 2014). A number of studies indicate that anti-inflammatory NR transrepression is dependent on SUMOylation and coregulator action. The first of these studies has shown that modification of the fatty acid receptor PPARγ by SUMO1 causes docking of the SUMOylated receptor to the NCOR-HDAC3 corepressor complex at inflammatory genes and prevents the removal of this complex upon pro-inflammatory signaling, maintaining target gene expression in the repressed state (Pascual et al. 2005). A follow-up study has demonstrated that SUMOylated (SUMO 2/3) LXRs, important receptors for cholesterol metabolites, repress a distinct subset of pro-inflammatory genes via related mechanisms (Ghisletti et al. 2007). While both of these studies have addressed NR transrepression in mouse macrophages, related mechanisms may occur in many different cell types. In fact, data from mouse and human hepatocytes suggest SUMOylated LRH-1 (a candidate phospholipid receptor) and LXRβ to exert the same inhibitory action at hepatic acute phase response genes, thereby acting in an anti-inflammatory way in the liver. This study has further identified the coregulator GPS2 (subunit of the NCOR-HDAC3 corepressor complex) as a likely SUMO sensor and essential NR transrepression mediator, at least in hepatocytes (Venteclef et al. 2010) (Fig. 2). The modulatory role of SUMOylation and coregulators in inflammatory pathways has been further supported by a recent study on the bile acid receptor FXR, identifying a high-fat-diet (HFD)-dependent acetylation-SUMO switch in regulating FXR activities in the liver (Kim et al. 2015).

Phosphorylation Given the common and fundamental nature of phosphorylation, many NRs are modified and modulated by this modification, likely responding to metabolic alterations. However, whether and how coregulators communicate with the many kinases and phosphatases involved is largely unknown. As an exception, it has been shown that phosphorylation of PPARγ (Ser 273) by CDK5 in adipose tissue in obesity is modified by NCOR, thereby affecting fundamental target genes such as adiponectin. Intriguingly, this NR-PTM-coregulator network is inhibited as part of the anti-diabetic action of PPARγ ligands (Choi et al. 2010; Li et al. 2011).

Nuclear receptors (**NRs**) are ligand-regulated transcription factors that coordinate selective gene expression programs governing physiology and disease by directly binding to DNA. In humans, most of the 48 distinct receptors are differentially expressed in metabolic tissues and cell types. By acting as sensors for hormones, lipid metabolites, xenobiotics, and pharmaceuticals, NRs modulate metabolic and closely related inflammatory pathways in response to nutrients, environmental cues, disease-associated alterations, and therapeutic interventions. NRs share a conserved domain structure with a unique ligand-binding domain only found in this protein family. NRs execute three key functions necessary for genomic signaling: (1) binding to DNA response elements in the regulatory regions (promoters, enhancers) of target genes, (2) binding to small molecule ligands, and (3) recruitment of coregulators. They regulate transcription by a variety of distinct mechanisms *in cis* (e.g., ligand-dependent activation or repression) and *in trans* (e.g., anti-inflammatory transrepression).

NR coregulators are usually defined as transcription factors which do not directly bind to DNA but associate with DNA-bound NRs (and other transcription factors), to regulate their transcriptional activity. More broadly, any NR-associated protein that affects NR activity could function as a coregulator. In humans, the number of coregulators has been estimated to include at least 200 up to thousands of proteins, depending on the definition and classification applied. Coregulators are highly diversified proteins that often function in multi-protein complexes with chromatin-modifying activities linked to gene regulation, such as (de-)acetylation or (de-)methylation. Many coregulators contain conserved NR-binding peptide motifs, referred to as NR-box/LxxLL motifs and CoRNR-box, suggesting co-evolution and a specific role of these coregulators in NR signaling.

2.4 Coregulators: Sensors of Metabolism

Evidently, coregulator action itself is regulated by metabolic signals. Two major mechanisms for how metabolic signals can impact on coregulator action emerged: the regulation at the post-translational level, i.e., by PTMs (coregulator protein function), and the regulation at the transcriptional level, i.e., by modulating gene expression (coregulator mRNA levels) (Fig. 3).

Post-translational Regulation of Coregulators It can be assumed that every coregulator can become a target for multiple PTMs which modulate fate and function of the proteins in a very signal- and cell-type-dependent manner. The following examples shall illustrate how metabolic signals influence coregulators via PTMs.

1. *Phosphorylation*: SRC2 is phosphorylated and activated by AMPK, enhancing fat absorption depending on the energetic state (Chopra et al. 2011). Furthermore, HDACs 4/5/7 are found to be phosphorylated and exported to the

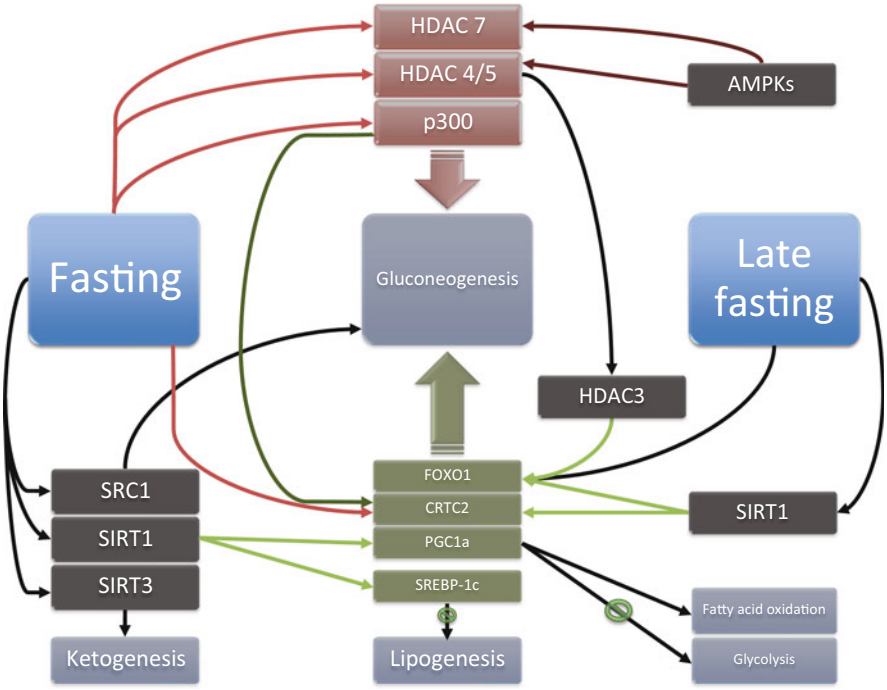

Fig. 3 Coregulators and PTMs during fasting. Coregulators play a pivotal role in the regulation of the different metabolic pathways occurring during the early and late phase of fasting. They can be directly activated by fasting (e.g., SRC1, SIRT1, SIRT3); they can be post-translationally modified by fasting (e.g., p300, HDAC 4/5, HDAC7, FOXO1, CRTC2) or by other coregulators (e.g., CRTC2, PGC1α, SREBP-1c, FOXO1), inducing different metabolic outcomes. *Dark red*, phosphorylation; *light red*, dephosphorylation; *dark green*, acetylation; *light green*, deacetylation

cytoplasm by AMPKs. In response to glucagon in fasting, these HDACs are dephosphorylated and recruited to the nucleus to associate with the promoters of gluconeogenic enzyme genes (Mihaylova Maria et al. 2011). Moreover, phosphorylated PGC1α by CLK2 is not able to interact with the mediator subunit MED1, leading to suppression of PPARα target gene activation in the liver, in turn inhibiting fatty acid oxidation and ketogenesis (Tabata et al. 2014).

2. *Acetylation*: PGC1α is acetylated on at least 10 lysine residues by GCN5 and can be deacetylated by SIRT1 (Rodgers et al. 2005). Acetylation of PGC1α represses its activity and has profound effects on target transcription factors such as NRF-1, and the NRs ERRα and HNF4α (Kelly et al. 2009). SRC3 has been found to be another factor that promotes this acetylation through the action of GCN5 (Coste et al. 2008). At the same time, SIRT6 is able to deacetylate and activate GCN5, which in turn exerts its acetyltransferase activity on PGC1α. Thus, SIRT6 induces PGC1α acetylation, suppressing hepatic glucose production, and this acetylation is dependent on SIRT6 interaction and enhancement of GCN5 activity (Dominy et al. 2012).

3. *Ubiquitination*: SHP is an atypical orphan NR that cannot bind DNA and lacks ligands. It functions as a corepressor of NRs involved in the feedback regulation of cholesterol to bile acid metabolism in the liver. Intriguingly, SHP is regulated by bile acids at both transcriptional and post-translational level. Bile acids can either upregulate SHP expression via FXR and via inhibition of the ubiquitin-proteasomal pathway or inhibit SHP degradation in an ERK-dependent manner, along with FGF15/19 (Miao et al. 2009).

Transcriptional Regulation of Coregulator Expression Second, the expression level of coregulator-encoding genes/mRNAs can be subject to metabolically related up- or downregulation. Notably, NRs and their metabolic ligands can regulate the expression of coregulators, as explained above for the bile acid regulation of SHP in the liver. Another example was identified in human adipocytes, where anti-diabetic TZDs via PPARγ regulate the expression of GPS2, along with epigenetic alterations that occur in the adipose tissue of obese subjects (Toubal et al. 2013). Yet another generally important mechanism is linked to the nutritional state that influences metabolism at different levels. For example, SRC1 is induced upon fasting and coordinates the gluconeogenic pathway (Louet et al. 2010).

Direct Metabolite Sensing by Coregulators A third mechanism has remained subject to controversy till now, namely, whether coregulators can also directly be regulated by small molecule ligands including metabolites. Recent findings suggest several HDAC complexes to require phospho-inositol Ins(1,4,5,6)P_4 as intramolecular pocket ligand for stability and function and would therefore respond to intracellular changes of phospho-inositol levels (Millard Christopher et al. 2013). Additionally, the role of sirtuins, class III HDACs, as intracellular NAD sensors is well established (Herranz and Serrano 2010; Houtkooper et al. 2012; Fiorino et al. 2014). Although these examples suggest an evolutionary conserved mechanism for sensing and maintaining intracellular energy homeostasis by essential chromatin-modifying complexes, it may not apply to the majority of coregulators (Fig. 3).

Coregulators Act in Concert to Sense and Regulate Metabolism The above example illustrates yet another important aspect of coregulator action, namely, that coregulators form complexes and regulatory networks to influence each other in a synergistic or antagonistic manner. Thus, different physiological states that alter coregulator expression will consequently also alter the coregulator equilibrium leading to different metabolic responses. The prototypical NR corepressor complex, consisting of the core subunits NCOR/SMRT, GPS2, TBL1/TBLR1, and HDAC3, is a good example for the interdependence of complex subunits with implications for metabolic diseases. It has recently been found that alterations in the expression of GPS2 in adipose tissue, in the context of obesity, or of TBL1 in liver, in the context of fatty liver disease, lead to dysfunction of the entire complex and downstream pathways (Kulozik et al. 2011; Toubal et al. 2013) (Fig. 2). Other subunits like SMRT and NCOR appear to be fundamental for HDAC3 function

within the complex in vivo, since HDAC activity is undetectable in mice bearing point mutations in the deacetylase-activating domain of NCOR/SMRT (You et al. 2013). Coregulators that do not function in a multi-protein complex also influence each other, as illustrated by the action of TLE3 and PRDM16 on PPARγ in adipose tissue. Both coregulators occupy exclusively different target promoters, and TLE3 is able to competitively counteract the interaction between PRMD16 and PPARγ, leading to different metabolic outcomes (Villanueva et al. 2013).

2.5 Additional Layers of Control: Aging and Circadian Rhythm

Aging is considered a metabolic risk factor due to the accumulation of transcriptional and epigenomic alterations that along with intracellular signaling defects dramatically disturb metabolic homeostasis at different levels (de Cabo et al. 2014). It is, for example, known that SMRT expression and repression of PPAR target genes in different tissues increases with age. This effect can be mimicked using a SMRT mutant defective for NR binding, in which the effects of SMRT on PPARs cause premature aging, mitochondrial dysfunction, and antioxidant gene expression (Reilly et al. 2010). On the other hand, aging induces derepression of PPARα, PPARγ, and LXRα in mouse liver, enhancing lipid synthesis and storage and leading to steatosis, and these changes are mostly driven by reduced expression of HDAC3 (Bochkis Irina et al. 2014).

The circadian clock influences metabolic pathways by many different mechanisms, and the expression of many NRs follows a circadian rhythm (Zhao et al. 2014). Intriguingly, the "clock gene"-encoded REV-ERBα is a member of the NR family and an established regulator of circadian gene expression in the liver. In search for underlying mechanisms, it was found that the periodic recruitment of HDAC3 to REV-ERBα at chromatin directs a circadian rhythm of histone (de-) acetylation and gene expression required for hepatic lipid homeostasis. In support of these genomic data, it was demonstrated that removal of HDAC3 from mouse liver causes steatosis (Feng et al. 2011). Additionally, genetic disruption of the HDAC3 interaction with NCOR in mice resulted in an aberrant regulation of clock genes, altering the oscillatory patterns of several metabolic genes and resulting in leaner and more insulin-sensitive phenotype (Alenghat et al. 2008).

3 Tissue-Specific Coregulator Functions in Metabolism

Coregulator functions have been dissected in the past years in the different metabolic districts. We will focus on coregulator pathways in liver, adipose tissue, macrophages, and skeletal muscle, which currently have been best characterized. However, studies should expand to other tissues such as intestine, pancreas, kidney, lung, and bone to gain further insights in coregulator functions in metabolic pathways.

3.1 Liver: From Energy Homeostasis to Insulin Resistance and Steatosis

As the factory in which fundamental metabolic pathways occur, the liver is a primary tissue for coregulator action and an important player in the development of metabolic disease. It was especially the development of conventional knockout (KO) and conditional liver-specific knockout (LKO) mouse models that have advanced our current understanding of tissue-specific coregulator function in metabolism.

Coactivators The members of SRC family seem to play important roles in the regulation of hepatic metabolism. SRC1 is a fundamental player in hepatic glucose homeostasis, modulating the expression and activity of C/EBPα for pyruvate carboxylase transactivation; thus lack of hepatic SRC1 leads to hypoglycemia (Louet et al. 2010). SRC2 LKO mice resemble the human Von Gierke's disease phenotype due to a decrease in RORα-dependent G6Pase and dysregulation in hepatic glucose release (Chopra et al. 2008). SRC2 has acquired different roles in metabolism than SRC1 as it promotes absorption of dietary fat from the gut. SRC2 LKO mice have intestinal fat malabsorption and less fat entry into the blood stream (Chopra et al. 2011). Even more the more promiscuous histone acetyltransferases CBP/p300 and GCN5/PCAF have quite distinct functions in the liver. CBP LKO mice are lean and more insulin sensitive than wild-type mice, although blood glucose levels remained unchanged (Bedford David et al. 2011). GCN5/PCAF LKO mice show enhanced PGC-1α activity, resulting in an increase in blood glucose levels and hepatic glucose production (gluconeogenesis), while adenoviral expression of GCN5/PCAF in obese mouse livers showed the opposite phenotype (Sun et al. 2014). Quite intriguingly, LKO mice that lacked the mediator subunit MED1/TRAP220 were protected from developing fatty liver even upon feed with a 60% HFD for up to 4 months (Bai et al. 2011). Moreover, PGC1β LKO leads to reduced SREBP-1 expression and downstream lipogenic genes, improving the metabolic phenotype induced by HFD and reversing hepatic insulin resistance induced by fructose (Nagai et al. 2009). All these examples suggest that a balanced coactivator action in the liver is crucial for metabolic health and that alterations in either direction (increase, decrease) can lead to metabolic disturbances and disease.

Corepressors Liver-specific functions have been particularly elucidated in case of the subunits of the HDAC3 corepressor complex, with some examples already discussed above (see Sect. 2.4). Additionally, GPS2 has been identified as a differential coregulator of CYP7A1 and CYP8B1 expression, directly interacting with SHP, LRH-1, HNF4α, and FXR and causing alternative coregulator recruitment that leads to the modulation of hepatic bile acid biosynthesis (Sanyal et al. 2007) (Fig. 2). Further, NCOR interacts with TR, modulating cholesterol metabolism and clearance in the liver, and mice expressing a mutant NR-binding-deficient NCOR show increased expression of TR- and LXR-regulated hepatic genes. In particular, the increased expression of TRβ1-regulated genes (Cyp27a1, Cyp3a11 and Abcb11) leads to less intestinal cholesterol absorption and changes in

bile salt pool composition (Astapova et al. 2014). It is noteworthy that NCOR LKO causes metabolic and transcriptional alterations resembling those of the HDAC3 LKO, demonstrating that the interaction between the corepressor complex subunits is fundamental to their action (Sun et al. 2013). Another intriguing example of how disturbance of the fine-tuning of physiological processes can cause dysfunctional liver metabolism is represented by SHP. KO mice display complex phenotypes, probably due to the fact that SHP targets many metabolic NRs. The mice display decreased lipogenesis and increased fatty acid oxidation and are protected from obesity and hepatic steatosis. The effects observed in the KO mice could be ascribed to both direct binding of metabolites to SHP or increased recruitment of SHP to the nutrient-activated NRs. SHP is not only an established feedback regulator of bile acid biosynthesis but also a regulator of diet-induced PPARα transactivation. When challenged with a western diet, SHP KO mice are leaner and accumulate less triglycerides in the liver. At the same time the redistribution of fat to the liver leads to the development of hepatic insulin resistance and to alterations in peripheral tissue lipid uptake, along with pancreatic islet dysfunction (Park et al. 2011). Hyperlipidemia can be induced also by dysregulation of the SHP circadian clock. CLOCK upregulates SHP, which in turn represses HNF4α/LRH1-dependent expression of microsomal triglyceride transfer protein (MTP). Evidently, dysregulation of this process can induce hyperlipidemia (Pan et al. 2010).

HDACs/Sirtuins HDAC3 LKO mice develop steatosis without any change in body weight and insulin sensitivity. Presumably, lack of HDAC3 enhances lipid synthesis and storage and reduces hepatic glucose production. Perilipin 2 has been identified as the key target gene in this process (Sun et al. 2012). The intracellular shuttling class II HDACs 4 and 5, after being imported to the nucleus during fasting conditions, recruit HDAC3 that deacetylates and activates FOXO family members. In HDAC4/5 LKO mice FOXO target genes are less expressed, lowering blood glucose and increasing glycogen storage (Mihaylova Maria et al. 2011).

Sirtuins, referred to as NAD-dependent class III HDACs, play a pivotal role in the modulation of hepatic homeostasis. SIRT1 is induced by pyruvate during fasting and then deacetylates PGC1α, inducing gluconeogenic genes and hepatic glucose output, thereby repressing glycolytic genes (Rodgers et al. 2005). The SIRT1 activation of PGC1α has profound effects also on lipid homeostasis by positively regulating PPARα. SIRT1 LKO mice show decreased fatty acid β-oxidation, and when challenged with a HFD, they develop steatosis and inflammation (Purushotham et al. 2009). These effects are evident also in SIRT1 heterozygous mice challenged with a 40% HFD: hepatomegaly, increased serum cytokine levels, obesity, and insulin resistance (Purushotham et al. 2012a). The overexpression of SIRT1 (SirBACO mice) leads to improved glucose homeostasis on an atherogenic diet but worsens the lipoprotein profile being the cause of large atherosclerotic lesions. This in part depends on SIRT1-dependent deacetylation of CREB, preventing its cAMP-dependent phosphorylation and leading to reduced expression of glucogenic genes, promoting hepatic lipid accumulation and secretion (Qiang et al. 2011). Cholesterol metabolism is also modulated by the action of SIRT1. During fasting SIRT1 deacetylates and inhibits SREBP-1c transactivation,

decreasing its stability and occupancy at lipogenic genes, while feeding can reverse this scenario (Ponugoti et al. 2010). SIRT1 also modulates bile acid homeostasis through deacetylation and activation of FXR. Consequently, SIRT1 deficiency leads to deleterious metabolic outcomes (Kemper et al. 2009), predisposing mice to development of cholesterol gallstones when challenged with a lithogenic diet (Purushotham et al. 2012b).

The role of SIRT3 in mitochondrial metabolism affects metabolic pathways in the liver. SIRT3 enhances ketone body production during fasting, deacetylating and activating HMGCS2 at lysines 310, 447, and 473, and mice lacking SIRT3 show decreased β-hydroxybutyrate levels during fasting (Shimazu et al. 2010). These mice show also higher levels of fatty acid oxidation intermediates and triglycerides, associated with decreased levels of fatty acid oxidation (Hirschey et al. 2010). The urea cycle is also affected: these mice fail to deacetylate and stimulate OTC in response to caloric restriction, and this leads to a failure in reducing orotic acid levels (Hallows et al. 2011). Finally, SIRT6 LKO mice display increased glycolysis, triglyceride synthesis, reduced β-oxidation, and fatty liver formation. This is due to the fact that SIRT6 negatively regulates these pathways by deacetylating H3K9 at the promoters of many genes involved in these processes (Kim et al. 2010).

3.2 Adipose Tissue: From Lipid Homeostasis to Inflammation and Obesity

Adipose tissue rose in the last years as a fundamental metabolic district with key roles in energy storage and release, endocrine regulation, and metabolically induced inflammation (metaflammation), all of them linked to obesity. Coregulators act in the different adipose depots to modulate gene expression linked to distinctive pathways, and different nutritional or inflammatory states seem to have a predominant role for coregulator activation in these tissues.

WAT Coactivators White adipose tissue (WAT) plasticity is fundamental for lipid storage. Adipocyte-specific knockout mice (AKO) removing TBLR1 have impaired cAMP signaling, leading to defects in fasting-induced lipid mobilization and, on HFD, glucose intolerance and insulin resistance (Rohm et al. 2013). PGC1α and PGC1β have prominent roles both in white and brown adipose tissue (BAT) functionality. PGC1β KO induces glucose disposal in the whole body, due to an increase in glucose uptake in WAT (Nagai et al. 2009). PGC1α is acetylated and thus inhibited by SRC3 (through GCN5); the action of SRC3 is caloric-dependent, enhanced by caloric excess (Coste et al. 2008). SRC3 KO mice show reduced body weight and adipose tissue mass, due to the lack of interaction of SRC3 with C/EBPα to control PPARγ2 expression in WAT (Louet et al. 2006).

WAT Corepressors Different protagonists play dominant roles in WAT. Mice in which SMRT is mutated to disrupt its interaction with NRs show altered insulin sensitivity and increased adiposity (Nofsinger et al. 2008). When challenged with

HFD, they have increased lipid accumulation, inflammation, and adipocyte hypertrophy in visceral WAT, BAT (along with reduced thermogenic capacity), and liver (triglycerides and cholesterol). These mice are refractory to treatments with antidiabetic TZDs and AICAR and show increased serum cholesterol and triglyceride levels, increased leptin, and decreased adiponectin (Fang et al. 2011). Surprising phenotypes were reported for NCOR AKO mice: increased obesity but improved glucose tolerance and insulin sensitivity in liver, muscle, and fat. Strikingly, inflammation and macrophage infiltration were decreased in WAT. This is probably due to a constitutively active PPARγ state, with reduced CDK5 mediated Ser-273 phosphorylation, in the NCOR AKO mice (Li et al. 2011). Independent studies in mice and human adipose tissue suggest that GPS2 has a dual function in affecting metabolic and inflammatory processes. In mouse WAT, GPS2 appears to be required for PPARγ function, likely through the inhibition of the ubiquitin ligase RNF8 and the stabilization of the H3K9 histone demethylase KDM4A/JMJD2 at the lipolytic enzymes ATGL and HSL, thus exerting a fundamental role in adipose tissue lipid mobilization (Cardamone et al. 2014). Intriguingly, AKO mice lacking TBLR1 (a subunit of the corepressor complex that directly interacts with GPS2) are similarly defective in fasting-induced lipid mobilization and show aggravated adiposity, glucose intolerance, and insulin resistance upon HDF. TBLR1 levels were increased under lipolytic conditions in mouse and human WAT (Rohm et al. 2013). Also, in human WAT, GPS2 has been characterized as an anti-inflammatory corepressor that along with SMRT is downregulated during obesity, thus suggesting a causative role in promoting adipose tissue inflammation (Toubal et al. 2013) (Fig. 2).

WAT HDACs and Sirtuins After overnight fasting, increasing levels of cAMP enhance the association between HDAC4/5 and GLUT4 promoter; the repression of GLUT4 correlates thus with insulin resistance in WAT (Weems et al. 2012). Besides, HFD induces the cleavage of SIRT1 in WAT by the inflammation-activated Caspase-1, and SIRT1 AKO mice show changes that highly overlap with the HFD challenge (Chalkiadaki and Guarente 2012).

Coregulators in Browning of WAT Another fundamental process that links WAT, BAT, and oxidative metabolism is the "browning" of WAT. The resulting "brite" or "beige" fat depots with BAT-like features (energy burning via UCP1) within WAT (energy storage) have been proposed as a therapeutic tool to reverse obesity complications such as to improve whole body insulin sensitivity. Different coregulators have been demonstrated to have a role in the modulation of this important process. RIP140 AKO mice have less weight gain and enhanced glucose tolerance and insulin sensitivity upon HFD; in vitro tests characterize RIP140 as a negative regulator of adipocyte oxidative metabolism and mitochondrial biogenesis (Powelka et al. 2006). The adipogenesis process seems not to be affected, while there is a great increase of UCP1 and different genes involved in energy dissipation (Leonardsson et al. 2004). Recent evidence demonstrates that RIP140 blocks the browning of WAT reducing the expression of brown fat genes and inhibiting a triacylglycerol futile cycle (Kiskinis et al. 2014). On the other hand, the browning

of WAT is promoted by SIRT1 through deacetylation of PPARγ on K268 and K293, which leads to the recruitment of PRDM16 on PPARγ and to the induction of BAT specific genes (Qiang et al. 2012). The recruitment of PRDM16 on PPARγ is in competition with TLE3 recruitment for the same site. TLE3 drives PPARγ function to activate lipid storage, and to represses thermogenic gene programs, resulting in impaired fatty acid oxidation and thermogenesis. TLE3 AKO mice thus show enhanced thermogenesis in WAT (Villanueva et al. 2013).

BAT Coregulators Coregulators are fundamental also for the development and the function of BAT. Despite that PRDM16 seems to be dispensable for embryonic BAT development, it is required in young mice for the action of the histone methyltransferase EHMT1 and the suppression of WAT specific genes (Harms Matthew et al. 2014). Moreover, SRC1 and SRC3 KO mice fail to induce PPARγ target genes involved in adipogenesis and mitochondrial uncoupling; the mice then undergo a developmental arrest in interscapular BAT and are defective in adaptive thermogenesis. Despite an increased food intake, these mice are lean and resistant to HFD-induced obesity, due to increased basal metabolic rates and physical activity (Wang et al. 2006).

3.3 Skeletal Muscle: Peripheral Insulin Sensitivity and Exercise

Skeletal muscle is another important metabolic district, which can help the whole body to switch metabolism depending on the energetic demand. Different coregulators have been shown to play fundamental roles in the modulation of skeletal muscle metabolic pathways.

Coactivators PGC1α and PGC1β are fundamental for muscle functionality; the double muscle-specific KO (MuKO) mice (PGC1α/β -/-) show a reduction in exercise performance due to a decreased oxidative capacity, increased depletion of glycogen storage, and mitochondrial dysfunction, surprisingly not accompanied by insulin resistance or glucose intolerance (Zechner et al. 2010). An intriguing finding was reported using a transgenic mouse model expressing the PGC-1α1 isoform in skeletal muscle. The study revealed a mechanism by which exercise changes PPARα/δ-dependent kynurenine metabolism and protects from stress-induced depression (Agudelo Leandro et al. 2014). Confirmatory human data suggest therapeutic avenues for the treatment of depression by targeting the PGC-1α1-PPAR axis in skeletal muscle. SRC3, as in adipose tissue, exert its acetylating action on PGC1α also in the skeletal muscle, thereby inhibiting its activity during caloric excess (Coste et al. 2008). CARM1/PRMT4 seems to be necessary for the modulation of glycogen metabolism in muscle (Wang et al. 2012). Different MuKO mice were developed to dissect the function of other coregulators in skeletal muscle. TIF2 MuKO mice are protected from decrease in muscle oxidative capacities in sedentariness and from obesity and T2D; in these mice SRC1 level increases antagonizing the expression of UCP3 (Duteil et al. 2010).

MED1 MuKO mice show a similar phenotype, with enhanced insulin sensitivity and glucose tolerance leading to resistance to HFD-induced obesity. Moreover, a fast-to-slow fiber switch occurs, with increased mitochondrial density and Ucp1 and Cidea expression, in white muscle fibers (Chen et al. 2010).

Corepressors RIP140 MuKO, heterozygous, and transgenic mice were generated. These models showed that low RIP140 levels are associated with the formation of oxidative fibers, promoting changes in the genes implicated in myofiber phenotype and metabolic function, upregulating fatty acid oxidation, oxidative phosphorylation, and mitochondrial biogenesis (Seth et al. 2007). NCOR MuKO leads to increase in muscle mass and mitochondrial number and activity, activating (de-repressing) MEF2, PPARβ/δ, and ERRs, finally enhancing oxidative metabolism and oxygen consumption (Yamamoto et al. 2011). It is worth noticing that the phenotypes of NCOR deletion and PGC1α overexpression highly overlap. PPARβ/δ and ERRα have been identified as common targets of these coregulators, with opposite effects on their transcriptional activity depending on the antagonizing actions of corepressor and coactivator (Pérez-Schindler et al. 2012).

HDACs and Sirtuins As in the adipose tissue, AMPK regulates HDAC5 phosphorylation also in the skeletal muscle, reducing its association with the GLUT4 promoter and thereby increasing Glut4 gene expression (McGee et al. 2008). SIRT1 acts in the muscle by deacetylating PGC1α and FOXO1 under the influence of AMPK during fasting or after exercise, thereby enhancing lipid metabolism to preserve glycogen storage and blood glucose levels (Cantó et al. 2010). Additionally, SIRT1 improves insulin sensitivity in skeletal muscle by repressing PTP1B (Sun et al. 2007). Finally, another sirtuin, SIRT3, is involved in a complex with FOXO3a and mitochondrial RNA polymerase to increase mitochondrial respiration during glucose restriction (Peserico et al. 2013).

3.4 Cardiovascular System

The profound effects that coregulators have in skeletal muscle can be also observed in the cardiovascular system (heart, smooth muscle cells). SRC3 and PGC1α exert fundamental roles in this district, the first one regulating CACT gene expression and thus modulating long chain fatty acid metabolism (York et al. 2012) and the second one inducing Notch signaling and blunting the activation of Rac/Akt/eNOS, thereby blocking vasculogenesis and contributing to vascular dysfunction in diabetes (Sawada et al. 2014). Concerning the heart, heart-specific PGC1α/β HKO mice exhibit mitochondrial abnormalities linked to dysregulation of ERR-dependent phospholipid metabolism. Strikingly, this mouse phenotype mimics the human Barth syndrome (Lai et al. 2014). Moreover, HDAC3 HKO mice show increased mortality due to cardiac hypertrophy depending on increased fatty acid uptake and oxidation, oxidative phosphorylation, and myocardial lipid and triglyceride accumulation, due to hyperactivation of PPARα (Montgomery et al. 2008). Finally,

SIRT6 HKO mice show cardiac hypertrophy and heart failure, due to hyperactivation of IGF signaling-related genes (Sundaresan et al. 2012).

3.5 Immune System: Linking Metabolism and Inflammation in Metabolic Disorders

It is well known that metabolic dysfunctions and inflammation act together and influence each other in the pathophysiology of metabolic disorders. To distinguish this metabolically driven chronic low-grade inflammation from the classic acute inflammation linked to infections and defense mechanisms, the term "metaflammation" (similar to the meaning of the term "immunometabolism") was coined (Gregor and Hotamisligil 2011). Metaflammatory coregulator pathways have been so far well characterized in macrophages but also in cell types of metabolic tissues, such as hepatocytes and adipocytes, which play distinctive roles in the attraction of immune cells.

A key observation was that immune cells switch their metabolism during activation: during the early inflammatory phase glycolysis is the major energy source, while in the later adaptation fatty acid oxidation is fundamental to maintain the inflammation. SIRT1 and SIRT6 modulate this switch from glycolysis to fatty acid oxidation during inflammation. In particular, SIRT6 reduces glycolysis, acting on HIF1α, GLUT1, and PDK1, while SIRT1 increases fatty acid oxidation through PGC1α, PGC1β, CD36, and CPT1 (Liu et al. 2012).

Another key observation was, at the genomic level, that many corepressors (and the corresponding complex) are shared components of NR and inflammatory signaling, some of which are essential for anti-inflammatory NR crosstalk (termed *trans*repression) (Glass and Saijo 2010). In vitro studies using cultured macrophages have in particular demonstrated that NCOR and SMRT, along with the remaining corepressor complex, are present at promoters and enhancers of classic pro-inflammatory genes (such as NOS2/INOS, CCL2/MCP1, IL6) upon pro-inflammatory activation, suggesting that these corepressors act as an "epigenomic brake" to prevent undesired activation of the immune system. However, the in vivo situation remains more complicated since macrophage-specific NCOR mice (MKO) show an anti-inflammatory phenotype and insulin sensitization, instead of the expected pro-inflammatory phenotype due to derepression of gene expression. The study provides indications that NCOR MKO leads to LXR derepression, which in turn elevates the expression of genes required for the biosynthesis of anti-inflammatory ω3-fatty acids, which in turn inhibit NF-κB-dependent pro-inflammatory responses (Li et al. 2013).

Other studies point at distinct metabolic and anti-inflammatory actions of GPS2, another subunit of the same corepressor complex. GPS2 acts in human macrophages to facilitate LXR recruitment to the ABCG1 promoter through an epigenomic mechanism that involves KDM-dependent histone demethylation at H3K9, thereby regulating cholesterol efflux (Jakobsson et al. 2009) (Fig. 2). GPS2 additionally inhibits TNFα signaling towards NF-κB activation in macrophages probably by a combination of non-genomic (cytoplasmic) and genomic (nuclear)

mechanisms (Cardamone et al. 2012). This study also demonstrated that aP2/FABP4 promoter-mediated transgenic GPS2 mice in adipose tissue (specifically in adipocytes and presumably also in macrophages where the aP2/FABP4 promoter is also active) display improved insulin signaling, increased circulating levels of resistin, and development of hepatic steatosis (Cardamone et al. 2012). As with NCOR, these phenotypes have to be re-evaluated using complementary and comparable MKO models.

Two different HDAC3 MKO models were generated for HDAC3 (Mxi-Cre versus Lys2-Cre), revealing quite distinct phenotypes. They suggest a requirement of HDAC3 in the alternative M2 activation of macrophages (Mullican et al. 2011) but also for PPARα repression (Chen et al. 2012). A rather unexpected observation was that pro-inflammatory LPS/TLR4 responses were reduced in the HDAC3 MKO mice. As the NCOR MKO phenotype, this finding is apparently inconsistent with the current model in which NCOR/HDAC3 act as corepressors of pro-inflammatory gene expression in macrophages. One intriguing possibility that has to be addressed is that sub-complexes of the prototypic corepressor complex exist, which lack NCOR or HDAC3, and thereby control inflammatory pathways in macrophages via distinct mechanisms.

The study of HDAC4 MKO mice revealed a mechanism by which the second messenger cAMP attenuates macrophage activity in response to a variety of hormonal signals including leptin (Luan et al. 2014). It was found that acute overnutrition, via leptin, triggers catecholamine-dependent increase in cAMP signaling that reduces inflammatory gene expression via the activation of HDAC4, through the alteration of its phosphorylation-dependent nuclear/cytoplasmic localization.

3.6 Brain/CNS

Recent evidences point out the importance of the central nervous system (CNS) and of the hypothalamic liver and adipose tissue axis for the modulation of the metabolic responses depending on the nutritional status. Surprisingly little is currently known about the role of coregulators in the CNS, with the exception of sirtuins: SIRT1 seems to play a fundamental role in the nervous system: transgenic mice overexpressing SIRT1 in striatum and hippocampus show increased fat accumulation and upregulation of adipogenic genes in WAT, together with impaired glucose tolerance and decreased Glut4 mRNA levels in skeletal muscle, leading to decreased energy expenditure and physical activity (Wu et al. 2011). SIRT1 acts also in the peripheral nervous system: lack of SIRT1 in POMC neurons compromises the ability of leptin to start the PI3K signaling and to remodel perigonadal WAT (with reduction of pWAT browning); this leads to reduced energy expenditure and hypersensitivity to diet-induced obesity (Ramadori et al. 2010).

The analysis of coregulators in the brain may be delayed also because most conventional (full body) coregulator KO mice are embryonic lethal and brain-

specific KO mice have not yet been described. The lessons learned from many conventional NR KO mice yielding metabolic phenotypes suggest that the central brain control of metabolism was disturbed in these mice. Subsequently, the phenotypes were confirmed using brain-specific NR KO mice, as shown in the case of PPARγ or ERα. Thus, some metabolic NRs emerge as crucial players in these processes, and they might cooperate with coregulators, yet to be characterized, in the CNS to execute their functions.

4 Coregulators as Therapeutic Targets in Human Metabolic Diseases

4.1 The Coregulator Code in Human Metabolism and Disease

Mouse models are widely used to dissect coregulator functions in metabolic pathways. Despite the concern about significant species differences in metabolism, several of the identified coregulator pathways in mice are already supported by alterations of coregulator expression and function due to genotypic variation and epigenomic reprogramming events linked to metabolic disorders. A CRTC3 common variant in Mexican subjects is associated with adiposity and obesity (Song et al. 2010). The rs12409277 SNP located in the 5′-flanking region of PRDM16 is associated with higher lean body mass in postmenopausal Japanese women (Urano et al. 2014). Two PGC1α variants (Thr394Thr, rs2970847) (Gly482Ser, rs8192673) are associated with T2D in two North Indian populations (Bhat et al. 2007); the second variant (Gly482Ser, rs8192673) was also found to be associated with hypertension among Danes (Andersen et al. 2005). CARM1/PRMT4 has been found to be fundamental for glycogen metabolism (and dysregulated in glycogen storage diseases) in humans, controlling GYS1, PGAM2, and PYGM gene expression (Wang et al. 2012). SRCs are considered as potential contributors to CACT deficiency syndrome (York et al. 2012). Decrease in SIRT6 expression has been found both in human fatty liver samples (Kim et al. 2010) and in human failing hearts (Sundaresan et al. 2012). TBL1 expression is inversely correlated with fatty liver in humans (Kulozik et al. 2011), and TBLR1 is increased under lipolytic conditions in human WAT, correlating with serum FFAs (Rohm et al. 2013). In this context, several SMRT gene polymorphisms are found to be associated with T2D and adiponectin levels (Reilly et al. 2010). Moreover, the expression of SMRT and GPS2 was reduced in adipose tissue of obese humans, inversely correlated with the inflammatory status, and restored upon TZD treatment or by clinical intervention, i.e., via gastric bypass surgery-induced weight loss in morbid obesity (Toubal et al. 2013).

4.2 Therapeutic Modulation of Coregulator Action in Metabolism

Due to their fundamental modulating activities, different coregulators have been suggested as possible targets for therapeutic intervention with common metabolic

disorders and age-related metabolic alterations. SIRT1 has been proposed as a promising therapeutic target for metabolic disorders (Kemper et al. 2009), especially for its role in the liver (Purushotham et al. 2009). SIRT6 has overlapping effects with SIRT1 in fatty liver and has been proposed as an equal promising therapeutic target (Kim et al. 2010). In this context, inhibitors of class I/II HDACs have been suggested as potential therapeutics (Mihaylova Maria et al. 2011). MED1 modulators have been proposed to improve metabolic syndrome especially in peripheral districts due to their actions in skeletal muscle (Chen et al. 2010). Counteracting obesity in white adipose tissue has been proposed through RIP140 agonists (Leonardsson et al. 2004). As already discussed, NCOR removal from adipocytes phenocopies the effect of anti-diabetic TZDs (PPARγ agonists), suggesting that intervention with NCOR expression or function should have a therapeutic benefit (Li et al. 2011). The phosphorylation of PPARγ by CDK5 is involved in the pathogenesis of insulin resistance, representing a potential therapeutic target for T2D (Choi et al. 2010), and recent evidence identified the coregulator THRAP3 as modulator of this process (Choi et al. 2014). Different coregulators have been pointed out for the treatment of dysfunctions in the liver: small molecules that attenuate the CREB-CRTC2 pathway (Wang et al. 2010), modulators of GCN5 (Lerin et al. 2006), MED1 inhibitors (Bai et al. 2011), and PGC1β inhibitors (Nagai et al. 2009).

Despite these indications most coregulators remain challenging therapeutic targets, and non-pharmacological ways of intervention must be considered.

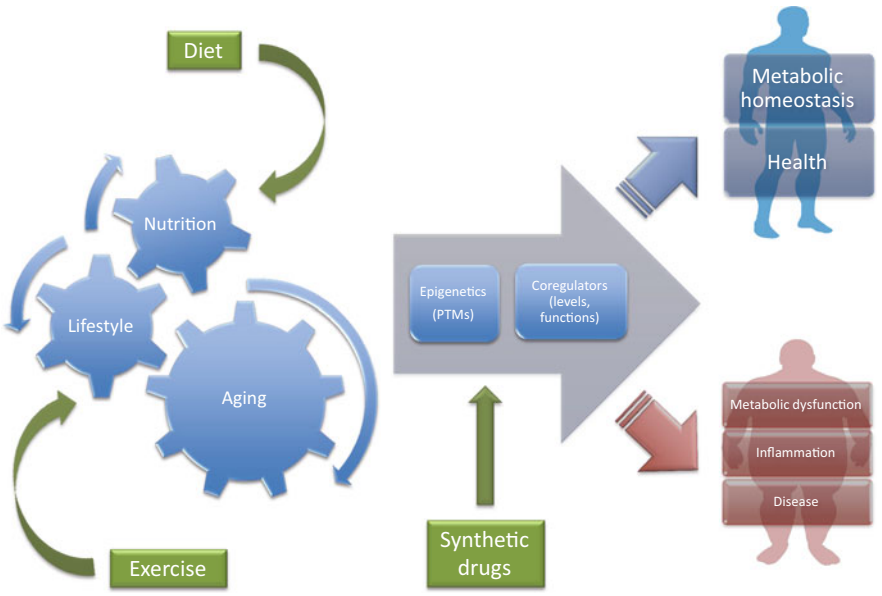

Fig. 4 Factors that influence coregulator action in health and disease. Human epigenetics, environment, lifestyle, and therapeutic interventions shape coregulator levels and function to generate different outcomes, from health (in *blue*) to disease (in *red*)

As such alternatives, diet and exercise have emerged as they can induce epigenomic changes linked to altered coregulator expression and metabolism in humans. For instance, after caloric restriction, DNA methylation differences appear in 35 regions containing genes involved in weight control and insulin secretion (Bouchard et al. 2010). Moreover, 6 months exercise has been demonstrated to induce DNA methylation changes in human adipose tissue, altering among the others RALBP1, HDAC4, SMRT, TCF7L2, and KCNQ1 methylation (Rönn et al. 2013). Finally, short time exercise has been found to alter DNA methylation in skeletal muscle, leading to altered expression of PGC1α (Barrès et al. 2012) (Fig. 4).

5 Future Perspectives

Together with the well-known importance of NR action to modulate metabolic and dysmetabolic pathways in the body, coregulators emerged in the past decade as a fundamental modulatory component of NR signaling. Changes in lifestyle, such as diets and exercise, along with future drugs that target tissue-specific coregulator action or promote specific PTMs seem to be promising approaches to modulate metabolic pathways linked to disease. Despite this, different fundamental issues still need to be addressed prior to targeting coregulators for therapeutic interventions.

First of all, despite the increasing number of promising results obtained in mice, it must be considered that important species differences straiten these discoveries for the use of coregulators as therapeutic targets in humans. From this point of view, it will be extremely important to find more species correlations and associated coregulator influences in human tissues under physiological and pathophysiological conditions, using high-throughput approaches in samples from different disease contexts and different individuals (Fig. 5).

Second, coregulators are themselves part of an equilibrium dependent on: partners (e.g., subunits within a complex, target transcription factors), different energetic status, circadian clock, aging, inflammation, and pathological states. Moreover, the coregulator balance changes in different tissues, leading one force to prevail and influence others. This underestimated complexity, along with current methodological limits in analyzing complex and dynamic systems, is probably the reason why many seemingly unexpected and sometimes controversial results were reported from individual coregulator mouse models.

Third, among the hundreds of suspected coregulators, the metabolic actions and disease links of only a few are sufficiently dissected. This may in part relate to the obvious historical bias in research, meaning that researchers tend to focus on very few of the early identified and thus better-studied master coregulators (including SRCs, PGC1s, the NCOR/SMRT/HDAC3 complex), similar to the situation previously recognized for NRs and other drug targets (Edwards et al. 2011). Evidently, just as synthetic NR ligands turned out to be powerful tools for the experimental analysis of in vivo functions, future drugs specifically targeting individual coregulators would substantially accelerate their analysis.

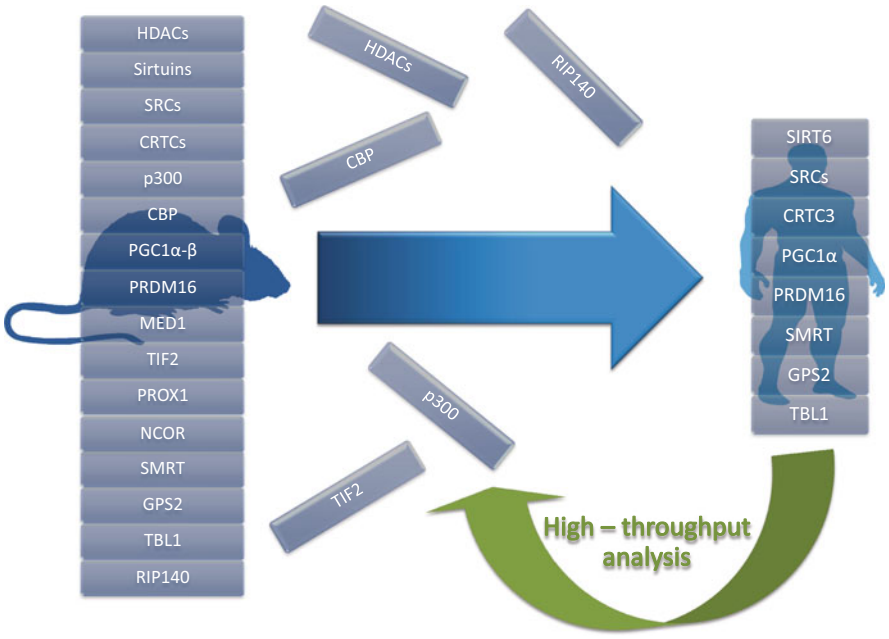

Fig. 5 Translational aspects of coregulator research. Many different transgenic and KO mouse models support the involvement of coregulators in metabolic and inflammatory pathways; nevertheless, only few coregulator variants or dysfunctions have been found to be associated with pathological conditions in humans. High-throughput (epi-)genomic and proteomic analysis of tissues from patients could help gaining more clues on the role of coregulators in dysregulated pathways in human diseases, leading to future therapeutic approaches, including novel drug and dietary interventions

Without doubt, fulfilling these issues, along with the analysis and integration of genomic, epigenomic, proteomic, and metabolomic high-throughput data, will be fundamental to gain further insights into the action and the therapeutic potential of coregulators in human metabolic diseases.

References

Agudelo Leandro Z, Femenía T, Orhan F, Porsmyr-Palmertz M, Goiny M, Martinez-Redondo V, Correia Jorge C, Izadi M, Bhat M, Schuppe-Koistinen I, Pettersson Amanda T, Ferreira Duarte MS, Krook A, Barres R, Zierath Juleen R, Erhardt S, Lindskog M, Ruas Jorge L (2014) Skeletal muscle PGC-1α1 modulates kynurenine metabolism and mediates resilience to stress-induced depression. Cell 159(1):33–45. doi:10.1016/j.cell.2014.07.051

Alenghat T, Meyers K, Mullican SE, Leitner K, Adeniji-Adele A, Avila J, Bucan M, Ahima RS, Kaestner KH, Lazar MA (2008) Nuclear receptor corepressor and histone deacetylase 3 govern circadian metabolic physiology. Nature 456(7224):997–1000, http://www.nature.com/nature/journal/v456/n7224/suppinfo/nature07541_S1.html

Alenghat T, Osborne LC, Saenz SA, Kobuley D, Ziegler CGK, Mullican SE, Choi I, Grunberg S, Sinha R, Wynosky-Dolfi M, Snyder A, Giacomin PR, Joyce KL, Hoang TB, Bewtra M, Brodsky IE, Sonnenberg GF, Bushman FD, Won K-J, Lazar MA, Artis D (2013) Histone deacetylase 3 coordinates commensal-bacteria-dependent intestinal homeostasis. Nature 504 (7478):153–157. doi:10.1038/nature12687

Andersen G, Wegner L, Jensen DP, Glümer C, Tarnow L, Drivsholm T, Poulsen P, Hansen SK, Nielsen E-MD, Ek J, Mouritzen P, Vaag A, Parving H-H, Borch-Johnsen K, Jørgensen T, Hansen T, Pedersen O (2005) PGC-1α Gly482Ser polymorphism associates with hypertension among Danish whites. Hypertension 45(4):565–570. doi:10.1161/01.hyp.0000158946.53289.24

Astapova I, Ramadoss P, Costa-e-Sousa RH, Ye F, Holtz KA, Li Y, Niepel MW, Cohen DE, Hollenberg AN (2014) Hepatic nuclear corepressor 1 regulates cholesterol absorption through a TRβ1-governed pathway. J Clin Invest 124(5):1976–1986. doi:10.1172/JCI73419

Bai L, Jia Y, Viswakarma N, Huang J, Vluggens A, Wolins NE, Jafari N, Rao MS, Borensztajn J, Yang G, Reddy JK (2011) Transcription coactivator mediator subunit MED1 Is required for the development of fatty liver in the mouse. Hepatology 53(4):1164–1174. doi:10.1002/hep.24155

Banks AS, Kon N, Knight C, Matsumoto M, Gutiérrez-Juárez R, Rossetti L, Gu W, Accili D (2008) SirT1 gain-of-function increases energy efficiency and prevents diabetes in mice. Cell Metab 8(4):333–341. doi:10.1016/j.cmet.2008.08.014

Barrès R, Yan J, Egan B, Treebak Jonas T, Rasmussen M, Fritz T, Caidahl K, Krook A, O'Gorman Donal J, Zierath Juleen R (2012) Acute exercise remodels promoter methylation in human skeletal muscle. Cell Metab 15(3):405–411. doi:10.1016/j.cmet.2012.01.001

Bedford David C, Kasper Lawryn H, Wang R, Chang Y, Green Douglas R, Brindle Paul K (2011) Disrupting the CH1 domain structure in the acetyltransferases CBP and p300 results in lean mice with increased metabolic control. Cell Metab 14(2):219–230. doi:10.1016/j.cmet.2011.06.010

Bhat A, Koul A, Rai E, Sharma S, Dhar MK, Bamezai RNK (2007) PGC-1α Thr394Thr and Gly482Ser variants are significantly associated with T2DM in two North Indian populations: a replicate case-control study. Hum Genet 121(5):609–614. doi:10.1007/s00439-007-0352-0

Bochkis Irina M, Przybylski D, Chen J, Regev A (2014) Changes in nucleosome occupancy associated with metabolic alterations in aged mammalian liver. Cell Rep 9(3):996–1006. doi:10.1016/j.celrep.2014.09.048

Bordone L, Cohen D, Robinson A, Motta MC, Van Veen E, Czopik A, Steele AD, Crowe H, Marmor S, Luo J, Gu W, Guarente L (2007) SIRT1 transgenic mice show phenotypes resembling calorie restriction. Aging Cell 6(6):759–767. doi:10.1111/j.1474-9726.2007.00335.x

Bouchard L, Rabasa-Lhoret R, Faraj M, Lavoie M-È, Mill J, Pérusse L, Vohl M-C (2010) Differential epigenomic and transcriptomic responses in subcutaneous adipose tissue between low and high responders to caloric restriction. Am J Clin Nutr 91(2):309–320. doi:10.3945/ajcn.2009.28085

Cantó C, Jiang LQ, Deshmukh AS, Mataki C, Coste A, Lagouge M, Zierath JR, Auwerx J (2010) Interdependence of AMPK and SIRT1 for metabolic adaptation to fasting and exercise in skeletal muscle. Cell Metab 11(3):213–219. doi:10.1016/j.cmet.2010.02.006

Cardamone MD, Krones A, Tanasa B, Taylor H, Ricci L, Ohgi Kenneth A, Glass Christopher K, Rosenfeld Michael G, Perissi V (2012) A protective strategy against hyperinflammatory responses requiring the nontranscriptional actions of GPS2. Mol Cell 46(1):91–104. doi:10.1016/j.molcel.2012.01.025

Cardamone MD, Tanasa B, Chan M, Cederquist Carly T, Andricovich J, Rosenfeld Michael G, Perissi V (2014) GPS2/KDM4A pioneering activity regulates promoter-specific recruitment of PPARγ. Cell Rep 8(1):163–176. doi:10.1016/j.celrep.2014.05.041

Chalkiadaki A, Guarente L (2012) High-fat diet triggers inflammation-induced cleavage of SIRT1 in adipose tissue to promote metabolic dysfunction. Cell Metab 16(2):180–188. doi:10.1016/j.cmet.2012.07.003

Chang S, McKinsey TA, Zhang CL, Richardson JA, Hill JA, Olson EN (2004) Histone deacetylases 5 and 9 govern responsiveness of the heart to a subset of stress signals and play redundant roles in heart development. Mol Cell Biol 24(19):8467–8476. doi:10.1128/MCB.24.19.8467-8476.2004

Chen W, Zhang X, Birsoy K, Roeder RG (2010) A muscle-specific knockout implicates nuclear receptor coactivator MED1 in the regulation of glucose and energy metabolism. Proc Natl Acad Sci 107(22):10196–10201. doi:10.1073/pnas.1005626107

Chen X, Barozzi I, Termanini A, Prosperini E, Recchiuti A, Dalli J, Mietton F, Matteoli G, Hiebert S, Natoli G (2012) Requirement for the histone deacetylase Hdac3 for the inflammatory gene expression program in macrophages. Proc Natl Acad Sci 109(42):E2865–E2874. doi:10.1073/pnas.1121131109

Choi JH, Banks AS, Estall JL, Kajimura S, Bostrom P, Laznik D, Ruas JL, Chalmers MJ, Kamenecka TM, Bluher M, Griffin PR, Spiegelman BM (2010) Anti-diabetic drugs inhibit obesity-linked phosphorylation of PPAR[ggr] by Cdk5. Nature 466(7305):451–456, http://www.nature.com/nature/journal/v466/n7305/abs/nature09291.html

Choi JH, Choi S-S, Kim ES, Jedrychowski MP, Yang YR, Jang H-J, Suh P-G, Banks AS, Gygi SP, Spiegelman BM (2014) Thrap3 docks on phosphoserine 273 of PPARγ and controls diabetic gene programming. Genes Dev. doi:10.1101/gad.249367.114

Chopra AR, Louet J-F, Saha P, An J, DeMayo F, Xu J, York B, Karpen S, Finegold M, Moore D, Chan L, Newgard CB, O'Malley BW (2008) Absence of the SRC-2 coactivator results in a glycogenopathy resembling Von Gierke's disease. Science 322(5906):1395–1399. doi:10.1126/science.1164847

Chopra AR, Kommagani R, Saha P, Louet J-F, Salazar C, Song J, Jeong J, Finegold M, Viollet B, DeMayo F, Chan L, Moore DD, O'Malley BW (2011) Cellular energy depletion resets whole-body energy by promoting coactivator-mediated dietary fuel absorption. Cell Metab 13(1):35–43. doi:10.1016/j.cmet.2010.12.001

Cohen DE, Supinski AM, Bonkowski MS, Donmez G, Guarente LP (2009) Neuronal SIRT1 regulates endocrine and behavioral responses to calorie restriction. Genes Dev 23(24):2812–2817. doi:10.1101/gad.1839209

Coste A, Louet J-F, Lagouge M, Lerin C, Antal MC, Meziane H, Schoonjans K, Puigserver P, O'Malley BW, Auwerx J (2008) The genetic ablation of SRC-3 protects against obesity and improves insulin sensitivity by reducing the acetylation of PGC-1α. Proc Natl Acad Sci 105(44):17187–17192. doi:10.1073/pnas.0808207105

Dasgupta S, Lonard DM, O'Malley BW (2014) Nuclear receptor coactivators: master regulators of human health and disease. Annu Rev Med 65(1):279–292. doi:10.1146/annurev-med-051812-145316

de Cabo R, Carmona-Gutierrez D, Bernier M, Hall Michael N, Madeo F (2014) The search for antiaging interventions: from elixirs to fasting regimens. Cell 157(7):1515–1526. doi:10.1016/j.cell.2014.05.031

Dominy JE Jr, Lee Y, Jedrychowski MP, Chim H, Jurczak MJ, Camporez JP, Ruan H-B, Feldman J, Pierce K, Mostoslavsky R, Denu JM, Clish CB, Yang X, Shulman GI, Gygi S, Puigserver P (2012) The deacetylase Sirt6 activates the acetyltransferase GCN5 and suppresses hepatic gluconeogenesis. Mol Cell 48(6):900–913. doi:10.1016/j.molcel.2012.09.030

Duteil D, Chambon C, Ali F, Malivindi R, Zoll J, Kato S, Geny B, Chambon P, Metzger D (2010) The transcriptional coregulators TIF2 and SRC-1 regulate energy homeostasis by modulating mitochondrial respiration in skeletal muscles. Cell Metab 12(5):496–508. doi:10.1016/j.cmet.2010.09.016

Edwards AM, Isserlin R, Bader GD, Frye SV, Willson TM, Yu FH (2011) Too many roads not taken. Nature 470(7333):163–165

Fang S, Suh JM, Atkins AR, Hong S-H, Leblanc M, Nofsinger RR, Yu RT, Downes M, Evans RM (2011) Corepressor SMRT promotes oxidative phosphorylation in adipose tissue and protects against diet-induced obesity and insulin resistance. Proc Natl Acad Sci 108(8):3412–3417. doi:10.1073/pnas.1017707108

Feng D, Liu T, Sun Z, Bugge A, Mullican SE, Alenghat T, Liu XS, Lazar MA (2011) A circadian rhythm orchestrated by histone deacetylase 3 controls hepatic lipid metabolism. Science 331 (6022):1315–1319. doi:10.1126/science.1198125

Fiorino E, Giudici M, Ferrari A, Mitro N, Caruso D, De Fabiani E, Crestani M (2014) The sirtuin class of histone deacetylases: regulation and roles in lipid metabolism. IUBMB Life. doi:10.1002/iub.1246

Ghisletti S, Huang W, Ogawa S, Pascual G, Lin M-E, Willson TM, Rosenfeld MG, Glass CK (2007) Parallel SUMOylation-dependent pathways mediate gene- and signal-specific transrepression by LXRs and PPARγ. Mol Cell 25(1):57–70. doi:10.1016/j.molcel.2006.11.022

Glass CK, Saijo K (2010) Nuclear receptor transrepression pathways that regulate inflammation in macrophages and T cells. Nat Rev Immunol 10(5):365–376

Gregor MF, Hotamisligil GS (2011) Inflammatory mechanisms in obesity. Annu Rev Immunol 29 (1):415–445. doi:10.1146/annurev-immunol-031210-101322

Hallows WC, Yu W, Smith BC, Devires MK, Ellinger JJ, Someya S, Shortreed MR, Prolla T, Markley JL, Smith LM, Zhao S, Guan K-L, Denu JM (2011) Sirt3 promotes the urea cycle and fatty acid oxidation during dietary restriction. Mol Cell 41(2):139–149. doi:10.1016/j.molcel.2011.01.002

Harms Matthew J, Ishibashi J, Wang W, Lim H-W, Goyama S, Sato T, Kurokawa M, Won K-J, Seale P (2014) Prdm16 is required for the maintenance of brown adipocyte identity and function in adult mice. Cell Metab 19(4):593–604. doi:10.1016/j.cmet.2014.03.007

Herranz D, Serrano M (2010) SIRT1: recent lessons from mouse models. Nat Rev Cancer 10 (12):819–823

Hirschey MD, Shimazu T, Goetzman E, Jing E, Schwer B, Lombard DB, Grueter CA, Harris C, Biddinger S, Ilkayeva OR, Stevens RD, Li Y, Saha AK, Ruderman NB, Bain JR, Newgard CB, Farese RV Jr, Alt FW, Kahn CR, Verdin E (2010) SIRT3 regulates mitochondrial fatty-acid oxidation by reversible enzyme deacetylation. Nature 464(7285):121–125, http://www.nature.com/nature/journal/v464/n7285/suppinfo/nature08778_S1.html

Ho P-C, Lin Y-W, Tsui Y-C, Gupta P, Wei L-N (2009) A negative regulatory pathway of GLUT4 trafficking in adipocyte: new function of RIP140 in the cytoplasm via AS160. Cell Metab 10 (6):516–523. doi:10.1016/j.cmet.2009.09.012

Hohl M, Wagner M, Reil J-C, Muller S-A, Tauchnitz M, Zimmer AM, Lehmann LH, Thiel G, Bohm M, Backs J, Maack C (2013) HDAC4 controls histone methylation in response to elevated cardiac load. J Clin Invest 123(3):1359–1370. doi:10.1172/JCI61084

Houtkooper RH, Pirinen E, Auwerx J (2012) Sirtuins as regulators of metabolism and healthspan. Nat Rev Mol Cell Biol 13(4):225–238

Jakobsson T, Venteclef N, Toresson G, Damdimopoulos AE, Ehrlund A, Lou X, Sanyal S, Steffensen KR, Gustafsson J-Ö, Treuter E (2009) GPS2 is required for cholesterol efflux by triggering histone demethylation, LXR recruitment, and coregulator assembly at the ABCG1 locus. Mol Cell 34(4):510–518. doi:10.1016/j.molcel.2009.05.006

Jin Q, Yu LR, Wang L, Zhang Z, Kasper LH, Lee JE, Wang C, Brindle PK, Dent SYR, Ge K (2011) Distinct roles of GCN5/PCAF mediated H3K9ac and CBP/p300 mediated H3K18/27ac in nuclear receptor transactivation. EMBO J 30(2):249–262. doi:10.1038/emboj.2010.318

Kelly TJ, Lerin C, Haas W, Gygi SP, Puigserver P (2009) GCN5-mediated transcriptional control of the metabolic coactivator PGC-1β through lysine acetylation. J Biol Chem 284 (30):19945–19952. doi:10.1074/jbc.M109.015164

Kemper JK, Xiao Z, Ponugoti B, Miao J, Fang S, Kanamaluru D, Tsang S, Wu S-Y, Chiang C-M, Veenstra TD (2009) FXR acetylation is normally dynamically regulated by p300 and SIRT1 but constitutively elevated in metabolic disease states. Cell Metab 10(5):392–404. doi:10.1016/j.cmet.2009.09.009

Kerr TA, Saeki S, Schneider M, Schaefer K, Berdy S, Redder T, Shan B, Russell DW, Schwarz M (2002) Loss of nuclear receptor shp impairs but does not eliminate negative feedback regulation of bile acid synthesis. Dev Cell 2(6):713–720. doi:10.1016/S1534-5807(02)00154-5

Kim H-S, Xiao C, Wang R-H, Lahusen T, Xu X, Vassilopoulos A, Vazquez-Ortiz G, Jeong W-I, Park O, Ki SH, Gao B, Deng C-X (2010) Hepatic-specific disruption of SIRT6 in mice results in fatty liver formation due to enhanced glycolysis and triglyceride synthesis. Cell Metab 12 (3):224–236. doi:10.1016/j.cmet.2010.06.009

Kim DH, Xiao Z, Kwon S, Sun X, Ryerson D, Tkac D, Ma P, Wu SY, Chiang CM, Zhou E, Xu HE, Palvimo JJ, Chen LF, Kemper B, Kemper JK (2015) A dysregulated acetyl/SUMO switch of FXR promotes hepatic inflammation in obesity. EMBO J 34(2):184–199. doi:10.15252/embj.201489527

Kiskinis E, Chatzeli L, Curry E, Kaforou M, Frontini A, Cinti S, Montana G, Parker MG, Christian M (2014) RIP140 represses the "brown-in-white" adipocyte program including a futile cycle of triacyclglycerol breakdown and synthesis. Mol Endocrinol 28(3):344–356. doi:10.1210/me.2013-1254

Kulozik P, Jones A, Mattijssen F, Rose Adam J, Reimann A, Strzoda D, Kleinsorg S, Raupp C, Kleinschmidt J, Müller-Decker K, Wahli W, Sticht C, Gretz N, von Loeffelholz C, Stockmann M, Pfeiffer A, Stöhr S, Dallinga-Thie Geesje M, Nawroth Peter P, Diaz Mauricio B, Herzig S (2011) Hepatic deficiency in transcriptional cofactor TBL1 promotes liver steatosis and hypertriglyceridemia. Cell Metab 13(4):389–400. doi:10.1016/j.cmet.2011.02.011

Lai L, Wang M, Martin OJ, Leone TC, Vega RB, Han X, Kelly DP (2014) A role for peroxisome proliferator-activated receptor γ coactivator 1 (PGC-1) in the regulation of cardiac mitochondrial phospholipid biosynthesis. J Biol Chem 289(4):2250–2259. doi:10.1074/jbc.M113.523654

Lee Y, Dominy JE, Choi YJ, Jurczak M, Tolliday N, Camporez JP, Chim H, Lim J-H, Ruan H-B, Yang X, Vazquez F, Sicinski P, Shulman GI, Puigserver P (2014) Cyclin D1-Cdk4 controls glucose metabolism independently of cell cycle progression. Nature 510(7506):547–551. doi:10.1038/nature13267

Lehman JJ, Barger PM, Kovacs A, Saffitz JE, Medeiros DM, Kelly DP (2000) Peroxisome proliferator–activated receptor γ coactivator-1 promotes cardiac mitochondrial biogenesis. J Clin Invest 106(7):847–856. doi:10.1172/JCI10268

Lelliott CJ, Medina-Gomez G, Petrovic N, Kis A, Feldmann HM, Bjursell M, Parker N, Curtis K, Campbell M, Hu P, Zhang D, Litwin SE, Zaha VG, Fountain KT, Boudina S, Jimenez-Linan M, Blount M, Lopez M, Meirhaeghe A, Bohlooly-Y M, Storlien L, Strömstedt M, Snaith M, Orešič M, Abel ED, Cannon B, Vidal-Puig A (2006) Ablation of PGC-1β results in defective mitochondrial activity, thermogenesis, hepatic function, and cardiac performance. PLoS Biol 4 (11):e369. doi:10.1371/journal.pbio.0040369

Leonardsson G, Steel JH, Christian M, Pocock V, Milligan S, Bell J, So P-W, Medina-Gomez G, Vidal-Puig A, White R, Parker MG (2004) Nuclear receptor corepressor RIP140 regulates fat accumulation. Proc Natl Acad Sci U S A 101(22):8437–8442. doi:10.1073/pnas.0401013101

Lerin C, Rodgers JT, Kalume DE, S-h K, Pandey A, Puigserver P (2006) GCN5 acetyltransferase complex controls glucose metabolism through transcriptional repression of PGC-1α. Cell Metab 3(6):429–438. doi:10.1016/j.cmet.2006.04.013

Leuenberger N, Pradervand S, Wahli W (2009) Sumoylated PPARα mediates sex-specific gene repression and protects the liver from estrogen-induced toxicity in mice. J Clin Invest 119 (10):3138–3148. doi:10.1172/JCI39019

Li X, Zhang S, Blander G, Tse JG, Krieger M, Guarente L (2007) SIRT1 deacetylates and positively regulates the nuclear receptor LXR. Mol Cell 28(1):91–106. doi:10.1016/j.molcel.2007.07.032

Li P, Fan W, Xu J, Lu M, Yamamoto H, Auwerx J, Sears DD, Talukdar S, Oh D, Chen A, Bandyopadhyay G, Scadeng M, Ofrecio JM, Nalbandian S, Olefsky JM (2011) Adipocyte NCoR knockout decreases PPARγ phosphorylation and enhances PPARγ activity and insulin sensitivity. Cell 147(4):815–826. doi:10.1016/j.cell.2011.09.050

Li P, Spann Nathanael J, Kaikkonen Minna U, Lu M, Da Oh Y, Fox Jesse N, Bandyopadhyay G, Talukdar S, Xu J, Lagakos William S, Patsouris D, Armando A, Quehenberger O, Dennis

Edward A, Watkins Steven M, Auwerx J, Glass Christopher K, Olefsky Jerrold M (2013) NCoR repression of LXRs restricts macrophage biosynthesis of insulin-sensitizing omega 3 fatty acids. Cell 155(1):200–214. doi:10.1016/j.cell.2013.08.054

Lin J, Wu P-H, Tarr PT, Lindenberg KS, St-Pierre J, C-y Z, Mootha VK, Jäger S, Vianna CR, Reznick RM, Cui L, Manieri M, Donovan MX, Wu Z, Cooper MP, Fan MC, Rohas LM, Zavacki AM, Cinti S, Shulman GI, Lowell BB, Krainc D, Spiegelman BM (2004) Defects in adaptive energy metabolism with CNS-linked hyperactivity in PGC-1α null mice. Cell 119 (1):121–135. doi:10.1016/j.cell.2004.09.013

Liu TF, Vachharajani VT, Yoza BK, McCall CE (2012) NAD + -dependent sirtuin 1 and 6 proteins coordinate a switch from glucose to fatty acid oxidation during the acute inflammatory response. J Biol Chem 287(31):25758–25769. doi:10.1074/jbc.M112.362343

Louet J-F, Coste A, Amazit L, Tannour-Louet M, Wu R-C, Tsai SY, Tsai M-J, Auwerx J, O'Malley BW (2006) Oncogenic steroid receptor coactivator-3 is a key regulator of the white adipogenic program. Proc Natl Acad Sci 103(47):17868–17873. doi:10.1073/pnas.0608711103

Louet J-F, Chopra AR, Sagen JV, An J, York B, Tannour-Louet M, Saha PK, Stevens RD, Wenner BR, Ilkayeva OR, Bain JR, Zhou S, DeMayo F, Xu J, Newgard CB, O'Malley BW (2010) The coactivator SRC-1 is an essential coordinator of hepatic glucose production. Cell Metab 12 (6):606–618. doi:10.1016/j.cmet.2010.11.009

Luan B, Goodarzi Mark O, Phillips Naomi G, Guo X, Chen Y-Der I, Yao J, Allison M, Rotter Jerome I, Shaw R, Montminy M (2014) Leptin-mediated increases in catecholamine signaling reduce adipose tissue inflammation via activation of macrophage HDAC4. Cell Metab 19 (6):1058–1065. doi:10.1016/j.cmet.2014.03.024

Malovannaya A, Lanz RB, Jung SY, Bulynko Y, Le NT, Chan DW, Ding C, Shi Y, Yucer N, Krenciute G, Kim B-J, Li C, Chen R, Li W, Wang Y, O'Malley BW, Qin J (2011) Analysis of the human endogenous coregulator complexome. Cell 145(5):787–799. doi:10.1016/j.cell.2011.05.006

McGee SL, van Denderen BJW, Howlett KF, Mollica J, Schertzer JD, Kemp BE, Hargreaves M (2008) AMP-activated protein kinase regulates GLUT4 transcription by phosphorylating histone deacetylase 5. Diabetes 57(4):860–867. doi:10.2337/db07-0843

McKenna NJ, O'Malley BW (2010) SnapShot: NR coregulators. Cell 143(1):172–172.e171. doi:10.1016/j.cell.2010.09.032

Miao J, Xiao Z, Kanamaluru D, Min G, Yau PM, Veenstra TD, Ellis E, Strom S, Suino-Powell K, Xu HE, Kemper JK (2009) Bile acid signaling pathways increase stability of Small Heterodimer Partner (SHP) by inhibiting ubiquitin–proteasomal degradation. Genes Dev 23 (8):986–996. doi:10.1101/gad.1773909

Mihaylova Maria M, Vasquez Debbie S, Ravnskjaer K, Denechaud P-D, Yu Ruth T, Alvarez Jacqueline G, Downes M, Evans Ronald M, Montminy M, Shaw Reuben J (2011) Class IIa histone deacetylases Are hormone-activated regulators of FOXO and mammalian glucose homeostasis. Cell 145(4):607–621. doi:10.1016/j.cell.2011.03.043

Millard Christopher J, Watson Peter J, Celardo I, Gordiyenko Y, Cowley Shaun M, Robinson Carol V, Fairall L, Schwabe John WR (2013) Class I HDACs share a common mechanism of regulation by inositol phosphates. Mol Cell 51(1):57–67. doi:10.1016/j.molcel.2013.05.020

Montgomery RL, Potthoff MJ, Haberland M, Qi X, Matsuzaki S, Humphries KM, Richardson JA, Bassel-Duby R, Olson EN (2008) Maintenance of cardiac energy metabolism by histone deacetylase 3 in mice. J Clin Invest 118(11):3588–3597. doi:10.1172/JCI35847

Moynihan KA, Grimm AA, Plueger MM, Bernal-Mizrachi E, Ford E, Cras-Méneur C, Permutt MA, Imai S-I (2005) Increased dosage of mammalian Sir2 in pancreatic β cells enhances glucose-stimulated insulin secretion in mice. Cell Metab 2(2):105–117. doi:10.1016/j.cmet.2005.07.001

Mullican SE, Gaddis CA, Alenghat T, Nair MG, Giacomin PR, Everett LJ, Feng D, Steger DJ, Schug J, Artis D, Lazar MA (2011) Histone deacetylase 3 is an epigenomic brake in macrophage alternative activation. Genes Dev 25(23):2480–2488. doi:10.1101/gad.175950.111

Nagai Y, Yonemitsu S, Erion DM, Iwasaki T, Stark R, Weismann D, Dong J, Zhang D, Jurczak MJ, Löffler MG, Cresswell J, Yu XX, Murray SF, Bhanot S, Monia BP, Bogan JS, Samuel V, Shulman GI (2009) The role of peroxisome proliferator-activated receptor γ coactivator-1 β in the pathogenesis of fructose-induced insulin resistance. Cell Metab 9(3):252–264. doi:10.1016/j.cmet.2009.01.011

Nofsinger RR, Li P, Hong S-H, Jonker JW, Barish GD, Ying H, Cheng S-Y, LeBlanc M, Xu W, Pei L, Kang Y-J, Nelson M, Downes M, Yu RT, Olefsky JM, Lee C-H, Evans RM (2008) SMRT repression of nuclear receptors controls the adipogenic set point and metabolic homeostasis. Proc Natl Acad Sci 105(50):20021–20026. doi:10.1073/pnas.0811012105

O'Malley BW, Malovannaya A, Qin J (2012) Minireview: nuclear receptor and coregulator proteomics—2012 and beyond. Mol Endocrinol 26(10):1646–1650. doi:10.1210/me.2012-1114

Pan X, Zhang Y, Wang L, Hussain MM (2010) Diurnal regulation of MTP and plasma triglyceride by CLOCK is mediated by SHP. Cell Metab 12(2):174–186. doi:10.1016/j.cmet.2010.05.014

Park YJ, Kim SC, Kim J, Anakk S, Lee JM, Tseng H-T, Yechoor V, Park J, Choi J-S, Jang HC, Lee K-U, Novak CM, Moore DD, Lee YK (2011) Dissociation of diabetes and obesity in mice lacking orphan nuclear receptor small heterodimer partner. J Lipid Res 52(12):2234–2244. doi:10.1194/jlr.M016048

Pascual G, Fong AL, Ogawa S, Gamliel A, Li AC, Perissi V, Rose DW, Willson TM, Rosenfeld MG, Glass CK (2005) A SUMOylation-dependent pathway mediates transrepression of inflammatory response genes by PPAR-[gamma]. Nature 437(7059):759–763, http://www.nature.com/nature/journal/v437/n7059/suppinfo/nature03988_S1.html

Pérez-Schindler J, Summermatter S, Salatino S, Zorzato F, Beer M, Balwierz PJ, van Nimwegen E, Feige JN, Auwerx J, Handschin C (2012) The corepressor NCoR1 antagonizes PGC-1α and estrogen-related receptor α in the regulation of skeletal muscle function and oxidative metabolism. Mol Cell Biol 32(24):4913–4924. doi:10.1128/mcb. 00877-12

Peserico A, Chiacchiera F, Grossi V, Matrone A, Latorre D, Simonatto M, Fusella A, Ryall J, Finley LS, Haigis M, Villani G, Puri P, Sartorelli V, Simone C (2013) A novel AMPK-dependent FoxO3A-SIRT3 intramitochondrial complex sensing glucose levels. Cell Mol Life Sci 70(11):2015–2029. doi:10.1007/s00018-012-1244-6

Ponugoti B, Kim D-H, Xiao Z, Smith Z, Miao J, Zang M, Wu S-Y, Chiang C-M, Veenstra TD, Kemper JK (2010) SIRT1 deacetylates and inhibits SREBP-1C activity in regulation of hepatic lipid metabolism. J Biol Chem 285(44):33959–33970. doi:10.1074/jbc.M110.122978

Powelka AM, Seth A, Virbasius JV, Kiskinis E, Nicoloro SM, Guilherme A, Tang X, Straubhaar J, Cherniack AD, Parker MG, Czech MP (2006) Suppression of oxidative metabolism and mitochondrial biogenesis by the transcriptional corepressor RIP140 in mouse adipocytes. J Clin Invest 116(1):125–136. doi:10.1172/JCI26040

Purushotham A, Schug TT, Xu Q, Surapureddi S, Guo X, Li X (2009) Hepatocyte-specific deletion of SIRT1 alters fatty acid metabolism and results in hepatic steatosis and inflammation. Cell Metab 9(4):327–338. doi:10.1016/j.cmet.2009.02.006

Purushotham A, Xu Q, Li X (2012a) Systemic SIRT1 insufficiency results in disruption of energy homeostasis and steroid hormone metabolism upon high-fat-diet feeding. FASEB J 26 (2):656–667. doi:10.1096/fj.11-195172

Purushotham A, Xu Q, Lu J, Foley JF, Yan X, Kim D-H, Kemper JK, Li X (2012b) Hepatic deletion of SIRT1 decreases hepatocyte nuclear factor 1α/farnesoid X receptor signaling and induces formation of cholesterol gallstones in mice. Mol Cell Biol 32(7):1226–1236. doi:10.1128/mcb. 05988-11

Qi C, Zhu Y, Pan J, Yeldandi AV, Rao MS, Maeda N, Subbarao V, Pulikuri S, Hashimoto T, Reddy JK (1999) Mouse steroid receptor coactivator-1 is not essential for peroxisome proliferator-activated receptor α-regulated gene expression. Proc Natl Acad Sci U S A 96 (4):1585–1590

Qiang L, Lin HV, Kim-Muller JY, Welch CL, Gu W, Accili D (2011) Proatherogenic abnormalities of lipid metabolism in SirT1 transgenic mice Are mediated through creb deacetylation. Cell Metab 14(6):758–767. doi:10.1016/j.cmet.2011.10.007

Qiang L, Wang L, Kon N, Zhao W, Lee S, Zhang Y, Rosenbaum M, Zhao Y, Gu W, Farmer SR, Accili D (2012) Brown remodeling of white adipose tissue by SirT1-dependent deacetylation of Pparγ. Cell 150(3):620–632. doi:10.1016/j.cell.2012.06.027

Ramadori G, Fujikawa T, Fukuda M, Anderson J, Morgan DA, Mostoslavsky R, Stuart RC, Perello M, Vianna CR, Nillni EA, Rahmouni K, Coppari R (2010) SIRT1 deacetylase in POMC neurons is required for homeostatic defenses against diet-induced obesity. Cell Metab 12(1):78–87. doi:10.1016/j.cmet.2010.05.010

Reilly SM, Bhargava P, Liu S, Gangl MR, Gorgun C, Nofsinger RR, Evans RM, Qi L, Hu FB, Lee C-H (2010) Nuclear receptor corepressor SMRT regulates mitochondrial oxidative metabolism and mediates aging-related metabolic deterioration. Cell Metab 12(6):643–653. doi:10.1016/j.cmet.2010.11.007

Rodgers JT, Lerin C, Haas W, Gygi SP, Spiegelman BM, Puigserver P (2005) Nutrient control of glucose homeostasis through a complex of PGC-1α and SIRT1. Nature 434(7029):113–118, http://www.nature.com/nature/journal/v434/n7029/suppinfo/nature03354_S1.html

Rohm M, Sommerfeld A, Strzoda D, Jones A, Sijmonsma TP, Rudofsky G, Wolfrum C, Sticht C, Gretz N, Zeyda M, Leitner L, Nawroth PP, Stulnig TM, Diaz Mauricio B, Vegiopoulos A, Herzig S (2013) Transcriptional cofactor TBLR1 controls lipid mobilization in white adipose tissue. Cell Metab 17(4):575–585. doi:10.1016/j.cmet.2013.02.010

Rönn T, Volkov P, Davegårdh C, Dayeh T, Hall E, Olsson AH, Nilsson E, Tornberg Å, Dekker Nitert M, Eriksson K-F, Jones HA, Groop L, Ling C (2013) A six months exercise intervention influences the genome-wide DNA methylation pattern in human adipose tissue. PLoS Genet 9 (6):e1003572. doi:10.1371/journal.pgen.1003572

Rosenfeld MG, Lunyak VV, Glass CK (2006) Sensors and signals: a coactivator/corepressor/epigenetic code for integrating signal-dependent programs of transcriptional response. Genes Dev 20(11):1405–1428. doi:10.1101/gad.1424806

Russell LK, Mansfield CM, Lehman JJ, Kovacs A, Courtois M, Saffitz JE, Medeiros DM, Valencik ML, McDonald JA, Kelly DP (2004) Cardiac-specific induction of the transcriptional coactivator peroxisome proliferator-activated receptor γ coactivator-1α promotes mitochondrial biogenesis and reversible cardiomyopathy in a developmental stage-dependent manner. Circ Res 94(4):525–533. doi:10.1161/01.res.0000117088.36577.eb

Sanyal S, Båvner A, Haroniti A, Nilsson L-M, Lundåsen T, Rehnmark S, Witt MR, Einarsson C, Talianidis I, Gustafsson J-Å, Treuter E (2007) Involvement of corepressor complex subunit GPS2 in transcriptional pathways governing human bile acid biosynthesis. Proc Natl Acad Sci 104(40):15665–15670. doi:10.1073/pnas.0706736104

Sawada N, Jiang A, Takizawa F, Safdar A, Manika A, Tesmenitsky Y, Kang K-T, Bischoff J, Kalwa H, Sartoretto Juliano L, Kamei Y, Benjamin Laura E, Watada H, Ogawa Y, Higashikuni Y, Kessinger Chase W, Jaffer Farouc A, Michel T, Sata M, Croce K, Tanaka R, Arany Z (2014) Endothelial PGC-1α mediates vascular dysfunction in diabetes. Cell Metab 19 (2):246–258. doi:10.1016/j.cmet.2013.12.014

Schug TT, Xu Q, Gao H, Peres-da-Silva A, Draper DW, Fessler MB, Purushotham A, Li X (2010) Myeloid deletion of SIRT1 induces inflammatory signaling in response to environmental stress. Mol Cell Biol 30(19):4712–4721. doi:10.1128/mcb. 00657-10

Seale P, Kajimura S, Yang W, Chin S, Rohas LM, Uldry M, Tavernier G, Langin D, Spiegelman BM (2007) Transcriptional control of brown fat determination by PRDM16. Cell Metab 6 (1):38–54. doi:10.1016/j.cmet.2007.06.001

Seale P, Conroe HM, Estall J, Kajimura S, Frontini A, Ishibashi J, Cohen P, Cinti S, Spiegelman BM (2011) Prdm16 determines the thermogenic program of subcutaneous white adipose tissue in mice. J Clin Invest 121(1):96–105. doi:10.1172/JCI44271

Seth A, Steel JH, Nichol D, Pocock V, Kumaran MK, Fritah A, Mobberley M, Ryder TA, Rowlerson A, Scott J, Poutanen M, White R, Parker M (2007) The transcriptional corepressor

RIP140 regulates oxidative metabolism in skeletal muscle. Cell Metab 6(3):236–245. doi:10.1016/j.cmet.2007.08.004

Shimazu T, Hirschey MD, Hua L, Dittenhafer-Reed KE, Schwer B, Lombard DB, Li Y, Bunkenborg J, Alt FW, Denu JM, Jacobson MP, Verdin E (2010) SIRT3 deacetylates mitochondrial 3-hydroxy-3-methylglutaryl CoA synthase 2 and regulates ketone body production. Cell Metab 12(6):654–661. doi:10.1016/j.cmet.2010.11.003

Siersbæk MS, Loft A, Aagaard MM, Nielsen R, Schmidt SF, Petrovic N, Nedergaard J, Mandrup S (2012) Genome-wide profiling of peroxisome proliferator-activated receptor γ in primary epididymal, inguinal, and brown adipocytes reveals depot-selective binding correlated with gene expression. Mol Cell Biol 32(17):3452–3463. doi:10.1128/mcb. 00526-12

Song Y, Altarejos J, Goodarzi MO, Inoue H, Guo X, Berdeaux R, Kim J-H, Goode J, Igata M, Paz JC, Hogan MF, Singh PK, Goebel N, Vera L, Miller N, Cui J, Jones MR, ConsortiumCharge, ConsortiumGiant, Chen Y-DI, Taylor KD, Hsueh WA, Rotter JI, Montminy M (2010) CRTC3 links catecholamine signalling to energy balance. Nature 468(7326):933–939, http://www.nature.com/nature/journal/v468/n7326/abs/nature09564.html

Stein S, Oosterveer Maaike H, Mataki C, Xu P, Lemos V, Havinga R, Dittner C, Ryu D, Menzies Keir J, Wang X, Perino A, Houten Sander M, Melchior F, Schoonjans K (2014) SUMOylation-dependent LRH-1/PROX1 interaction promotes atherosclerosis by decreasing hepatic reverse cholesterol transport. Cell Metab 20(4):603–613. doi:10.1016/j.cmet.2014.07.023

Sun C, Zhang F, Ge X, Yan T, Chen X, Shi X, Zhai Q (2007) SIRT1 improves insulin sensitivity under insulin-resistant conditions by repressing PTP1B. Cell Metab 6(4):307–319. doi:10.1016/j.cmet.2007.08.014

Sun Z, Singh N, Mullican SE, Everett LJ, Li L, Yuan L, Liu X, Epstein JA, Lazar MA (2011) Diet-induced lethality due to deletion of the Hdac3 gene in heart and skeletal muscle. J Biol Chem 286(38):33301–33309. doi:10.1074/jbc.M111.277707

Sun Z, Miller RA, Patel RT, Chen J, Dhir R, Wang H, Zhang D, Graham MJ, Unterman TG, Shulman GI, Sztalryd C, Bennett MJ, Ahima RS, Birnbaum MJ, Lazar MA (2012) Hepatic Hdac3 promotes gluconeogenesis by repressing lipid synthesis and sequestration. Nat Med 18 (6):934–942, http://www.nature.com/nm/journal/v18/n6/abs/nm.2744.html

Sun Z, Feng D, Fang B, Mullican Shannon E, You S-H, Lim H-W, Everett Logan J, Nabel Christopher S, Li Y, Selvakumaran V, Won K-J, Lazar Mitchell A (2013) Deacetylase-independent function of HDAC3 in transcription and metabolism requires nuclear receptor corepressor. Mol Cell 52(6):769–782. doi:10.1016/j.molcel.2013.10.022

Sun C, Wang M, Liu X, Luo L, Li K, Zhang S, Wang Y, Yang Y, Ding F, Gu X (2014) PCAF improves glucose homeostasis by suppressing the gluconeogenic activity of PGC-1α. Cell Rep 9(6):2250–2262. doi:10.1016/j.celrep.2014.11.029

Sundaresan NR, Vasudevan P, Zhong L, Kim G, Samant S, Parekh V, Pillai VB, Ravindra PV, Gupta M, Jeevanandam V, Cunningham JM, Deng C-X, Lombard DB, Mostoslavsky R, Gupta MP (2012) The sirtuin SIRT6 blocks IGF-Akt signaling and development of cardiac hypertrophy by targeting c-Jun. Nat Med 18(11):1643–1650, http://www.nature.com/nm/journal/v18/n11/abs/nm.2961.html

Tabata M, Rodgers JT, Hall JA, Lee Y, Jedrychowski MP, Gygi SP, Puigserver P (2014) Cdc2-like kinase 2 suppresses hepatic fatty acid oxidation and ketogenesis through disruption of the PGC-1α and MED1 complex. Diabetes 63(5):1519–1532. doi:10.2337/db13-1304

Toubal A, Clement K, Fan R, Ancel P, Pelloux V, Rouault C, Veyrie N, Hartemann A, Treuter E, Venteclef N (2013) SMRT-GPS2 corepressor pathway dysregulation coincides with obesity-linked adipocyte inflammation. J Clin Invest 123(1):362–379

Treuter E, Venteclef N (2011) Transcriptional control of metabolic and inflammatory pathways by nuclear receptor SUMOylation. Biochim Biophys Acta 1812(8):909–918. doi:10.1016/j.bbadis.2010.12.008

Urano T, Shiraki M, Sasaki N, Ouchi Y, Inoue S (2014) Large-scale analysis reveals a functional single-nucleotide polymorphism in the 5′-flanking region of PRDM16 gene associated with lean body mass. Aging Cell 13(4):739–743. doi:10.1111/acel.12228

Venteclef N, Jakobsson T, Ehrlund A, Damdimopoulos A, Mikkonen L, Ellis E, Nilsson L-M, Parini P, Janne OA, Gustafsson J-A, Steffensen KR, Treuter E (2010) GPS2-dependent corepressor/SUMO pathways govern anti-inflammatory actions of LRH-1 and LXRβ in the hepatic acute phase response. Genes Dev 24(4):381–395. doi:10.1101/gad.545110

Vidal M, Fields S (2014) The yeast two-hybrid assay: still finding connections after 25 years. Nat Methods 11(12):1203–1206. doi:10.1038/nmeth.3182

Villanueva Claudio J, Waki H, Godio C, Nielsen R, Chou W-L, Vargas L, Wroblewski K, Schmedt C, Chao Lily C, Boyadjian R, Mandrup S, Hevener A, Saez E, Tontonoz P (2011) TLE3 is a dual-function transcriptional coregulator of adipogenesis. Cell Metab 13 (4):413–427. doi:10.1016/j.cmet.2011.02.014

Villanueva CJ, Vergnes L, Wang J, Drew BG, Hong C, Tu Y, Hu Y, Peng X, Xu F, Saez E, Wroblewski K, Hevener AL, Reue K, Fong LG, Young SG, Tontonoz P (2013) Adipose subtype-selective recruitment of TLE3 or Prdm16 by PPARγ specifies lipid storage versus thermogenic gene programs. Cell Metab 17(3):423–435. doi:10.1016/j.cmet.2013.01.016

Wang Z, Qi C, Krones A, Woodring P, Zhu X, Reddy JK, Evans RM, Rosenfeld MG, Hunter T (2006) Critical roles of the p160 transcriptional coactivators p/CIP and SRC-1 in energy balance. Cell Metab 3(2):111–122. doi:10.1016/j.cmet.2006.01.002

Wang Y, Inoue H, Ravnskjaer K, Viste K, Miller N, Liu Y, Hedrick S, Vera L, Montminy M (2010) Targeted disruption of the CREB coactivator Crtc2 increases insulin sensitivity. Proc Natl Acad Sci 107(7):3087–3092. doi:10.1073/pnas.0914897107

Wang SCM, Dowhan DH, Eriksson NA, Muscat GEO (2012) CARM1/PRMT4 is necessary for the glycogen gene expression programme in skeletal muscle cells. Biochem J 444(2):323–331. doi:10.1042/bj20112033

Weems JC, Griesel BA, Olson AL (2012) Class II histone deacetylases downregulate GLUT4 transcription in response to increased cAMP signaling in cultured adipocytes and fasting mice. Diabetes 61(6):1404–1414. doi:10.2337/db11-0737

Wu D, Qiu Y, Gao X, Yuan X-B, Zhai Q (2011) Overexpression of SIRT1 in mouse forebrain impairs lipid/glucose metabolism and motor function. PLoS One 6(6):e21759. doi:10.1371/journal.pone.0021759

Yamamoto H, Williams Evan G, Mouchiroud L, Cantó C, Fan W, Downes M, Héligon C, Barish Grant D, Desvergne B, Evans Ronald M, Schoonjans K, Auwerx J (2011) NCoR1 is a conserved physiological modulator of muscle mass and oxidative function. Cell 147 (4):827–839. doi:10.1016/j.cell.2011.10.017

York B, Reineke Erin L, Sagen Jørn V, Nikolai Bryan C, Zhou S, Louet J-F, Chopra Atul R, Chen X, Reed G, Noebels J, Adesina Adekunle M, Yu H, Wong L-Jun C, Tsimelzon A, Hilsenbeck S, Stevens Robert D, Wenner Brett R, Ilkayeva O, Xu J, Newgard Christopher B, O'Malley Bert W (2012) Ablation of steroid receptor coactivator-3 resembles the human CACT metabolic myopathy. Cell Metab 15(5):752–763. doi:10.1016/j.cmet.2012.03.020

You S-H, Lim H-W, Sun Z, Broache M, Won K-J, Lazar MA (2013) Nuclear receptor co-repressors are required for the histone-deacetylase activity of HDAC3 in vivo. Nat Struct Mol Biol 20(2):182–187, http://www.nature.com/nsmb/journal/v20/n2/abs/nsmb.2476.html

Yuk J-M, Shin D-M, Lee H-M, Kim J-J, Kim S-W, Jin HS, Yang C-S, Park KA, Chanda D, Kim D-K, Huang SM, Lee SK, Lee C-H, Kim J-M, Song C-H, Lee SY, Hur GM, Moore DD, Choi H-S, Jo E-K (2011) The orphan nuclear receptor SHP acts as a negative regulator in inflammatory signaling triggered by Toll-like receptors. Nat Immunol 12(8):742–751, http://www.nature.com/ni/journal/v12/n8/abs/ni.2064.html

Zechner C, Lai L, Zechner JF, Geng T, Yan Z, Rumsey JW, Collia D, Chen Z, Wozniak DF, Leone TC, Kelly DP (2010) Total skeletal muscle PGC-1 deficiency uncouples mitochondrial derangements from fiber type determination and insulin sensitivity. Cell Metab 12 (6):633–642. doi:10.1016/j.cmet.2010.11.008

Zhao X, Cho H, Yu RT, Atkins AR, Downes M, Evans RM (2014) Nuclear receptors rock around the clock. EMBO Rep 15(5):518–528. doi:10.1002/embr.201338271

Incretins

Tongzhi Wu, Christopher K. Rayner, and Michael Horowitz

Contents

Abstract

Glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are the known incretin hormones in humans, released predominantly from the enteroendocrine K and L cells within the gut. Their secretion is regulated by a complex of integrated mechanisms involving direct contact for

T. Wu
Discipline of Medicine, The University of Adelaide, Royal Adelaide Hospital, North Terrace, Adelaide, South Australia 5000, Australia

C.K. Rayner (✉) • M. Horowitz
Discipline of Medicine, The University of Adelaide, Royal Adelaide Hospital, North Terrace, Adelaide, South Australia 5000, Australia

Centre of Research Excellence in Translating Nutritional Science into Good Health, The University of Adelaide, Adelaide, Australia
e-mail: chris.rayner@adelaide.edu.au

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the activation of different chemo-sensors on the brush boarder of K and L cells and several indirect neuro-immuno-hormonal loops. The biological actions of GIP and GLP-1 are fundamental determinants of islet function and blood glucose homeostasis in health and type 2 diabetes. Moreover, there is increasing recognition that GIP and GLP-1 also exert pleiotropic extra-glycaemic actions, which may represent therapeutic targets for human diseases. In this review, we summarise current knowledge of the biology of incretin hormones in health and metabolic disorders and highlight the therapeutic potential of incretin hormones in metabolic regulation.

Keywords
Extra-glycaemic actions · Glucagon-like peptide-1 · Glucose-dependent insulinotropic polypeptide · Glycaemic actions · Incretin hormones · Obesity · Type 2 diabetes

1 Introduction

In humans, glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are the two known "incretin" hormones released from the gut in response to nutrient ingestion, responsible for amplified insulin release after an oral or enteral glucose load when compared to an "isoglycaemic" intravenous glucose infusion – the so-called incretin effect (Wu et al. 2010). In health, GIP and GLP-1 together may account for up to 70% of postprandial insulin secretion after oral ingestion of glucose, dependent on the glucose load (Nauck et al. 1986). While plasma concentrations of GLP-1 after oral glucose are often lower compared with GIP, the insulinotropic potency of the former (on a molar basis) is markedly greater (Kreymann et al. 1987). Accordingly, GLP-1 and GIP appear to contribute approximately equally to the incretin effect in health (Vilsboll et al. 2003b).

Since the discovery of GIP in the 1970s (Brown 1971; Brown et al. 1975) and GLP-1 in the next decade (Drucker et al. 1987; Kreymann et al. 1987), the two hormones have enjoyed extensive investigation, which has resulted in the substantial evolution of the concept of the incretin axis. It has been increasingly recognised that the two incretin hormones do not only stimulate pancreatic β-cell secretion in a glucose-dependent manner (Creutzfeldt 1979) but also regulate numerous physiological functions within and outside the pancreas, which are key to metabolic homeostasis and, hence, are of great therapeutic interest. A major advance has been the emergence of incretin-based pharmacological and nutritional approaches to the management of type 2 diabetes, which, because of the pleiotropic actions of both incretin hormones, have further stimulated interest in

their extra-glycaemic effects. In this review, we summarise the current knowledge on the biology of incretin hormones relating to metabolic control in health and metabolic disorders and highlight the therapeutic potential of incretin hormones in metabolic regulation.

2 Synthesis, Secretion and Metabolism

Both GLP-1 and GIP are primarily synthesised in, and secreted from, the gastrointestinal tract, although the production of both hormones in pancreatic α-cells (Fujita et al. 2010; Marchetti et al. 2012) and a variety of brain regions (Gu et al. 2013; Nyberg et al. 2005) has also been described. In the human gut, GLP-1 is a product of the proglucagon gene of enteroendocrine L cells, predominantly distributed in the ileum and colon (Eissele et al. 1992), whereas GIP is mainly produced by enteroendocrine K cells, which are largely located in the duodenum and jejunum (Mortensen et al. 2003). There is also evidence that GLP-1 and GIP are co-expressed in a subset of "L/K cells", located mainly in the upper small intestine (Mortensen et al. 2003; Theodorakis et al. 2006). The translational products of proglucagon and GIP genes are processed by the prohormone convertase 1/3 (PC1/3), which results in the production of the C-terminally intact incretin hormones, i.e. GLP-1(7–37) and GIP(1–42); both forms are biologically active and stored within secretory granules. During fasting, there is a low basal secretion of GLP-1 and GIP, which is markedly increased following enteral, but not intravenous, nutrient administration (Wu et al. 2010). Upon secretion, both hormones are rapidly "inactivated" to their N-terminal metabolites, i.e. GLP-1(9–37) and GIP(3–42), by dipeptidyl peptidase-4 (DPP-4) located on the surface of the endothelial cells that are in close proximity to intestinal L and K cells (Hansen et al. 1999) and in the liver, as well as by soluble DPP-4 in the circulation (Holst 2007). Accordingly, only about 25% of newly released incretin hormones enter the portal vein in intact forms, 40–50% of which are further degraded in the liver, resulting in only 10–15% intact hormones reaching the peripheral circulation. Therefore, the half-lives for both peptides are extremely short – less than 2 min for GLP-1 (Deacon et al. 1996; Vilsboll et al. 2003a) and 2–3 min for GIP (Deacon et al. 2000; Vilsboll et al. 2006).

3 Regulation of Incretin Hormone Secretion

The low rate of GLP-1 and GIP secretion during fasting may be related to genetic predisposition (Gjesing et al. 2012) and can be modulated by exercise, inflammation (Ellingsgaard et al. 2011; Kahles et al. 2014) and circadian rhythm (Gil-Lozano et al. 2014). For example, fasting and oral glucose-stimulated GLP-1 and GIP concentrations tend to be comparable between type 2 diabetic

patients and their nondiabetic relatives (Gjesing et al. 2012). In rodents, GLP-1 secretion from both the intestine and pancreatic α-cells is increased in response to the administration of endotoxin, interleukin-1 (IL-1) and interleukin-6 (IL-6) (Kahles et al. 2014), and the exercise-induced GLP-1 secretion is IL-6 dependent (Ellingsgaard et al. 2011). Furthermore, in patients with critical illness, plasma GLP-1 concentrations are closely associated with levels of inflammatory markers (Kahles et al. 2014). Therefore, the activation of the immune system appears to be linked to the regulation of enteroendocrine secretion, although the underlying mechanisms remain unknown. A circadian secretion of GLP-1 has been reported in rodents, especially in response to oral glucose challenge (Gil-Lozano et al. 2014). In healthy humans, the responses of GLP-1 and GIP to an identical meal were shown to be greater in the morning than in the afternoon (Lindgren et al. 2009), although this may potentially be attributable to differences in gastric emptying (Goo et al. 1987).

The magnitude of variations in plasma GLP-1 and GIP concentrations during fasting is quite modest, relative to those after a meal; the latter are highly dependent on meal composition and caloric content (Vilsboll et al. 2003c), suggesting that the direct exposure of K and L cells to the luminal contents is a primary route for GIP and GLP-1 stimulation. This concept is further supported by the discovery of a diversity of chemo-sensors expressed on the luminal side of K and L cells (to be discussed) and the patterns of GIP and GLP-1 secretion in relation to the rate of gastric emptying and small intestinal transit, digestion and absorption of ingested nutrients (Wu et al. 2013e). When glucose is administered intraduodenally to mimic the range of physiological rates of gastric emptying (i.e. 1–4 kcal/min), plasma GIP increases linearly with increasing infusion rates, while the GLP-1 response is minimal to glucose infusion at rates between 1 and 2 kcal/min (Wu et al. 2014a) but increases substantially in response to infusions of 3 and 4 kcal/min (Ma et al. 2012; Trahair et al. 2012). The latter suggests that a threshold of duodenal glucose delivery of about 2 kcal/min is required to allow for the interaction with L cells located in the relatively distal region of the gut. Furthermore, the inhibition of glucose flow in the duodenum by hyoscine attenuated the secretion of GIP and GLP-1 (Chaikomin et al. 2007), and the blockade of glucose absorption from the proximal small intestine by sodium glucose co-transport-1 (SGLT-1) inhibitors resulted in a delayed, but overall substantially increased, GLP-1 response to oral glucose ingestion (Powell et al. 2013; Zambrowicz et al. 2013) – a phenomenon also seen when the malabsorption of a non-glucose carbohydrate is induced by α-glucosidase inhibitors (e.g. acarbose and miglitol) (Arakawa et al. 2008; Enc et al. 2001; Qualmann et al. 1995).

Both GIP and GLP-1 are secreted in response to each of the macronutrients – carbohydrates, fats and proteins – with carbohydrate and fat generally being more potent (Wu et al. 2010). It is worth mentioning that fructose, a widely used

sweetener in the food industry, exhibits quite distinct effects on incretin hormone secretion compared with glucose. It modestly stimulates GLP-1, with little, if any, effect on GIP secretion (Kuhre et al. 2014). In rodents, the combination of fat and carbohydrate is reported to have an additive effect on incretin secretion, suggesting the possible involvement of different sensing pathways between macronutrients (Lu et al. 2007). Indeed, chemo-sensors present on the brush border of K and L cells have been found to be responsible for sensing different nutrient components. For example, carbohydrates can be detected by sweet taste receptors (STRs) and SGLT-1 (Wu et al. 2012; Young et al. 2013), although the activation of STRs alone is insufficient to induce incretin secretion in humans (Ma et al. 2009a; Wu et al. 2013a). G-protein-coupled receptors (GPRs), including GPR119, GPR120 and GPR40, have now been identified to play a role in fatty acid sensing, particularly long-chain monounsaturated nonesterified fatty acids (NEFA) (Brennan et al. 2011; Little et al. 2014). The mechanism of protein sensing by enteroendocrine cells is poorly defined, although a proton-coupled di- and tripeptide transporter, oligopeptide transporter 1 (PepT1), is a potential candidate based on in vitro studies (Wu et al. 2010). Notably, many ingested macronutrients require digestion in order to activate enteroendocrine cells; therefore, the immediate responses of GIP and GLP-1 to complex carbohydrates and fats are attenuated by α-glucosidase and lipase inhibitors, respectively, whereas the supplementation of pancreatic enzymes to patients with pancreatic exocrine insufficiency augments postprandial incretin hormone secretion after a meal (Kuo et al. 2011; Perano et al. 2014). Accordingly, the pattern and amplitude of incretin hormone secretion during the postprandial phase are modulated by nutrient transit (i.e. gastric emptying and small intestinal transit) and digestion, the products of which activate specific chemo-sensors on the enteroendocrine cells to induce enteroendocrine secretion.

It may appear paradoxical that an early and transient increase in plasma GLP-1 levels can occur even when glucose is administered at a relatively low physiological rate (1 kcal/min) into the duodenum (Kuo et al. 2008); in this setting, L cells in the distal gut are unlikely to be exposed to glucose. One explanation is that there might be a small, but sufficient, quantity of enteroendocrine cells located in the proximal small intestine expressing GLP-1 (Theodorakis et al. 2006). Moreover, complex neuroendocrine loops have also been suggested to be indirectly involved in the regulation of incretin hormone secretion after meals. In rodents, a vagal cholinergic tone is essential for both basal and nutrient-stimulated GLP-1 secretion (Mulherin et al. 2011; Rocca and Brubaker 1999). However, in truncally vagotomised human subjects with pyloroplasty, basal incretin concentrations are unaffected, and oral glucose-induced GIP and GLP-1 secretion is increased when compared to healthy controls, presumably as a result of more rapid gastric emptying due to pyloroplasty (Plamboeck et al. 2013). Therefore, vagal innervation does not appear to play a major role in the regulation of incretin hormone secretion in

humans. In agreement with this notion, plasma GLP-1 concentrations are not affected by sham feeding (Wettergren et al. 1994). A proximal-distal endocrine loop that stimulates GLP-1 secretion has also been suggested. Although GIP was shown to increase GLP-1 secretion from L cells in vitro and in vivo in rodent models (Rocca and Brubaker 1999), exogenous GIP, even at high doses, does not affect plasma GLP-1 concentrations in humans (Edholm et al. 2010; Lee et al. 2013). In contrast, the antagonism of cholecystokinin (CCK) type 1 receptor attenuates the GLP-1 response to intraduodenal fat infusion (Beglinger et al. 2010), indicating that CCK is an "upper gut signal" for the stimulation of GLP-1 secretion.

There is evidence that incretin hormone secretion is tonically regulated by a negative feedback loop. For example, intravenous infusion of GLP-1 was shown to suppress endogenous GLP-1 production (Toft-Nielsen et al. 1999). Furthermore, DPP-4 inhibition, which results in increased plasma concentrations of intact incretins, is associated with reductions in total incretin levels, indicative of overall suppression on incretin secretion (Wu et al. 2014a). Conversely, the antagonism of GLP-1 signalling by exendin(9–39) results in an exaggerated peptide YY (PYY) secretion in response to intraduodenal lipid infusion in healthy humans (Steinert et al. 2014b), indicating that the feedback regulation is likely to be cell-, but not hormone-, specific, since PYY is synthesised and released from different organelles of L cells (Cho et al. 2014). Consistent with this concept, intravenous administration of PYY decreases plasma GLP-1 concentrations in humans (De Silva et al. 2011).

In summary, the secretion of the incretin hormones is controlled by a complex of direct and indirect mechanisms that remain incompletely understood and may be further complicated by the potential influence of disease conditions, including obesity and type 2 diabetes.

4 Incretin Secretion in Obesity and Type 2 Diabetes

Several pooled analyses that allow stratifying by different confounders (Calanna et al. 2013; Nauck et al. 2011b), and more controlled studies with fewer confounding factors, e.g. employment of intraduodenal infusion of nutrients at fixed physiological rates (Ma et al. 2012; Wu et al. 2014a), indicate that GLP-1 secretion is unlikely to be altered by the presence of diabetes per se but may be modestly attenuated in obesity by unclear mechanism(s) (Fig. 1). Nonetheless, it is tempting to reason that the apparent volume of distribution of GLP-1 may be greater in obesity, resulting in greater dilution of concentrations measured from the peripheral circulation. In terms of GIP, its secretion is generally found not to be affected by type 2 diabetes or obesity (Deacon and Ahren 2011).

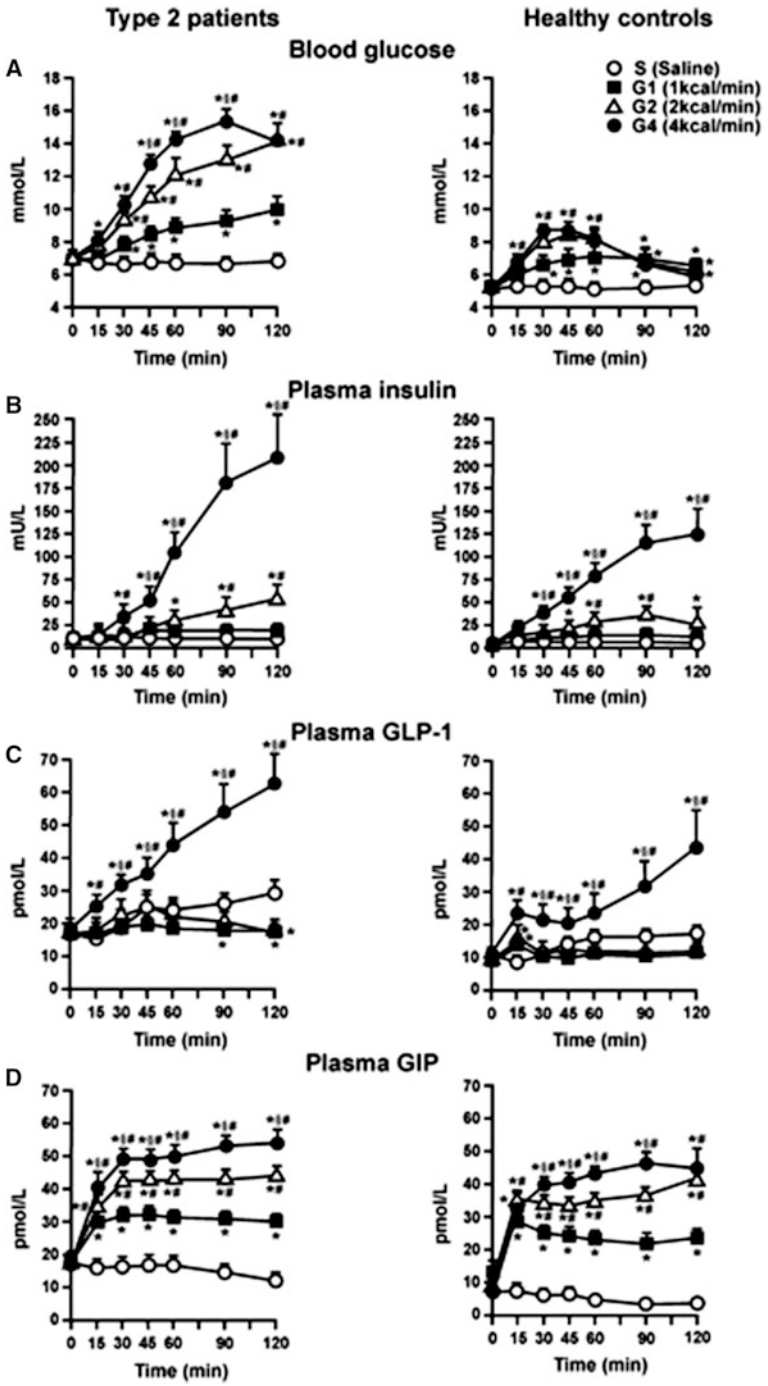

Fig. 1 Blood glucose (**a**), plasma insulin (**b**), glucagon-like peptide-1 (GLP-1) (**c**) and glucose-dependent insulinotropic polypeptide (GIP) (**d**) concentrations in response to a 120 min intraduodenal glucose infusion at 1 kcal/min (G1), 2 kcal/min (G2), 4 kcal/min (G4) or saline control (S) in healthy subjects ($n = 10$) and patients with type 2 diabetes ($n = 8$). Data are presented as mean ± SEM. *$P < 0.05$ vs. control, $^{\#}P < 0.05$ vs. G1, $^{\S}P < 0.05$ vs. G2 (adapted from Ma et al. Ma et al. 2012)

5 Metabolic Actions of Incretin Hormones

After secretion, the mechanism of incretin hormone action is by the activation of corresponding G-protein-coupled receptors (GCR), which in turn mediate pleiotropic glycaemic and extra-glycaemic metabolic actions (Table 1).

Table 1 Actions and sites of GLP-1 and GIP

	GLP-1	GIP
Glycaemic effect	Exerts complementary glucose-lowering actions via a complex of endocrine and neurocrine pathways	Exerts glucose-stabilising actions mainly in an endocrine fashion
Pancreas	• Stimulates insulin secretion in a glucose-dependent manner	• Stimulates insulin secretion in a glucose-dependent manner
	• Enhances insulin biosynthesis	• Increases insulin biosynthesis
	• Improves β-cell proliferation and survival	• Improves β-cell proliferation and survival
	• Maintains glucose competency of β-cells	• Stimulates glucagon secretion in a glucose-dependent manner
	• Suppresses glucagon secretion in a glucose-dependent manner	
Liver	• Reduces hepatic glucose output	• Increases hepatic glucose output in the context of hypoglycaemia
Central and peripheral nervous system	• Mediates insulin secretion and suppression of endogenous glucose output	• Has no effect on appetite or food intake
	• Suppresses appetite and food intake that leads to reduction in body weight and improvement in insulin resistance	
Gastrointestinal tract	• Slows gastric emptying	• Has no effect on gastric emptying
	• Inhibits small intestinal transit and hence absorption of glucose	
Extra-glycaemic effects	Mediates a number of interrelated extra-glycaemic metabolic benefits	Exerts a number of tissue-specific extra-glycaemic effects
Adipose tissue	• Stimulates lipolysis in white adipose tissue	• Inhibits lipolysis
	• Induces thermogenesis in brown adipose tissue	• Increases lipid uptake and lipogenesis, resulting in accumulation of body fat and development of obesity
		• Has no effect on thermogenic activity of brown adipose tissue

(continued)

Table 1 (continued)

	GLP-1	GIP
Liver	• Reduces hepatic fat content by controlling hepatic lipid transport, oxidation, synthesis and secretion	• Promotes hepatic steatosis
	• Ameliorates hepatocyte apoptosis	
Cardiovascular system	• Metabolic control-associated cardiovascular benefits	• Increases splanchnic blood pooling, which may lead to a hypotensive response and an increase in heart rate
	• Improves myocardial function	
	• Improves endothelial dysfunction	
	• Elevates heart rate	
	• Regulates blood pressure in relation to meal ingestion	
Bone	• Has no clear clinical evidence on bone metabolism, although an indirect anabolic effect has been suggested in preclinical studies	• Exerts anti-osteoporotic effects in preclinical studies, which are yet to be established in humans
Others	• Exhibits neuroprotective/ neurotropic properties in preclinical studies	• Exhibits neuroprotective/ neurotropic properties in preclinical studies
	• Not clearly implicated in causing acute pancreatitis, C-cell hyperplasia or medullary thyroid cancer in humans	

5.1 Glucose Regulatory Effect

5.1.1 GLP-1

GLP-1 signalling maintains blood glucose homeostasis via a number of actions that include direct and indirect stimulation of insulin secretion, increases in insulin sensitivity and peripheral glucose disposal, suppression of glucagon secretion and inhibition of upper gastrointestinal motility (particularly gastric emptying) and food intake.

GLP-1 stimulates insulin secretion in a strict glucose-dependent manner, being magnified during hyperglycaemia and attenuated in the context of hypoglycaemia – an important feature accounting for the substantially lower risk of inducing hypoglycaemia by GLP-1-based diabetes therapies. In type 2 diabetic patients with hyperglycaemia, the stimulation of insulin secretion by intravenous GLP-1 at a pharmacological dose decreases rapidly along with glycaemic improvement and is completely abolished when plasma glucose is normalised (Mentis et al. 2011). Thus, a threshold of plasma glucose of about 4.3 mmol/L appears to be required for GLP-1 to exert an insulinotropic activity (Nauck et al. 2002).

However, the glucose dependence of this effect by GLP-1 may be uncoupled in conjunction with sulfonylurea compounds (de Heer and Holst 2007), since the administration of exenatide once a week to patients who are treated with a sulfonylurea in addition to metformin results in a substantially higher incidence of hypoglycaemia, compared with those on metformin alone (Diamant et al. 2012). GLP-1 signalling also appears to be essential to maintain the glucose competency of β-cells, i.e. the secretory response of β-cells to changes in ambient glycaemia requires intact GLP-1 signalling. In both health and type 2 diabetes, the antagonism of GLP-1 receptors with exendin(9–39) during fasting, when there is little endogenous GLP-1 secretion, attenuates insulin secretion in response to hyperglycaemia (Salehi et al. 2010; Schirra et al. 1998). Moreover, GLP-1 can restore β-cell responsiveness to sulfonylureas in patients with type 2 diabetes.

It has become clear that the insulinotropic activity of GLP-1 is mediated through the activation of receptors both within and outside pancreatic β-cells (Wu et al. 2010); the activation of neurons in the central nervous system (CNS) and the stimulation of afferent vagal fibres are implicated in the latter (Baggio and Drucker 2007; Holst 2007). However, the relative importance of each component to the insulinaemic response remains to be clarified. The binding of GLP-1 to its receptors on β-cells initiates a signal transduction cascade, involving the formation of intracellular cAMP, alteration of ion channel activity and elevation of intracellular calcium, thereby enhancing the exocytotic process of the β-cells (reviewed in Holst 2007). The activation of GLP-1 receptors on the β-cells also promotes the biosynthesis of insulin. Moreover, preclinical studies suggest that the GLP-1 signalling pathway is involved in β-cell survival and proliferation. For example, in a rodent model of diabetes, the application of the GLP-1 receptor agonist, exendin-4, was shown to enhance β-cell proliferation and neogenesis and inhibit β-cell apoptosis, thereby leading to improved β-cell mass and glucose tolerance (Maida et al. 2009). Conversely, mice lacking GLP-1 and GLP-1 receptors exhibit defective β-cell regeneration and are more susceptible to streptozotocin-induced β-cell apoptosis (Maida et al. 2009). However, the effects of GLP-1 or GLP-1 receptor agonists on β-cell mass/regeneration in humans – which would intuitively have potential for stabilising or even restoring the progressive loss of β-cell mass in the course of diabetes – have not been established. In patients with type 2 diabetes (aged in their late 50s), treatment with exenatide escalated to a maximum dose of 20 μg t.i.d. or the maximum tolerated dose for 3 years failed to exert a clinically meaningful improvement on β-cell function (Bunck et al. 2011a). However, the proliferative response of β-cells to stimuli, including GLP-1 receptor agonists, appears to be diminished with ageing (Rankin and Kushner 2009), and the turnover time for human β-cells may be up to 5 years (Butler et al. 2004). Longer-term trials are, accordingly, required to determine whether GLP-1 receptor agonists can restore β-cell mass/function in patients with type 2 diabetes.

It is noteworthy that ~90% of GLP-1 is rapidly metabolised by DPP-4 prior to entering the peripheral circulation, raising the possibility of an indirect pathway, probably involving GLP-1 sensory neurons in the intestine and hepatic portal system, underlying GLP-1-potentiated insulin release (Holst 2007). In the portal

vein of rodents, GLP-1 activates hepatic vagal afferent fibres, which in turn increases the activity of pancreatic vagal efferents leading to glucose-stimulated insulin secretion and glucose disposal (Balkan and Li 2000). In line with this view, mice with β-cell-specific GLP-1 receptor knockdown maintain normal insulinaemic and glycaemic responses to oral glucose, but both insulin secretion and glucose tolerance are impaired during intravenous glucose challenge in this model (Smith et al. 2014). Therefore, there are distinct levels of β-cell regulation by GLP-1, with a component of direct stimulation and indirect mediation through paracrine or neurocrine actions.

Of comparable importance to the regulation of blood glucose homeostasis is the glucagonostatic action of GLP-1 (Hare et al. 2010; Nicolaus et al. 2011). The effect of GLP-1 to suppress glucagon secretion is also glucose dependent, so that GLP-1 does not impair the counter-regulatory release of glucagon in the face of hypoglycaemia (Nauck et al. 2002). The mechanisms through which GLP-1 controls α-cell secretion are incompletely understood. Several pathways may be involved. Firstly, a small subset of α-cells (~20%) are found to express GLP-1 receptors (Heller et al. 1997), and GLP-1 may suppress glucagon secretion by direct activation of GLP-1 receptors on α-cells. Secondly, the stimulation of β-cell secretion has the capacity to reduce glucagon in a paracrine manner (Franklin et al. 2005), although the glucagonostatic effect of GLP-1 is preserved in patients with type 1 diabetes who by definition have markedly diminished or absent β-cell activity (Creutzfeldt et al. 1996; Dupre et al. 2004). Thirdly, GLP-1 stimulates somatostatin secretion from δ-cells, and the latter is a potent suppressor of glucagon secretion through the expression of somatostatin receptor 2 on α-cells (de Heer et al. 2008).

While GLP-1 receptor agonists (e.g. exenatide) and DPP-4 inhibitors (e.g. sitagliptin) ameliorate hyperglycaemia via inhibiting glucagon secretion, a remarkable expansion of α-cell mass associated with the use of sitagliptin or exenatide was reported in a recent study in which morphological differences in pancreata from diabetic human subjects who received incretin therapy for at least 1 year were compared to those treated with other therapies and nondiabetic subjects, raising a concerning issue of α-cell hyperplasia (Butler et al. 2013). However, there is considerable heterogeneity with regard to age, duration of diabetes, gender, BMI and history of ketoacidosis within and between the two diabetic groups, confounding the interpretation of the data. Accordingly, the data have largely been discredited. Although complete elimination of glucagon production may result in compensatory proliferation of α-cells, the rather modest reduction in plasma glucagon (20–50%) associated with DPP-4 inhibitors or GLP-1 receptor agonists has not been shown to exert a hypertrophic effect on α-cells in a large number of preclinical studies (Drucker 2013).

Pancreatic clamp experiments, where the secretion of islet hormones is tightly controlled, have shed light on the importance of the extrapancreatic actions of GLP-1 in the regulation of blood glucose homeostasis. In health, endogenous glucose production is remarkably suppressed when GLP-1 is infused intravenously to achieve physiological and supraphysiological concentrations, independent of

changes in islet hormones (Prigeon et al. 2003; Seghieri et al. 2013). Studies in rodents have established that this action is initiated predominantly at the level of the gastrointestinal tract and/or hepatic portal system and mediated via GLP-1-dependent neural communication between peripheral and central nervous systems (CNS) (Vahl et al. 2007; Waget et al. 2011). As a result, a low dose of the GLP-1 receptor antagonist, [des-His(1),Glu(9)] exendin-4, given by intraportal, but not intrajugular, infusion, induces glucose intolerance (Vahl et al. 2007). Central administration of GLP-1 also reduces hepatic glucose production and glycaemic excursions during intravenous or intraperitoneal glucose challenge, whereas central [des-His(1),Glu(9)] exendin-4 administration exerts opposite effects (Sandoval et al. 2008). However, the importance of CNS GLP-1 signalling in humans remains to be established.

In patients with type 2 diabetes, continuous infusion of GLP-1 via the subcutaneous route for 6 weeks results in a remarkable increase in peripheral insulin sensitivity (Zander et al. 2002), an effect likely to be secondary to weight loss and amelioration in glucotoxicity, rather than a direct enhancement of insulin action on peripheral glucose disposal by GLP-1 (Vella et al. 2000). The weight-lowering effect of GLP-1 is not surprising, as both GLP-1 and GLP-1 receptor agonists potently suppress energy intake (apparently unrelated to the induction of nausea) (Holst 2007). More recently, GLP-1 signalling has also been implicated in the downregulation of liquid drinking behaviour (McKay et al. 2014). These effects appear to be mediated primarily via the activation of central GLP-1 receptors in the paraventricular nucleus of the hypothalamus and central nucleus of the amygdala (Abbott et al. 2005; Turton et al. 1996). While acute administration of GLP-1 reduced diet-induced thermogenesis probably as a result of the slowing of gastric emptying and subsequent absorption in health (Flint et al. 2000), a 12-month therapy with GLP-1 receptor agonists has been reported to increase energy expenditure in patients with type 2 diabetes (Beiroa et al. 2014).

GLP-1 is also one of the key regulators of gastrointestinal motor function during the postprandial phase (Wu et al. 2013e). In health, the antagonism of GLP-1 receptors by exendin(9–39) leads to an increase in antroduodenal contractility, a reduction in pyloric motility (Schirra et al. 2006) and, accordingly, an acceleration of gastric emptying (Deane et al. 2010b). These observations support a physiological role of endogenous GLP-1 in slowing gastric emptying, although several other studies, employing small test meals or suboptimal methods to assess gastric emptying, failed to show a significant effect (Nicolaus et al. 2011; Salehi et al. 2008; Witte et al. 2011). It should also be noted that exendin(9–39), as discussed, while blocking the effect of GLP-1, elevates GLP-1 and PYY secretion in response to nutrient ingestion (Steinert et al. 2014b). Because PYY slows gastric emptying (Wu et al. 2013e), it would, to some extent, have masked the prokinetic action expected from the blockade of GLP-1 receptors in these experimental settings. Studies employing the "physiological" infusion of exogenous GLP-1 have demonstrated more consistently an inhibitory effect of GLP-1 on gastric emptying. That the reduction in postprandial glycaemia induced by exogenous GLP-1 in health and type 2 diabetes is associated with less, rather than greater, postprandial insulin secretion indicates that the slowing of gastric emptying outweighs the

insulinotropic effect on controlling postprandial glycaemia in this setting (Little et al. 2006; Nauck et al. 1997). Not surprisingly, the administration of the prokinetic agent, erythromycin, which counteracts GLP-1 action on gastric emptying, attenuates the glucose-lowering effect of GLP-1 after a meal (Meier et al. 2005b). Similarly, the slowing of gastric emptying represents a major mechanism of the short-acting GLP-1 receptor agonists, such as exenatide bd and lixisenatide, to reduce postprandial glycaemia in type 2 diabetes (Cervera et al. 2008; Lorenz et al. 2013). Moreover, the magnitude of reduction in postprandial glycaemia by exogenous GLP-1, exenatide bd or lixisenatide was related to the slowing of gastric emptying that it induced (Little et al. 2006; Lorenz et al. 2013).

The effect of GLP-1 on gastric emptying appears to be less prominent when "baseline" gastric emptying is relatively slower, with minimal or no effect when the latter is abnormally delayed (Deane et al. 2010a). There is also recent evidence that continuous exposure to GLP-1 is associated with tachyphylaxis for its effects on gastric emptying, which may occur at the level of vagal nerve (Nauck et al. 2011a; Umapathysivam et al. 2014). Consequently, the efficacy of exogenous GLP-1 for slowing gastric emptying and reducing postprandial glycaemic excursions is attenuated with prolonged infusion but can be maintained with intermittent infusion (Umapathysivam et al. 2014). Accordingly, "short-acting" GLP-1 agonists appear to have a substantial, sustained action to slow gastric emptying and be superior to "long-acting" agonists for the control of postprandial glycaemic excursions, while long-acting agonists act predominantly on preprandial blood glucose by suppression of endogenous glucose production, as well as insulinotropic and glucagonostatic effects (Horowitz et al. 2013).

Emerging evidence has also linked the activation of GLP-1 receptors to the regulation of intestinal motility (Camilleri et al. 2012; Kunkel et al. 2011). Diarrhoea is a common side effect of therapy with GLP-1 receptor agonists in type 2 diabetes, while in patients with short bowel syndrome, exenatide was reported to ameliorate diarrhoea (Kunkel et al. 2011). We have recently demonstrated that acute, intravenous infusion of exenatide to pharmacological concentrations leads to a remarkable inhibition in small intestinal motility, transit and absorption of intraduodenal glucose in both healthy and type 2 diabetic humans, indicating that changes in small intestinal motility represent an additional mechanism underlying the glucose-lowering effect of GLP-1 and its agonists (Thazhath et al. 2014). Whether these effects are subject to tachyphylaxis remains to be established.

5.1.2 GIP

Unlike GLP-1, which exerts many of its actions via both endocrine and neurocrine pathways, GIP acts mainly in an endocrine fashion through binding to the GIP receptor on islet cells to regulate blood glucose and has no effect on gastric emptying in humans (Meier et al. 2004). While the mode of its action on the β-cell is similar to that of GLP-1, GIP enhances glucagon secretion from the α-cell (Christensen et al. 2015; Christensen et al. 2014; Meier et al. 2003a). Thus,

the net glycaemic effect of GIP is largely dependent on the balance of actions on both pancreatic α- and β-cells. Furthermore, evidence from rodent studies has linked GIP signalling to fat accumulation, adipose inflammation and weight gain, which may exacerbate insulin resistance (Chen et al. 2014).

The administration of exogenous GIP is usually associated with reduced glycaemic excursions in response to either intravenous glucose or oral meals in health, due predominantly to its insulinotropic action (Asmar et al. 2010b; Edholm et al. 2010). Through binding to its receptors on the β-cell, GIP upregulates intracellular cAMP, which activates signalling cascades involving both PKA and Epac 2 for the exocytosis of insulin-containing granules. Like GLP-1, GIP in rodent models also enhances insulin gene expression and biosynthesis, as well as the survival and the mass of pancreatic β-cells, leading to improved β-cell function and glucose tolerance (Baggio and Drucker 2007; Widenmaier et al. 2010), although these effects are less robust compared with GLP-1 (Maida et al. 2009). The antagonism of GIP receptors or ablation of GIP receptors has been shown to result in impaired insulin secretion and oral glucose tolerance (Lewis et al. 2000; Maida et al. 2009; Miyawaki et al. 1999). The effect of GIP to stimulate insulin secretion is also glucose dependent. While many studies reported little, if any, effect of GIP on insulin secretion in the presence of blood glucose concentrations less than 8 mmol/L, one report showed a marked insulinaemic response to "physiological" infusion of exogenous GIP in healthy subjects when blood glucose concentrations were tightly clamped at 5, 6 and 7 mmol/L (Vilsboll et al. 2003b). In support of the latter, an incretin effect is also evident after oral fatty acid or amino acid ingestion in health when compared with intravenous iso-lipidaemic or iso-amino acidaemic infusion, in which there was little change in blood glucose concentrations (Lindgren et al. 2011; Lindgren et al. 2014). GIP may be of greater relevance to mediating the incretin effect in the case of amino acids since GLP-1 secretion is less affected in response to oral ingestion of an amino acid mixture (Lindgren et al. 2014), although that is not the case for whey protein or certain purified amino acids (Ma et al. 2009b; Steinert et al. 2014a).

The glucose-lowering effect of GIP is, however, markedly impaired in type 2 diabetes, due predominantly to impairment in insulinotropic action (Nauck et al. 1993; Wu et al. 2014a). Acute administration of GIP with GLP-1 to hyperglycaemic patients with type 2 diabetes does not stimulate greater insulin secretion or lead to better glycaemic reduction when compared with GLP-1 infusion alone (Mentis et al. 2011). Similarly, xenin-25, a neurotensin-related peptide co-released with GIP from enteroendocrine K cells and known to potentiate the stimulation of insulin secretion by GIP in health, also failed to restore the insulinotropic action of GIP in type 2 diabetes acutely (Wice et al. 2012). Therefore, the activation of the GIP receptor has not been pursued as a therapeutic target for the management of type 2 diabetes to the same extent as GLP-1.

The mechanism(s) underlying the loss of the β-cell responsivity to GIP in diabetes involves the downregulation of GIP receptor expression and/or impairment

of receptor signalling, which may be associated with glucotoxicity-induced suppression on peroxisome proliferator-activated receptor (PPAR)-γ activity (Gupta et al. 2010). In a partial pancreatectomy-induced diabetic rodent model, improving glycaemic control by phlorizin was effective in preventing reductions in islet GIP receptor mRNA transcripts and expression of receptors (Xu et al. 2007). Genome-wide association studies indicate that the genetic phenotype is also critical to the effects of GIP signalling on insulin secretion (Saxena et al. 2010). Individuals with an rs10423928 on chromosome 19q13.3 exhibit diminished GIP receptors on the β-cell and impaired insulin secretion in response to exogenous GIP, as well as glucose intolerance following an oral glucose challenge (Lyssenko et al. 2011). Therefore, both adaptive changes and genetic variations are associated with the functionality of GIP signalling in the control of β-cell secretion.

The loss of response to GIP is unlikely to represent a primary cause of diabetes but rather a consequence of the disease. Although the insulin response to GIP during a hyperglycaemic clamp was found impaired in half of the first-degree relatives of patients with type 2 diabetes (Meier et al. 2001), these subjects also demonstrated a similar impairment in the insulin response to intravenous glucose, indicating that the diminished insulin secretion observed may well reflect general impairment in β-cell function, rather than a specific defect in GIP action (Meier et al. 2003b). Moreover, women with a history of gestational diabetes exhibit a normal insulinaemic response to GIP (Meier et al. 2005a). More recently, it has been shown that the induction of even mild insulin resistance and glucose intolerance by administration of prednisolone for 12 days in healthy young subjects, without affecting β-cell function or glucose disposition index, attenuates the potentiation of insulin secretion by both exogenous GLP-1 and GIP (Hansen et al. 2012). Conversely, the normalisation of hyperglycaemia with insulin therapy for 4 weeks has the capacity to improve the insulinotropic action of GIP in patients with type 2 diabetes (Hojberg et al. 2009). Therefore, the loss of GIP action on insulin secretion is potentially reversible with improvements in glycaemic control. Nevertheless, the modest improvement in insulin response to GIP in this hyperglycaemic clamp setting (~15 mmol/L) was not associated with significantly enhanced glucose disposal, probably because GIP also stimulates glucagon secretion.

GIP receptors are localised on pancreatic α-cells, and activation of GIP receptors releases glucagon in a glucose-dependent manner in health – being prominent during euglycaemia (Meier et al. 2003a) and diminished during hyperglycaemia even within the physiological range (~8 mmol/L) (Meier et al. 2001). Similarly, the glucagonotropic action of exogenous GIP was observed to be attenuated (but not abolished) during a hyperglycaemic clamp (~12 mmol/L) in type 2 diabetes (Christensen et al. 2014). In addition, the infusion of exogenous GIP results in enhanced glucagon secretion and endogenous glucose production in response to insulin-induced hypoglycaemia in both type 1 and 2 diabetes (Christensen et al. 2015; Christensen et al. 2014), indicating that GIP may be useful for maintaining blood glucose concentrations in the face of hypoglycaemia.

5.2 Extra-glycaemic Metabolic Effects

There is increasing recognition that the activation of GLP-1 and GIP receptors in tissues essential for blood glucose homeostasis also mediates a number of interrelated extra-glycaemic actions.

5.2.1 Adipose Tissue

The expression of both GLP-1 and GIP receptors in adipose tissue remains controversial, but exposure to GLP-1 and GIP exerts distinct effects on adipocytes in both in vitro and in vivo studies. In vitro studies using both adipose 3T3-L1 cells and human primary adipocytes observed a stimulatory effect of GLP-1 on lipolysis, mediated directly via the GLP-1 receptor expressed on adipocytes (Vendrell et al. 2011). However, subcutaneous administration of GLP-1 into abdominal adipose tissue did not show any lipolytic effect in healthy humans, at least acutely (Bertin et al. 2001). Emerging data from rodent studies are indicative of a critical role for GLP-1-dependent communication between CNS and peripheral tissues in the control of white adipose tissue lipolysis, energy expenditure associated with thermogenesis in brown adipose tissue and appetite (Baggio and Drucker 2014; Campbell and Drucker 2013). In rodents, the activation of central GLP-1 receptors by intracerebroventricular (i.c.v.) GLP-1 infusion decreased adiposity, whereas the antagonism of these receptors by exendin(9–39) led to an increase in fat mass (Nogueiras et al. 2009). The reduction in peripheral fat mass in response to central GLP-1 receptor activation can also be independent of changes in energy intake and body weight, and be mediated through increased sympathetic outflow, a pathway also underlying the central control of thermogenesis in brown adipose tissue (Lockie et al. 2012). In mice, targeted injection of the GLP-1 receptor agonist, liraglutide, in the hypothalamic ventromedial nucleus (VMH) was shown to not only stimulate thermogenic activity of brown adipose tissue but also to induce the browning of white adipose tissue via the downregulation of hypothalamic AMPK (Beiroa et al. 2014). More importantly, in obese patients with type 2 diabetes, the addition of a GLP-1 receptor agonist (exenatide and liraglutide) to metformin over 1 year leads to an increase in energy expenditure, providing clinical evidence for increased energy expenditure, in addition to the inhibitory effect on energy intake, underlying the weight-lowering effect of GLP-1 receptor agonists.

While GIP does not affect the thermogenic activity of brown adipose tissue or energy intake, it promotes lipid uptake in the liver and muscle and increases lipoprotein lipase activity and lipogenesis, particularly in the presence of insulin (Irwin and Flatt 2009). In rats pretreated with a fat-rich diet, exogenous GIP markedly decreased plasma triglyceride in response to an intraduodenal lipid infusion as a result of increased uptake and deposition, whereas GIP antiserum that neutralised the actions of GIP increased plasma triglyceride (Ebert et al. 1991). In health, the administration of exogenous GIP during a hyperinsulinaemic-hyperglycaemic clamp was associated with increases in abdominal adipose tissue blood flow, glucose uptake and free fatty acid re-esterification, by which GIP may result in increased triglyceride deposition in abdominal adipose tissue (Asmar

et al. 2010a). When infused alone in the absence of an enteral nutrient load, GIP was also reported to lower plasma free fatty acids by inhibiting lipolysis and increasing re-esterification in health, although it did not reduce plasma triglyceride (Gogebakan et al. 2012). Therefore, GIP signalling, in contrast to GLP-1, is anabolic. In preclinical studies, the elimination or antagonism of GIP receptors has been shown consistently to prevent diet-induced obesity (Althage et al. 2008; Pathak et al. 2014). In high-fat-fed mice, even the partial impairment of GIP secretion induced by the truncation of the prepro-GIP gene alleviates obesity and insulin resistance (Nasteska et al. 2014). There is, accordingly, a rationale for the use of diets that have little effect on GIP secretion for the prevention/management of obesity, fatty liver and insulin resistance (Keyhani-Nejad et al. 2015).

5.2.2 Liver

In addition to their effects on adipose tissue and body weight, GLP-1 and GIP may participate directly in the regulation of hepatic fat content by controlling hepatic lipid transport, oxidation, synthesis and secretion and are becoming emerging targets for treating nonalcoholic fatty liver disease (NAFLD), although the expression of functional GLP-1 and GIP receptors in the liver remains controversial (Seino and Yabe 2013).

In both preclinical and clinical studies of NAFLD and type 2 diabetes, GLP-1 receptor agonists have been reported to reduce hepatic steatosis along with improvement in plasma levels of liver enzymes (Eguchi et al. 2014; Jendle et al. 2009; Klonoff et al. 2008; Sathyanarayana et al. 2011). Although the reduction in hepatic steatosis was often associated with substantial weight loss, one study reported a significant reduction in hepatic fat content and improvements in plasma liver enzyme levels without changes in body weight after treatment with exenatide and pioglitazone, when compared with pioglitazone monotherapy, over 12 months in patients with type 2 diabetes (Sathyanarayana et al. 2011). Exenatide was also associated with an increase in adiponectin levels, indicating that direct effect of GLP-1 signalling on hepatic lipid oxidation may underlie the attenuation of hepatic fat accumulation. In support of this concept, exendin-4 increased fatty acid β-oxidation and reduced intracellular fatty acid levels in primary hepatocytes of high-fat-fed rats (Svegliati-Baroni et al. 2011). Furthermore, exendin-4 ameliorated endoplasmic reticulum (ER) stress-related hepatocyte apoptosis induced by fatty acids (Sharma et al. 2011).

In contrast, the lipogenic/anabolic actions of GIP may be associated with progression of weight gain and hepatic steatosis. Accordingly, the administration of a GIP antagonist, (Pro(3))GIP, reduced hepatic fat content significantly and partially restored liver morphology in high-fat- and cafeteria-fed mice (Gault et al. 2007). Replacement of sucrose with palatinose, which is absorbed distally and therefore minimally stimulates GIP secretion, reduced hepatic steatosis by ~50% in wild-type mice, an effect recapitulated by the knockdown of GIP receptors (Keyhani-Nejad et al. 2015). However, the metabolic benefits by interrupting GIP signalling in humans remain to be established.

5.2.3 Cardiovascular System

The metabolic actions of incretin hormones on glycaemia, lipidaemia, obesity, insulin resistance and NAFLD discussed above are important determinants of cardiovascular risks. That variations in heart rate and blood pressure, as with the release of incretin hormones, occur after oral, rather than intravenous, glucose is indicative of the physiological importance of the incretin hormones to the regulation of cardiovascular function during the postprandial state (Wu et al. 2014b). There is compelling evidence that GLP-1 participates in the regulation of myocardial and endothelial function, heart rate and blood pressure, independent of above cardiovascular risks (extensively reviewed in Ussher and Drucker (2012) and Ussher and Drucker (2014)), while little is known with respect to GIP.

Recent studies have established that GLP-1 receptors within the cardiovascular system are localised to the atria and vascular smooth muscle cells but not the ventricle or endothelium (Kim et al. 2013; Pyke and Knudsen 2013; Pyke et al. 2014; Richards et al. 2014). These findings may partly explain the small tachycardic effect (~2–3 beats/min increase) of GLP-1 and GLP-1 receptor agonists seen in preclinical and clinical studies (Ussher and Drucker 2012) but are against a classical GLP-1 receptor-dependent improvements in ventricular or endothelial function. In support of the latter concept, the GLP-1 metabolite, GLP-1(9–36), which has an extremely low binding affinity to GLP-1 receptors and is generally thought to be "inactive", was shown to exhibit profound cardioprotective and vasodilatory properties in both wild-type and GLP-1 receptor-knockout rodents (Ban et al. 2008). In addition, native GLP-1, but not exendin-4 (a DPP-4 resistant GLP-1 receptor agonist), increased cGMP release from isolated preconstricted blood vessels and induced vasodilation (Ban et al. 2008). Therefore, the GLP-1 receptor-dependent protection of ventricular cardiomyocytes and improvements in endothelial function in vivo are likely to be indirect, mediated through mechanisms that require further clarification.

In contrast to the elevation of blood pressure evident in rodents exposed to GLP-1 and GLP-1 receptor agonists, the majority of clinical trials in type 2 diabetes and obesity have reported a reduction in both systolic and diastolic blood pressure (~4–6 mmHg), particularly in individuals with higher baseline blood pressure, and often been associated with a reduction in body weight and an increase in urinary sodium excretion (Seufert and Gallwitz 2014). The blood pressure-lowering effect is often evident before significant weight loss (Gallwitz et al. 2010). In rodents, the effects of GLP-1 and GLP-1 receptor agonists on blood pressure and heart rate are likely to be related to the GLP-1 receptor-dependent central regulation of sympathetic outflow (Yamamoto et al. 2002), while peripheral activation of GLP-1 receptors within the cardiovascular system may promote ANP secretion from the atria which would favour a reduction in blood pressure due to inhibition of renal sodium reabsorption (Kim et al. 2013). In humans, acute administration of GLP-1, even at supraphysiological doses, has no effect on heart rate, blood pressure or cardio-parasympathetic and cardio-sympathetic activity (Bharucha et al. 2008). That the expression of GLP-1 receptors is predominantly within the sinoatrial node rather than the entire atria in humans does not support a major role for

GLP-1-ANP axis in the regulation blood pressure in humans (Pyke et al. 2014). Indeed, liraglutide administration for 21 days in hypertensive patients with type 2 diabetes promoted natriuresis independent of changes on circulating ANP or blood pressure (Lovshin et al. 2015). Accordingly, the natriuretic effect associated with GLP-1 or GLP-1 receptor agonists may be related to as yet undefined actions on renal tubular cells and sodium transporters (Gutzwiller et al. 2004).

It is noteworthy that few studies have discriminated the cardiovascular actions of the incretins between the fasting and postprandial states. The latter is, however, arguably of more relevance, since GLP-1 and GIP are predominantly released postprandially. When a "physiological" dose of GLP-1 was administered with an enteral glucose load known to induce a fall in blood pressure in healthy older human subjects, the hypotensive response to an enteral glucose load was attenuated, despite an increase in superior mesenteric artery (SMA) blood flow (Trahair et al. 2014), raising a potential therapeutic implication for GLP-1 and GLP-1 receptor agonists in the management of postprandial hypotension – a common disorder, particularly in older subjects and patients with disordered autonomic function (e.g. diabetes and Parkinson's disease).

Studies investigating the cardiovascular actions of GIP are limited. While the expression of GIP receptors within the cardiovascular system remains to be further validated, intravenous GIP has been reported to increase vagal activity (Amland et al. 1985) and cause splanchnic blood pooling (Fara and Salazar 1978; Svensson et al. 1997), an elevation in heart rate and a reduction in blood pressure (Wice et al. 2012). In our recent study, in which enteral glucose was infused at a rate that induced predominantly GIP secretion, the DPP-4 inhibitor, sitagliptin, increased heart rate in patients with type 2 diabetes (Wu et al. 2014b), supporting the need for further clarification of a potential role for GIP in the control of the "gut-heart" axis.

5.2.4 Bone

GLP-1 and GIP signalling have also been implicated in bone formation. In rodents, GLP-1 does not exert a direct effect on osteoclasts or osteoblasts but activates the GLP-1 receptor on thyroid C cells to induce calcitonin secretion (Yamada et al. 2008). The latter suppresses bone resorption by osteoclasts. However, GLP-1 receptor expression on thyroid C cells is substantially less in humans than in rodents (Waser et al. 2011). Prolonged exposure to GLP-1 receptor agonists in human subjects with type 2 diabetes has not shown any effects on bone strength, bone mineral density or serum levels of calcitonin (Bunck et al. 2011b; Hegedus et al. 2011). GIP receptors are localised on both osteoblasts and osteoclasts. The activation of these receptors may lead to bone formation by osteoblasts and suppression of bone resorption by osteoclasts. Complete elimination of GIP signalling, while producing resistance to high-fat diet-induced obesity, also leads to osteoporosis in rodents (Nasteska et al. 2014; Tsukiyama et al. 2006). However, rodents with partial impairment (~50%) of GIP secretion maintained normal bone formation (Nasteska et al. 2014). Nevertheless, like GLP-1, there is little evidence

for a role of GIP in the control of bone formation in humans. In the acute setting at least, the infusion of GIP failed to affect markers of bone resorption in postmenopausal women (Henriksen et al. 2003).

5.2.5 Others

Preclinical data suggest that GLP-1 and GIP have non-metabolic neuroprotective/neurotrophic properties, which suggest therapeutic potential for neurodegenerative and cognitive CNS disorders, such as Alzheimer's disease (Campbell and Drucker 2013). These aspects, however, will not be discussed in this review due to the primary focus on metabolic control. Although rodent data raised concerns of potential of incretin signalling to induce acute pancreatitis, C-cell hyperplasia and medullary thyroid cancer, there is a lack of evidence to support these effects in humans (Drucker et al. 2011; Drucker 2013).

6 Therapeutic Implications

The recognition that there are major defects in the action of GIP to mediate insulin secretion and lower blood glucose in type 2 diabetes, and that GLP-1 maintains a considerable insulinotropic effect in type 2 diabetes and has the capacity to normalise blood glucose homeostasis at pharmacological doses via complementary mechanisms (as discussed), has promoted the focus on GLP-1-based incretin therapies for the management of type 2 diabetes. Intravenous GLP-1 may normalise glucose in type 2 diabetes, while subcutaneous administration does not (Nauck et al. 2013). The reasons for this clinically important issue are unclear but may be related to modifications of the peptide molecules and/or poor tolerance of high dose associated with subcutaneous administration. Recent observations indicate that the glucagonotropic action of GIP may serve to "rescue" hypoglycaemia in both type 1 and 2 diabetes (Christensen et al. 2015; Christensen et al. 2014), warranting the evaluation of the clinical effects of GIP receptor agonists on blood glucose homeostasis in diabetes. Paradoxically, however, the "obesogenic" potential of GIP observed in preclinical studies suggests that antagonism of GIP signalling may be useful for treating obesity.

The incretin-based pharmaceuticals currently available include GLP-1 receptor agonists that are resistant to rapid degradation by DPP-4 and DPP-4 inhibitors, which preserve plasma concentrations of endogenous intact GLP-1 after its secretion. Distinct from many traditional antidiabetic drugs (e.g. insulin), GLP-1 receptor agonists and DPP-4 inhibitors do not increase the risk of hypoglycaemia or weight gain. While the range of available DPP-4 inhibitors appears to have comparable potency on glycaemia (as well as DPP-4 inhibition) (Deacon 2011), the "short-acting" GLP-1 receptor agonists are superior to the "long-acting" agents on reducing postprandial glycaemic excursions, probably as a result of their impact on gastric emptying; the "long-acting" agents are more effective for reducing fasting hyperglycaemia and body weight (Horowitz et al. 2013). This discrepancy is important in that in the majority of patients with type 2 diabetes, who have

relatively good overall glycaemic control, postprandial glycaemia predominates over fasting blood glucose in contributing to HbA1c, and deterioration in postprandial glycaemic control precedes any substantial elevation of fasting blood glucose (Monnier et al. 2007). Therefore, optimal selection of appropriate GLP-1 receptor agonists may require careful evaluation of patient phenotype and therapeutic goals.

Until the recent approval for liraglutide in addition to lifestyle intervention as a treatment option for chronic weight management by the US Food and Drug Administration (FDA), GLP-1 receptor agonists and DPP-4 inhibitors were restricted to being a second-line option for managing inadequately controlled hyperglycaemia in type 2 diabetes managed with metformin alone (American Diabetes Association 2015). Whether incretin-based pharmaceuticals fulfil the extra-glycaemic effects (discussed above) and, accordingly, treat metabolic targets in addition to body weight and blood glucose will become apparent only from the outcomes of well-designed, long-term prospective studies powered adequately to detect superiority. Cardiovascular outcome studies of two DPP-4 inhibitors (i.e. saxagliptin and alogliptin) failed to demonstrate improvement in cardiovascular endpoints (Scirica et al. 2013; White et al. 2013). Rather, saxagliptin was associated with increased hospitalisation rates for heart failure in a small subset of patients (Scirica et al. 2013). DPP-4 inhibition potentially has a complex influence on "cardio-vaso-active" compounds in vivo; for example, it increases plasma intact GLP-1 levels but reduces overall GLP-1 secretion as a result of negative feedback control on the L cell, thereby reducing the formation of GLP-1 metabolites known to have GLP-1-receptor-independent vasodilator properties (discussed previously). Ongoing clinical trials of different GLP-1 receptor agonists will provide further insights into the cardiovascular outcomes related to manipulation of the incretin axis.

As an alternative to pharmacological compounds, approaches to enhance endogenous GLP-1 secretion appear promising, particularly as the presence of obesity or type 2 diabetes per se does not seem to be associated with a major defect of GLP-1 secretion. Because the majority of GLP-1-producing L cells are located distally within the ileum and colon, the creation of a shortcut for nutrients to access the distal gut by gastric bypass surgery, facilitated by more rapid small intestinal transit, is associated with marked improvements in GLP-1 secretion (Laferrere et al. 2007; Laferrere et al. 2008), accounting at least in part for the improvement of glucose homeostasis in type 2 diabetes after this operation (Shah et al. 2014). Conversely, the blockade of the GLP-1 receptor with exendin(9–39) can effectively correct recurrent hypoglycaemia associated with gastric bypass surgery (Salehi et al. 2014). Alternatively, targeted delivery of even a small nutrient stimulus to the distal gut may provide sufficiently great GLP-1 stimulation to reduce postprandial glycaemic excursions. This concept is supported by the finding of enhanced GLP-1 secretion with acarbose-induced malabsorption of carbohydrates (Gentilcore et al. 2005; Qualmann et al. 1995; Seifarth et al. 1998) and, more recently, the effects of delivery of a small amount of lauric acid to the distal gut via enteric coated pellets in patients with type 2 diabetes (Ma et al. 2013). In addition, a small nutrient-based "preload" can be consumed, in order to stimulate secretion of

enteroendocrine hormones such as GLP-1, in advance of the main meal, with resultant slowing of gastric emptying and attenuation of the postprandial glycaemic excursion (Wu et al. 2012; Wu et al. 2013d). Other physiological ligands of enteroendocrine L cells (e.g. bile acids) are worthy of evaluation for their potential to restore metabolic homeostasis in obesity and type 2 diabetes (Adrian et al. 2012; Wu et al. 2013b; Wu et al. 2013c). Moreover, the glucose-lowering effect of initiatives to stimulate endogenous GLP-1 secretion can potentially be further optimised by concurrent administration of a DPP-4 inhibitor (Wu et al. 2013d), highlighting the potential for complementary actions between dietary and pharmacological interventions (Fig. 2). However, long-term studies are required to evaluate the clinical efficacy of these novel approaches in the management of type 2 diabetes and obesity. While the stimulation of endogenous GIP secretion seems to have little gluco-regulatory advantage in type 2 diabetes, the manipulation of nutrient composition in order to attenuate secretion of GIP was recently reported to prevent diet-induced obesity, fatty liver and insulin resistance in a rodent model, recapitulating the metabolic phenotype seen in GIP receptor-knockout animals

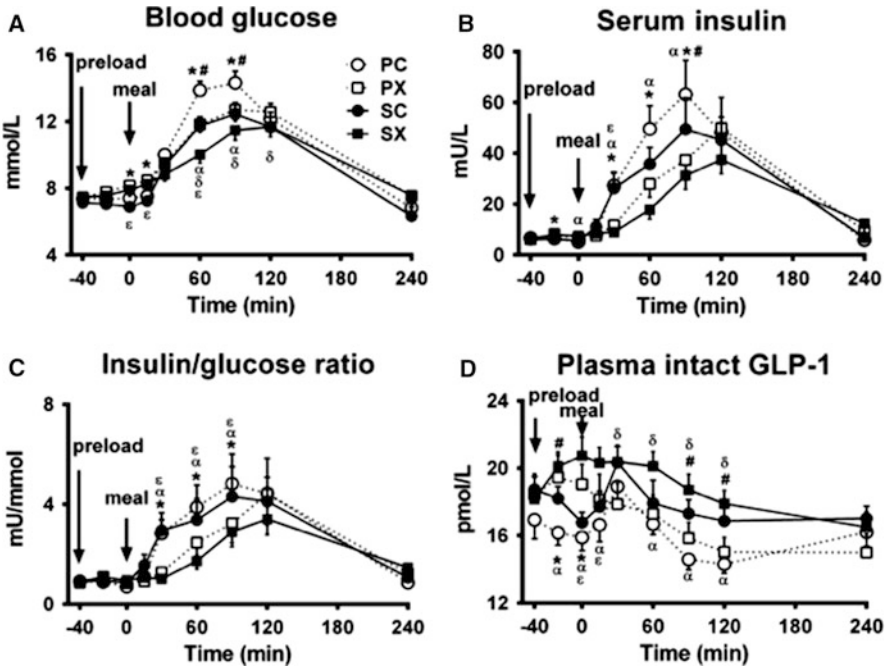

Fig. 2 Effects of D-xylose or sucralose (control), with or without sitagliptin, on blood glucose (**a**), serum insulin (**b**), insulin/glucose ratio (**c**) and plasma intact glucagon-like peptide-1 (GLP-1) (**d**) in response to a carbohydrate meal ($n = 12$). The four treatments were sitagliptin + D-xylose (SX), sitagliptin + control (SC), placebo + D-xylose (PX) and placebo + control (PC), respectively. Data are means ± SEM. $P = 0.000$ for each treatment × time interaction; $^{*}P < 0.05$, PX vs. PC; $^{\#}P < 0.05$, SC vs. PC; $^{\alpha}P < 0.05$, SX vs. PC; $^{\delta}P < 0.05$, SX vs. PX; $^{\varepsilon}P < 0.05$, SX vs. SC (adapted from Wu et al. 2013d)

(Keyhani-Nejad et al. 2015). Therefore, future human studies should address the therapeutic potential of lowering GIP secretion for weight loss, with careful monitoring of bone health given the potential effect of GIP on bone formation.

7 Conclusions

Subsequent to the discovery of GLP-1 and GIP as the primary incretin hormones in humans, incretin-based interventions are translated rapidly to clinical therapies, owing to scientific advances in the understanding of the biology of incretin synthesis, metabolism, secretion and actions. Secretion of incretin hormones during both fasting and postprandial states is regulated by integrated direct and indirect mechanisms. Although several neuro-immuno-hormonal loops are likely to be of relevance to the release of incretins, the major determinant appears to be the direct stimulation by nutrients in contact with enteroendocrine K and L cells, with the magnitude and pattern of responses dependent on the load and properties (i.e. nutrient composition) of the meals, gastrointestinal transit and the capacity of digestion of macronutrients. While the secretion of GLP-1 and GIP does not appear to be greatly impaired in obesity and diabetes, the latter are recognised to result in impaired insulinotropic and glucose-lowering effects of GIP. GLP-1 now has an established role as an effective glucose-lowering agent based on complementary actions to improve blood glucose control in health and type 2 diabetes. That GLP-1 and GIP receptors are expressed in a variety of tissues not limited to glycaemic control has further stimulated interest in extra-glycaemic actions that may have translational potential. In addition to the two therapeutic classes currently available, there has been substantial interest in the development of nutritional approaches to modulate endogenous GLP-1 and GIP secretion in order to restore metabolic homeostasis. These novel concepts await further evaluation of their efficacy and safety in long-term, prospective clinical trials.

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References

Abbott CR, Monteiro M, Small CJ, Sajedi A, Smith KL, Parkinson JR, Ghatei MA, Bloom SR (2005) The inhibitory effects of peripheral administration of peptide YY(3-36) and glucagon-like peptide-1 on food intake are attenuated by ablation of the vagal-brainstem-hypothalamic pathway. Brain Res 1044:127–131

Adrian TE, Gariballa S, Parekh KA, Thomas SA, Saadi H, Al Kaabi J, Nagelkerke N, Gedulin B, Young AA (2012) Rectal taurocholate increases L cell and insulin secretion, and decreases blood glucose and food intake in obese type 2 diabetic volunteers. Diabetologia 55:2343–2347

Althage MC, Ford EL, Wang S, Tso P, Polonsky KS, Wice BM (2008) Targeted ablation of glucose-dependent insulinotropic polypeptide-producing cells in transgenic mice reduces obesity and insulin resistance induced by a high fat diet. J Biol Chem 283:18365–18376

American Diabetes Association (2015) Approaches to glycemic treatment. Diabetes Care 38:S41–S48

Amland PF, Jorde R, Aanderud S, Burhol PG, Giercksky KE (1985) Effects of intravenously infused porcine GIP on serum insulin, plasma C-peptide, and pancreatic polypeptide in non-insulin-dependent diabetes in the fasting state. Scand J Gastroenterol 20:315–320

Arakawa M, Ebato C, Mita T, Fujitani Y, Shimizu T, Watada H, Kawamori R, Hirose T (2008) Miglitol suppresses the postprandial increase in interleukin 6 and enhances active glucagon-like peptide 1 secretion in viscerally obese subjects. Metabolism 57:1299–1306

Asmar M, Simonsen L, Madsbad S, Stallknecht B, Holst JJ, Bulow J (2010a) Glucose-dependent insulinotropic polypeptide may enhance fatty acid re-esterification in subcutaneous abdominal adipose tissue in lean humans. Diabetes 59:2160–2163

Asmar M, Tangaa W, Madsbad S, Hare K, Astrup A, Flint A, Bulow J, Holst JJ (2010b) On the role of glucose-dependent insulinotropic polypeptide in postprandial metabolism in humans. Am J Physiol Endocrinol Metab 298:E614–E621

Baggio LL, Drucker DJ (2007) Biology of incretins: GLP-1 and GIP. Gastroenterology 132:2131–2157

Baggio LL, Drucker DJ (2014) Glucagon-like peptide-1 receptors in the brain: controlling food intake and body weight. J Clin Invest 124:4223–4226

Balkan B, Li X (2000) Portal GLP-1 administration in rats augments the insulin response to glucose via neuronal mechanisms. Am J Physiol Regul Integr Comp Physiol 279:R1449–R1454

Ban K, Noyan-Ashraf MH, Hoefer J, Bolz SS, Drucker DJ, Husain M (2008) Cardioprotective and vasodilatory actions of glucagon-like peptide 1 receptor are mediated through both glucagon-like peptide 1 receptor-dependent and -independent pathways. Circulation 117:2340–2350

Beglinger S, Drewe J, Schirra J, Goke B, D'Amato M, Beglinger C (2010) Role of fat hydrolysis in regulating glucagon-like Peptide-1 secretion. J Clin Endocrinol Metab 95:879–886

Beiroa D, Imbernon M, Gallego R, Senra A, Herranz D, Villarroya F, Serrano M, Ferno J, Salvador J, Escalada J, Dieguez C, Lopez M, Fruhbeck G, Nogueiras R (2014) GLP-1 agonism stimulates brown adipose tissue thermogenesis and browning through hypothalamic AMPK. Diabetes 63:3346–3358

Bertin E, Arner P, Bolinder J, Hagstrom-Toft E (2001) Action of glucagon and glucagon-like peptide-1-(7-36) amide on lipolysis in human subcutaneous adipose tissue and skeletal muscle in vivo. J Clin Endocrinol Metab 86:1229–1234

Bharucha AE, Charkoudian N, Andrews CN, Camilleri M, Sletten D, Zinsmeister AR, Low PA (2008) Effects of glucagon-like peptide-1, yohimbine, and nitrergic modulation on sympathetic and parasympathetic activity in humans. Am J Physiol Regul Integr Comp Physiol 295:R874–R880

Brennan IM, Seimon RV, Luscombe-Marsh ND, Otto B, Horowitz M, Feinle-Bisset C (2011) Effects of acute dietary restriction on gut motor, hormone and energy intake responses to duodenal fat in obese men. Int J Obes (Lond) 35:448–456

Brown JC (1971) A gastric inhibitory polypeptide. I. The amino acid composition and the tryptic peptides. Can J Biochem 49:255–261

Brown JC, Dryburgh JR, Ross SA, Dupre J (1975) Identification and actions of gastric inhibitory polypeptide. Recent Prog Horm Res 31:487–532

Bunck MC, Corner A, Eliasson B, Heine RJ, Shaginian RM, Taskinen MR, Smith U, Yki-Jarvinen H, Diamant M (2011a) Effects of exenatide on measures of beta-cell function after 3 years in metformin-treated patients with type 2 diabetes. Diabetes Care 34:2041–2047

Bunck MC, Eliasson B, Corner A, Heine RJ, Shaginian RM, Taskinen MR, Yki-Jarvinen H, Smith U, Diamant M (2011b) Exenatide treatment did not affect bone mineral density despite body weight reduction in patients with type 2 diabetes. Diabetes Obes Metab 13:374–377

Butler PC, Ritzel R, Butler AE, Ritzel RA (2004) Islet turnover in lean and obese humans. Diabetes 53:A356

Butler AE, Campbell-Thompson M, Gurlo T, Dawson DW, Atkinson M, Butler PC (2013) Marked expansion of exocrine and endocrine pancreas with incretin therapy in humans with increased exocrine pancreas dysplasia and the potential for glucagon-producing neuroendocrine tumors. Diabetes 62:2595–2604

Calanna S, Christensen M, Holst JJ, Laferrere B, Gluud LL, Vilsboll T, Knop FK (2013) Secretion of glucagon-like peptide-1 in patients with type 2 diabetes mellitus: systematic review and meta-analyses of clinical studies. Diabetologia 56:965–972

Camilleri M, Vazquez-Roque M, Iturrino J, Boldingh A, Burton D, McKinzie S, Wong BS, Rao AS, Kenny E, Mansson M, Zinsmeister AR (2012) Effect of a glucagon-like peptide 1 analog, ROSE-010, on GI motor functions in female patients with constipation-predominant irritable bowel syndrome. Am J Physiol Gastrointest Liver Physiol 303:G120–G128

Campbell JE, Drucker DJ (2013) Pharmacology, physiology, and mechanisms of incretin hormone action. Cell Metab 17(6):819–837

Cervera A, Wajcberg E, Sriwijitkamol A, Fernandez M, Zuo P, Triplitt C, Musi N, DeFronzo RA, Cersosimo E (2008) Mechanism of action of exenatide to reduce postprandial hyperglycemia in type 2 diabetes. Am J Physiol Endocrinol Metab 294:E846–E852

Chaikomin R, Wu KL, Doran S, Jones KL, Smout AJ, Renooij W, Holloway RH, Meyer JH, Horowitz M, Rayner CK (2007) Concurrent duodenal manometric and impedance recording to evaluate the effects of hyoscine on motility and flow events, glucose absorption, and incretin release. Am J Physiol Gastrointest Liver Physiol 292:G1099–G1104

Chen S, Okahara F, Osaki N, Shimotoyodome A (2014) Increased GIP signaling induces adipose inflammation via a HIF-1alpha-dependent pathway and impairs insulin sensitivity in mice. Am J Physiol Endocrinol Metab 308(5):E414–E425

Cho HJ, Robinson ES, Rivera LR, McMillan PJ, Testro A, Nikfarjam M, Bravo DM, Furness JB (2014) Glucagon-like peptide 1 and peptide YY are in separate storage organelles in enteroendocrine cells. Cell Tissue Res 357(1):63–69

Christensen MB, Calanna S, Holst JJ, Vilsboll T, Knop FK (2014) Glucose-dependent insulinotropic polypeptide: blood glucose stabilizing effects in patients with type 2 diabetes. J Clin Endocrinol Metab 99:E418–E426

Christensen M, Calanna S, Sparre-Ulrich AH, Kristensen PL, Rosenkilde MM, Faber J, Purrello F, van Hall G, Holst JJ, Vilsboll T, Knop FK (2015) Glucose-dependent insulinotropic polypeptide augments glucagon responses to hypoglycemia in type 1 diabetes. Diabetes 64:72–78

Creutzfeldt W (1979) The incretin concept today. Diabetologia 16:75–85

Creutzfeldt WO, Kleine N, Willms B, Orskov C, Holst JJ, Nauck MA (1996) Glucagonostatic actions and reduction of fasting hyperglycemia by exogenous glucagon-like peptide I(7-36) amide in type I diabetic patients. Diabetes Care 19:580–586

de Heer J, Holst JJ (2007) Sulfonylurea compounds uncouple the glucose dependence of the insulinotropic effect of glucagon-like peptide 1. Diabetes 56:438–443

de Heer J, Rasmussen C, Coy DH, Holst JJ (2008) Glucagon-like peptide-1, but not glucose-dependent insulinotropic peptide, inhibits glucagon secretion via somatostatin (receptor subtype 2) in the perfused rat pancreas. Diabetologia 51:2263–2270

De Silva A, Salem V, Long CJ, Makwana A, Newbould RD, Rabiner EA, Ghatei MA, Bloom SR, Matthews PM, Beaver JD, Dhillo WS (2011) The gut hormones PYY 3-36 and GLP-1 7-36 amide reduce food intake and modulate brain activity in appetite centers in humans. Cell Metab 14:700–706

Deacon CF (2011) Dipeptidyl peptidase-4 inhibitors in the treatment of type 2 diabetes: a comparative review. Diabetes Obes Metab 13:7–18

Deacon CF, Ahren B (2011) Physiology of incretins in health and disease. Rev Diabet Stud 8:293–306

Deacon CF, Pridal L, Klarskov L, Olesen M, Holst JJ (1996) Glucagon-like peptide 1 undergoes differential tissue-specific metabolism in the anesthetized pig. Am J Physiol 271:E458–E464

Deacon CF, Nauck MA, Meier J, Hucking K, Holst JJ (2000) Degradation of endogenous and exogenous gastric inhibitory polypeptide in healthy and in type 2 diabetic subjects as revealed using a new assay for the intact peptide. J Clin Endocrinol Metab 85:3575–3581

Deane AM, Chapman MJ, Fraser RJ, Summers MJ, Zaknic AV, Storey JP, Jones KL, Rayner CK, Horowitz M (2010a) Effects of exogenous glucagon-like peptide-1 on gastric emptying and glucose absorption in the critically ill: relationship to glycemia. Crit Care Med 38:1261–1269

Deane AM, Nguyen NQ, Stevens JE, Fraser RJ, Holloway RH, Besanko LK, Burgstad C, Jones KL, Chapman MJ, Rayner CK, Horowitz M (2010b) Endogenous glucagon-like peptide-1 slows gastric emptying in healthy subjects, attenuating postprandial glycemia. J Clin Endocrinol Metab 95:215–221

Diamant M, Van Gaal L, Stranks S, Guerci B, MacConell L, Haber H, Scism-Bacon J, Trautmann M (2012) Safety and efficacy of once-weekly exenatide compared with insulin glargine titrated to target in patients with type 2 diabetes over 84 weeks. Diabetes Care 35:683–689

Drucker DJ (2013) Incretin action in the pancreas: potential promise, possible perils, and pathological pitfalls. Diabetes 62:3316–3323

Drucker DJ, Philippe J, Mojsov S, Chick WL, Habener JF (1987) Glucagon-like peptide I stimulates insulin gene expression and increases cyclic AMP levels in a rat islet cell line. Proc Natl Acad Sci U S A 84:3434–3438

Drucker DJ, Sherman SI, Bergenstal RM, Buse JB (2011) The safety of incretin-based therapies–review of the scientific evidence. J Clin Endocrinol Metab 96:2027–2031

Dupre J, Behme MT, McDonald TJ (2004) Exendin-4 normalized postcibal glycemic excursions in type 1 diabetes. J Clin Endocrinol Metab 89:3469–3473

Ebert R, Nauck M, Creutzfeldt W (1991) Effect of exogenous or endogenous gastric inhibitory polypeptide (GIP) on plasma triglyceride responses in rats. Horm Metab Res 23:517–521

Edholm T, Degerblad M, Gryback P, Hilsted L, Holst JJ, Jacobsson H, Efendic S, Schmidt PT, Hellstrom PM (2010) Differential incretin effects of GIP and GLP-1 on gastric emptying, appetite, and insulin-glucose homeostasis. Neurogastroenterol Motil 22(1191–200):e315

Eguchi Y, Kitajima Y, Hyogo H, Takahashi H, Kojima M, Ono M, Araki N, Tanaka K, Yamaguchi M, Matsuda Y, Ide Y, Otsuka T, Ozaki I, Ono N, Eguchi T, Anzai K, Japan Study Group for NAFLD (JSG-NAFLD) (2014) Pilot study of liraglutide effects in non-alcoholic steatohepatitis and non-alcoholic fatty liver disease with glucose intolerance in Japanese patients (LEAN-J). Hepatol Res 45(3):269–278

Eissele R, Goke R, Willemer S, Harthus HP, Vermeer H, Arnold R, Goke B (1992) Glucagon-like peptide-1 cells in the gastrointestinal tract and pancreas of rat, pig and man. Eur J Clin Invest 22:283–291

Ellingsgaard H, Hauselmann I, Schuler B, Habib AM, Baggio LL, Meier DT, Eppler E, Bouzakri K, Wueest S, Muller YD, Hansen AM, Reinecke M, Konrad D, Gassmann M, Reimann F, Halban PA, Gromada J, Drucker DJ, Gribble FM, Ehses JA, Donath MY (2011) Interleukin-6 enhances insulin secretion by increasing glucagon-like peptide-1 secretion from L cells and alpha cells. Nat Med 17:1481–1489

Enc FY, Imeryuz N, Akin L, Turoglu T, Dede F, Haklar G, Tekesin N, Bekiroglu N, Yegen BC, Rehfeld JF, Holst JJ, Ulusoy NB (2001) Inhibition of gastric emptying by acarbose is correlated with GLP-1 response and accompanied by CCK release. Am J Physiol Gastrointest Liver Physiol 281:G752–G763

Fara JW, Salazar AM (1978) Gastric inhibitory polypeptide increases mesenteric blood flow. Proc Soc Exp Biol Med 158:446–448

Flint A, Raben A, Rehfeld JF, Holst JJ, Astrup A (2000) The effect of glucagon-like peptide-1 on energy expenditure and substrate metabolism in humans. Int J Obes Relat Metab Disord 24:288–298

Franklin I, Gromada J, Gjinovci A, Theander S, Wollheim CB (2005) Beta-cell secretory products activate alpha-cell ATP-dependent potassium channels to inhibit glucagon release. Diabetes 54:1808–1815

Fujita Y, Wideman RD, Asadi A, Yang GK, Baker R, Webber T, Zhang T, Wang R, Ao Z, Warnock GL, Kwok YN, Kieffer TJ (2010) Glucose-dependent insulinotropic polypeptide is expressed in pancreatic islet alpha-cells and promotes insulin secretion. Gastroenterology 138:1966–1975

Gallwitz B, Vaag A, Falahati A, Madsbad S (2010) Adding liraglutide to oral antidiabetic drug therapy: onset of treatment effects over time. Int J Clin Pract 64:267–276

Gault VA, McClean PL, Cassidy RS, Irwin N, Flatt PR (2007) Chemical gastric inhibitory polypeptide receptor antagonism protects against obesity, insulin resistance, glucose intolerance and associated disturbances in mice fed high-fat and cafeteria diets. Diabetologia 50:1752–1762

Gentilcore D, Bryant B, Wishart JM, Morris HA, Horowitz M, Jones KL (2005) Acarbose attenuates the hypotensive response to sucrose and slows gastric emptying in the elderly. Am J Med 118:1289

Gil-Lozano M, Mingomataj EL, Wu WK, Ridout SA, Brubaker PL (2014) Circadian secretion of the intestinal hormone GLP-1 by the rodent L cell. Diabetes 63:3674–3685

Gjesing AP, Ekstrom CT, Eiberg H, Urhammer SA, Holst JJ, Pedersen O, Hansen T (2012) Fasting and oral glucose-stimulated levels of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are highly familial traits. Diabetologia 55:1338–1345

Gogebakan O, Andres J, Biedasek K, Mai K, Kuhnen P, Krude H, Isken F, Rudovich N, Osterhoff MA, Kintscher U, Nauck M, Pfeiffer AF, Spranger J (2012) Glucose-dependent insulinotropic polypeptide reduces fat-specific expression and activity of 11beta-hydroxysteroid dehydrogenase type 1 and inhibits release of free fatty acids. Diabetes 61:292–300

Goo RH, Moore JG, Greenberg E, Alazraki NP (1987) Circadian variation in gastric emptying of meals in humans. Gastroenterology 93:515–518

Gu G, Roland B, Tomaselli K, Dolman CS, Lowe C, Heilig JS (2013) Glucagon-like peptide-1 in the rat brain: distribution of expression and functional implication. J Comp Neurol 521:2235–2261

Gupta D, Peshavaria M, Monga N, Jetton TL, Leahy JL (2010) Physiologic and pharmacologic modulation of glucose-dependent insulinotropic polypeptide (GIP) receptor expression in beta-cells by peroxisome proliferator-activated receptor (PPAR)-gamma signaling: possible mechanism for the GIP resistance in type 2 diabetes. Diabetes 59:1445–1450

Gutzwiller JP, Tschopp S, Bock A, Zehnder CE, Huber AR, Kreyenbuehl M, Gutmann H, Drewe J, Henzen C, Goeke B, Beglinger C (2004) Glucagon-like peptide 1 induces natriuresis in healthy subjects and in insulin-resistant obese men. J Clin Endocrinol Metab 89:3055–3061

Hansen L, Deacon CF, Orskov C, Holst JJ (1999) Glucagon-like peptide-1-(7-36)amide is transformed to glucagon-like peptide-1-(9-36)amide by dipeptidyl peptidase IV in the capillaries supplying the L cells of the porcine intestine. Endocrinology 140:5356–5363

Hansen KB, Vilsboll T, Bagger JI, Holst JJ, Knop FK (2012) Impaired incretin-induced amplification of insulin secretion after glucose homeostatic dysregulation in healthy subjects. J Clin Endocrinol Metab 97:1363–1370

Hare KJ, Vilsboll T, Asmar M, Deacon CF, Knop FK, Holst JJ (2010) The glucagonostatic and insulinotropic effects of glucagon-like peptide 1 contribute equally to its glucose-lowering action. Diabetes 59:1765–1770

Hegedus L, Moses AC, Zdravkovic M, Le Thi T, Daniels GH (2011) GLP-1 and calcitonin concentration in humans: lack of evidence of calcitonin release from sequential screening in over 5000 subjects with type 2 diabetes or nondiabetic obese subjects treated with the human GLP-1 analog, liraglutide. J Clin Endocrinol Metab 96:853–860

Heller RS, Kieffer TJ, Habener JF (1997) Insulinotropic glucagon-like peptide I receptor expression in glucagon-producing alpha-cells of the rat endocrine pancreas. Diabetes 46:785–791

Henriksen DB, Alexandersen P, Bjarnason NH, Vilsboll T, Hartmann B, Henriksen EE, Byrjalsen I, Krarup T, Holst JJ, Christiansen C (2003) Role of gastrointestinal hormones in postprandial reduction of bone resorption. J Bone Miner Res 18:2180–2189

Hojberg PV, Vilsboll T, Rabol R, Knop FK, Bache M, Krarup T, Holst JJ, Madsbad S (2009) Four weeks of near-normalisation of blood glucose improves the insulin response to glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide in patients with type 2 diabetes. Diabetologia 52:199–207

Holst JJ (2007) The physiology of glucagon-like peptide 1. Physiol Rev 87:1409–1439

Horowitz M, Rayner CK, Jones KL (2013) Mechanisms and clinical efficacy of lixisenatide for the management of type 2 diabetes. Adv Ther 30:81–101

Irwin N, Flatt PR (2009) Evidence for beneficial effects of compromised gastric inhibitory polypeptide action in obesity-related diabetes and possible therapeutic implications. Diabetologia 52:1724–1731

Jendle J, Nauck MA, Matthews DR, Frid A, Hermansen K, During M, Zdravkovic M, Strauss BJ, Garber AJ, LEAD-2 and LEAD-3 Study Groups (2009) Weight loss with liraglutide, a once-daily human glucagon-like peptide-1 analogue for type 2 diabetes treatment as monotherapy or added to metformin, is primarily as a result of a reduction in fat tissue. Diabetes Obes Metab 11:1163–1172

Kahles F, Meyer C, Mollmann J, Diebold S, Findeisen HM, Lebherz C, Trautwein C, Koch A, Tacke F, Marx N, Lehrke M (2014) GLP-1 secretion is increased by inflammatory stimuli in an IL-6-dependent manner, leading to hyperinsulinemia and blood glucose lowering. Diabetes 63 (10):3221–3229

Keyhani-Nejad F, Irmler M, Isken F, Wirth EK, Beckers J, Birkenfeld AL, Pfeiffer AF (2015) Nutritional strategy to prevent fatty liver and insulin resistance independent of obesity by reducing glucose-dependent insulinotropic polypeptide responses in mice. Diabetologia 58:374–383

Kim M, Platt MJ, Shibasaki T, Quaggin SE, Backx PH, Seino S, Simpson JA, Drucker DJ (2013) GLP-1 receptor activation and Epac2 link atrial natriuretic peptide secretion to control of blood pressure. Nat Med 19:567–575

Klonoff DC, Buse JB, Nielsen LL, Guan X, Bowlus CL, Holcombe JH, Wintle ME, Maggs DG (2008) Exenatide effects on diabetes, obesity, cardiovascular risk factors and hepatic biomarkers in patients with type 2 diabetes treated for at least 3 years. Curr Med Res Opin 24:275–286

Kreymann B, Williams G, Ghatei MA, Bloom SR (1987) Glucagon-like peptide-1 7-36: a physiological incretin in man. Lancet 2:1300–1304

Kuhre RE, Gribble FM, Hartmann B, Reimann F, Windelov JA, Rehfeld JF, Holst JJ (2014) Fructose stimulates GLP-1 but not GIP secretion in mice, rats, and humans. Am J Physiol Gastrointest Liver Physiol 306:G622–G630

Kunkel D, Basseri B, Low K, Lezcano S, Soffer EE, Conklin JL, Mathur R, Pimentel M (2011) Efficacy of the glucagon-like peptide-1 agonist exenatide in the treatment of short bowel syndrome. Neurogastroenterol Motil 23:739-e328

Kuo P, Chaikomin R, Pilichiewicz A, O'Donovan D, Wishart JM, Meyer JH, Jones KL, Feinle-Bisset C, Horowitz M, Rayner CK (2008) Transient, early release of glucagon-like peptide-1 during low rates of intraduodenal glucose delivery. Regul Pept 146:1–3

Kuo P, Stevens JE, Russo A, Maddox A, Wishart JM, Jones KL, Greville H, Hetzel D, Chapman I, Horowitz M, Rayner CK (2011) Gastric emptying, incretin hormone secretion, and postprandial glycemia in cystic fibrosis–effects of pancreatic enzyme supplementation. J Clin Endocrinol Metab 96:E851–E855

Laferrere B, Heshka S, Wang K, Khan Y, McGinty J, Teixeira J, Hart AB, Olivan B (2007) Incretin levels and effect are markedly enhanced 1 month after Roux-en-Y gastric bypass surgery in obese patients with type 2 diabetes. Diabetes Care 30:1709–1716

Laferrere B, Teixeira J, McGinty J, Tran H, Egger JR, Colarusso A, Kovack B, Bawa B, Koshy N, Lee H, Yapp K, Olivan B (2008) Effect of weight loss by gastric bypass surgery versus hypocaloric diet on glucose and incretin levels in patients with type 2 diabetes. J Clin Endocrinol Metab 93:2479–2485

Lee MY, Fraser JD, Chapman MJ, Sundararajan K, Umapathysivam MM, Summers MJ, Zaknic AV, Rayner CK, Meier JJ, Horowitz M, Deane AM (2013) The effect of exogenous glucose-

dependent insulinotropic polypeptide in combination with glucagon-like peptide-1 on glycemia in the critically ill. Diabetes Care 36:3333–3336
Lewis JT, Dayanandan B, Habener JF, Kieffer TJ (2000) Glucose-dependent insulinotropic polypeptide confers early phase insulin release to oral glucose in rats: demonstration by a receptor antagonist. Endocrinology 141:3710–3716
Lindgren O, Mari A, Deacon CF, Carr RD, Winzell MS, Vikman J, Ahren B (2009) Differential islet and incretin hormone responses in morning versus afternoon after standardized meal in healthy men. J Clin Endocrinol Metab 94:2887–2892
Lindgren O, Carr RD, Deacon CF, Holst JJ, Pacini G, Mari A, Ahren B (2011) Incretin hormone and insulin responses to oral versus intravenous lipid administration in humans. J Clin Endocrinol Metab 96:2519–2524
Lindgren O, Pacini G, Tura A, Holst JJ, Deacon CF, Ahren B (2014) Incretin effect after oral amino acid ingestion in humans. J Clin Endocrinol Metab 100(3):1172–1176
Little TJ, Pilichiewicz AN, Russo A, Phillips L, Jones KL, Nauck MA, Wishart J, Horowitz M, Feinle-Bisset C (2006) Effects of intravenous glucagon-like peptide-1 on gastric emptying and intragastric distribution in healthy subjects: relationships with postprandial glycemic and insulinemic responses. J Clin Endocrinol Metab 91:1916–1923
Little TJ, Isaacs NJ, Young RL, Ott R, Nguyen NQ, Rayner CK, Horowitz M, Feinle-Bisset C (2014) Characterization of duodenal expression and localization of fatty acid-sensing receptors in humans: relationships with body mass index. Am J Physiol Gastrointest Liver Physiol 307 (10):G958–G967
Lockie SH, Heppner KM, Chaudhary N, Chabenne JR, Morgan DA, Veyrat-Durebex C, Ananthakrishnan G, Rohner-Jeanrenaud F, Drucker DJ, DiMarchi R, Rahmouni K, Oldfield BJ, Tschop MH, Perez-Tilve D (2012) Direct control of brown adipose tissue thermogenesis by central nervous system glucagon-like peptide-1 receptor signaling. Diabetes 61:2753–2762
Lorenz M, Pfeiffer C, Steinstrasser A, Becker RH, Rutten H, Ruus P, Horowitz M (2013) Effects of lixisenatide once daily on gastric emptying in type 2 diabetes - relationship to postprandial glycemia. Regul Pept 185C:1–8
Lovshin JA, Barnie A, DeAlmeida A, Logan A, Zinman B, Drucker DJ (2015) Liraglutide promotes natriuresis but does not increase circulating levels of atrial natriuretic peptide in hypertensive subjects with type 2 diabetes. Diabetes Care 38:132–139
Lu WJ, Yang Q, Sun W, Woods SC, D'Alessio D, Tso P (2007) The regulation of the lymphatic secretion of glucagon-like peptide-1 (GLP-1) by intestinal absorption of fat and carbohydrate. Am J Physiol Gastrointest Liver Physiol 293:G963–G971
Lyssenko V, Eliasson L, Kotova O, Pilgaard K, Wierup N, Salehi A, Wendt A, Jonsson A, De Marinis YZ, Berglund LM, Taneera J, Balhuizen A, Hansson O, Osmark P, Duner P, Brons C, Stancakova A, Kuusisto J, Bugliani M, Saxena R, Ahlqvist E, Kieffer TJ, Tuomi T, Isomaa B, Melander O, Sonestedt E, Orho-Melander M, Nilsson P, Bonetti S, Bonadonna R, Miccoli R, Delprato S, Marchetti P, Madsbad S, Poulsen P, Vaag A, Laakso M, Gomez MF, Groop L (2011) Pleiotropic effects of GIP on islet function involve osteopontin. Diabetes 60:2424–2433
Ma J, Bellon M, Wishart JM, Young R, Blackshaw LA, Jones KL, Horowitz M, Rayner CK (2009a) Effect of the artificial sweetener, sucralose, on gastric emptying and incretin hormone release in healthy subjects. Am J Physiol Gastrointest Liver Physiol 296:G735–G739
Ma J, Stevens JE, Cukier K, Maddox AF, Wishart JM, Jones KL, Clifton PM, Horowitz M, Rayner CK (2009b) Effects of a protein preload on gastric emptying, glycemia, and gut hormones after a carbohydrate meal in diet-controlled type 2 diabetes. Diabetes Care 32:1600–1602
Ma J, Pilichiewicz AN, Feinle-Bisset C, Wishart JM, Jones KL, Horowitz M, Rayner CK (2012) Effects of variations in duodenal glucose load on glycaemic, insulin, and incretin responses in type 2 diabetes. Diabet Med 29:604–608
Ma J, Checklin HL, Wishart JM, Stevens JE, Jones KL, Horowitz M, Meyer JH, Rayner CK (2013) A randomised trial of enteric-coated nutrient pellets to stimulate gastrointestinal peptide release and lower glycaemia in type 2 diabetes. Diabetologia 56:1236–1242

Maida A, Hansotia T, Longuet C, Seino Y, Drucker DJ (2009) Differential importance of glucose-dependent insulinotropic polypeptide vs glucagon-like peptide 1 receptor signaling for beta cell survival in mice. Gastroenterology 137:2146–2157

Marchetti P, Lupi R, Bugliani M, Kirkpatrick CL, Sebastiani G, Grieco FA, Del Guerra S, D'Aleo V, Piro S, Marselli L, Boggi U, Filipponi F, Tinti L, Salvini L, Wollheim CB, Purrello F, Dotta F (2012) A local glucagon-like peptide 1 (GLP-1) system in human pancreatic islets. Diabetologia 55:3262–3272

McKay NJ, Galante DL, Daniels D (2014) Endogenous glucagon-like peptide-1 reduces drinking behavior and is differentially engaged by water and food intakes in rats. J Neurosci 34:16417–16423

Meier JJ, Hucking K, Holst JJ, Deacon CF, Schmiegel WH, Nauck MA (2001) Reduced insulinotropic effect of gastric inhibitory polypeptide in first-degree relatives of patients with type 2 diabetes. Diabetes 50:2497–2504

Meier JJ, Gallwitz B, Siepmann N, Holst JJ, Deacon CF, Schmidt WE, Nauck MA (2003a) Gastric inhibitory polypeptide (GIP) dose-dependently stimulates glucagon secretion in healthy human subjects at euglycaemia. Diabetologia 46:798–801

Meier JJ, Nauck MA, Siepmann N, Greulich M, Holst JJ, Deacon CF, Schmidt WE, Gallwitz B (2003b) Similar insulin secretory response to a gastric inhibitory polypeptide bolus injection at euglycemia in first-degree relatives of patients with type 2 diabetes and control subjects. Metabolism 52:1579–1585

Meier JJ, Goetze O, Anstipp J, Hagemann D, Holst JJ, Schmidt WE, Gallwitz B, Nauck MA (2004) Gastric inhibitory polypeptide does not inhibit gastric emptying in humans. Am J Physiol Endocrinol Metab 286:E621–E625

Meier JJ, Gallwitz B, Askenas M, Vollmer K, Deacon CF, Holst JJ, Schmidt WE, Nauck MA (2005a) Secretion of incretin hormones and the insulinotropic effect of gastric inhibitory polypeptide in women with a history of gestational diabetes. Diabetologia 48:1872–1881

Meier JJ, Kemmeries G, Holst JJ, Nauck MA (2005b) Erythromycin antagonizes the deceleration of gastric emptying by glucagon-like peptide 1 and unmasks its insulinotropic effect in healthy subjects. Diabetes 54:2212–2218

Mentis N, Vardarli I, Kothe LD, Holst JJ, Deacon CF, Theodorakis M, Meier JJ, Nauck MA (2011) GIP does not potentiate the antidiabetic effects of GLP-1 in hyperglycemic patients with type 2 diabetes. Diabetes 60:1270–1276

Miyawaki K, Yamada Y, Yano H, Niwa H, Ban N, Ihara Y, Kubota A, Fujimoto S, Kajikawa M, Kuroe A, Tsuda K, Hashimoto H, Yamashita T, Jomori T, Tashiro F, Miyazaki J, Seino Y (1999) Glucose intolerance caused by a defect in the entero-insular axis: a study in gastric inhibitory polypeptide receptor knockout mice. Proc Natl Acad Sci U S A 96:14843–14847

Monnier L, Colette C, Dunseath GJ, Owens DR (2007) The loss of postprandial glycemic control precedes stepwise deterioration of fasting with worsening diabetes. Diabetes Care 30:263–269

Mortensen K, Christensen LL, Holst JJ, Orskov C (2003) GLP-1 and GIP are colocalized in a subset of endocrine cells in the small intestine. Regul Pept 114:189–196

Mulherin AJ, Oh AH, Kim H, Grieco A, Lauffer LM, Brubaker PL (2011) Mechanisms underlying metformin-induced secretion of glucagon-like peptide-1 from the intestinal L cell. Endocrinology 152:4610–4619

Nasteska D, Harada N, Suzuki K, Yamane S, Hamasaki A, Joo E, Iwasaki K, Shibue K, Harada T, Inagaki N (2014) Chronic reduction of GIP secretion alleviates obesity and insulin resistance under high-fat diet conditions. Diabetes 63:2332–2343

Nauck MA, Homberger E, Siegel EG, Allen RC, Eaton RP, Ebert R, Creutzfeldt W (1986) Incretin effects of increasing glucose loads in man calculated from venous insulin and C-peptide responses. J Clin Endocrinol Metab 63:492–498

Nauck MA, Heimesaat MM, Orskov C, Holst JJ, Ebert R, Creutzfeldt W (1993) Preserved incretin activity of glucagon-like peptide 1 [7-36 amide] but not of synthetic human gastric inhibitory polypeptide in patients with type-2 diabetes mellitus. J Clin Invest 91:301–307

Nauck MA, Niedereichholz U, Ettler R, Holst JJ, Orskov C, Ritzel R, Schmiegel WH (1997) Glucagon-like peptide 1 inhibition of gastric emptying outweighs its insulinotropic effects in healthy humans. Am J Physiol 273:E981–E988

Nauck MA, Heimesaat MM, Behle K, Holst JJ, Nauck MS, Ritzel R, Hufner M, Schmiegel WH (2002) Effects of glucagon-like peptide 1 on counterregulatory hormone responses, cognitive functions, and insulin secretion during hyperinsulinemic, stepped hypoglycemic clamp experiments in healthy volunteers. J Clin Endocrinol Metab 87:1239–1246

Nauck MA, Kemmeries G, Holst JJ, Meier JJ (2011a) Rapid tachyphylaxis of the glucagon-like peptide 1-induced deceleration of gastric emptying in humans. Diabetes 60:1561–1565

Nauck MA, Vardarli I, Deacon CF, Holst JJ, Meier JJ (2011b) Secretion of glucagon-like peptide-1 (GLP-1) in type 2 diabetes: what is up, what is down? Diabetologia 54:10–18

Nauck MA, Baranov O, Ritzel RA, Meier JJ (2013) Do current incretin mimetics exploit the full therapeutic potential inherent in GLP-1 receptor stimulation? Diabetologia 56:1878–1883

Nicolaus M, Brodl J, Linke R, Woerle HJ, Goke B, Schirra J (2011) Endogenous GLP-1 regulates postprandial glycemia in humans: relative contributions of insulin, glucagon, and gastric emptying. J Clin Endocrinol Metab 96:229–236

Nogueiras R, Perez-Tilve D, Veyrat-Durebex C, Morgan DA, Varela L, Haynes WG, Patterson JT, Disse E, Pfluger PT, Lopez M, Woods SC, DiMarchi R, Dieguez C, Rahmouni K, Rohner-Jeanrenaud F, Tschop MH (2009) Direct control of peripheral lipid deposition by CNS GLP-1 receptor signaling is mediated by the sympathetic nervous system and blunted in diet-induced obesity. J Neurosci 29:5916–5925

Nyberg J, Anderson MF, Meister B, Alborn AM, Strom AK, Brederlau A, Illerskog AC, Nilsson O, Kieffer TJ, Hietala MA, Ricksten A, Eriksson PS (2005) Glucose-dependent insulinotropic polypeptide is expressed in adult hippocampus and induces progenitor cell proliferation. J Neurosci 25:1816–1825

Pathak V, Gault VA, Flatt PR, Irwin N (2014) Antagonism of gastric inhibitory polypeptide (GIP) by palmitoylation of GIP analogues with N- and C-terminal modifications improves obesity and metabolic control in high fat fed mice. Mol Cell Endocrinol 401:120–129

Perano SJ, Couper JJ, Horowitz M, Martin AJ, Kritas S, Sullivan T, Rayner CK (2014) Pancreatic enzyme supplementation improves the incretin hormone response and attenuates postprandial glycemia in adolescents with cystic fibrosis: a randomized crossover trial. J Clin Endocrinol Metab 99:2486–2493

Plamboeck A, Veedfald S, Deacon CF, Hartmann B, Wettergren A, Svendsen LB, Meisner S, Hovendal C, Knop FK, Vilsboll T, Holst JJ (2013) Characterisation of oral and i.v. glucose handling in truncally vagotomised subjects with pyloroplasty. Eur J Endocrinol 169:187–201

Powell DR, Smith M, Greer J, Harris A, Zhao S, Dacosta C, Mseeh F, Shadoan MK, Sands A, Zambrowicz B, Ding ZM (2013) LX4211 increases serum GLP-1 and PYY levels by reducing SGLT-1-mediated absorption of intestinal glucose. J Pharmacol Exp Ther 345(2):250–259

Prigeon RL, Quddusi S, Paty B, D'Alessio DA (2003) Suppression of glucose production by GLP-1 independent of islet hormones: a novel extrapancreatic effect. Am J Physiol Endocrinol Metab 285:E-701–E-707

Pyke C, Knudsen LB (2013) The glucagon-like peptide-1 receptor–or not? Endocrinology 154:4–8

Pyke C, Heller RS, Kirk RK, Orskov C, Reedtz-Runge S, Kaastrup P, Hvelplund A, Bardram L, Calatayud D, Knudsen LB (2014) GLP-1 receptor localization in monkey and human tissue: novel distribution revealed with extensively validated monoclonal antibody. Endocrinology 155:1280–1290

Qualmann C, Nauck MA, Holst JJ, Orskov C, Creutzfeldt W (1995) Glucagon-like peptide 1 (7-36 amide) secretion in response to luminal sucrose from the upper and lower gut. A study using alpha-glucosidase inhibition (acarbose). Scand J Gastroenterol 30:892–896

Rankin MM, Kushner JA (2009) Adaptive beta-cell proliferation is severely restricted with advanced age. Diabetes 58:1365–1372

Richards P, Parker HE, Adriaenssens AE, Hodgson JM, Cork SC, Trapp S, Gribble FM, Reimann F (2014) Identification and characterization of GLP-1 receptor-expressing cells using a new transgenic mouse model. Diabetes 63:1224–1233

Rocca AS, Brubaker PL (1999) Role of the vagus nerve in mediating proximal nutrient-induced glucagon-like peptide-1 secretion. Endocrinology 140:1687–1694

Salehi M, Vahl TP, D'Alessio DA (2008) Regulation of islet hormone release and gastric emptying by endogenous glucagon-like peptide 1 after glucose ingestion. J Clin Endocrinol Metab 93:4909–4916

Salehi M, Aulinger B, Prigeon RL, D'Alessio DA (2010) Effect of endogenous GLP-1 on insulin secretion in type 2 diabetes. Diabetes 59:1330–1337

Salehi M, Gastaldelli A, D'Alessio DA (2014) Blockade of glucagon-like peptide 1 receptor corrects postprandial hypoglycemia after gastric bypass. Gastroenterology 146(669–680):e2

Sandoval DA, Bagnol D, Woods SC, D'Alessio DA, Seeley RJ (2008) Arcuate glucagon-like peptide 1 receptors regulate glucose homeostasis but not food intake. Diabetes 57:2046–2054

Sathyanarayana P, Jogi M, Muthupillai R, Krishnamurthy R, Samson SL, Bajaj M (2011) Effects of combined exenatide and pioglitazone therapy on hepatic fat content in type 2 diabetes. Obesity (Silver Spring) 19:2310–2315

Saxena R, Hivert MF, Langenberg C, Tanaka T, Pankow JS, Vollenweider P, Lyssenko V, Bouatia-Naji N, Dupuis J, Jackson AU, Kao WH, Li M, Glazer NL, Manning AK, Luan J, Stringham HM, Prokopenko I, Johnson T, Grarup N, Boesgaard TW, Lecoeur C, Shrader P, O'Connell J, Ingelsson E, Couper DJ, Rice K, Song K, Andreasen CH, Dina C, Kottgen A, Le Bacquer O, Pattou F, Taneera J, Steinthorsdottir V, Rybin D, Ardlie K, Sampson M, Qi L, van Hoek M, Weedon MN, Aulchenko YS, Voight BF, Grallert H, Balkau B, Bergman RN, Bielinski SJ, Bonnefond A, Bonnycastle LL, Borch-Johnsen K, Bottcher Y, Brunner E, Buchanan TA, Bumpstead SJ, Cavalcanti-Proenca C, Charpentier G, Chen YD, Chines PS, Collins FS, Cornelis M, J Crawford G, Delplanque J, Doney A, Egan JM, Erdos MR, Firmann M, Forouhi NG, Fox CS, Goodarzi MO, Graessler J, Hingorani A, Isomaa B, Jorgensen T, Kivimaki M, Kovacs P, Krohn K, Kumari M, Lauritzen T, Levy-Marchal C, Mayor V, McAteer JB, Meyre D, Mitchell BD, Mohlke KL, Morken MA, Narisu N, Palmer CN, Pakyz R, Pascoe L, Payne F, Pearson D, Rathmann W, Sandbaek A, Sayer AA, Scott LJ, Sharp SJ, Sijbrands E, Singleton A, Siscovick DS, Smith NL, Sparso T, Swift AJ, Syddall H, Thorleifsson G, Tonjes A, Tuomi T, Tuomilehto J, Valle TT, Waeber G, Walley A, Waterworth DM, Zeggini E, Zhao JH, GIANT consortium, MAGIC investigators, Illig T, Wichmann HE, Wilson JF, van Duijn C, Hu FB, Morris AD, Frayling TM, Hattersley AT, Thorsteinsdottir U, Stefansson K, Nilsson P, Syvanen AC, Shuldiner AR, Walker M, Bornstein SR, Schwarz P, Williams GH, Nathan DM, Kuusisto J, Laakso M, Cooper C, Marmot M, Ferrucci L, Mooser V, Stumvoll M, Loos RJ, Altshuler D, Psaty BM, Rotter JI, Boerwinkle E, Hansen T, Pedersen O, Florez JC, McCarthy MI, Boehnke M, Barroso I, Sladek R, Froguel P, Meigs JB, Groop L, Wareham NJ, Watanabe RM (2010) Genetic variation in GIPR influences the glucose and insulin responses to an oral glucose challenge. Nat Genet 42:142–148

Schirra J, Sturm K, Leicht P, Arnold R, Goke B, Katschinski M (1998) Exendin(9-39)amide is an antagonist of glucagon-like peptide-1(7-36)amide in humans. J Clin Invest 101:1421–1430

Schirra J, Nicolaus M, Roggel R, Katschinski M, Storr M, Woerle HJ, Goke B (2006) Endogenous glucagon-like peptide 1 controls endocrine pancreatic secretion and antro-pyloro-duodenal motility in humans. Gut 55:243–251

Scirica BM, Bhatt DL, Braunwald E, Steg PG, Davidson J, Hirshberg B, Ohman P, Frederich R, Wiviott SD, Hoffman EB, Cavender MA, Udell JA, Desai NR, Mosenzon O, McGuire DK, Ray KK, Leiter LA, Raz I, SAVOR-TIMI 53 Steering Committee and Investigators (2013) Saxagliptin and cardiovascular outcomes in patients with type 2 diabetes mellitus. N Engl J Med 369:1317–1326

Seghieri M, Rebelos E, Gastaldelli A, Astiarraga BD, Casolaro A, Barsotti E, Pocai A, Nauck M, Muscelli E, Ferrannini E (2013) Direct effect of GLP-1 infusion on endogenous glucose production in humans. Diabetologia 56:156–161

Seifarth C, Bergmann J, Holst JJ, Ritzel R, Schmiegel W, Nauck MA (1998) Prolonged and enhanced secretion of glucagon-like peptide 1 (7-36 amide) after oral sucrose due to alpha-glucosidase inhibition (acarbose) in Type 2 diabetic patients. Diabet Med 15:485–491

Seino Y, Yabe D (2013) Glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1: incretin actions beyond the pancreas. J Diabetes Investig 4:108–130

Seufert J, Gallwitz B (2014) The extra-pancreatic effects of GLP-1 receptor agonists: a focus on the cardiovascular, gastrointestinal and central nervous systems. Diabetes Obes Metab 16:673–688

Shah M, Law JH, Micheletto F, Sathananthan M, Dalla Man C, Cobelli C, Rizza RA, Camilleri M, Zinsmeister AR, Vella A (2014) Contribution of endogenous glucagon-like peptide 1 to glucose metabolism after Roux-en-Y gastric bypass. Diabetes 63:483–493

Sharma S, Mells JE, Fu PP, Saxena NK, Anania FA (2011) GLP-1 analogs reduce hepatocyte steatosis and improve survival by enhancing the unfolded protein response and promoting macroautophagy. PLoS One 6:e25269

Smith EP, An Z, Wagner C, Lewis AG, Cohen EB, Li B, Mahbod P, Sandoval D, Perez-Tilve D, Tamarina N, Philipson LH, Stoffers DA, Seeley RJ, D'Alessio DA (2014) The role of beta cell glucagon-like peptide-1 signaling in glucose regulation and response to diabetes drugs. Cell Metab 19:1050–1057

Steinert RE, Luscombe-Marsh ND, Little TJ, Standfield S, Otto B, Horowitz M, Feinle-Bisset C (2014a) Effects of intraduodenal infusion of L-tryptophan on ad libitum eating, antropylorodouodenal motility, glycemia, insulinemia, and gut peptide secretion in healthy men. J Clin Endocrinol Metab 99:3275–3284

Steinert RE, Schirra J, Meyer-Gerspach AC, Kienle P, Fischer H, Schulte F, Goeke B, Beglinger C (2014b) Effect of glucagon-like peptide-1 receptor antagonism on appetite and food intake in healthy men. Am J Clin Nutr 100:514–523

Svegliati-Baroni G, Saccomanno S, Rychlicki C, Agostinelli L, De Minicis S, Candelaresi C, Faraci G, Pacetti D, Vivarelli M, Nicolini D, Garelli P, Casini A, Manco M, Mingrone G, Risaliti A, Frega GN, Benedetti A, Gastaldelli A (2011) Glucagon-like peptide-1 receptor activation stimulates hepatic lipid oxidation and restores hepatic signalling alteration induced by a high-fat diet in nonalcoholic steatohepatitis. Liver Int 31:1285–1297

Svensson AM, Efendic S, Ostenson CG, Jansson L (1997) Gastric inhibitory polypeptide and splanchnic blood perfusion: augmentation of the islet blood flow increase in hyperglycemic rats. Peptides 18:1055–1059

Thazhath SS, Marathe C, Wu T, Chang J, Khoo J, Kuo P, Checklin H, Bound M, Russo A, Rigda RS, Jones KL, Horowitz M, Rayner CK (2014) Effects of the glucagon-like peptide-1 (GLP-1) receptor agonist, exenatide, on small intestinal motility, flow, and glucose absorption in healthy subjects and in type 2 diabetes (Abstract). United Eur Gastroenterol J 2(1S):A29

Theodorakis MJ, Carlson O, Michopoulos S, Doyle ME, Juhaszova M, Petraki K, Egan JM (2006) Human duodenal enteroendocrine cells: source of both incretin peptides, GLP-1 and GIP. Am J Physiol Endocrinol Metab 290:E550–E559

Toft-Nielsen MB, Madsbad S, Holst JJ (1999) Continuous subcutaneous infusion of glucagon-like peptide 1 lowers plasma glucose and reduces appetite in type 2 diabetic patients. Diabetes Care 22:1137–1143

Trahair LG, Horowitz M, Rayner CK, Gentilcore D, Lange K, Wishart JM, Jones KL (2012) Comparative effects of variations in duodenal glucose load on glycemic, insulinemic, and incretin responses in healthy young and older subjects. J Clin Endocrinol Metab 97:844–851

Trahair LG, Horowitz M, Hausken T, Feinle-Bisset C, Rayner CK, Jones KL (2014) Effects of exogenous glucagon-like peptide-1 on the blood pressure, heart rate, mesenteric blood flow and glycemic responses to intraduodenal glucose in healthy older subjects. J Clin Endocrinol Metab 99(12):E2628–E2634

Tsukiyama K, Yamada Y, Yamada C, Harada N, Kawasaki Y, Ogura M, Bessho K, Li M, Amizuka N, Sato M, Udagawa N, Takahashi N, Tanaka K, Oiso Y, Seino Y (2006) Gastric inhibitory polypeptide as an endogenous factor promoting new bone formation after food ingestion. Mol Endocrinol 20:1644–1651

Turton MD, O'Shea D, Gunn I, Beak SA, Edwards CM, Meeran K, Choi SJ, Taylor GM, Heath MM, Lambert PD, Wilding JP, Smith DM, Ghatei MA, Herbert J, Bloom SR (1996) A role for glucagon-like peptide-1 in the central regulation of feeding. Nature 379:69–72

Umapathysivam MM, Lee MY, Jones KL, Annink CE, Cousins CE, Trahair LG, Rayner CK, Chapman MJ, Nauck MA, Horowitz M, Deane AM (2014) Comparative effects of prolonged and intermittent stimulation of the glucagon-like peptide 1 receptor on gastric emptying and glycemia. Diabetes 63:785–790

Ussher JR, Drucker DJ (2012) Cardiovascular biology of the incretin system. Endocr Rev 33:187–215

Ussher JR, Drucker DJ (2014) Cardiovascular actions of incretin-based therapies. Circ Res 114:1788–1803

Vahl TP, Tauchi M, Durler TS, Elfers EE, Fernandes TM, Bitner RD, Ellis KS, Woods SC, Seeley RJ, Herman JP, D'Alessio DA (2007) Glucagon-like peptide-1 (GLP-1) receptors expressed on nerve terminals in the portal vein mediate the effects of endogenous GLP-1 on glucose tolerance in rats. Endocrinology 148:4965–4973

Vella A, Shah P, Basu R, Basu A, Holst JJ, Rizza RA (2000) Effect of glucagon-like peptide 1 (7-36) amide on glucose effectiveness and insulin action in people with type 2 diabetes. Diabetes 49:611–617

Vendrell J, El Bekay R, Peral B, Garcia-Fuentes E, Megia A, Macias-Gonzalez M, Fernandez Real J, Jimenez-Gomez Y, Escote X, Pachon G, Simo R, Selva DM, Malagon MM, Tinahones FJ (2011) Study of the potential association of adipose tissue GLP-1 receptor with obesity and insulin resistance. Endocrinology 152:4072–4079

Vilsboll T, Agerso H, Krarup T, Holst JJ (2003a) Similar elimination rates of glucagon-like peptide-1 in obese type 2 diabetic patients and healthy subjects. J Clin Endocrinol Metab 88:220–224

Vilsboll T, Krarup T, Madsbad S, Holst JJ (2003b) Both GLP-1 and GIP are insulinotropic at basal and postprandial glucose levels and contribute nearly equally to the incretin effect of a meal in healthy subjects. Regul Pept 114:115–121

Vilsboll T, Krarup T, Sonne J, Madsbad S, Volund A, Juul AG, Holst JJ (2003c) Incretin secretion in relation to meal size and body weight in healthy subjects and people with type 1 and type 2 diabetes mellitus. J Clin Endocrinol Metab 88:2706–2713

Vilsboll T, Agerso H, Lauritsen T, Deacon CF, Aaboe K, Madsbad S, Krarup T, Holst JJ (2006) The elimination rates of intact GIP as well as its primary metabolite, GIP 3-42, are similar in type 2 diabetic patients and healthy subjects. Regul Pept 137:168–172

Waget A, Cabou C, Masseboeuf M, Cattan P, Armanet M, Karaca M, Castel J, Garret C, Payros G, Maida A, Sulpice T, Holst JJ, Drucker DJ, Magnan C, Burcelin R (2011) Physiological and pharmacological mechanisms through which the DPP-4 inhibitor sitagliptin regulates glycemia in mice. Endocrinology 152:3018–3029

Waser B, Beetschen K, Pellegata NS, Reubi JC (2011) Incretin receptors in non-neoplastic and neoplastic thyroid C cells in rodents and humans: relevance for incretin-based diabetes therapy. Neuroendocrinology 94:291–301

Wettergren A, Petersen H, Orskov C, Christiansen J, Sheikh SP, Holst JJ (1994) Glucagon-like peptide-1 7-36 amide and peptide YY from the L-cell of the ileal mucosa are potent inhibitors of vagally induced gastric acid secretion in man. Scand J Gastroenterol 29:501–505

White WB, Cannon CP, Heller SR, Nissen SE, Bergenstal RM, Bakris GL, Perez AT, Fleck PR, Mehta CR, Kupfer S, Wilson C, Cushman WC, Zannad F, Investigators E (2013) Alogliptin after acute coronary syndrome in patients with type 2 diabetes. N Engl J Med 369:1327–1335

Wice BM, Reeds DN, Tran HD, Crimmins DL, Patterson BW, Dunai J, Wallendorf MJ, Ladenson JH, Villareal DT, Polonsky KS (2012) Xenin-25 amplifies GIP-mediated insulin secretion in humans with normal and impaired glucose tolerance but not type 2 diabetes. Diabetes 61:1793–1800

Widenmaier SB, Kim SJ, Yang GK, De Los Reyes T, Nian C, Asadi A, Seino Y, Kieffer TJ, Kwok YN, McIntosh CH (2010) A GIP receptor agonist exhibits beta-cell anti-apoptotic actions in rat models of diabetes resulting in improved beta-cell function and glycemic control. PLoS One 5: e9590

Witte AB, Gryback P, Jacobsson H, Naslund E, Hellstrom PM, Holst JJ, Hilsted L, Schmidt PT (2011) Involvement of endogenous glucagon-like peptide-1 in regulation of gastric motility and pancreatic endocrine secretion. Scand J Gastroenterol 46:428–435

Wu T, Rayner CK, Jones K, Horowitz M (2010) Dietary effects on incretin hormone secretion. Vitam Horm 84:81–110

Wu T, Zhao BR, Bound MJ, Checklin HL, Bellon M, Little TJ, Young RL, Jones KL, Horowitz M, Rayner CK (2012) Effects of different sweet preloads on incretin hormone secretion, gastric emptying, and postprandial glycemia in healthy humans. Am J Clin Nutr 95:78–83

Wu T, Bound MJ, Standfield SD, Bellon M, Young RL, Jones KL, Horowitz M, Rayner CK (2013a) Artificial sweeteners have no effect on gastric emptying, glucagon-like peptide-1, or glycemia after oral glucose in healthy humans. Diabetes Care 36:e202–e203

Wu T, Bound MJ, Standfield SD, Gedulin B, Jones KL, Horowitz M, Rayner CK (2013b) Effects of rectal administration of taurocholic acid on glucagon-like peptide-1 and peptide YY secretion in healthy humans. Diabetes Obes Metab 15:474–477

Wu T, Bound MJ, Standfield SD, Jones KL, Horowitz M, Rayner CK (2013c) Effects of taurocholic acid on glycemic, glucagon-like peptide-1, and insulin responses to small intestinal glucose infusion in healthy humans. J Clin Endocrinol Metab 98:E718–E722

Wu T, Bound MJ, Zhao BR, Standfield SD, Bellon M, Jones KL, Horowitz M, Rayner CK (2013d) Effects of a D-xylose preload with or without sitagliptin on gastric emptying, glucagon-like peptide-1, and postprandial glycemia in type 2 diabetes. Diabetes Care 36:1913–1918

Wu T, Rayner CK, Young RL, Horowitz M (2013e) Gut motility and enteroendocrine secretion. Curr Opin Pharmacol 13:928–934

Wu T, Ma J, Bound MJ, Checklin H, Deacon CF, Jones KL, Horowitz M, Rayner CK (2014a) Effects of sitagliptin on glycemia, incretin hormones, and antropyloroduodenal motility in response to intraduodenal glucose infusion in healthy lean and obese humans and patients with type 2 diabetes treated with or without metformin. Diabetes 63:2776–2787

Wu T, Trahair LG, Bound MJ, Deacon CF, Horowitz M, Rayner CK, Jones KL (2014b) Effects of sitagliptin on blood pressure and heart rate in response to intraduodenal glucose infusion in patients with Type 2 diabetes: a potential role for glucose-dependent insulinotropic polypeptide? Diabet Med. doi:10.1111/dme.12622

Xu G, Kaneto H, Laybutt DR, Duvivier-Kali VF, Trivedi N, Suzuma K, King GL, Weir GC, Bonner-Weir S (2007) Downregulation of GLP-1 and GIP receptor expression by hyperglycemia: possible contribution to impaired incretin effects in diabetes. Diabetes 56:1551–1558

Yamada C, Yamada Y, Tsukiyama K, Yamada K, Udagawa N, Takahashi N, Tanaka K, Drucker DJ, Seino Y, Inagaki N (2008) The murine glucagon-like peptide-1 receptor is essential for control of bone resorption. Endocrinology 149:574–579

Yamamoto H, Lee CE, Marcus JN, Williams TD, Overton JM, Lopez ME, Hollenberg AN, Baggio L, Saper CB, Drucker DJ, Elmquist JK (2002) Glucagon-like peptide-1 receptor stimulation increases blood pressure and heart rate and activates autonomic regulatory neurons. J Clin Invest 110:43–52

Young RL, Chia B, Isaacs NJ, Ma J, Khoo J, Wu T, Horowitz M, Rayner CK (2013) Disordered control of intestinal sweet taste receptor expression and glucose absorption in type 2 diabetes. Diabetes 62:3532–3541

Zambrowicz B, Ogbaa I, Frazier K, Banks P, Turnage A, Freiman J, Boehm KA, Ruff D, Powell D, Sands A (2013) Effects of LX4211, a dual sodium-dependent glucose cotransporters 1 and 2 inhibitor, on postprandial glucose, insulin, glucagon-like peptide 1, and peptide tyrosine tyrosine in a dose-timing study in healthy subjects. Clin Ther 35(1162–1173):e8

Zander M, Madsbad S, Madsen JL, Holst JJ (2002) Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and beta-cell function in type 2 diabetes: a parallel-group study. Lancet 359:824–830

Neural Control of Energy Expenditure

Heike Münzberg, Emily Qualls-Creekmore, Hans-Rudolf Berthoud, Christopher D. Morrison, and Sangho Yu

Contents

Abstract

The continuous rise in obesity is a major concern for future healthcare management. Many strategies to control body weight focus on a permanent modification of food intake with limited success in the long term. Metabolism or energy expenditure is the other side of the coin for the regulation of body weight, and strategies to enhance energy expenditure are a current focus for obesity treatment, especially since the (re)-discovery of the energy depleting brown adipose tissue in adult humans. Conversely, several human illnesses like neurodegenerative diseases, cancer, or autoimmune deficiency syndrome suffer from increased energy expenditure and severe weight loss. Thus, strategies to modulate energy expenditure to target weight gain or loss would improve life expectancies and quality of life in many human patients. The aim of this book chapter is to give an

H. Münzberg (✉) • E. Qualls-Creekmore • H.-R. Berthoud • C.D. Morrison • S. Yu
Pennington Biomedical Research Center, Louisiana State University System, 6400 Perkins Rd, Baton Rouge, LA 70808, USA
e-mail: Heike.Munzberg@pbrc.edu

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overview of our current understanding and recent progress in energy expenditure control with specific emphasis on central control mechanisms.

Keywords
Neuronal circuits · Hypothalamus · Hormones · Leptin · FGF21 · Dorsomedial hypothalamus · Body weight

1 Introduction

The nervous system has evolved to regulate and coordinate bodily functions and behavior in a changing environment. The mammalian brain thus extensively communicates not only with the external world, but also with all aspects of internal physiology. While the neural controls of cardiovascular and gastrointestinal functions have been most intensively studied, the neural control of metabolism is less well understood and appreciated, probably owing to its complexity. Neural control of metabolism includes energy production, storage, mobilization, conversion, and utilization, as accomplished by the coordinated actions of the gastrointestinal tract, liver, pancreas, muscle, white adipose tissue (WAT), and brown adipose tissue (BAT).

The neural control of metabolism can be conceived as consisting of sensory inputs, central integrative circuits, and motor outputs (sympathetic and parasympathetic), allowing for typical feedback regulation of specific functions (Fig. 1). Sensory input to the brain is accomplished by primary afferents innervating and by humoral factors secreted from relevant peripheral organs (Cechetto 1987; Craig 2002; Fealey 2013; Janig 1996). In turn, the brain controls specific functions of these organs through the autonomic nervous system and endocrine outflow. Most of these sensory and motor neural pathways have been well documented since the inception of neural tract tracing methods some 40 years ago (Ricardo 1983) and particularly the ascent of trans-synaptic viral tracing techniques (Card and Enquist 2014; Loewy 1998). Much less is known about the organization of integrative circuits in the brain. This is probably due to the complexity of these circuits and lack of appropriate methodology to untangle them. However, the recent availability of neuron-specific stimulation and recording techniques in animals and functional neuroimaging techniques in humans is starting to provide exciting new insights (Rezai-Zadeh and Munzberg 2013; Williams and Elmquist 2012).

One of the first accounts demonstrating brain-evoked changes in a metabolic parameter can be traced back almost 150 years to Claude Bernard's "piqûre diabetique," in which he showed a slow rise in blood glucose following lesions in the caudal brainstem (Bernard 1957). More systematic investigations followed much later in the context of the classical studies on the hypothalamic control of energy balance and body weight of the mid-1900's (Brobeck 1946; Kennedy 1951; Mayer and Barrnett 1955). A key observation was the increase in body weight and adiposity in rats with ventromedial hypothalamic (VMH) lesions, even when food intake was restricted to sham-operated rats (Cox and Powley 1981); it is a clear

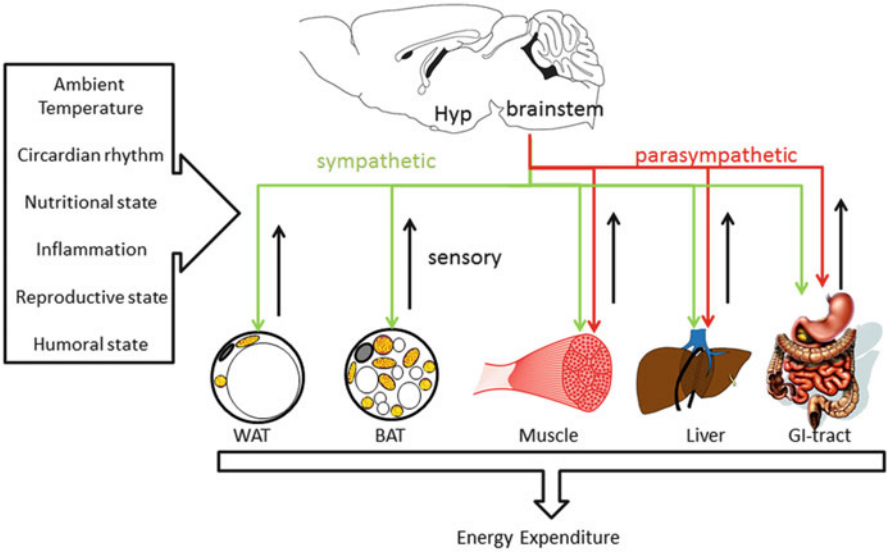

Fig. 1 Schematic view of the complex interaction of brain, peripheral tissues, and environment

evidence that the hypothalamus not only controls energy intake but also energy expenditure to achieve energy balance. This led to the discovery of BAT and its role in heat production and body weight regulation (Rothwell and Stock 1979). The discovery of leptin some 20 years ago provided the final push toward identification of neural circuitry in the hypothalamus and beyond, responsible for the regulation of energy balance and control of metabolism (Halaas et al. 1995). In this chapter, we review recent progress in the identification of brain circuits and pathways that control energy expenditure via peripheral organs.

2 Input and Output Systems for Energy Expenditure Control

Energy expenditure depends on several external and internal factors such as ambient temperature, nutritional or reproductive state, circadian rhythms, and levels of circulating hormones (Fig. 1). These external and internal modulators have sometimes opposing physiological effects and need to be integrated and translated via the brain to allow appropriate physiological changes and ensure homeostasis. Cold exposure is an excellent example that demonstrates the quick increase in energy expenditure within minutes after such an external challenge (Fig. 2a). Conversely, increased ambient temperature results in decreased energy expenditure (Fig. 2b).

Three components of whole-body energy expenditure can be distinguished: basal metabolic rate (BMR), adaptive thermogenesis, and physical activity (Fig. 3). BMR is the energy expenditure that is measured at thermoneutrality

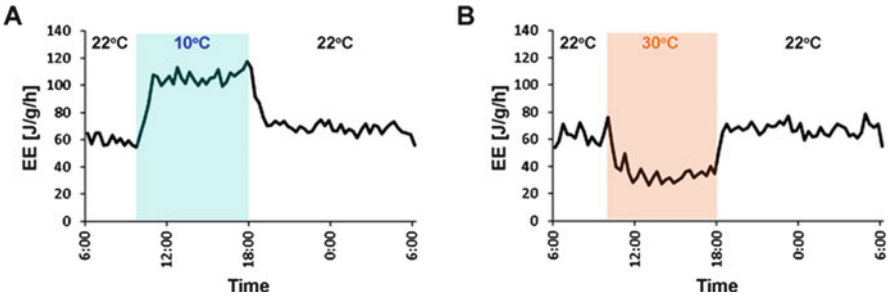

Fig. 2 Temperature changes induce robust adaptations in energy expenditure. (**a**) Acute decreases in ambient temperature quickly and robustly increase energy expenditure. (**b**) Acute increase in ambient temperature results in adaptive decrease in energy expenditure

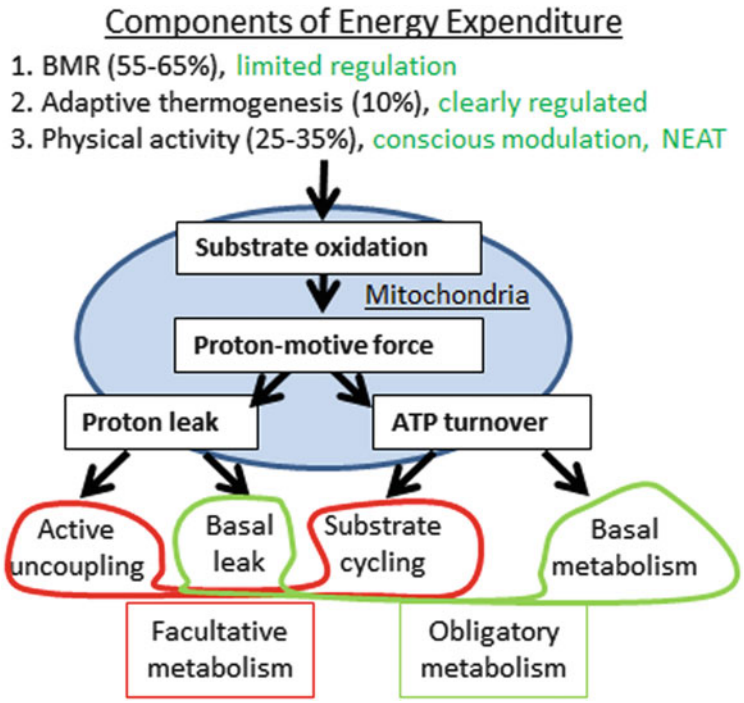

Fig. 3 Components of energy expenditure. Oxidative processes result in a proton-motive force in the mitochondrion used to generate ATP, even though basal proton leaks are observed that "wastes" energy. ATP production and basal proton leaks together account for obligatory metabolism, required for minimal bodily functions. The active uncoupling of proton-motive force from ATP production is used to generate heat, e.g., in the brown adipose tissue. And uncoupling protein 1 (UCP1) is a well-studied example for active uncoupling. Substrate cycling also actively contributes to heat production. Together these mechanisms account for facultative metabolism, which is optional and not used for baseline maintenance of bodily functions, e.g., at thermoneutral conditions

(no extra energy needed for cold- or warm-defensive adaptations), postprandially (after active meal digestion), and at rest (minimal muscle movement) and defines the oxygen consumed for ATP production. The coupling of oxidative processes to ATP production is not perfect, and some energy is lost by proton leaks (basal leak) across the mitochondrial membrane (Brand et al. 1999). Thus, BMR also includes such basal leaks and is also called obligatory metabolism as it is required to maintain minimal bodily functions.

During external challenges such as cold exposure, additional systems are activated and increase energy expenditure beyond BMR. This can be conscious and voluntary exercise, and involuntary muscle shivering (Rowland et al. 2014). In addition, adaptive systems are activated – specifically during chronic cold exposure – that are known as cold-induced thermogenesis or adaptive thermogenesis. These processes use active uncoupling of the mitochondrial proton-motive force from ATP production or futile cycling to "waste" energy and to release energy as heat (Rowland et al. 2014). This active energy wasting is also known as facultative metabolism, because it is optional and not required to maintain minimal bodily functions under thermoneutral conditions. BAT is an invention of euthermic animals such as birds and mammals and is a heat-generating tissue that is specialized in adaptive thermogenesis.

2.1 BAT and Adaptive Thermogenesis

In rodents, the interscapular BAT is the largest depot, with smaller depots in the mediastinum, along the cervical and thoracic aorta, and around the kidney (Giordano et al. 2004). In humans, BAT is less centralized than in rodents, with significant depots in supraclavicular, neck, and paraspinal regions (Cypess et al. 2009; Lidell et al. 2013; Saito et al. 2009). Based on numerous experiments with denervation of the interscapular pads in rodents, as well as pharmacological studies using ß3-adrenergic agonists and blockers, the main driver of BAT thermogenesis seems to be its noradrenergic sympathetic innervation (Andrews et al. 1985; Bartness and Wade 1984; Himms-Hagen et al. 1990; Takahashi et al. 1992; Tsukazaki et al. 1995). Retrograde tracing and other studies in rats and Siberian hamsters have identified postganglionic perikarya innervating BAT in the stellate ganglia (Grkovic and Anderson 1997; Oldfield et al. 2002), known to receive input from preganglionic neurons in the intermediolateral cell column of the cervical and thoracic spinal cord (Nozdrachev et al. 2002; Tanche and Therminarias 1967) (Fig. 4).

BAT is also innervated by dorsal root sensory nerve fibers (De et al. 1998; Himms-Hagen et al. 1990; Vaughan et al. 2014), but based on the lack of cholinergic markers, BAT is not innervated by the parasympathetic nervous system (Norman et al. 1988). Sympathetic activation of BAT leads to the activation and gene expression of the uncoupling protein-1 (UCP1), which is well accepted as a true uncoupler with heat-producing properties (Shabalina and Nedergaard 2011). The

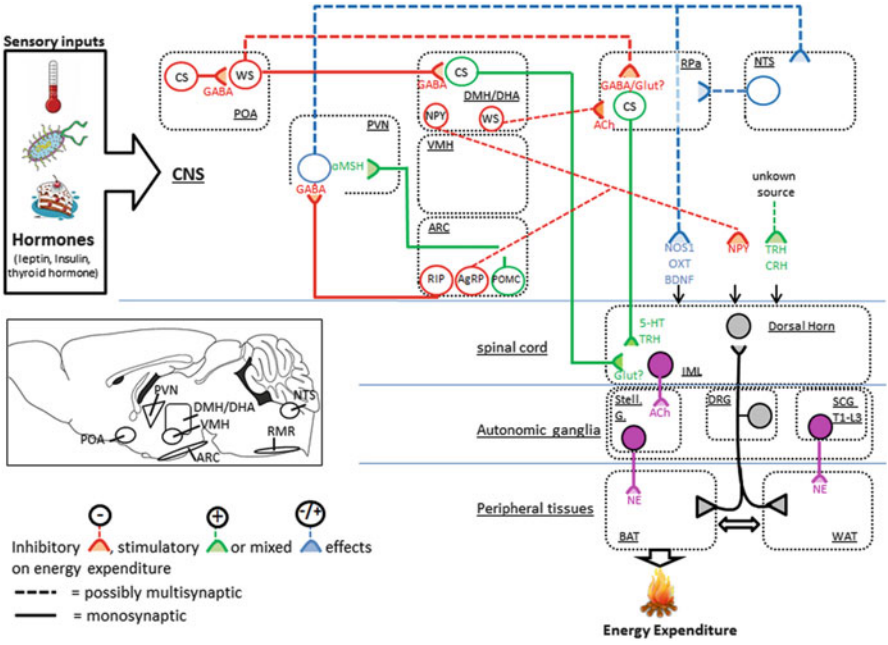

Fig. 4 Schematic overview of central circuits that modulate energy expenditure

idea to burn excess calories by activating BAT in the fight against obesity flared up 35 years ago (Rothwell and Stock 1979) and, after a long hiatus, returned only recently because the existence of functional and inducible BAT was convincingly demonstrated in adult humans (Cypess et al. 2009; Saito et al. 2009; van Marken Lichtenbelt et al. 2009; Virtanen et al. 2009).

2.2 Muscle, Energy Expenditure, and Thermogenesis

Physical activity greatly contributes to whole-body energy expenditure and can be distinguished into exercise and non-exercise activity thermogenesis (NEAT). NEAT involves occupational activities, walking, sitting, standing, fidgeting, talking, leisure activities, etc., but excluding voluntary exercise, sleeping, or eating (Levine 2004) and can substantially contribute to total energy expenditure (15–50%). Therefore, NEAT has been studied as a malleable variable for body weight regulation through increasing energy expenditure.

Physical activity is at least in part genetically encoded, because selective breeding for physical activity in rats resulted in the genetic distinction of high- and low-capacity runners (HCR, LCR, respectively) with low and high incidences of obesity, respectively (Wisloff et al. 2005). HCR had higher total energy expenditure, even though resting energy expenditure was similar between HCR and LCR,

suggesting that increased locomotion largely accounts for changes in total energy expenditure. However, HCR rats had increased mitochondria content and increased sympathetic drive in their skeletal muscle, and the existence of skeletal muscle thermogenesis, not exercise per se, has been suggested as a contributing factor for weight gain resistance on high-fat diets (Gavini et al. 2014; Wisloff et al. 2005). HCR also have increased expression of uncoupling protein 2 and 3 (UCP2 and UCP3). However, in contrast to UCP1, UCP2 and UCP3 do not have true uncoupling functions (Brand and Esteves 2005; Shabalina and Nedergaard 2011). Another potential mechanism to mediate muscle non-shivering thermogenesis is sarco-/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) by uncoupling ATP hydrolysis from Ca^{2+} transport (Bal et al. 2012). Whether SERCA uncoupling is also controlled by sympathetic nerve activity (SNA) similar to UCP1 is unknown. Thus, muscle thermogenesis and its regulatory properties, e.g., during diet-induced obesity, remains ill defined and is an urgent future topic.

2.3 Sensory System

The skin is a very important sensory organ for thermoregulatory control of energy expenditure. It works as a feed-forward system, so that ambient temperature changes are communicated to the brain for temperature defensive adaptations, even though core temperature is not immediately compromised. Temperature-sensing receptors (superfamily of transient receptor potential (TRP) channels) are located in the nerve endings of sensory cells throughout the skin. The cell bodies of these bipolar sensory neurons are found in trigeminal and dorsal root ganglia from where they further communicate with central structures in the spinal cord (dorsal horn lamina) and brain (Damann et al. 2008; Julius and Nathans 2012). Temperature changes in the skin cause the opening of TRP channels and promote the activation of sensory neurons (Voets 2014). This is further communicated to the CNS to modulate SNA in peripheral tissues (e.g., BAT, WAT), which is further addressed in Sect. 3.

BAT and WAT also send feedback information to the CNS via sensory nerves that connect adipocytes via dorsal root ganglia with the brain (Bartness et al. 2010a; b; De et al. 1998; Himms-Hagen et al. 1990; Vaughan et al. 2014). Incoming (afferent) sympathetic nerves can be distinguished from outgoing (efferent) sensory nerves with multisynaptic anterograde (Herpes virus) and retrograde (pseudorabies virus) viral tracers that are injected into BAT or WAT. Surprisingly, many CNS sites showed both sympathetic and sensory connectivity, so that an extensive feedback system for incoming and outgoing signals is likely (Ryu et al. 2015; Ryu and Bartness 2014). Adipose tissue sensory nerves are responsive to metabolic changes (e.g., lipolysis) (Song et al. 2009) and the adipokine leptin (Murphy et al. 2013). In the BAT, sensory input may specifically influence the trophic adaptations to changes in ambient temperature (Himms-Hagen et al. 1990). However, we still do not have a firm understanding of the extent and how sensory feedback loops influence physiological function.

2.4 Endocrine Systems and Energy Expenditure Regulation

The modulation of energy expenditure in response to external and internal challenges involves neuronal input to and outputs from the brain to perform energy expenditure changes in peripheral tissues (e.g., BAT). In addition to this neural loop, various endocrine hormones are important communicators between peripheral tissues and the central sites to regulate energy expenditure.

2.4.1 Cold-Induced Endocrine Hormones

The thyroid hormone is perhaps the most important humoral regulator of metabolism and energy expenditure. Its production is regulated by the brain via the hypothalamic-pituitary-thyroid (HPT) axis, in which the activation of thyrotropin-releasing hormone (TRH) neurons within the hypothalamus ultimately leads to increased thyroid hormone (T4/T3) signaling at peripheral tissues (for review, see Fekete and Lechan 2014; Joseph-Bravo et al. 2015). Thyroid hormone acts on many tissues to promote cellular metabolism and energy expenditure, including effects on heart function, skeletal muscle, and BAT, and as such thyroid hormone is a critical positive regulator of BMR. Changes in ambient temperature or nutritional state influence the activity of TRH neurons within the paraventricular hypothalamus (PVH), resulting in increased release of thyroid hormone from the thyroid gland (Bianco et al. 2002; Zoeller et al. 1990). Leptin also directly stimulates TRH neurons while fasting inhibits these neurons (Huo et al. 2004; Legradi et al. 1997, 1998). Thus, TRH neurons and the HPT axis are critically involved in the regulation of whole-body energy expenditure in response to changes in the external and internal milieu. The thyroid axis may also modulate BAT thermogenesis via central thermoregulatory circuits as discussed in Sect. 3.

Ongoing efforts aim to discover additional "peripheral" endocrine modulators of energy expenditure that could promote weight loss. The muscle-derived hormone irisin (produced by the *fndc5* gene) has received considerable attention. Irisin is increased by exercise to promote the transition of lipid-storing WAT to energy expending BAT-like properties, also known as "browning" of WAT, and is also induced by cold exposure (Bostrom et al. 2012; Lee et al. 2014). Another notable metabolic hormone is fibroblast growth factor 21(FGF21) (Lee et al. 2014). FGF21 is mainly secreted from the liver (Markan et al. 2014) but is also robustly induced by cold exposure in the BAT (Chartoumpekis et al. 2011). Whether FGF21 in BAT is solely induced by cold exposure or instead requires additional metabolic stressors as observed in UCP1-deficient mice (Keipert et al. 2015) remains to be clarified. Also, it is unclear if cold-induced production and secretion of irisin (from muscle) or FGF21 (e.g., BAT) depends on increased sympathetic outflow to skeletal muscle and BAT, respectively.

2.4.2 Endocrine Signals and Adaptive Responses to Energy Restriction

Changes in energy availability (e.g., during fasting) also induce adaptive changes in energy expenditure. This process of energy homeostasis requires the CNS to detect and respond to endocrine hormones (and possibly sensory inputs from peripheral

tissues) that are triggered by negative or positive energy balances (Morrison and Berthoud 2007). Such a decrease in energy expenditure typically accompanies fasting and starvation (Dulloo and Jacquet 1998; Leibel et al. 1995), even though acute fasting may initially rather trigger an increased sympathetic tone to mobilize fat stores in WAT (Goodner et al. 1973; Havel 1968; Koerker et al. 1975). Fasting-induced hypometabolism involves a variety of circulating hormones with central actions, including the adipose-derived hormone leptin. Circulating leptin levels rapidly fall with negative energy balance, and the resulting hypometabolism can be prevented by restoring serum or central leptin levels (Ahima et al. 1996; Rosenbaum et al. 2002, 2005). Taken together, falling leptin levels during starvation are detected by the CNS to change the motivation to eat and to reduce energy expenditure.

The gut hormone ghrelin also contributes to starvation-induced adaptive responses. Ghrelin release is increased during starvation and suppresses energy expenditure (Muller et al. 2015). Also insulin and glucagon are highly regulated by energy intake and contribute substantially to the starvation response, e.g., induction of lipolysis. Considering the variety of hormones that act in the brain to suppress food intake and energy expenditure simultaneously, it is suggested that a precise interaction of feeding and thermoregulatory neuronal circuits exist. However, comprehensive knowledge of how these systems are coordinated is missing and a key goal for the future.

2.4.3 Overfeeding and Energy Expenditure: Diet-Induced Thermogenesis

A negative energy balance (e.g., during fasting) is associated with a reduction in energy expenditure, while increased food intake (e.g., during high-fat feeding) induces thermogenic responses, also known as diet-induced thermogenesis (DIT) (Rothwell et al. 1983). Rothwell and Stock also demonstrated that low-protein diet increased energy expenditure, suggesting that both overfeeding and protein restriction triggered DIT (Rothwell et al. 1983). The circulating hormone FGF21 is well known to increase energy expenditure and promote the browning of WAT (Douris et al. 2015; Fisher et al. 2012), but only recent work showed that FGF21 is required for the low protein-induced energy expenditure (Laeger et al. 2014; Morrison and Laeger 2015). Whether FGF21 promotes these effects within the periphery and/or through the brain remains unclear (Kharitonenkov and Adams 2014; Owen et al. 2015).

In summary, the maintenance of body weight and thermoregulation in response changes in external temperature and food availability are mediated by an intricate neural and endocrine network.

3 Neural Circuits That Modulate Energy Expenditure

The brain network that regulates adaptive thermogenesis receives inputs from temperature- and energy-sensing neurons through hypothalamic and brain stem areas such as the preoptic area (POA), arcuate nucleus (ARC), and nucleus of the

solitary tract (NTS) (Morrison et al. 2014). Naturally, many physiological states such as fever, stress-induced hyperthermia, and diurnal fluctuation of body temperature require attention from these central thermoregulatory circuits. Some physiological states may require opposing adaptations of energy expenditure, e.g., cold exposure increases energy expenditure, but fasting requires energy preservation and decreased energy expenditure. Thus, if cold exposure and fasting challenges are combined, this conflict needs to be solved by the brain for an appropriate modulation of energy expenditure, manifested in a change of SNA and secretion of neurohormones.

The anatomical location of BAT-related CNS neurons that control BAT thermogenesis stems from multisynaptic, retrograde tracing studies with PRV infections of the BAT (Cano et al. 2003). Another tool to identify thermoregulatory neurons is to track which neurons are activated by changes in ambient temperature. The early response gene cFos is rapidly induced by neuronal firing and is a reliable and efficient marker for neurons that are activated in response to temperature changes. (Bamshad et al. 1999; Cano et al. 2003; Oldfield et al. 2002). However, molecular identities and synaptic connections of these neurons are not entirely understood. In this section, we focus on central circuits that govern BAT SNA. We also briefly discuss neural circuits that modulate the release of neurohormones that affect adaptive thermogenesis.

3.1 Hypothalamus

Much of the literature that defines central sites that control BAT thermogenesis stems from research on pyrogenic stimuli and cold-defense behavior. The POA has received specific attention in the control of such thermoregulatory processes (Nakamura 2011). Thermoregulatory neurons in the POA receive thermosensory neuronal inputs from the skin, but many POA neurons are internally temperature sensitive. They change their firing activity with local temperature changes, thus enabling the POA to detect both peripheral and brain temperature changes (Boulant and Dean 1986; Nakamura and Morrison 2008, 2010). These neurons are proposed to be mostly warm-sensitive GABAergic (inhibitory) neurons that directly inhibit BAT sympathetic premotor neurons in the rostral medullary raphe (RMR) or indirectly through the dorsomedial hypothalamus/dorsal hypothalamic area (DMH/DHA) (Yoshida et al. 2009). Therefore, during a cold exposure, warm-sensitive POA neurons are inhibited and enable thermogenic neurons in the DMH/DHA and RMR to increase BAT SNA.

Some warm-sensitive GABAergic POA neurons express prostaglandin E receptor subtype EP3 and mediate febrile responses by using the same POA > DMH/DHA > RMR circuits to BAT (Lazarus et al. 2007; Nakamura et al. 2009; Scammell et al. 1996; Ushikubi et al. 1998). However, stimulatory glutamatergic inputs to the DMH/DHA have been proposed as well (Madden and Morrison 2004), and cold-sensitive glutamatergic POA neurons may provide these inputs (Dimitrov et al. 2011). Because there are also warm-activated cholinergic neurons in the DMH

that directly inhibit thermogenic RMR neurons (Jeong et al. 2015), the POA is very likely to contain warm-sensitive glutamatergic neurons that directly innervate these DMH neurons.

Other lines of research that are more concerned with body weight regulation have focused on additional neuronal sites and their effect on energy expenditure and body weight regulation. These energy homeostatic sites have not been well characterized for their responsiveness to thermal inputs, but clearly modulate BAT SNA. The ARC is highly responsive to changes in energy/nutritional state (e.g., fasting) and mediates changes in BAT SNA. Pro-opiomelanocortin (POMC)-expressing neurons in the ARC are anorexigenic neurons that increase BAT thermogenesis. The secretion of ∝-melanocyte-stimulating hormone, a byproduct of POMC, or melanotan II (MTII), an MC4R agonist, activates melanocortin 4 receptors (MC4R) to increase energy expenditure and UCP1 expression via BAT SNA, while loss of MC4R decreases energy expenditure and promotes weight gain (Chen et al. 2000; Haynes et al. 1999; Ste Marie et al. 2000). Although the exact sites of MC4R-mediated BAT activation is not completely understood, MC4Rs in the PVH are not involved in the regulation of energy expenditure, while cholinergic neurons in the intermediolateral nucleus (IML) within the spinal cord are sufficient to restore energy expenditure in whole-body MC4R-deficient mice (Berglund et al. 2014; Rossi et al. 2011; Sohn et al. 2013).

GABAergic ARC neurons agouti-related peptide/neuropeptide Y (AgRP/NPY) neurons and RIP-Cre neurons both affect BAT SNA. NPY derived from ARC AgRP/NPY neurons inhibits BAT SNA via activation of Y1 receptor in unknown target neurons (Shi et al. 2013). Similarly, DMH NPY neurons also inhibit BAT sympathetic control (Bi et al. 2003; Chao et al. 2011), and the central administration of NPY induces torpor-like hypothermia (Dark and Pelz 2008), suggesting overall sympathoinhibitory NPY function in the brain. Another set of sympathoinhibitory neurons exist in the DMH that are warm-activated neurons and project to the RMR (Jeong et al. 2015). RIP-Cre neurons are a distinct population of GABAergic neurons within the ARC that inhibit PVH neurons to enhance BAT activation (Kong et al. 2012).

Interestingly, several central thermoregulatory and energy homeostatic neurons express leptin receptors or are controlled by LepRb neurons (Bachman et al. 2002). Leptin action within the DMH has a clear sympathostimulatory effect on BAT thermogenesis and associated cardiovascular responses, which are largely independent of anorexic leptin effects (Enriori et al. 2011; Rezai-Zadeh et al. 2014). Some of these leptin-mediated effects on energy expenditure requires glutamate signaling (Xu et al. 2013), even though leptin likely exerts its effects via complex inhibition and stimulation of several neuronal populations, including its interaction with insulin via POMC neurons to promote browning of WAT (Dodd et al. 2015).

The PVH is an essential output center for neuronal and humoral signals and has been rediscovered as an important thermoregulatory site. General PVH activation prevents cold- and prostaglandin E2-increased BAT SNA by increasing GABAergic inputs to the RMR (Madden and Morrison 2009). Because the PVH consists of mainly glutamatergic neurons, this may involve multisynaptic circuits,

possibly involving identified PVH > NTS > RMR circuits that are modulated by ARC RIP-Cre neurons (Kong et al. 2012). With the use of the modern genetic toolbox, some thermoregulatory PVH subpopulations have been further identified as nitric oxide synthase 1 (NOS1), oxytocin (OXT), or brain-derived neurotrophic factor (BDNF)-expressing neurons that project directly to sympathetic preganglionic neurons in the spinal cord to increase BAT activity (An et al. 2015; Sutton et al. 2014).

PVH OXT neurons are a subpopulation of NOS1 neurons, and activation of either population activates BAT thermogenesis. Interestingly, simple-minded homolog 1 expressing PVH neurons, which marks most PVH neurons, also increase BAT thermogenesis when activated (Sutton et al. 2014), contradicting earlier findings mentioned above. Similarly, posterior PVH BDNF neurons seem to regulate BAT thermogenesis because PVH-specific BDNF deletion reduced energy expenditure and increased body weight (An et al. 2015). Whether PVH BDNF neurons also express NOS1 is not known. These findings are in line with earlier studies showing that cFos in the PVH is induced by both cold and warm exposures (Cano et al. 2003; Yoshida et al. 2002). Taken together, the PVH seems to be involved in both directional controls of BAT sympathetic activity, and future research needs to identify the neurochemical properties of sympathoinhibitory PVH neurons.

In addition to the neural control of sympathetic BAT inputs, the PVH also modulates humoral effectors of BAT thermogenesis. Cold exposure increases while warm exposure decreases thyroid hormone levels via TRH neurons to stimulate BAT activity and BMR (Andersson et al. 1963; Eastman et al. 1974; Kim 2008). Interestingly, in addition to the intensely studied peripheral effects of thyroid hormone, more recent data also indicate a central function of thyroid hormone to increase BAT SNA (Coppola et al. 2007; Lopez et al. 2010). Furthermore, TRH neurons are found outside the PVH in important thermoregulatory sites like the DMH and RMR (based on Allen Brain Atlas data, http://mouse.brain-map.org/), and compelling functional data support thermoregulatory synergistic effects of TRH and leptin via brainstem circuits (Hermann et al. 2006; Rogers et al. 2009, 2011).

Another thermoregulatory PVH neurohormone is corticotropin-releasing hormone (CRH), which is increased by low glucose levels or other stressor. CRH increased pituitary adrenocorticotropic hormone release to induce stress hormones like glucocorticoids that upon other functions inhibit BAT activity peripherally (Moriscot et al. 1993). Like TRH neurons, CRH neurons are also found outside the PVH (based on Allen Brain Atlas data) and may function within thermoregulatory central circuitries, e.g., central CRH infusions into the POA and other hypothalamic sites stimulate BAT SNA output (Egawa et al. 1990a,b).

The VMH has long been implicated in the control of BAT SNA and energy expenditure even though the pathways leading to BAT sympathetic neurons have not been identified (Perkins et al. 1981), due to the typical lack of PRV labeling. Nonetheless, insulin, thyroid hormone, and estrogen affect BAT SNA through the VMH (Klockener et al. 2011; Lopez et al. 2010; Musatov et al. 2007). Estrogen

signals via its receptor ER$\propto$ and promotes different aspects of thermogenesis via distinct ER$\propto$-expressing VMH neurons (Correa et al. 2015; Xu et al. 2011). Future studies are needed to explore the involved downstream targets within these thermoregulatory circuitries.

Finally, a subpopulation of orexin/hypocretin neurons in the lateral hypothalamic area (LHA) project to BAT sympathetic premotor neurons in the RMR, and the secretion of orexin seems to potentiate already existing BAT sympathostimulatory signals onto the RMR (Berthoud et al. 2005; Tupone et al. 2011). Interestingly, LHA orexin neurons are not involved in cold- or pyrogen-induced BAT thermogenesis (Nakamura et al. 2005) but are rather critical for stress-induced BAT thermogenesis (Zhang et al. 2010), even though the DMH may be more dominantly involved in stress-induced thermogenesis (Kataoka et al. 2014).

3.2 Brainstem

Hypothalamic areas that receive BAT-related inputs send efferent fibers to sympathetic premotor neurons in the RMR or project directly to spinal preganglionic neurons as mentioned above for the PVH. The RMR includes the rostral raphe pallidus (rRPa), raphe magnus, parapyramidal area, and ventrolateral medulla (VLM) and contains main sympathetic premotor neurons for BAT, vasculature, and heart (Nakamura 2011). The rRPa is especially important for BAT thermogenesis and innervated by many excitatory and inhibitory neuronal fibers that are originated from the hypothalamus and brainstem. rRPa neurons receive tonic inhibitory inputs at neutral conditions, most notably by warm-sensitive GABAergic POA neurons, and disinhibition of rRPa neurons by various thermogenic signals increases BAT SNA. Catecholaminergic neurons in the VLM including the A1/C1 neurons inhibit rRPa BAT premotor neurons through the activation of $\propto$2 adrenergic receptor, possibly explaining the systemic $\propto$2 adrenergic agonist-mediated hypothermia (Cao et al. 2010; Madden et al. 2013). C1 neurons have been proposed to respond to emergencies such as hypoxia and glucoprivation (Guyenet et al. 2013), and this VLM > rRPa pathway may account for inhibition of BAT during those situations (Madden 2012; Madden and Morrison 2005).

Neurons in the NTS receive viscerosensory information and inhibit BAT SNA when activated (Cao et al. 2010). The NTS also regulates BAT activity independent of hypothalamic inputs via hindbrain leptin/TRH signaling (Rogers et al. 2009) and ARC RIP-Cre neurons (Kong et al. 2012), implying it as a potential integrative site of viscerosensory and metabolic signals.

Other brainstem areas such as the lateral parabrachial nucleus, periaqueductal gray, and locus coeruleus have been associated with BAT sympathetic control as sensory afferent or effector efferent relay stations, but more studies are required to precisely identify their involvement in specific conditions (Almeida et al. 2004; Chen et al. 2002; Nakamura and Morrison 2008, 2010; Rathner and Morrison 2006; Yoshida et al. 2005).

Overall, many central sites possess a mixture of sympathostimulatory and sympathoinhibitory sets of neurons that may interact with the RMR as a master modulator for sympathetic BAT inputs. The hypothalamus is central for regulation of adaptive thermogenesis. Recent technical advancements of various neural mapping, recording, and manipulation tools at a spatially, genetically, and temporally controlled manner have accelerated our understanding of how brain works. The next step of understanding how the brain regulates energy expenditure would be deciphering where and how various environmental and internal signals are integrated and ramified to different thermoeffectors.

4 Remaining Questions and Conclusion

Metabolic diseases like obesity and diabetes are still increasing and remain a serious healthcare problem. Despite considerable efforts to treat obesity, it has been widely recognized that any treatment is flawed by the powerful ability of the body to adapt to dietary changes. A considerable part of the population is affected by the opposite problem: the failure to maintain healthy body weight and excessive weight loss due to enhanced energy expenditure in cachexic patients. This state is observed in neurodegenerative diseases and cancer and in patients with progressing acquired immune deficiency syndrome (AIDS) (Argiles et al. 2015; Dupuis et al. 2011; Salomon et al. 2002). Thus, targeting the central or peripheral nervous system to modulate energy expenditure is a realistic goal that would benefit many human patients and is currently under intense investigation.

One major roadblock is that the molecular basis of defended homeostatic levels (e.g., body weight and body temperature) remains unclear. Also, how changes in the defended levels (e.g., during obesity) are realized at the molecular level. Specifically, the interplay of energy expenditure and food intake is important to defend homeostatic levels. The reviewed work in this chapter compiles research from the thermoregulation and body weight regulation fields. However, more studies are required with emphasis on the interaction of thermoregulatory and food intake regulating neuronal circuits. Only if we are able to modulate the defended homeostatic body weight, we a will be able to achieve sustainable corrections in body weight.

References

Ahima RS, Prabakaran D, Mantzoros C, Qu D, Lowell B, Maratos-Flier E, Flier JS (1996) Role of leptin in the neuroendocrine response to fasting. Nature 382:250–252

Almeida MC, Steiner AA, Coimbra NC, Branco LG (2004) Thermoeffector neuronal pathways in fever: a study in rats showing a new role of the locus coeruleus. J Physiol 558:283–294

An JJ, Liao GY, Kinney CE, Sahibzada N, Xu B (2015) Discrete BDNF neurons in the paraventricular hypothalamus control feeding and energy expenditure. Cell Metab 22:175–88

Andersson B, Ekman L, Gale CC, Sundsten JW (1963) Control of thyrotrophic hormone (Tsh) secretion by the "Heat Loss Center". Acta Physiol Scand 59:12–33

Andrews PL, Rothwell NJ, Stock MJ (1985) Influence of subdiaphragmatic vagotomy and brown fat sympathectomy on thermogenesis in rats. Am J Physiol 249:E239–E243

Argiles JM, Lopez-Soriano FJ, Busquets S (2015) Muscle wasting in cancer: the role of mitochondria. Curr Opin Clin Nutr Metab Care 18:221–225

Bachman ES, Dhillon H, Zhang CY, Cinti S, Bianco AC, Kobilka BK, Lowell BB (2002) betaAR signaling required for diet-induced thermogenesis and obesity resistance. Science 297:843–845

Bal NC, Maurya SK, Sopariwala DH, Sahoo SK, Gupta SC, Shaikh SA, Pant M, Rowland LA, Bombardier E, Goonasekera SA, Tupling AR, Molkentin JD, Periasamy M (2012) Sarcolipin is a newly identified regulator of muscle-based thermogenesis in mammals. Nat Med 18:1575–1579

Bamshad M, Song CK, Bartness TJ (1999) CNS origins of the sympathetic nervous system outflow to brown adipose tissue. Am J Physiol 276:R1569–R1578

Bartness TJ, Wade GN (1984) Effects of interscapular brown adipose tissue denervation on body weight and energy metabolism in ovariectomized and estradiol-treated rats. Behav Neurosci 98:674–685

Bartness TJ, Shrestha YB, Vaughan CH, Schwartz GJ, Song CK (2010a) Sensory and sympathetic nervous system control of white adipose tissue lipolysis. Mol Cell Endocrinol 318:34–43

Bartness TJ, Vaughan CH, Song CK (2010b) Sympathetic and sensory innervation of brown adipose tissue. Int J Obes (Lond) 34(Suppl 1):S36–S42

Berglund ED, Liu T, Kong X, Sohn JW, Vong L, Deng Z, Lee CE, Lee S, Williams KW, Olson DP, Scherer PE, Lowell BB, Elmquist JK (2014) Melanocortin 4 receptors in autonomic neurons regulate thermogenesis and glycemia. Nat Neurosci 17:911–913

Bernard C (1957) An introduction to the study of experimental medicine. Dover, New York, Originally published in 1865; first English translation by Henry Copley Greene, published by Macmillan & Co., Ltd., 1927. Reprint Edn. Dover edn

Berthoud HR, Patterson LM, Sutton GM, Morrison C, Zheng H (2005) Orexin inputs to caudal raphe neurons involved in thermal, cardiovascular, and gastrointestinal regulation. Histochem Cell Biol 123:147–156

Bi S, Robinson BM, Moran TH (2003) Acute food deprivation and chronic food restriction differentially affect hypothalamic NPY mRNA expression. Am J Physiol Regul Integr Comp Physiol 285:R1030–R1036

Bianco AC, Salvatore D, Gereben B, Berry MJ, Larsen PR (2002) Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. Endocr Rev 23:38–89

Bostrom P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC, Rasbach KA, Bostrom EA, Choi JH, Long JZ, Kajimura S, Zingaretti MC, Vind BF, Tu H, Cinti S, Hojlund K, Gygi SP, Spiegelman BM (2012) A PGC1-alpha-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. Nature 481:463–468

Boulant JA, Dean JB (1986) Temperature receptors in the central nervous system. Annu Rev Physiol 48:639–654

Brand MD, Esteves TC (2005) Physiological functions of the mitochondrial uncoupling proteins UCP2 and UCP3. Cell Metab 2:85–93

Brand MD, Brindle KM, Buckingham JA, Harper JA, Rolfe DF, Stuart JA (1999) The significance and mechanism of mitochondrial proton conductance. Int J Obes Relat Metab Disord 23(Suppl 6):S4–11

Brobeck JR (1946) Mechanism of the development of obesity in animals with hypothalamic lesions. Physiol Rev 26:541–559

Cano G, Passerin AM, Schiltz JC, Card JP, Morrison SF, Sved AF (2003) Anatomical substrates for the central control of sympathetic outflow to interscapular adipose tissue during cold exposure. J Comp Neurol 460:303–326

Cao WH, Madden CJ, Morrison SF (2010) Inhibition of brown adipose tissue thermogenesis by neurons in the ventrolateral medulla and in the nucleus tractus solitarius. Am J Physiol Regul Integr Comp Physiol 299:R277–R290

Card JP, Enquist LW (2014) Transneuronal circuit analysis with pseudorabies viruses. Curr Protoc Neurosci 68:1

Cechetto DF (1987) Central representation of visceral function. Fed Proc 46:17–23

Chao PT, Yang L, Aja S, Moran TH, Bi S (2011) Knockdown of NPY expression in the dorsomedial hypothalamus promotes development of brown adipocytes and prevents diet-induced obesity. Cell Metab 13:573–583

Chartoumpekis DV, Habeos IG, Ziros PG, Psyrogiannis AI, Kyriazopoulou VE, Papavassiliou AG (2011) Brown adipose tissue responds to cold and adrenergic stimulation by induction of FGF21. Mol Med 17:736–740

Chen AS, Metzger JM, Trumbauer ME, Guan XM, Yu H, Frazier EG, Marsh DJ, Forrest MJ, Gopal-Truter S, Fisher J, Camacho RE, Strack AM, Mellin TN, MacIntyre DE, Chen HY, Van der Ploeg LH (2000) Role of the melanocortin-4 receptor in metabolic rate and food intake in mice. Transgenic Res 9:145–154

Chen XM, Nishi M, Taniguchi A, Nagashima K, Shibata M, Kanosue K (2002) The caudal periaqueductal gray participates in the activation of brown adipose tissue in rats. Neurosci Lett 331:17–20

Coppola A, Liu ZW, Andrews ZB, Paradis E, Roy MC, Friedman JM, Ricquier D, Richard D, Horvath TL, Gao XB, Diano S (2007) A central thermogenic-like mechanism in feeding regulation: an interplay between arcuate nucleus T3 and UCP2. Cell Metab 5:21–33

Correa SM, Newstrom DW, Warne JP, Flandin P, Cheung CC, Lin-Moore AT, Pierce AA, Xu AW, Rubenstein JL, Ingraham HA (2015) An estrogen-responsive module in the ventromedial hypothalamus selectively drives sex-specific activity in females. Cell Rep 10:62–74

Cox JE, Powley TL (1981) Intragastric pair feeding fails to prevent VMH obesity or hyperinsulinemia. Am J Physiol 240:E566–E572

Craig AD (2002) How do you feel? Interoception: the sense of the physiological condition of the body. Nat Rev Neurosci 3:655–666

Cypess AM, Lehman S, Williams G, Tal I, Rodman D, Goldfine AB, Kuo FC, Palmer EL, Tseng YH, Doria A, Kolodny GM, Kahn CR (2009) Identification and importance of brown adipose tissue in adult humans. N Engl J Med 360:1509–1517

Damann N, Voets T, Nilius B (2008) TRPs in our senses. Curr Biol 18:R880–R889

Dark J, Pelz KM (2008) NPY Y1 receptor antagonist prevents NPY-induced torpor-like hypothermia in cold-acclimated Siberian hamsters. Am J Physiol Regul Integr Comp Physiol 294: R236–R245

De MR, Ricquier D, Cinti S (1998) TH-, NPY-, SP-, and CGRP-immunoreactive nerves in interscapular brown adipose tissue of adult rats acclimated at different temperatures: an immunohistochemical study. J Neurocytol 27:877–886

Dimitrov EL, Kim YY, Usdin TB (2011) Regulation of hypothalamic signaling by tuberoinfundibular peptide of 39 residues is critical for the response to cold: a novel peptidergic mechanism of thermoregulation. J Neurosci 31:18166–18179

Dodd GT, Decherf S, Loh K, Simonds SE, Wiede F, Balland E, Merry TL, Munzberg H, Zhang ZY, Kahn BB, Neel BG, Bence KK, Andrews ZB, Cowley MA, Tiganis T (2015) Leptin and insulin act on POMC neurons to promote the browning of white fat. Cell 160:88–104

Douris N, Stevanovic DM, Fisher FM, Cisu TI, Chee MJ, Nguyen NL, Zarebidaki E, Adams AC, Kharitonenkov A, Flier JS, Bartness TJ, Maratos-Flier E (2015) Central fibroblast growth factor 21 browns white fat via sympathetic action in male mice. Endocrinology 156:2470–2481

Dulloo AG, Jacquet J (1998) Adaptive reduction in basal metabolic rate in response to food deprivation in humans: a role for feedback signals from fat stores. Am J Clin Nutr 68:599–606

Dupuis L, Pradat PF, Ludolph AC, Loeffler JP (2011) Energy metabolism in amyotrophic lateral sclerosis. Lancet Neurol 10:75–82

Eastman CJ, Ekins RP, Leith IM, Williams ES (1974) Thyroid hormone response to prolonged cold exposure in man. J Physiol 241:175–181

Egawa M, Yoshimatsu H, Bray GA (1990a) Effect of corticotropin releasing hormone and neuropeptide Y on electrophysiological activity of sympathetic nerves to interscapular brown adipose tissue. Neuroscience 34:771–775

Egawa M, Yoshimatsu H, Bray GA (1990b) Preoptic area injection of corticotropin-releasing hormone stimulates sympathetic activity. Am J Physiol 259:R799–R806

Enriori P, Sinnayah P, Simonds S, Garcia Rudaz C, Cowley M (2011) Leptin action in the dorsomedial hypothalamus increases sympathetic tone to brown adipose tissue in spite of systemic leptin resistance. J Neurosci 31:12189–12197

Fealey RD (2013) Interoception and autonomic nervous system reflexes thermoregulation. Handb Clin Neurol 117:79–88

Fekete C, Lechan RM (2014) Central regulation of hypothalamic-pituitary-thyroid axis under physiological and pathophysiological conditions. Endocr Rev 35:159–194

Fisher FM, Kleiner S, Douris N, Fox EC, Mepani RJ, Verdeguer F, Wu J, Kharitonenkov A, Flier JS, Maratos-Flier E, Spiegelman BM (2012) FGF21 regulates PGC-1alpha and browning of white adipose tissues in adaptive thermogenesis. Genes Dev 26:271–281

Gavini CK, Mukherjee S, Shukla C, Britton SL, Koch LG, Shi H, Novak CM (2014) Leanness and heightened nonresting energy expenditure: role of skeletal muscle activity thermogenesis. Am J Physiol Endocrinol Metab 306:E635–E647

Giordano A, Frontini A, Castellucci M, Cinti S (2004) Presence and distribution of cholinergic nerves in rat mediastinal brown adipose tissue. J Histochem Cytochem 52:923–930

Goodner CJ, Koerker DJ, Werbach JH, Toivola P, Gale CC (1973) Adrenergic regulation of lipolysis and insulin secretion in the fasted baboon. Am J Physiol 224:534–539

Grkovic I, Anderson CR (1997) Calbindin D28K-immunoreactivity identifies distinct subpopulations of sympathetic pre- and postganglionic neurons in the rat. J Comp Neurol 386:245–259

Guyenet PG, Stornetta RL, Bochorishvili G, Depuy SD, Burke PG, Abbott SB (2013) C1 neurons: the body's EMTs. Am J Physiol Regul Integr Comp Physiol 305:R187–R204

Halaas JL, Gajiwala KS, Maffei M, Cohen SL, Chait BT, Rabinowitz D, Lallone RL, Burley SK, Friedman JM (1995) Weight-reducing effects of the plasma protein encoded by the obese gene. Science 269:543–546

Havel RJ (1968) The autonomic nervous system and intermediary carbohydrate and fat metabolism. Anesthesiology 29:702–713

Haynes WG, Morgan DA, Djalali A, Sivitz WI, Mark AL (1999) Interactions between the melanocortin system and leptin in control of sympathetic nerve traffic. Hypertension 33:542–547

Hermann GE, Barnes MJ, Rogers RC (2006) Leptin and thyrotropin-releasing hormone: cooperative action in the hindbrain to activate brown adipose thermogenesis. Brain Res 1117:118–124

Himms-Hagen J, Cui J, Lynn SS (1990) Sympathetic and sensory nerves in control of growth of brown adipose tissue: Effects of denervation and of capsaicin. Neurochem Int 17:271–279

Huo L, Munzberg H, Nillni EA, Bjorbaek C (2004) Role of signal transducer and activator of transcription 3 in regulation of hypothalamic trh gene expression by leptin. Endocrinology 145:2516–2523

Janig W (1996) Neurobiology of visceral afferent neurons: neuroanatomy, functions, organ regulations and sensations. Biol Psychol 42:29–51

Jeong JH, Lee DK, Blouet C, Ruiz HH, Buettner C, Chua S Jr, Schwartz GJ, Jo YH (2015) Cholinergic neurons in the dorsomedial hypothalamus regulate mouse brown adipose tissue metabolism. Mol Metab 4:483–492

Joseph-Bravo P, Jaimes-Hoy L, Maria UR, Charli JL (2015) 60 YEARS OF NEUROENDOCRINOLOGY: TRH, the first hypophysiotropic releasing hormone isolated: control of the pituitary-thyroid axis. J Endocrinol 226:T85–T100

Julius D, Nathans J (2012) Signaling by sensory receptors. Cold Spring Harb Perspect Biol 4: a005991

Kataoka N, Hioki H, Kaneko T, Nakamura K (2014) Psychological stress activates a dorsomedial hypothalamus-medullary raphe circuit driving brown adipose tissue thermogenesis and hyperthermia. Cell Metab 20:346–358

Keipert S, Kutschke M, Lamp D, Brachthauser L, Neff F, Meyer CW, Oelkrug R, Kharitonenkov A, Jastroch M (2015) Genetic disruption of uncoupling protein 1 in mice renders brown adipose tissue a significant source of FGF21 secretion. Mol Metab 4:537–542

Kennedy GC (1951) Experimental hypothalamic obesity. Proc R Soc Med 44:899–902

Kharitonenkov A, Adams AC (2014) Inventing new medicines: The FGF21 story. Mol Meta 3:221–229

Kim B (2008) Thyroid hormone as a determinant of energy expenditure and the basal metabolic rate. Thyroid 18:141–144

Klockener T, Hess S, Belgardt BF, Paeger L, Verhagen LA, Husch A, Sohn JW, Hampel B, Dhillon H, Zigman JM, Lowell BB, Williams KW, Elmquist JK, Horvath TL, Kloppenburg P, Bruning JC (2011) High-fat feeding promotes obesity via insulin receptor/PI3K-dependent inhibition of SF-1 VMH neurons. Nat Neurosci 14:911–918

Koerker DJ, Goodner CJ, Chideckel EW, Ensinck JW (1975) Adaptation to fasting in baboon. II. Regulation of lipolysis early and late in fasting. Am J Physiol 229:350–354

Kong D, Tong Q, Ye C, Koda S, Fuller PM, Krashes MJ, Vong L, Ray RS, Olson DP, Lowell BB (2012) GABAergic RIP-Cre neurons in the arcuate nucleus selectively regulate energy expenditure. Cell 151:645–657

Laeger T, Henagan TM, Albarado DC, Redman LM, Bray GA, Noland RC, Munzberg H, Hutson SM, Gettys TW, Schwartz MW, Morrison CD (2014) FGF21 is an endocrine signal of protein restriction. J Clin Invest 124:3913–3922

Lazarus M, Yoshida K, Coppari R, Bass CE, Mochizuki T, Lowell BB, Saper CB (2007) EP3 prostaglandin receptors in the median preoptic nucleus are critical for fever responses. Nat Neurosci 10:1131–1133

Lee P, Linderman JD, Smith S, Brychta RJ, Wang J, Idelson C, Perron RM, Werner CD, Phan GQ, Kammula US, Kebebew E, Pacak K, Chen KY, Celi FS (2014) Irisin and FGF21 are cold-induced endocrine activators of brown fat function in humans. Cell Metab 19:302–309

Legradi G, Emerson CH, Ahima RS, Flier JS, Lechan RM (1997) Leptin prevents fasting-induced suppression of prothyrotropin-releasing hormone messenger ribonucleic acid in neurons of the hypothalamic paraventricular nucleus. Endocrinology 138:2569–2576

Legradi G, Emerson CH, Ahima RS, Rand WM, Flier JS, Lechan RM (1998) Arcuate nucleus ablation prevents fasting-induced suppression of ProTRH mRNA in the hypothalamic paraventricular nucleus. Neuroendocrinology 68:89–97

Leibel RL, Rosenbaum M, Hirsch J (1995) Changes in energy expenditure resulting from altered body weight. N Engl J Med 332:621–628

Levine JA (2004) Non-exercise activity thermogenesis (NEAT). Nutr Rev 62:S82–S97

Lidell ME, Betz MJ, Dahlqvist LO, Heglind M, Elander L, Slawik M, Mussack T, Nilsson D, Romu T, Nuutila P, Virtanen KA, Beuschlein F, Persson A, Borga M, Enerback S (2013) Evidence for two types of brown adipose tissue in humans. Nat Med 19:631–634

Loewy AD (1998) Viruses as transneuronal tracers for defining neural circuits. Neurosci Biobehav Rev 22:679–684

Lopez M, Varela L, Vazquez MJ, Rodriguez-Cuenca S, Gonzalez CR, Velagapudi VR, Morgan DA, Schoenmakers E, Agassandian K, Lage R, Martinez de Morentin PB, Tovar S, Nogueiras R, Carling D, Lelliott C, Gallego R, Oresic M, Chatterjee K, Saha AK, Rahmouni K, Dieguez C, Vidal-Puig A (2010) Hypothalamic AMPK and fatty acid metabolism mediate thyroid regulation of energy balance. Nat Med 16:1001–1008

Madden CJ (2012) Glucoprivation in the ventrolateral medulla decreases brown adipose tissue sympathetic nerve activity by decreasing the activity of neurons in raphe pallidus. Am J Physiol Regul Integr Comp Physiol 302:R224–R232

Madden CJ, Morrison SF (2004) Excitatory amino acid receptors in the dorsomedial hypothalamus mediate prostaglandin-evoked thermogenesis in brown adipose tissue. Am J Physiol Regul Integr Comp Physiol 286:R320–R325

Madden CJ, Morrison SF (2005) Hypoxic activation of arterial chemoreceptors inhibits sympathetic outflow to brown adipose tissue in rats. J Physiol 566:559–573

Madden CJ, Morrison SF (2009) Neurons in the paraventricular nucleus of the hypothalamus inhibit sympathetic outflow to brown adipose tissue. Am J Physiol Regul Integr Comp Physiol 296:R831–R843

Madden C, Tupone D, Cano G, Morrison S (2013) Î}\^{I}{ ± 2 Adrenergic receptor-mediated inhibition of thermogenesis. J Neurosci 33:2017–2028

Markan KR, Naber MC, Ameka MK, Anderegg MD, Mangelsdorf DJ, Kliewer SA, Mohammadi M, Potthoff MJ (2014) Circulating FGF21 is liver derived and enhances glucose uptake during refeeding and overfeeding. Diabetes 63:4057–4063

Mayer J, Barrnett RJ (1955) Obesity following unilateral hypothalamic lesions in rats. Science 121:599–600

Moriscot A, Rabelo R, Bianco AC (1993) Corticosterone inhibits uncoupling protein gene expression in brown adipose tissue. Am J Physiol 265:E81–E87

Morrison CD, Berthoud HR (2007) Neurobiology of nutrition and obesity. Nutr Rev 65:517–534

Morrison CD, Laeger T (2015) Protein-dependent regulation of feeding and metabolism. Trends Endocrinol Metab 26:256–262

Morrison S, Madden C, Tupone D (2014) Central neural regulation of brown adipose tissue thermogenesis and energy expenditure. Cell Metab 19:741–756

Muller TD, Nogueiras R, Andermann ML, Andrews ZB, Anker SD, Argente J, Batterham RL, Benoit SC, Bowers CY, Broglio F, Casanueva FF, D'Alessio D, Depoortere I, Geliebter A, Ghigo E, Cole PA, Cowley M, Cummings DE, Dagher A, Diano S, Dickson SL, Dieguez C, Granata R, Grill HJ, Grove K, Habegger KM, Heppner K, Heiman ML, Holsen L, Holst B, Inui A, Jansson JO, Kirchner H, Korbonits M, Laferrere B, LeRoux CW, Lopez M, Morin S, Nakazato M, Nass R, Perez-Tilve D, Pfluger PT, Schwartz TW, Seeley RJ, Sleeman M, Sun Y, Sussel L, Tong J, Thorner MO, van der Lely AJ, Van der Ploeg LH, Zigman JM, Kojima M, Kangawa K, Smith RG, Horvath T, Tschop MH (2015) Ghrelin. Mol Metab 4:437–460

Murphy KT, Schwartz GJ, Nguyen NL, Mendez JM, Ryu V, Bartness TJ (2013) Leptin-sensitive sensory nerves innervate white fat. Am J Physiol Endocrinol Metab 304:E1338–E1347

Musatov S, Chen W, Pfaff DW, Mobbs CV, Yang XJ, Clegg DJ, Kaplitt MG, Ogawa S (2007) Silencing of estrogen receptor alpha in the ventromedial nucleus of hypothalamus leads to metabolic syndrome. Proc Natl Acad Sci USA 104:2501–2506

Nakamura K (2011) Central circuitries for body temperature regulation and fever. Am J Physiol Regul Integr Comp Physiol 301:207–228

Nakamura K, Morrison SF (2008) A thermosensory pathway that controls body temperature. Nat Neurosci 11:62–71

Nakamura K, Morrison SF (2010) A thermosensory pathway mediating heat-defense responses. Proc Natl Acad Sci U S A 107:8848–8853

Nakamura Y, Nakamura K, Matsumura K, Kobayashi S, Kaneko T, Morrison SF (2005) Direct pyrogenic input from prostaglandin EP3 receptor-expressing preoptic neurons to the dorsomedial hypothalamus. Eur J Neurosci 22:3137–3146

Nakamura Y, Nakamura K, Morrison SF (2009) Different populations of prostaglandin EP3 receptor-expressing preoptic neurons project to two fever-mediating sympathoexcitatory brain regions. Neuroscience 161:614–620

Norman D, Mukherjee S, Symons D, Jung RT, Lever JD (1988) Neuropeptides in interscapular and perirenal brown adipose tissue in the rat: a plurality of innervation. J Neurocytol 17:305–311

Nozdrachev AD, Jimenez B, Morales MA, Fateev MM (2002) Neuronal organization and cell interactions of the cat stellate ganglion. Auton Neurosci 95:43–56

Oldfield BJ, Giles ME, Watson A, Anderson C, Colvill LM, McKinley MJ (2002) The neurochemical characterisation of hypothalamic pathways projecting polysynaptically to brown adipose tissue in the rat. Neuroscience 110:515–526

Owen BM, Mangelsdorf DJ, Kliewer SA (2015) Tissue-specific actions of the metabolic hormones FGF15/19 and FGF21. Trends Endocrinol Metab 26:22–29

Perkins MN, Rothwell NJ, Stock MJ, Stone TW (1981) Activation of brown adipose tissue thermogenesis by the ventromedial hypothalamus. Nature 289:401–402

Rathner JA, Morrison SF (2006) Rostral ventromedial periaqueductal gray: A source of inhibition of the sympathetic outflow to brown adipose tissue. Brain Res 1077:99–107

Rezai-Zadeh K, Munzberg H (2013) Integration of sensory information via central thermoregulatory leptin targets. Physiol Behav 121:49–55

Rezai-Zadeh K, Yu S, Jiang Y, Laque A, Schwartzenburg C, Morrison CD, Derbenev AV, Zsombok A, Munzberg H (2014) Leptin receptor neurons in the dorsomedial hypothalamus are key regulators of energy expenditure and body weight, but not food intake. Mol Metab 3:681–693

Ricardo JA (1983) Hypothalamic pathways involved in metabolic regulatory functions, as identified by track-tracing methods. Adv Metab Disord 10:1–30

Rogers RC, Barnes MJ, Hermann GE (2009) Leptin "gates" thermogenic action of thyrotropin-releasing hormone in the hindbrain. Brain Res 1295:135–141

Rogers RC, McDougal DH, Hermann GE (2011) Leptin amplifies the action of thyrotropin-releasing hormone in the solitary nucleus: an in vitro calcium imaging study. Brain Res 1385:47–55

Rosenbaum M, Murphy EM, Heymsfield SB, Matthews DE, Leibel RL (2002) Low dose leptin administration reverses effects of sustained weight-reduction on energy expenditure and circulating concentrations of thyroid hormones. J Clin Endocrinol Metab 87:2391–2394

Rosenbaum M, Goldsmith R, Bloomfield D, Magnano A, Weimer L, Heymsfield S, Gallagher D, Mayer L, Murphy E, Leibel RL (2005) Low-dose leptin reverses skeletal muscle, autonomic, and neuroendocrine adaptations to maintenance of reduced weight. J Clin Invest 115:3579–3586

Rossi J, Balthasar N, Olson D, Scott M, Berglund E, Lee CE, Choi MJ, Lauzon D, Lowell BB, Elmquist JK (2011) Melanocortin-4 receptors expressed by cholinergic neurons regulate energy balance and glucose homeostasis. Cell Metab 13:195–204

Rothwell NJ, Stock MJ (1979) A role for brown adipose tissue in diet-induced thermogenesis. Nature 281:31–35

Rothwell NJ, Stock MJ, Tyzbir RS (1983) Mechanisms of thermogenesis induced by low protein diets. Metabolism 32:257–261

Rowland LA, Bal NC, Periasamy M (2014) The role of skeletal-muscle-based thermogenic mechanisms in vertebrate endothermy. Biol Rev Camb Philos Soc 90:1279–1297

Ryu V, Bartness TJ (2014) Short and long sympathetic-sensory feedback loops in white fat. Am J Physiol Regul Integr Comp Physiol 306:R886–R900

Ryu V, Garretson JT, Liu Y, Vaughan CH, Bartness TJ (2015) Brown adipose tissue has sympathetic-sensory feedback circuits. J Neurosci 35:2181–2190

Saito M, Okamatsu-Ogura Y, Matsushita M, Watanabe K, Yoneshiro T, Nio-Kobayashi J, Iwanaga T, Miyagawa M, Kameya T, Nakada K, Kawai Y, Tsujisaki M (2009) High incidence of metabolically active brown adipose tissue in healthy adult humans: effects of cold exposure and adiposity. Diabetes 58:1526–1531

Salomon J, de Truchis P, Melchior JC (2002) Body composition and nutritional parameters in HIV and AIDS patients. Clin Chem Lab Med 40:1329–1333

Scammell TE, Elmquist JK, Griffin JD, Saper CB (1996) Ventromedial preoptic prostaglandin E2 activates fever-producing autonomic pathways. J Neurosci 16:6246–6254

Shabalina IG, Nedergaard J (2011) Mitochondrial ('mild') uncoupling and ROS production: physiologically relevant or not? Biochem Soc Trans 39:1305–1309

Shi YC, Lau J, Lin Z, Zhang H, Zhai L, Sperk G, Heilbronn R, Mietzsch M, Weger S, Huang XF, Enriquez RF, Baldock PA, Zhang L, Sainsbury A, Herzog H, Lin S (2013) Arcuate NPY controls sympathetic output and BAT function via a relay of tyrosine hydroxylase neurons in the PVN. Cell Metab 17:236–248

Sohn JW, Harris L, Berglund E, Liu T, Vong L, Lowell B, Balthasar N, Williams K, Elmquist J (2013) Melanocortin 4 receptors reciprocally regulate sympathetic and parasympathetic preganglionic neurons. Cell 152:612–619

Song CK, Schwartz GJ, Bartness TJ (2009) Anterograde transneuronal viral tract tracing reveals central sensory circuits from white adipose tissue. Am J Physiol Regul Integr Comp Physiol 296:R501–R511

Ste Marie L, Miura GI, Marsh DJ, Yagaloff K, Palmiter RD (2000) A metabolic defect promotes obesity in mice lacking melanocortin-4 receptors. Proc Natl Acad Sci USA 97:12339–12344

Sutton AK, Pei H, Burnett KH, Myers MG Jr, Rhodes CJ, Olson DP (2014) Control of food intake and energy expenditure by Nos1 neurons of the paraventricular hypothalamus. J Neurosci 34:15306–15318

Takahashi A, Shimazu T, Maruyama Y (1992) Importance of sympathetic nerves for the stimulatory effect of cold exposure on glucose utilization in brown adipose tissue. Jpn J Physiol 42:653–664

Tanche M, Therminarias A (1967) Participation of stellate ganglions in rewarming thermogenesis in the dog. Arch Sci Physiol (Paris) 21:67–80

Tsukazaki K, Nikami H, Shimizu Y, Kawada T, Yoshida T, Saito M (1995) Chronic administration of beta-adrenergic agonists can mimic the stimulative effect of cold exposure on protein synthesis in rat brown adipose tissue. J Biochem 117:96–100

Tupone D, Madden CJ, Cano G, Morrison SF (2011) An orexinergic projection from perifornical hypothalamus to raphe pallidus increases rat brown adipose tissue thermogenesis. J Neurosci 34:15944–15955

Ushikubi F, Segi E, Sugimoto Y, Murata T, Matsuoka T, Kobayashi T, Hizaki H, Tuboi K, Katsuyama M, Ichikawa A, Tanaka T, Yoshida N, Narumiya S (1998) Impaired febrile response in mice lacking the prostaglandin E receptor subtype EP3. Nature 395:281–284

van Marken Lichtenbelt WD, Vanhommerig JW, Smulders NM, Drossaerts JM, Kemerink GJ, Bouvy ND, Schrauwen P, Teule GJ (2009) Cold-activated brown adipose tissue in healthy men. N Engl J Med 360:1500–1508

Vaughan CH, Zarebidaki E, Ehlen JC, Bartness TJ (2014) Analysis and measurement of the sympathetic and sensory innervation of white and brown adipose tissue. Methods Enzymol 537:199–225

Virtanen KA, Lidell ME, Orava J, Heglind M, Westergren R, Niemi T, Taittonen M, Laine J, Savisto NJ, Enerback S, Nuutila P (2009) Functional brown adipose tissue in healthy adults. N Engl J Med 360:1518–1525

Voets T (2014) TRP channels and thermosensation. Handb Exp Pharmacol 223:729–741

Williams KW, Elmquist JK (2012) From neuroanatomy to behavior: central integration of peripheral signals regulating feeding behavior. Nat Neurosci 15:1350–1355

Wisloff U, Najjar SM, Ellingsen O, Haram PM, Swoap S, Al-Share Q, Fernstrom M, Rezaei K, Lee SJ, Koch LG, Britton SL (2005) Cardiovascular risk factors emerge after artificial selection for low aerobic capacity. Science 307:418–420

Xu Y, Nedungadi TP, Zhu L, Sobhani N, Irani BG, Davis KE, Zhang X, Zou F, Gent LM, Hahner LD, Khan SA, Elias CF, Elmquist JK, Clegg DJ (2011) Distinct hypothalamic neurons mediate estrogenic effects on energy homeostasis and reproduction. Cell Metab 14:453–465

Xu Y, Kim ER, Zhao R, Myers MG Jr, Munzberg H, Tong Q (2013) Glutamate release mediates leptin action on energy expenditure. Mol Metab 2:109–115

Yoshida K, Maruyama M, Hosono T, Nagashima K, Fukuda Y, Gerstberger R, Kanosue K (2002) Fos expression induced by warming the preoptic area in rats. Brain Res 933:109–117

Yoshida K, Konishi M, Nagashima K, Saper CB, Kanosue K (2005) Fos activation in hypothalamic neurons during cold or warm exposure: Projections to periaqueductal gray matter. Neuroscience 133:1039–1046

Yoshida K, Li X, Cano G, Lazarus M, Saper CB (2009) Parallel Preoptic Pathways for Thermoregulation. J Neurosci 29:11954–11964

Zhang W, Sunanaga J, Takahashi Y, Mori T, Sakurai T, Kanmura Y, Kuwaki T (2010) Orexin neurons are indispensable for stress-induced thermogenesis in mice. J Physiol 588:4117–4129

Zoeller RT, Kabeer N, Albers HE (1990) Cold exposure elevates cellular levels of messenger ribonucleic acid encoding thyrotropin-releasing hormone in paraventricular nucleus despite elevated levels of thyroid hormones. Endocrinology 127:2955–2962

Signalling Networks Governing Metabolic Inflammation

Nassim Dali-Youcef and Romeo Ricci

Contents

Abstract

Low-grade inflammation is an established pathological condition that contributes to the development of obesity, insulin resistance and type 2 diabetes. Metabolic inflammation is dependent on multiple signalling events. In an over-nutrition state, canonical inflammatory pathways are induced by inflammatory cytokines and lipid species. They can also be triggered through inflammasome activation as well as through cellular stress provoked by the unfolded protein response at the endoplasmic reticulum as well as by reactive oxygen species.

N. Dali-Youcef (✉) • R. Ricci (✉)
Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC)/CNRS UMR 7104 - Inserm U 964/Université de Strasbourg, 1 rue Laurent Fries, 67404 Illkirch, France

Laboratoire de Biochimie et de Biologie Moléculaire, Hôpitaux Universitaires de Strasbourg, 1 place de l'hôpital, 67091 Strasbourg Cedex, France
e-mail: dali@igbmc.fr; ricci@igbmc.fr

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In this chapter, we summarize the current knowledge about signalling events within the cell and describe how they impact on metabolic inflammation and whole-body metabolism. We particularly highlight the interplay between different signalling pathways that link low-grade inflammation responses to the inactivation of the insulin receptor pathway, ultimately leading to insulin resistance, a hallmark of type 2 diabetes.

Keywords
Ceramide • Fatty acids • IKKβ • Inflammatory cytokines • Insulin resistance • JNK • Macrophages • NFκB • Obesity • Toll-like receptors • White adipose tissue

1 Introduction

There is pivotal evidence that a cross talk between canonical immune and metabolic pathways exists. While malnutrition compromises proper immune responses, overnutrition activates them (Hotamisligil 2006). On the other hand, it is well known that acute and chronic inflammatory conditions can impact on whole-body metabolism (Odegaard and Chawla 2013).

At the molecular level, nutrient sensing can occur directly through immune modulators. Prominent examples are lipid-induced toll-like receptor (Tlr) (Shi et al. 2006; Tsukumo et al. 2007) and peroxisome proliferator-activated receptor (PPAR) signalling (Chawla 2010). On the other hand, cytokines such as tumour necrosis factor α (TNFα) or interleukin-1β (IL-1β) through intracellular inflammatory signalling molecules including the inhibitor of nuclear factor kappa B (IκB) kinase (IKK) or the mitogen-activated protein kinase (MAPK) c-Jun N-terminal kinase (JNK) attenuate insulin receptor signalling, thus inhibiting insulin-mediated anabolic functions in different organs. While such an intricate network of interconnected inflammatory and metabolic signalling modules has evolved during evolution and thus has important adaptive roles in cells, it may also contribute to disease development. In fact, it is now very well established that low-grade inflammation in the context of obesity contributes to insulin resistance (Gregor and Hotamisligil 2011).

In simplistic terms, obesity essentially reflects the expansion of the white adipose tissue (WAT) through accumulation of lipids triggered by chronic overnutrition. As several lipid species are particularly prone to enhance inflammation, the WAT is primarily predisposed to the induction of the above signalling networks. This may occur in WAT-resident immune cells as well as in white adipocytes that can secrete themselves a whole arsenal of pro-inflammatory cytokines (Ouchi et al. 2011). However, infiltration and activation of immune cells were also observed in other metabolically active tissues including the skeletal muscle, central nervous system, liver and pancreatic islets (Tishinsky et al. 2014; Posey et al. 2009;

Meli et al. 2014; Donath et al. 2013). These events may engage systemic low-grade inflammation that interferes with insulin signalling in different organs, thus causing systemic insulin resistance.

In this chapter, we discuss some principal molecules involved in low-grade metabolic inflammation and the underlying cellular signalling networks. We also explain how these inflammatory signals impair insulin receptor signalling, providing a valuable basis as to how insulin resistance may develop during obesity.

2 PPARs and M2-Type Macrophage Activation in Obesity

WAT-resident macrophages play an important role in establishing low-grade metabolic inflammation during obesity. In a simple scheme proposed by Siamon Gordon, macrophages are divided into states of classical activation (also known as M1-type macrophages), alternative activation (also known as M2-type macrophages) or deactivation (Gordon 2003). Macrophage activation by type 1 helper-T lymphocyte (T_{H1}) cytokines such as interferon gamma (IFNγ) or by bacterial lipopolysaccharide (LPS) favours their polarization into the "classical" M1-type macrophages with a high pro-inflammatory profile (Mosser and Edwards 2008). M2-type macrophages secrete anti-inflammatory molecules such as interleukin-10 (IL10) and arginase I upon release of IL4 and IL13 by type 2 helper-T lymphocyte (T_{H2}) (Gordon 2003; Tilg and Moschen 2006). Macrophage deactivation is a state in which classical activation and alternative activation are switched off. This shows the remarkable plasticity of macrophages that can be interchangeably activated into a given phenotype depending on the stimuli received.

In normal conditions, the WAT mainly contains M2-type resident macrophages keeping adipose inflammation low (Fig. 1). M2 macrophage activation requires expression of the PPARγ as well as PPARβ/δ. Myeloid-specific PPARγ or PPARβ/δ as well as PPARβ/δ-null bone marrow chimeric mice displayed impaired M2 activation, enhanced adipose tissue inflammation and insulin resistance (Odegaard et al. 2007; Kang et al. 2008; Odegaard and Chawla 2008; Hevener et al. 2007). Interestingly, it has been shown that only native monocytes can be primed to an increased M2 phenotype by PPARγ activation (Bouhlel et al. 2007). In obesity, adipocytes become hypertrophied and secrete pro-inflammatory cytokines such as TNFα, interleukin-6 (IL-6) as well as the chemokine (C–C motif) ligand 2 (CCL-2) (Tilg and Moschen 2006). A cross talk between adipocytes and macrophages occurs and macrophages undergo a change in polarity from the M2 to the M1 phenotype. M1 macrophages express pro-inflammatory molecules including interferon γ (IFNγ), IFNγ receptor, inducible nitric oxide synthase (iNOS), IL-1β, TNFα and IL-6, all of which have been shown to impact on insulin sensitivity in different organs (Odegaard and Chawla 2008) (Fig. 1).

At the molecular level, PPARγ and PPARβ/δ are both targets of the transcription factor STAT6. STAT6 is pivotal in controlling the transcriptional programme for

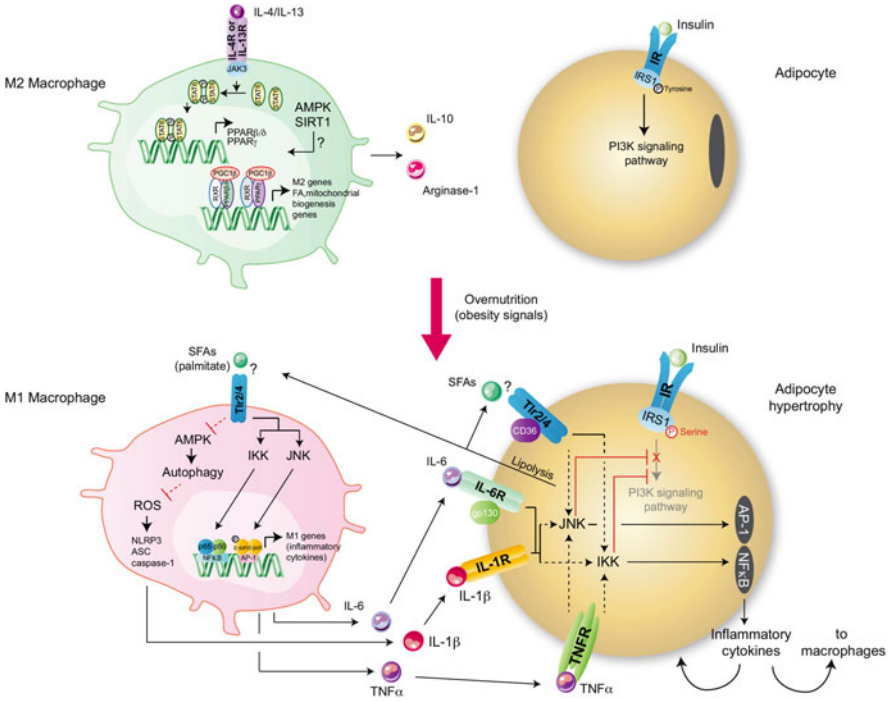

Fig. 1 *Interplay between macrophages and adipocytes in metabolic inflammation*. (**a**) Under normal nutritional conditions, M2 macrophages are highly abundant, secreting anti-inflammatory cytokines such as interleukin 10 and arginase 1. The M2 phenotype is maintained by the T_{H2} cytokines IL-4 and IL-13. Upon binding to their receptor, these cytokines stimulate the JAK/STAT6 pathway to induce the expression of PPARγ and PPARβ/δ, which upon binding of the PGC1β coactivator induce the transcription of M2 anti-inflammatory genes. AMPK and the SIRT1 NAD^+-dependent deacetylase are thought to favour the M2 phenotype. M2-resident macrophages do not interfere with insulin signalling in adjacent adipocytes. (**b**) In the presence of obesity signals, macrophages switch to an M1 phenotype. Saturated fatty acids (SFAs) released by adipocytes through lipolysis require Tlr signalling on macrophages to activate canonical inflammatory kinases (IKKβ, JNK), culminating in the NFκB- and AP-1-mediated transcription of pro-inflammatory genes, respectively. SFAs also stimulate a pathway that inactivates AMPK, resulting in the activation of autophagy that normally would prevent the formation of reactive oxygen species (ROS). ROS in turn activates the inflammasome components (NLRP3, ASC, Caspase-1) that allow the maturation of the pro-inflammatory IL-1β cytokine and its secretion. Pro-inflammatory cytokines as well as lipids signal through respective receptors, employing the same canonical pro-inflammatory kinases in white adipocytes to interfere with the insulin/PI3K signalling pathway (see also Fig. 2). Hypertrophied adipocytes will activate a pro-inflammatory gene programme by themselves and provide another important source of pro-inflammatory cytokines. *IR* insulin receptor, *TNFα* tumour necrosis factor α, *TNFR* TNFα receptor, *SFAs* saturated fatty acids, *Tlr2/4* toll-like receptors 2/4, *CD36/FAT* cluster of differentiation-36/fatty acid translocase, *IL-1β* interleukin-1β, *IL1-R* interleukin-1 receptor, *IL-4* interleukin-4, *IL-13* interleukin-13, *IL-4R* IL-4 receptor, *IL-13R* IL-13 receptor, *JAK3* Janus-associated kinase

the alternative activation of macrophages. The T_{H2} cytokines IL-4 and IL-13, which stimulate M2-type macrophage conversion, induce Janus-associated kinase (JAK) activity that phosphorylates STAT6, resulting in its nuclear translocation and STAT6-mediated transcription (Martinez et al. 2009). STAT6, PPARγ and PPARγ coactivator 1β (PGC-1β) cooperatively act to engage oxidative metabolism by enhancing fatty acid oxidation and mitochondrial biogenesis. PPARβ/δ, on the other hand, synergizes with STAT6 and PGC-1β to maintain the transcription of genes characterizing alternative macrophage activation (Fig. 1).

The AMP-activated protein kinase (AMPK) has also been shown to negatively regulate inflammation, thereby preventing whole-body insulin resistance in the adipose tissue (Gauthier et al. 2011). AMPK is a nutrient sensor responding to increased AMP/ATP ratio that regulates sirtuin 1 (SIRT1) NAD^+-dependent deacetylase (Canto et al. 2009). Indeed, other studies demonstrated that both AMPK and SIRT1 inhibit, probably in a cooperative manner, metabolic inflammation (Yang et al. 2010; Gillum et al. 2011). Importantly in this context, it was shown that treatment with quercetin, a dietary flavonoid, reduced obesity-associated metabolic inflammation and M2→M1 polarity in mice through activation of the activation of the AMPKα1/SIRT1 pathway (Dong et al. 2014). However, it is currently unclear how this pathway regulates macrophage polarity (Fig. 1).

These data strongly support the idea that M2-type macrophages protect against, while M1 macrophages promote obesity-related insulin resistance. Activation of PPARγ and β/δ or AMPK/SIRT1 is an important mechanism stimulating alternative macrophage activation and thus might attenuate low-grade inflammation in obesity.

Fig. 1 (continued) 3, *STAT3/6* signal transducer and activator of transcription 3/6, *IL-6* interleukin-6, *IL-6R* IL-6 receptor, *IL-18* interleukin-18, *IRS1* insulin receptor substrate 1, *JNK* Jun N-terminal kinase, *IκBα* inhibitor of kappa B α, *IKKα, β and ε* inhibitors of kappa B kinase, *NEMO* nuclear essential modulator, *NAP1* NAK-associated protein 1, *NFκB* nuclear factor kappa B, *TANK* TRAF-associated NFκB activator, *TBK1* TANK-binding kinase 1, *SINTBAD* similar to NAP and TBK1 adaptor, *NLRP3* nucleotide-binding domain with leucine-rich repeats (NLR) containing pyrin domain 3, *ASC* adaptor protein apoptosis-associated speck-like protein containing a caspase recruitment domain, *PPARγ or β/δ* peroxisome proliferator-activated receptor gamma or beta/delta, *PGC1β* PPARγ-coactivator 1beta, *AMPK* adenosine monophosphate-activated kinase, *SIRT1* sirtuin 1, *TAK1* transforming growth factor-beta-activated kinase 1, *TAB1* TAK1-binding protein 1, *IRAK1 and IRAK4* IL-1 receptor-associated kinases 1 and 4, *TRADD* TNFα receptor-associated death domain, *TRAF* TNF receptor-associated factor, *RIP* death domain kinase, *Tlr2/4* toll-like receptors 2/4, *TlRAP* toll-like receptor adaptor protein, *MyD88* myeloid differentiation protein-88 (a docking protein), *TOLLIP* toll-interacting protein, *MKK4/7* mitogen-activated protein kinase kinase 4/7, *IRF3* interferon regulatory factor 3, *AKT* protein kinase B, *PKR* double-stranded RNA-dependent protein kinase, *PTP1B* protein tyrosine phosphatase 1B, *SOCS3* suppressor of cytokine signalling 3, *PKCθ or PKCζ* protein kinase C theta or zeta, *PPA2* protein phosphatase A2, *GPR120* G-protein-coupled receptor 120, *ω-FAs* omega fatty acids, *FAHFAs* fatty acid esters of hydroxy fatty acids, *ROS* reactive oxygen species

3 Inflammatory Cytokines and Insulin Signalling

We have described above the source of different cytokines that come into play during metabolic inflammation. In the following, we explore several of them and discuss signalling pathways they induce leading to systemic insulin resistance.

3.1 Tumour Necrosis Factor α (TNFα)

TNFα was shown to play a prominent role in obesity-related diseases. A first important study was conducted in obese rats, demonstrating that neutralization of TNFα improved peripheral uptake of glucose in response to insulin (Hotamisligil et al. 1993). This was followed by various studies correlating TNFα expression with obesity and insulin resistance in experimental models and humans (Hotamisligil et al. 1993, 1995). Subsequently, it has been demonstrated that mice lacking TNFα were protected against obesity and insulin resistance provoked by a HFD (Uysal et al. 1997). Mechanistically, TNFα was the first cytokine that has been shown to inhibit insulin signalling and is thus exemplary in linking inflammation to insulin resistance in several cell types. Initial studies first showed that TNFα inhibits insulin signalling (Hotamisligil et al. 1994a, b). Next, it has been demonstrated that TNFα leads to IRS1 serine phosphorylation interfering with its tyrosine phosphorylation and signal propagation (Hotamisligil et al. 1996). In following studies several kinases including JNK and IKKβ (see below) activated by TNFα, other cytokines and lipids have been suggested to phosphorylate IRS1 on different serine residues (Schenk et al. 2008) (Figs. 1 and 2). TNFα upon binding to its receptor TNFR enables the adaptor protein TRADD to bind to the death domain of the receptor. TRADD recruits TRAF2 and RIP that employ distinct signalling molecules to activate IKK, JNK and moderately p38 (Aggarwal 2003) (Fig. 2).

Other mechanisms as to how TNFα inhibits insulin signalling have been proposed. TNFα was demonstrated to oppose the insulin-sensitizing effects of adiponectin by inhibiting its expression and release from adipocytes (Hajri et al. 2011). TNFα has also been demonstrated to stimulate the expression of protein tyrosine phosphatase 1B (PTB1B) in adipocytes and hepatocytes (Zabolotny et al. 2008). Engelman et al. (2000) reported abrogation of TNFα-induced IRS1 serine phosphorylation and insulin resistance through inhibition of the MEK1/2-p42/p44 MAPK pathway.

Overall, these data strongly suggest that TNFα signalling is at the heart of mechanisms leading to obesity-related insulin resistance.

3.2 Interleukin-1 (IL-1) Family of Cytokines

The IL-1 cytokine family is also considered as a key cytokine contributing to the development of insulin resistance in obese individuals. A recent study indeed showed that genetic variations in the IL-1 gene family (IL-1α, IL-1β and IL-1Ra)

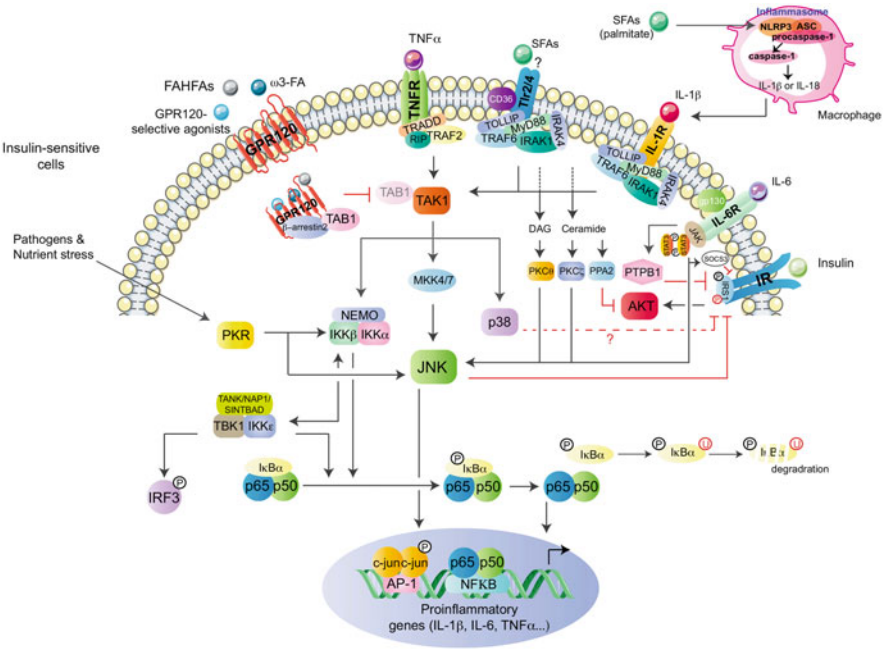

Fig. 2 *Signalling networks orchestrating low-grade inflammation in insulin-sensitive cells.* Saturated fatty acids (SFAs) and pro-inflammatory cytokines upon binding to their receptors recruit a number of adaptor proteins that initiate a signalling cascade converging into the activation of canonical inflammatory regulators JNK and IKKβ. JNK and IKKβ through phosphorylation of c-Jun and IκBα, respectively, activate AP-1- or NFκB-mediated transcription of pro-inflammatory genes, respectively. Phosphorylated IκBα is subsequently degraded through the proteasome system. The noncanonical IKKε can also phosphorylate IκBα to induce NFκB target genes but also phosphorylates IRF3 to mediate the latter transcription. JNK inactivates the insulin pathway through serine phosphorylation of IRS1 (phosphate in *red*). IRS1 phosphorylation on tyrosine residues (phosphate in *black*) is inactivated by the IL-6-mediated activation of the PTP1B phosphatase. IL-6 induces also the accumulation of SOCS-3, which interferes with IRS1 binding with the insulin receptor (IR). Fatty acid metabolites ceramide and diacylglycerol activate also JNK to amplify the inflammatory response through activation of PKCζ and PKCθ, respectively. Ceramide also interferes with insulin signalling through the PPA2-mediated inhibition of AKT/PKB. The stress-activated p38/MAPK is induced by several cytokines and is also thought to interfere with insulin action. The pathogen and nutrient-activated kinase PKR can stimulate JNK and IKKβ kinases and block the insulin pathway. A recently identified receptor GPR120, which binds ω-3 fatty acids, FAHFAs and selective agonists, inhibits metabolic inflammation through β-arrestin 2-mediated sequestration of the adaptor protein TAB1, thereby inactivating TAK1 and downstream kinases JNK as well as IKKβ. *IR* insulin receptor, *TNFα* tumour necrosis factor α, *TNFR* TNFα receptor, *SFAs* saturated fatty acids, *Tlr2/4* toll-like receptors 2/4, *CD36/FAT* cluster of differentiation-36/fatty acid translocase, *IL-1β* interleukin-1β, *IL1-R* interleukin-1 receptor, *IL-4* interleukin-4, *IL-13* interleukin-13, *IL-4R* IL-4 receptor, *IL-13R* IL-13 receptor, *JAK3* Janus-associated kinase 3, *STAT3/6* signal transducer and activator of transcription 3/6, *IL-6* interleukin-6, *IL-6R* IL-6 receptor, *IL-18* interleukin-18, *IRS1* insulin receptor substrate 1, *JNK* Jun N-terminal kinase, *IκBα* inhibitor of kappa B α, *IKKα, β and ε* inhibitors of kappa B kinase, *NEMO* nuclear essential modulator, *NAP1* NAK-associated protein 1, *NFκB* nuclear factor kappa B, *TANK* TRAF-associated NFκB activator, *TBK1* TANK-binding kinase 1, *SINTBAD* similar to

were associated with measures of glucose homeostasis and prevalent diabetes in the Finnish population (Luotola et al. 2009).

IL-1β is maturated through nucleotide-binding domain with leucine-rich repeats containing pyrin domain 3 (NLRP3) inflammasome-mediated caspase-1 activation, which results in its processing and secretion from macrophages and subsequent binding to its receptor IL1-R at the surface of target cells (Tschopp and Schroder 2010) (Fig. 1). Upon binding to its receptor, IL-1R, IL-1β induces activation of the MAPKs JNK and p38 as well as IKK beta (IKKβ), which cooperatively enhance the expression of IL-1 target genes such as IL-6, interleukin-8 (IL-8), CCL2, cyclooxygenase 2 (COX-2), IL-1α, IL-1β and MAPK phosphatase 1 (MKP-1) by transcriptional and posttranscriptional mechanisms (Solinas and Karin 2010; Garlanda et al. 2013). Activation of these kinases not only leads to the production of many pro-inflammatory factors but also will impair insulin signalling in target tissues (see below) (Figs. 1 and 2).

An interesting question is as to how the inflammasome is activated in an overnutrition state. An interesting recent study showed that the saturated fatty acid palmitate, but not unsaturated oleate, activates the NLRP3-ASC inflammasome in haematopoietic cells, leading to the production of caspase-1, IL-1β and IL-18. Inflammasome activation in haematopoietic cells promotes insulin resistance in several target tissues. Interestingly, activation of the inflammasome involves an AMPK- and UNC-51-like autophagy activating kinase 1 (ULK1)-dependent autophagy pathway and propagation of reactive oxygen species (ROS) (Wen et al. 2011) (Fig. 1). Even though ROS have been considered as important NLRP3-inflammasome activators, they were also shown to inhibit caspase-1 activation through redox signalling (Meissner et al. 2008). Thus, other potent NLRP3-inflammasome-stimulating mechanisms such as K^+-efflux, cathepsin B or lysosome rupture (Tschopp and Schroder 2010) need to be considered in the future in the context of metabolic inflammation. Furthermore, the role of other inflammasome complexes needs to be investigated.

Fig. 2 (continued) NAP and TBK1 adaptor, *NLRP3* nucleotide-binding domain with leucine-rich repeats (NLR) containing pyrin domain 3, *ASC* adaptor protein apoptosis-associated speck-like protein containing a caspase recruitment domain, *PPARγ or β/δ* peroxisome proliferator-activated receptor gamma or beta/delta, *PGC1β* PPARγ-coactivator 1beta, *AMPK* adenosine monophosphate-activated kinase, *SIRT1* sirtuin 1, *TAK1* transforming growth factor-beta-activated kinase 1, *TAB1* TAK1-binding protein 1, *IRAK1 and IRAK4* IL-1 receptor-associated kinases 1 and 4, *TRADD* TNFα receptor-associated death domain, *TRAF* TNF receptor-associated factor, *RIP* death domain kinase, *Tlr2/4* toll-like receptors 2/4, *TlRAP* toll-like receptor adaptor protein, *MyD88* myeloid differentiation protein-88 (a docking protein), *TOLLIP* toll-interacting protein, *MKK4/7* mitogen-activated protein kinase kinase 4/7, *IRF3* interferon regulatory factor 3, *AKT* protein kinase B, *PKR* double-stranded RNA-dependent protein kinase, *PTP1B* protein tyrosine phosphatase 1B, *SOCS3* suppressor of cytokine signalling 3, *PKCθ or PKCζ* protein kinase C theta or zeta, *PPA2* protein phosphatase A2, *GPR120* G-protein-coupled receptor 120, *ω-FAs* omega fatty acids, *FAHFAs* fatty acid esters of hydroxy fatty acids, *ROS* reactive oxygen species

Phenotypically, IL-1β-deficient mice showed a markedly decreased adipose tissue macrophage infiltration as compared to wild-type (wt) control animals upon high-fat diet (HFD) feeding, supporting an important role of IL-1β in metabolic inflammation. In line with IL-1β deficiency, IL-6 and TNFα transcripts were markedly lower as compared to wild-type mice under a HFD (Nov et al. 2013). Importantly, mice deficient in IL-1β, but also caspase-1 or NLRP3, were shown to have higher insulin sensitivity than the control mice (Stienstra et al. 2010, 2011; Vandanmagsar et al. 2011). IL-1β not only interferes with insulin signalling but also promotes pancreatic β cell dysfunction and loss (Donath et al. 2013). A recent study shed light on yet another function of the inflammasome system. Using a diet deficient in methionine and choline that induces non-alcoholic steatohepatitis (NASH), deletion of several components of the inflammasome system (including NLRP3 and NLRP6) aggravated NASH and several aspects of metabolic syndrome. Moreover, authors of this paper showed that in contrast to the studies by Stienstra et al. (2011) and Wen et al. (2011), mice lacking the inflammasome adaptor protein apoptosis-associated speck-like protein containing a carboxy-terminal CARD (ASC) on a HFD displayed an increased insulin resistance and glucose intolerance as compared to wt mice. Mechanistically, it has been proposed that the lack of inflammasome components impaired the gut microbiota in a way that predisposed for the development of NASH and traits of metabolic syndrome (Henao-Mejia et al. 2012). Even though earlier studies did not report alterations of the gut microbiota in mice lacking inflammasome components, the basic role of the inflammasome system in metabolic inflammation remains rather inconclusive, as so far described phenotypic outcomes on whole-body metabolism are inconsistent. It will therefore be necessary in the future to understand how different inflammasome components act on the molecular level in different immune compartments to impact on metabolic inflammation.

Another important pro-inflammatory member of the IL-1 family is IL-1α, which also signals through IL-1R, thereby activating the same signalling cascade. Interleukin-1α was also shown to inhibit insulin signalling through a similar mechanism (see below) (He et al. 2006).

Altogether, IL-1α and IL-1β appear to be key factors in several aspects in the pathogenesis of type 2 diabetes. Importantly, inhibition of IL-1R signalling using receptor blockade with anakinra, the recombinant form of the naturally occurring IL-1R antagonist (IL-1Ra), or using neutralizing IL-1β antibodies appears to be a very promising therapeutic approach in type 2 diabetes (Dinarello et al. 2010).

IL-18 represents another interesting member of the IL-1 cytokine family that should be discussed in the context of obesity and insulin resistance. It was first described as a cytokine that promotes INFγ production by T cells (Okamura et al. 1995). IL-18 is maturated and secreted similarly to IL-1β by NLRP3-inflammasome-mediated caspase-1 activation (Tschopp and Schroder 2010). IL-18 signals through the IL-18 receptor engaging essentially the same inflammatory signalling cascade as IL-1α and β that should promote insulin resistance (Ohnishi et al. 2012) and elevated IL-18 levels have been associated with obesity and insulin resistance (Leick et al. 2007). Unexpectedly, however, two independent

studies showed that mice with a global deletion of IL-18 become obese and insulin resistant. Injections of recombinant IL-18 reversed the latter effects caused by IL-18 deficiency. Both studies suggest that this paradoxical phenotype is mediated via neuronal appetite regulation (Netea et al. 2006; Zorrilla et al. 2007). Along the same line, a more recent report showed that IL-18 receptor (IL-18R) knockout mice have increased weight gain on a chow diet and showed increased insulin resistance and inflammation in metabolic organs (Lindegaard et al. 2013). A state of IL-18 resistance, similar to leptin resistance observed in obese individuals, can explain this paradox. The authors of this study showed that IL-18 increases AMPK signalling and fatty acid oxidation, thus providing another mechanism as to how insulin resistance can occur in the absence of IL-18.

Altogether, while it is fairly established that the inflammasome and related cytokines impact on metabolic inflammation, the functions of the individual components of this intricate system are very complex and context dependent.

3.3 Interleukin-6 (IL-6)

IL-6 is considered as one of the early markers of the acute inflammatory response (Ramadori and Christ 1999; Ramadori and Armbrust 2001). Circulating IL-6 levels were shown to be increased in obese (Mohamed-Ali et al. 1997; Straub et al. 2000; Fernandez-Real et al. 2001), in glucose-intolerant (Muller et al. 2002) and type 2 diabetic patients (Pickup et al. 1997; Kado et al. 1999). Another study also showed that IL-6 levels were robustly increased in fat cells from insulin-resistant individuals (Rotter et al. 2003). Results from the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam study showed that a combined elevation of IL-1 β and IL-6, rather than the isolated elevation of IL-6 alone, independently enhanced the risk of type 2 diabetes (Spranger et al. 2003). Very recently, circulating IL-6 levels, but not soluble IL-6 (sIL-6), were shown to significantly correlate with body mass index (BMI) and the homeostasis model assessment index for insulin resistance (HOMA-IR) also in obese children and adolescents (De Filippo et al. 2015). Despite these correlations, the role of IL-6 in low-grade inflammation and in the pathophysiology of insulin resistance is quite paradoxical since both IL-6 overexpression (Franckhauser et al. 2008) and deficiency (Matthews et al. 2010) resulted in liver inflammation and insulin resistance. These results can be explained by the fact that IL-6 exerts a dual role on insulin sensitivity (Sarvas et al. 2013). Acute IL-6 and insulin treatments of myotubes and mice have additive positive effects on increased glucose uptake and insulin sensitivity. In fact, IL-6 is considered as a myokine level which increases acutely up to 100 times during exercise. The mechanism by which elevated IL-6 ameliorates insulin sensitivity following physical activity might involve the regulation of AMPK activity, resulting in increased insulin-stimulated translocation of glucose transporter type 4 (GLUT4) to the plasma membrane (Kelly et al. 2004, 2009; Ruderman et al. 2006; Carey et al. 2006; Nieto-Vazquez et al. 2008). However, chronic exposure to IL-6 reduced insulin sensitivity and impaired GLUT4

translocation. The molecular mechanism behind this deleterious effect implicates the binding of IL-6 to its receptor IL-6R bound to a non-ligand-binding membrane glycoprotein, gp130, and signals through JAK-STAT3 to trigger JNK activation, the accumulation of suppressor of cytokine signalling 3 (SOCS3) and an increase in protein tyrosine phosphatase 1B (PTP1B) activity (Senn et al. 2002; Rotter et al. 2003; Nieto-Vazquez et al. 2008), all of which inhibit insulin signalling (Fig. 2). How JNK negatively regulates insulin signalling is described below. SOCS3 interferes with insulin action through binding to the insulin receptor (IR), thereby preventing its interaction with IR substrate 1 (IRS1) that is needed for signal propagation (Emanuelli et al. 2000). PTP1B removes tyrosine phosphorylation of the IR, thereby inactivating it (Ahmad et al. 1995; Romsicki et al. 2004). Moreover, PTP1B deficiency prevented the inhibitory effects of chronic IL-6 on insulin signalling and glucose uptake (Nieto-Vazquez et al. 2008). Overall, IL-6 signalling was shown to have positive and negative effects on insulin action depending on the context and target tissues. Mice with metabolic tissue-specific targeting of IL-6 or its receptor will improve our comprehension of IL-6-dependent pathophysiological roles in whole-body energy homeostasis.

4 Lipid Signalling, Inflammation and Insulin Resistance

4.1 Saturated Fatty Acids

Free fatty acid (FFA) levels were frequently found increased in obesity and insulin resistance (Cascio et al. 2012). Several studies showed that an acute rise of FFAs interfered with insulin sensitivity (Boden et al. 1991; Roden et al. 1996; Staehr et al. 2003). In an obesity state, white adipocytes increase their lipolytic activity and their storage capacity of lipids is overwhelmed. This not only provokes a spillover of FFA into the blood circulation but also leads to local accumulation of lipids that will stimulate local macrophages and other immune cells.

Saturated fatty acids (SFAs) increase inflammatory signalling in many cell types. Signalling converges into the activation of IKKβ and the MAPK JNK as well as p38 that further propagate the inflammatory response by enhancing cytokine expression and secretion (Figs. 1 and 2). A very important question in the field remains as to how lipids are sensed in cells to induce inflammation. It is now quite well established that lipids can signal through toll-like receptors 4 and 2 (Tlr4 and Tlr2) to evoke inflammatory responses (Konner and Bruning 2011). These receptors belong to the Tlr family that classically act to sense pathogen-associated molecular patterns (PAMPs) and damage-associated patterns (DAMPs) (Palm and Medzhitov 2009). Shi et al., however, demonstrated that Tlr4 was essential in the SFA-induced inflammatory response in adipocytes and macrophages. Furthermore, the lack of Tlr4 protected mice from diet-induced insulin resistance (Shi et al. 2006). In a subsequent study, Saberi et al. (2009) demonstrated that inactivation of Tlr4 signalling in the haematopoietic system is sufficient for these protective effects to occur. Tlr4 signalling requires the adaptor protein MyD88 and stimulates

the TAK1/TAB1 complex, which then activates IKKβ and MKK4 to stimulate the downstream NFκB and JNK inflammatory pathways, respectively (O'Neill 2008) (Fig. 2).

Knockdown of Tlr2 and MyD88 through RNA interference abrogated palmitate-induced insulin resistance and IL-6 production, while an antagonist Tlr2 antibody significantly reduced palmitate-induced IL-6 production and partially restored insulin signalling pathway (Senn 2006). Tlr2 also associates with MyD88 to induce the stress kinases p38, JNK as well as PKC, resulting in IκBα degradation and increased NFκB DNA binding (O'Neill 2008).

Interestingly, genetic variants in the Tlr4 gene have been associated with traits of metabolic syndrome and modified the association between dietary SFA and fasting HDL levels (Cuda et al. 2011). Of particular note, mice deficient in the Tlr4/Tlr2 co-receptor CD14 had improved glucose homeostasis and lower adiposity in the presence of intact Tlr4 and Tlr2 gene expression (Roncon-Albuquerque et al. 2008).

It is still very controversially discussed whether SFA bind directly Tlr4 or Tlr2 as structural analyses (Park et al. 2009) and in vitro binding assays (Schaeffler et al. 2009) do not support such a model. Several alternative possibilities based on new experiments have thus been suggested. One possibility is the involvement of interacting co-receptors. An interesting candidate is the lipid transporter *fatty acid translocase* (FAT/CD36) as CD36 has been shown to interact with Tlr2 and Tlr4, thereby inducing signalling in response to ox-LDL (Seimon et al. 2010; Stewart et al. 2010). CD36 is a class B scavenger lipid transporter ubiquitously expressed in adipocytes, myocytes, hepatocytes, enterocytes, macrophages, endothelial cells and platelets (Love-Gregory and Abumrad 2011). In metabolic organs, it allows for uptake of long-chain fatty acids (LCFAs) and oxidized low-density lipoproteins (ox-LDLs) and is recruited from intracellular compartments to the plasma membrane to allow FA storage within the cell and hence regulates FA metabolism (Koonen et al. 2011). A sustained relocation of CD36 to the plasma membrane was demonstrated to be an early phenomenon in insulin resistance. The turnover of CD36 was decreased in macrophages from *ob*/*ob* insulin-resistant mice. In addition, CD36 expression was increased when blocking insulin signalling in macrophages from *ob*/*ob* mice (Liang et al. 2004; Ouwens et al. 2007). CD36 deficiency in mice led to a significant reduction in adipose tissue inflammation and a marked improvement in insulin sensitivity as compared to wild-type animals under a HFD (Kennedy et al. 2011). Several lines of evidence demonstrated that FAT/CD36 contributes to the cross talk between adipocytes and macrophages, yielding insulin resistance via a JNK-dependent pathway (Kennedy et al. 2011; Nicholls et al. 2011). A soluble form of CD36 (sCD36) was identified in human plasma and was associated with markers of insulin resistance, type 2 diabetes and atherosclerosis (Handberg et al. 2012; Liani et al. 2012). Variants in the CD36 gene influence the susceptibility to develop cardio-metabolic diseases (Love-Gregory et al. 2008, 2011). Hence, CD36 might be an interesting candidate to mediate Tlr signalling in response to SFA.

Another mechanism that has been proposed might involve SFA-dependent Tlr4 dimerization. Dimerization is necessary to induce Tlr4 receptor signalling and

occurs within specialized lipid areas termed "lipid rafts". Lipid rafts in response to SFA stimulation also redistribute c-Src (Holzer et al. 2011), a known upstream activator of JNK. To our knowledge, it is, however, unclear thus far whether c-Src recruitment in response to SFA is dependent on Tlr4 dimerization or whether these two processes are unrelated.

A third option is that SFA induce tissue damage that would secondarily induce Tlr signalling as Tlrs are indeed sensing tissue damage. Obesity was shown to be accompanied by disturbances in the gut microflora, leading to a spillover of bacterial components into the circulation that might trigger Tlr-mediated signalling in different insulin-sensitive tissues (Henao-Mejia et al. 2012). Indeed, the gut microbiota was shown to be a key player in mediating insulin resistance in Tlr2-deficient mice (Caricilli et al. 2011). Overall, there is strong evidence that SFAs, through Tlr receptors, promote insulin resistance. It is less clear, however, how exactly SFAs induce Tlr signalling and additional work will therefore be required (Figs. 1 and 2).

Of note, SFAs have been shown to induce signalling independent of Tlr receptors. We have summarized above how SFA-stimulated ROS production activates the inflammasome and how this may lead to insulin resistance (Wen et al. 2011). Moreover, SFAs give rise to different lipid metabolites that act as second messengers. One prominent example is diacylglycerol (DAG) that activates protein kinase C that is yet another kinase, the activity of which can interfere with insulin signalling. Several lines of evidence have linked fatty acid-induced insulin resistance with the activation of protein kinase C theta (PKCθ) that phosphorylates insulin receptor substrate on serine via an increase in diacylglycerol (DAG) content (Amati 2012) (Fig. 2). Very recently, it has been shown that acute induction of muscle insulin resistance was associated with a transient increase in total and cytosolic DAG content that was temporally associated with PKCθ activation, increased IRS1 serine 1101 phosphorylation and inhibition of insulin-stimulated IRS1 tyrosine phosphorylation as well as AKT2 phosphorylation. Increased muscle DAG content, PKCθ activation and insulin resistance were also observed in healthy insulin-resistant obese individuals and in obese type 2 diabetes patients (Szendroedi et al. 2014).

Altogether, a large body of evidence suggests a role of nutritional SFAs triggering a whole plethora of signalling modules to promote metabolic inflammation during obesity.

4.2 Ceramide and Sphingosine-1-Phosphate

Ceramide is yet another very interesting metabolite synthesized out of the SFA palmitate and the amino acid serine, which contributes to insulin resistance. An in vivo study reported that diet-induced obesity (DIO) promotes ceramide accumulation in the gastrocnemius, which was completely reversed upon treatment with myriocin, a specific inhibitor of serine palmitoyltransferase (SPT), the rate-limiting enzyme in *de novo* ceramide synthesis (Chavez and Summers 2012). Importantly,

myriocin treatment reversed also glucose intolerance and insulin resistance in both DIO mice and in the diabetic *db*/*db* animals (Ussher et al. 2010). Ceramide impairs insulin signalling through PKCζ- and/or protein phosphatase A2-mediated AKT/PKB inactivation (Bikman and Summers 2011) (Fig. 2). It is particularly important to note in this chapter that metabolic inflammation via Tlr4 signalling is an important trigger of ceramide biosynthesis. In fact, Tlr4-dependent insulin resistance was shown to be at least partially dependent on ceramide (Holland et al. 2011a). Interestingly, the authors subsequently demonstrated that, in contrast to Tlr4 signalling, adiponectin reduced hepatic ceramide levels via the activation of ceramidase. Moreover, adiponectin deficiency increased ceramide levels and exacerbated insulin resistance. Instead, activation of adiponectin receptors 1 and 2 stimulated sphingosine and sphingosine-1-phosphate synthesis, which improved insulin sensitivity and reduced metabolic inflammation (Holland et al. 2011b).

In the future, it will be interesting to address the role of other sphingolipids in the context of obesity and insulin resistance and to understand better how they exert different effects on insulin action.

4.3 Anti-Inflammatory Lipids

Lipids do not only exert pro-inflammatory functions. For example, ω3-fatty acids (ω3-FAs) are known for their anti-inflammatory effects. In this context, a very interesting new mechanism has been put forward. The GPR120 receptor through ω3-FAs binding and subsequent internalization and association with β2-arrestin sequesters the kinase TAB1, thereby preventing TNFα-mediated activation of the TAK1/MKK4/JNK and of the TLR4/TAK1/IKK/NFκB pathway (Fig. 2). GPR120 inactivation abolished the beneficial anti-inflammatory and insulin-sensitizing effects of ω3-FAs in mice subjected to a HFD. Consistently, a selective GPR120 agonist was recently shown to improve insulin resistance and chronic inflammation in obese mice (Oh da et al. 2014). Most recently, new lipid species, fatty acid esters of hydroxy fatty acids (FAHFAs), also seem to signal through GPR120 to counteract inflammation-induced insulin resistance (Yore et al. 2014) (Fig. 2).

These recent discoveries offer very potential new therapeutic avenues targeting metabolic inflammation.

5 MAPK Signalling

Many components of the mitogen-activated protein kinase (MAPK) family are activated by environmental or intracellular stress, converting the latter into specific responses. MAPK-dependent adaption of cellular metabolism in response to stress, including that of inflammatory origin, has recently been widely investigated. Signalling over MAPKs has been shown to be pivotal in many aspects of metabolic disorders including type 2 diabetes.

Jun N-terminal kinases (JNK1 to 3) comprise one of the first important groups of the MAPK signalling cascade studied in the context of obesity and insulin resistance. JNK activity was found induced in many tissues of obese and insulin-resistant mice (Hirosumi et al. 2002) (Fig. 2). Mechanistically, JNK activation can occur through the induction of endoplasmic reticulum stress (Hotamisligil 2010), depending on the activity of double-stranded RNA-dependent protein kinase (PKR) (Nakamura et al. 2010). Alternatively, saturated fatty acids through binding of toll-like receptors (Shi et al. 2006; Tsukumo et al. 2007) or through activation of the protein kinase C-mediated activation of the mixed-lineage protein kinase (MLK) group of MAP kinase kinase kinases (MAPKKKs) (Kant et al. 2013) may also activate JNK. Last but not the least, low-grade inflammation and release of cytokines might additionally trigger JNK activation, which in turn will enhance the inflammatory response (Wellen and Hotamisligil 2005).

JNK1 is probably the main protein of JNKs involved in the development of insulin resistance. JNK1 knockout mice were protected against obesity and related insulin resistance (Hirosumi et al. 2002). However, subsequent studies using conditional JNK1 knockout mice demonstrated that metabolic consequences were different depending on where JNK1 was inactivated. Deletion of JNK1 in adipose tissue and muscle did not affect obesity but improved insulin sensitivity compared to the control mice. Interestingly, adipose JNK1 deletion resulted in markedly enhanced hepatic insulin sensitivity. Mechanistically, it has been suggested that IL-6 release from adipocytes was reduced and that the IL-6 insulin-desensitizing action on hepatocytes was therefore attenuated (Sabio et al. 2008). Deletion of JNK1 in the central nervous system protected against both, obesity and insulin resistance (Sabio et al. 2010). Unexpectedly, deletion of JNK1 in hepatocytes resulted in glucose intolerance and hepatic steatosis, suggesting that JNK1 activation in the liver rather attenuated disease development disease development (Sabio et al. 2009). Outcomes in haematopoietic- or myeloid-specific JNK1 knockout mice were not consistently described. While two studies do not support a major role for JNK1 in immune cells in the context of obesity and insulin resistance (Vallerie et al. 2008; Sabio et al. 2008), one study showed that it may contribute to the latter traits (Solinas et al. 2007). Conclusively, protective effects on obesity seen in total JNK1 knockout mice is most likely caused by the loss of JNK1 function in the brain as JNK1 deletion in the central nervous system was the only conditional knockout mouse model so far affecting obesity. Importantly, however, the JNK1-dependent regulation of insulin sensitivity in muscle, adipose tissue and liver seems to be independent of obesity.

An important question is as to how JNK signalling regulates insulin resistance. In vitro studies suggested that JNK phosphorylated insulin receptor substrate (IRS1) at serine 307 to inhibit insulin signalling (Aguirre et al. 2000, 2002; Lee et al. 2003) similarly to IKK-mediated IRS 1/2 phosphorylation (Figs. 1 and 2). An increase in serine 307 phosphorylation was correlated with obesity and insulin resistance in mice (Hirosumi et al. 2002). Unexpectedly, however, knock-in mice, in which serine 307 of IRS1 was mutated, showed more severe insulin resistance. Importantly, hepatic JNK1 deficiency was shown to lead to decreased serine

307 phosphorylation in liver but correlated with enhanced hepatic insulin resistance. This raises the possibility that the outcome of changes in serine 307 phosphorylation varies depending on the tissue. Furthermore, other phosphorylation sites in IRS1 have been shown to affect insulin action (Boucher et al. 2014). Further work is thus needed to understand the function of posttranslational modifications in IRS1 in insulin signalling in different organs.

p38 (p38α, β, γ and δ) kinases constitute yet another important MAPK subfamily that is activated by cellular stress. Compared to JNK signalling, much less is known about the role of p38 in metabolic diseases, in particular in an in vivo context. Essentially, all upstream mechanisms described above leading to JNK activation in obesity and insulin resistance have been shown to trigger p38 activation. One of the major issues of most published studies is the fact that tools used to modulate p38 signalling did not take into account that we deal with four different p38 proteins that show quite distinct expression patterns. Indeed, p38 activation has been reported in the skeletal muscle of diabetic patients (Koistinen et al. 2003). In strong contrast, one recent study showed that p38 activity in liver, skeletal muscle and white adipose tissue was decreased in leptin-deficient obese insulin-resistant mice (Lee et al. 2011). Moreover, enhancing p38 activity by overexpressing a constitutive active form of the upstream kinase MKK6 in liver improved glucose homeostasis. Mechanistically, p38-mediated phosphorylation of X-box binding protein 1 (Xbp1) enhanced the latter nuclear translocation, increasing the transcription of Xbp1 target genes that counteracted ER stress (Lee et al. 2011). It is indeed unexpected that the activity of a canonical stress kinase dropped under disease conditions where cellular stress is supposed to be higher. As a possible explanation, the authors report that inactivation of JNK enhanced p38 activity in cells and argue that positive effects on glucose homeostasis caused by JNK1 deletion in liver (Sabio et al. 2009) might indeed be mediated through increased p38 signalling. In other words, increased JNK activity found in the livers of obese mice may suppress p38 signalling even under stress conditions, thereby promoting disease development. Importantly, however, this study provided no evidence for the requirement of hepatic p38 signalling in the regulation of glucose homeostasis in metabolically challenged mice. Interestingly, several in vitro studies propose a role for p38 in the positive regulation of gluconeogenesis in liver, a process that contributes to enhanced glucose levels in obesity and insulin resistance (Cao et al. 2005; Qiao et al. 2006). In this sense, enhancing p38 activity in liver may not have only beneficial effects in a diabetic context. In the future, it needs to be determined how JNK signalling is connected with p38 signalling. The fact is that acute stress stimuli induce both pathways with quite similar kinetics through very similar upstream mechanisms. How chronic exposure to stress leads to the activity of only one pathway that suppresses the other will be indeed an interesting challenging question to be addressed in the future.

Efforts towards understanding the requirement of the function of individual p38 proteins are increasing. While in vivo functions of the canonical p38 proteins, p38α and p38β, in metabolism in different organs remain unexplored thus far, two recent studies shed some light on a possible function of p38γ and p38δ. p38γ appears to

play a role in skeletal muscle metabolism (Pogozelski et al. 2009). How this may affect glucose homeostasis in a disease context is yet to be determined. p38δ was shown to negatively regulate the activity of protein kinase D 1 (PKD1). This signalling module regulated insulin secretion from pancreatic β cells. Importantly, mice lacking p38δ were protected against obesity-related insulin resistance as well as β cell failure. In diabetes, there might be an exaggerated stress-induced p38-δ-dependent inhibition of PKD1 that would contribute to β cell failure (Sumara et al. 2009).

Many other signalling components of the MAPK kinase family have been explored in a metabolic context, which for space reasons cannot be discussed in the framework of this book chapter. We recommend reading the following recent review that covers more broadly the aspects of MAPK signalling in metabolism and associated diseases (Gehart et al. 2010).

Overall, the above studies put MAPK signalling in the centre of metabolic inflammation and associated insulin resistance.

6 IKK Signalling

Another key converging point in metabolic inflammation represents IKK that activates NFκB. NFκB is a complex formed by the p65 and p50 proteins that are sequestered in the cytoplasm by the inhibitor of kappa B protein (IκB). When an inflammatory response is initiated, IκB becomes phosphorylated (later IκB is targeted for degradation by the ubiquitin/proteasome system) and dissociates from the p65/p50 complex, which is phosphorylated and translocated to the nucleus to activate pro-inflammatory target genes (Barnes and Karin 1997) (Figs. 1 and 2). The role of NFκB in metabolic inflammation has been well covered by recent reviews (Tornatore et al. 2012; Solinas and Karin 2010). For reasons of space limitations, we will summarize here some but not all important points related to IKK signalling in the context of metabolic inflammation. IKKβ associates with IKKα and an adaptor protein named NEMO to phosphorylate IκBα, the negative regulator of NFκB allowing its translocation to the nucleus (Senftleben and Karin 2002). IKKβ activation was shown to impair insulin signalling, whereas its inactivation reversed insulin resistance in vitro. Heterozygous deletion of IKKβ protects mice from insulin resistance in diet-induced and genetic models of obesity (Yuan et al. 2001). Moreover, salicylate improved insulin signalling through IKKβ inhibition (Yuan et al. 2001). IKKβ was then explored further in different tissues using conditional knockout mice. While obese liver-specific knockout mice improved hepatic insulin sensitivity compared to controls, insulin resistance remained in muscle and adipose tissue as in the control mice. However, myeloid-specific deletion of IKKβ led to systemically improved insulin sensitivity (Arkan et al. 2005). Of note, hypothalamic IKKβ was also shown to contribute to energy imbalance and obesity (Zhang et al. 2008).

Interestingly, two noncanonical IKKs have been also discovered to play important roles in metabolic inflammation, IKKε and *TRAF-associated NFκB activator* (*TANK*)*-binding kinase 1* (TBK1). Similarly to the IKKβ/IKKα/NEMO complex, IKKε and TBK1 associate with three scaffold proteins (*NAK-associated protein-1* NAP1, TANK and *NAP and TBK1 adaptor* SINTBAD) to phosphorylate IRF3 (homodimerization and nuclear translocation) activating its target genes (Verhelst et al. 2013) (Fig. 2). Indeed, IKKε KO animals were protected against diet-induced obesity, exhibited less chronic liver inflammation and had improved insulin resistance as compared to wild-type mice under a HFD (Chiang et al. 2009). Moreover, its inhibition in the hypothalamus improves energy homeostasis and glucose metabolism (Weissmann et al. 2014). Consistently, the pharmacological inhibition of TBK1 and IKKε improves obesity-related metabolic abnormalities in mice (Reilly et al. 2013). Of particular interest, TBK1 was shown to phosphorylate the insulin receptor on serine 994 in genetically obese Zucker rats (Munoz et al. 2009). In line with these findings, IRF3 KO mice were also protected against diet-induced insulin resistance (Wang et al. 2014).

It has also been demonstrated that the fundamental metabolic regulators PPARs and liver X receptor (LXR) have anti-inflammatory effects through NFκB suppression (Wellen and Hotamisligil 2005; Kidani and Bensinger 2012). The anti-inflammatory effects of LXRs and PPARγ implicate their activation and subsequent SUMOylation, which induces the repression of inflammatory gene expression by maintaining corepressors on the promoters of NFκB target genes (Ghisletti et al. 2007).

Altogether, IKK signalling represents yet another very important signalling component that triggers insulin resistance through inflammatory mechanisms.

7 Concluding Remarks

This review provided an overview of the main signalling networks governing metabolic inflammation, emphasizing MAPKs and IKKs as major signalling hubs activated in immune cells as well as in metabolic target tissues. While these kinases propagate inflammatory inputs on the one hand, they also sense inflammation in target tissues linking the latter to decreased insulin action. They may therefore represent interesting drug targets in the treatment of obese patients with or without type 2 diabetes. However, more work is needed to understand more in details the exact role of these pathways in different cell types and tissues as crude systemic inhibition of these kinases may indeed entail many unwanted side effects. Even though metabolic inflammation acts systemically, attempts to discover cell-type- and tissue-specific signalling mechanisms and more sophisticated multi-targeting strategies thereof might be useful to overcome these drawbacks.

References

Aggarwal BB (2003) Signalling pathways of the TNF superfamily: a double-edged sword. Nat Rev Immunol 3:745–756

Aguirre V, Uchida T, Yenush L, Davis R, White MF (2000) The c-Jun NH(2)-terminal kinase promotes insulin resistance during association with insulin receptor substrate-1 and phosphorylation of Ser(307). J Biol Chem 275:9047–9054

Aguirre V, Werner ED, Giraud J, Lee YH, Shoelson SE, White MF (2002) Phosphorylation of Ser307 in insulin receptor substrate-1 blocks interactions with the insulin receptor and inhibits insulin action. J Biol Chem 277:1531–1537

Ahmad F, Li PM, Meyerovitch J, Goldstein BJ (1995) Osmotic loading of neutralizing antibodies demonstrates a role for protein-tyrosine phosphatase 1B in negative regulation of the insulin action pathway. J Biol Chem 270:20503–20508

Amati F (2012) Revisiting the diacylglycerol-induced insulin resistance hypothesis. Obes Rev 13 (Suppl 2):40–50

Arkan MC, Hevener AL, Greten FR, Maeda S, Li ZW, Long JM, Wynshaw-Boris A, Poli G, Olefsky J, Karin M (2005) IKK-beta links inflammation to obesity-induced insulin resistance. Nat Med 11:191–198

Barnes PJ, Karin M (1997) Nuclear factor-kappaB: a pivotal transcription factor in chronic inflammatory diseases. N Engl J Med 336:1066–1071

Bikman BT, Summers SA (2011) Ceramides as modulators of cellular and whole-body metabolism. J Clin Invest 121:4222–4230

Boden G, Jadali F, White J, Liang Y, Mozzoli M, Chen X, Coleman E, Smith C (1991) Effects of fat on insulin-stimulated carbohydrate metabolism in normal men. J Clin Invest 88:960–966

Boucher J, Kleinridders A, Kahn CR (2014) Insulin receptor signaling in normal and insulin-resistant states. Cold Spring Harb Perspect Biol 6(1). pii: a009191. doi: 10.1101/cshperspect.a009191

Bouhlel MA, Derudas B, Rigamonti E, Dievart R, Brozek J, Haulon S, Zawadzki C, Jude B, Torpier G, Marx N, Staels B, Chinetti-Gbaguidi G (2007) PPARgamma activation primes human monocytes into alternative M2 macrophages with anti-inflammatory properties. Cell Metab 6:137–143

Canto C, Gerhart-Hines Z, Feige JN, Lagouge M, Noriega L, Milne JC, Elliott PJ, Puigserver P, Auwerx J (2009) AMPK regulates energy expenditure by modulating NAD+ metabolism and SIRT1 activity. Nature 458:1056–1060

Cao W, Collins QF, Becker TC, Robidoux J, Lupo EG Jr, Xiong Y, Daniel KW, Floering L, Collins S (2005) p38 Mitogen-activated protein kinase plays a stimulatory role in hepatic gluconeogenesis. J Biol Chem 280:42731–42737

Carey AL, Steinberg GR, Macaulay SL, Thomas WG, Holmes AG, Ramm G, Prelovsek O, Hohnen-Behrens C, Watt MJ, James DE, Kemp BE, Pedersen BK, Febbraio MA (2006) Interleukin-6 increases insulin-stimulated glucose disposal in humans and glucose uptake and fatty acid oxidation in vitro via AMP-activated protein kinase. Diabetes 55:2688–2697

Caricilli AM, Picardi PK, de Abreu LL, Ueno M, Prada PO, Ropelle ER, Hirabara SM, Castoldi A, Vieira P, Camara NO, Curi R, Carvalheira JB, Saad MJ (2011) Gut microbiota is a key modulator of insulin resistance in TLR 2 knockout mice. PLoS Biol 9:e1001212

Cascio G, Schiera G, Di Liegro I (2012) Dietary fatty acids in metabolic syndrome, diabetes and cardiovascular diseases. Curr Diabetes Rev 8:2–17

Chavez JA, Summers SA (2012) A ceramide-centric view of insulin resistance. Cell Metab 15:585–594

Chawla A (2010) Control of macrophage activation and function by PPARs. Circ Res 106:1559–1569

Chiang SH, Bazuine M, Lumeng CN, Geletka LM, Mowers J, White NM, Ma JT, Zhou J, Qi N, Westcott D, Delproposto JB, Blackwell TS, Yull FE, Saltiel AR (2009) The protein kinase IKKepsilon regulates energy balance in obese mice. Cell 138:961–975

Cuda C, Badawi A, Karmali M, El-Sohemy A (2011) Polymorphisms in toll-like receptor 4 are associated with factors of the metabolic syndrome and modify the association between dietary saturated fat and fasting high-density lipoprotein cholesterol. Metabolism 60:1131–1135

De Filippo G, Rendina D, Moccia F, Rocco V, Campanozzi A (2015) Interleukin-6, soluble interleukin-6 receptor/interleukin-6 complex and insulin resistance in obese children and adolescents. J Endocrinol Invest 38(3):339–343

Dinarello CA, Donath MY, Mandrup-Poulsen T (2010) Role of IL-1beta in type 2 diabetes. Curr Opin Endocrinol Diabetes Obes 17:314–321

Donath MY, Dalmas E, Sauter NS, Boni-Schnetzler M (2013) Inflammation in obesity and diabetes: islet dysfunction and therapeutic opportunity. Cell Metab 17:860–872

Dong J, Zhang X, Zhang L, Bian HX, Xu N, Bao B, Liu J (2014) Quercetin reduces obesity-associated ATM infiltration and inflammation in mice: a mechanism including AMPKalpha1/SIRT1. J Lipid Res 55:363–374

Emanuelli B, Peraldi P, Filloux C, Sawka-Verhelle D, Hilton D, van Obberghen E (2000) SOCS-3 is an insulin-induced negative regulator of insulin signaling. J Biol Chem 275:15985–15991

Engelman JA, Berg AH, Lewis RY, Lisanti MP, Scherer PE (2000) Tumor necrosis factor alpha-mediated insulin resistance, but not dedifferentiation, is abrogated by MEK1/2 inhibitors in 3T3-L1 adipocytes. Mol Endocrinol 14:1557–1569

Fernandez-Real JM, Vayreda M, Richart C, Gutierrez C, Broch M, Vendrell J, Ricart W (2001) Circulating interleukin 6 levels, blood pressure, and insulin sensitivity in apparently healthy men and women. J Clin Endocrinol Metab 86:1154–1159

Franckhauser S, Elias I, Rotter Sopasakis V, Ferre T, Nagaev I, Andersson CX, Agudo J, Ruberte J, Bosch F, Smith U (2008) Overexpression of Il6 leads to hyperinsulinaemia, liver inflammation and reduced body weight in mice. Diabetologia 51:1306–1316

Garlanda C, Dinarello CA, Mantovani A (2013) The interleukin-1 family: back to the future. Immunity 39:1003–1018

Gauthier MS, O'Brien EL, Bigornia S, Mott M, Cacicedo JM, Xu XJ, Gokce N, Apovian C, Ruderman N (2011) Decreased AMP-activated protein kinase activity is associated with increased inflammation in visceral adipose tissue and with whole-body insulin resistance in morbidly obese humans. Biochem Biophys Res Commun 404:382–387

Gehart H, Kumpf S, Ittner A, Ricci R (2010) MAPK signalling in cellular metabolism: stress or wellness? EMBO Rep 11:834–840

Ghisletti S, Huang W, Ogawa S, Pascual G, Lin ME, Willson TM, Rosenfeld MG, Glass CK (2007) Parallel SUMOylation-dependent pathways mediate gene- and signal-specific transrepression by LXRs and PPARgamma. Mol Cell 25:57–70

Gillum MP, Kotas ME, Erion DM, Kursawe R, Chatterjee P, Nead KT, Muise ES, Hsiao JJ, Frederick DW, Yonemitsu S, Banks AS, Qiang L, Bhanot S, Olefsky JM, Sears DD, Caprio S, Shulman GI (2011) SirT1 regulates adipose tissue inflammation. Diabetes 60:3235–3245

Gordon S (2003) Alternative activation of macrophages. Nat Rev Immunol 3:23–35

Gregor MF, Hotamisligil GS (2011) Inflammatory mechanisms in obesity. Annu Rev Immunol 29:415–445

Hajri T, Tao H, Wattacheril J, Marks-Shulman P, Abumrad NN (2011) Regulation of adiponectin production by insulin: interactions with tumor necrosis factor-alpha and interleukin-6. Am J Physiol Endocrinol Metab 300:E350–E360

Handberg A, Hojlund K, Gastaldelli A, Flyvbjerg A, Dekker JM, Petrie J, Piatti P, Beck-Nielsen H, Investigators R (2012) Plasma sCD36 is associated with markers of atherosclerosis, insulin resistance and fatty liver in a nondiabetic healthy population. J Intern Med 271:294–304

He J, Usui I, Ishizuka K, Kanatani Y, Hiratani K, Iwata M, Bukhari A, Haruta T, Sasaoka T, Kobayashi M (2006) Interleukin-1alpha inhibits insulin signaling with phosphorylating insulin receptor substrate-1 on serine residues in 3T3-L1 adipocytes. Mol Endocrinol 20:114–124

Henao-Mejia J, Elinav E, Jin C, Hao L, Mehal WZ, Strowig T, Thaiss CA, Kau AL, Eisenbarth SC, Jurczak MJ, Camporez JP, Shulman GI, Gordon JI, Hoffman HM, Flavell RA (2012)

Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. Nature 482:179–185

Hevener AL, Olefsky JM, Reichart D, Nguyen MT, Bandyopadyhay G, Leung HY, Watt MJ, Benner C, Febbraio MA, Nguyen AK, Folian B, Subramaniam S, Gonzalez FJ, Glass CK, Ricote M (2007) Macrophage PPAR gamma is required for normal skeletal muscle and hepatic insulin sensitivity and full antidiabetic effects of thiazolidinediones. J Clin Invest 117:1658–1669

Hirosumi J, Tuncman G, Chang L, Gorgun CZ, Uysal KT, Maeda K, Karin M, Hotamisligil GS (2002) A central role for JNK in obesity and insulin resistance. Nature 420:333–336

Holland WL, Bikman BT, Wang LP, Yuguang G, Sargent KM, Bulchand S, Knotts TA, Shui G, Clegg DJ, Wenk MR, Pagliassotti MJ, Scherer PE, Summers SA (2011a) Lipid-induced insulin resistance mediated by the proinflammatory receptor TLR4 requires saturated fatty acid-induced ceramide biosynthesis in mice. J Clin Invest 121:1858–1870

Holland WL, Miller RA, Wang ZV, Sun K, Barth BM, Bui HH, Davis KE, Bikman BT, Halberg N, Rutkowski JM, Wade MR, Tenorio VM, Kuo MS, Brozinick JT, Zhang BB, Birnbaum MJ, Summers SA, Scherer PE (2011b) Receptor-mediated activation of ceramidase activity initiates the pleiotropic actions of adiponectin. Nat Med 17:55–63

Holzer RG, Park EJ, Li N, Tran H, Chen M, Choi C, Solinas G, Karin M (2011) Saturated fatty acids induce c-Src clustering within membrane subdomains, leading to JNK activation. Cell 147:173–184

Hotamisligil GS (2006) Inflammation and metabolic disorders. Nature 444:860–867

Hotamisligil GS (2010) Endoplasmic reticulum stress and the inflammatory basis of metabolic disease. Cell 140:900–917

Hotamisligil GS, Shargill NS, Spiegelman BM (1993) Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 259:87–91

Hotamisligil GS, Budavari A, Murray D, Spiegelman BM (1994a) Reduced tyrosine kinase activity of the insulin receptor in obesity-diabetes. Central role of tumor necrosis factor-alpha. J Clin Invest 94:1543–1549

Hotamisligil GS, Murray DL, Choy LN, Spiegelman BM (1994b) Tumor necrosis factor alpha inhibits signaling from the insulin receptor. Proc Natl Acad Sci U S A 91:4854–4858

Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM (1995) Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. J Clin Invest 95:2409–2415

Hotamisligil GS, Peraldi P, Budavari A, Ellis R, White MF, Spiegelman BM (1996) IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance. Science 271:665–668

Kado S, Nagase T, Nagata N (1999) Circulating levels of interleukin-6, its soluble receptor and interleukin-6/interleukin-6 receptor complexes in patients with type 2 diabetes mellitus. Acta Diabetol 36:67–72

Kang K, Reilly SM, Karabacak V, Gangl MR, Fitzgerald K, Hatano B, Lee CH (2008) Adipocyte-derived Th2 cytokines and myeloid PPARdelta regulate macrophage polarization and insulin sensitivity. Cell Metab 7:485–495

Kant S, Barrett T, Vertii A, Noh YH, Jung DY, Kim JK, Davis RJ (2013) Role of the mixed-lineage protein kinase pathway in the metabolic stress response to obesity. Cell Rep 4:681–688

Kelly M, Keller C, Avilucea PR, Keller P, Luo Z, Xiang X, Giralt M, Hidalgo J, Saha AK, Pedersen BK, Ruderman NB (2004) AMPK activity is diminished in tissues of IL-6 knockout mice: the effect of exercise. Biochem Biophys Res Commun 320:449–454

Kelly M, Gauthier MS, Saha AK, Ruderman NB (2009) Activation of AMP-activated protein kinase by interleukin-6 in rat skeletal muscle: association with changes in cAMP, energy state, and endogenous fuel mobilization. Diabetes 58:1953–1960

Kennedy DJ, Kuchibhotla S, Westfall KM, Silverstein RL, Morton RE, Febbraio M (2011) A CD36-dependent pathway enhances macrophage and adipose tissue inflammation and impairs insulin signalling. Cardiovasc Res 89:604–613

Kidani Y, Bensinger SJ (2012) Liver X receptor and peroxisome proliferator-activated receptor as integrators of lipid homeostasis and immunity. Immunol Rev 249:72–83
Koistinen HA, Chibalin AV, Zierath JR (2003) Aberrant p38 mitogen-activated protein kinase signalling in skeletal muscle from type 2 diabetic patients. Diabetologia 46:1324–1328
Konner AC, Bruning JC (2011) Toll-like receptors: linking inflammation to metabolism. Trends Endocrinol Metab 22:16–23
Koonen DP, Jensen MK, Handberg A (2011) Soluble CD36- a marker of the (pathophysiological) role of CD36 in the metabolic syndrome? Arch Physiol Biochem 117:57–63
Lee YH, Giraud J, Davis RJ, White MF (2003) c-Jun N-terminal kinase (JNK) mediates feedback inhibition of the insulin signaling cascade. J Biol Chem 278:2896–2902
Lee J, Sun C, Zhou Y, Lee J, Gokalp D, Herrema H, Park SW, Davis RJ, Ozcan U (2011) p38 MAPK-mediated regulation of Xbp1s is crucial for glucose homeostasis. Nat Med 17:1251–1260
Leick L, Lindegaard B, Stensvold D, Plomgaard P, Saltin B, Pilegaard H (2007) Adipose tissue interleukin-18 mRNA and plasma interleukin-18: effect of obesity and exercise. Obesity (Silver Spring) 15:356–363
Liang CP, Han S, Okamoto H, Carnemolla R, Tabas I, Accili D, Tall AR (2004) Increased CD36 protein as a response to defective insulin signaling in macrophages. J Clin Invest 113:764–773
Liani R, Halvorsen B, Sestili S, Handberg A, Santilli F, Vazzana N, Formoso G, Aukrust P, Davi G (2012) Plasma levels of soluble CD36, platelet activation, inflammation, and oxidative stress are increased in type 2 diabetic patients. Free Radic Biol Med 52:1318–1324
Lindegaard B, Matthews VB, Brandt C, Hojman P, Allen TL, Estevez E, Watt MJ, Bruce CR, Mortensen OH, Syberg S, Rudnicka C, Abildgaard J, Pilegaard H, Hidalgo J, Ditlevsen S, Alsted TJ, Madsen AN, Pedersen BK, Febbraio MA (2013) Interleukin-18 activates skeletal muscle AMPK and reduces weight gain and insulin resistance in mice. Diabetes 62:3064–3074
Love-Gregory L, Abumrad NA (2011) CD36 genetics and the metabolic complications of obesity. Curr Opin Clin Nutr Metab Care 14:527–534
Love-Gregory L, Sherva R, Sun L, Wasson J, Schappe T, Doria A, Rao DC, Hunt SC, Klein S, Neuman RJ, Permutt MA, Abumrad NA (2008) Variants in the CD36 gene associate with the metabolic syndrome and high-density lipoprotein cholesterol. Hum Mol Genet 17:1695–1704
Love-Gregory L, Sherva R, Schappe T, Qi JS, Mccrea J, Klein S, Connelly MA, Abumrad NA (2011) Common CD36 SNPs reduce protein expression and may contribute to a protective atherogenic profile. Hum Mol Genet 20:193–201
Luotola K, Paakkonen R, Alanne M, Lanki T, Moilanen L, Surakka I, Pietila A, Kahonen M, Nieminen MS, Kesaniemi YA, Peters A, Jula A, Perola M, Salomaa V, Health ASG (2009) Association of variation in the interleukin-1 gene family with diabetes and glucose homeostasis. J Clin Endocrinol Metab 94:4575–4583
Martinez FO, Helming L, Gordon S (2009) Alternative activation of macrophages: an immunologic functional perspective. Annu Rev Immunol 27:451–483
Matthews VB, Allen TL, Risis S, Chan MH, Henstridge DC, Watson N, Zaffino LA, Babb JR, Boon J, Meikle PJ, Jowett JB, Watt MJ, Jansson JO, Bruce CR, Febbraio MA (2010) Interleukin-6-deficient mice develop hepatic inflammation and systemic insulin resistance. Diabetologia 53:2431–2441
Meissner F, Molawi K, Zychlinsky A (2008) Superoxide dismutase 1 regulates caspase-1 and endotoxic shock. Nat Immunol 9:866–872
Meli R, Mattace Raso G, Calignano A (2014) Role of innate immune response in non-alcoholic Fatty liver disease: metabolic complications and therapeutic tools. Front Immunol 5:177
Mohamed-Ali V, Goodrick S, Rawesh A, Katz DR, Miles JM, Yudkin JS, Klein S, Coppack SW (1997) Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-alpha, in vivo. J Clin Endocrinol Metab 82:4196–4200
Mosser DM, Edwards JP (2008) Exploring the full spectrum of macrophage activation. Nat Rev Immunol 8:958–969

Muller S, Martin S, Koenig W, Hanifi-Moghaddam P, Rathmann W, Haastert B, Giani G, Illig T, Thorand B, Kolb H (2002) Impaired glucose tolerance is associated with increased serum concentrations of interleukin 6 and co-regulated acute-phase proteins but not TNF-alpha or its receptors. Diabetologia 45:805–812

Munoz MC, Giani JF, Mayer MA, Toblli JE, Turyn D, Dominici FP (2009) TANK-binding kinase 1 mediates phosphorylation of insulin receptor at serine residue 994: a potential link between inflammation and insulin resistance. J Endocrinol 201:185–197

Nakamura T, Furuhashi M, Li P, Cao H, Tuncman G, Sonenberg N, Gorgun CZ, Hotamisligil GS (2010) Double-stranded RNA-dependent protein kinase links pathogen sensing with stress and metabolic homeostasis. Cell 140:338–348

Netea MG, Joosten LA, Lewis E, Jensen DR, Voshol PJ, Kullberg BJ, Tack CJ, van Krieken H, Kim SH, Stalenhoef AF, van de Loo FA, Verschueren I, Pulawa L, Akira S, Eckel RH, Dinarello CA, van den Berg W, van der Meer JW (2006) Deficiency of interleukin-18 in mice leads to hyperphagia, obesity and insulin resistance. Nat Med 12:650–656

Nicholls HT, Kowalski G, Kennedy DJ, Risis S, Zaffino LA, Watson N, Kanellakis P, Watt MJ, Bobik A, Bonen A, Febbraio M, Lancaster GI, Febbraio MA (2011) Hematopoietic cell-restricted deletion of CD36 reduces high-fat diet-induced macrophage infiltration and improves insulin signaling in adipose tissue. Diabetes 60:1100–1110

Nieto-Vazquez I, Fernandez-Veledo S, de Alvaro C, Lorenzo M (2008) Dual role of interleukin-6 in regulating insulin sensitivity in murine skeletal muscle. Diabetes 57:3211–3221

Nov O, Shapiro H, Ovadia H, Tarnovscki T, Dvir I, Shemesh E, Kovsan J, Shelef I, Carmi Y, Voronov E, Apte RN, Lewis E, Haim Y, Konrad D, Bashan N, Rudich A (2013) Interleukin-1beta regulates fat-liver crosstalk in obesity by auto-paracrine modulation of adipose tissue inflammation and expandability. PLoS One 8:e53626

O'Neill LA (2008) When signaling pathways collide: positive and negative regulation of toll-like receptor signal transduction. Immunity 29:12–20

Odegaard JI, Chawla A (2008) Mechanisms of macrophage activation in obesity-induced insulin resistance. Nat Clin Pract Endocrinol Metab 4:619–626

Odegaard JI, Chawla A (2013) The immune system as a sensor of the metabolic state. Immunity 38:644–654

Odegaard JI, Ricardo-Gonzalez RR, Goforth MH, Morel CR, Subramanian V, Mukundan L, Red Eagle A, Vats D, Brombacher F, Ferrante AW, Chawla A (2007) Macrophage-specific PPARgamma controls alternative activation and improves insulin resistance. Nature 447:1116–1120

Oh da Y, Walenta E, Akiyama TE, Lagakos WS, Lackey D, Pessentheiner AR, Sasik R, Hah N, Chi TJ, Cox JM, Powels MA, Di Salvo J, Sinz C, Watkins SM, Armando AM, Chung H, Evans RM, Quehenberger O, Mcnelis J, Bogner-Strauss JG, Olefsky JM (2014) A Gpr120-selective agonist improves insulin resistance and chronic inflammation in obese mice. Nat Med 20:942–947

Ohnishi H, Tochio H, Kato Z, Kawamoto N, Kimura T, Kubota K, Yamamoto T, Funasaka T, Nakano H, Wong RW, Shirakawa M, Kondo N (2012) TRAM is involved in IL-18 signaling and functions as a sorting adaptor for MyD88. PLoS One 7:e38423

Okamura H, Tsutsi H, Komatsu T, Yutsudo M, Hakura A, Tanimoto T, Torigoe K, Okura T, Nukada Y, Hattori K et al (1995) Cloning of a new cytokine that induces IFN-gamma production by T cells. Nature 378:88–91

Ouchi N, Parker JL, Lugus JJ, Walsh K (2011) Adipokines in inflammation and metabolic disease. Nat Rev Immunol 11:85–97

Ouwens DM, Diamant M, Fodor M, Habets DD, Pelsers MM, El Hasnaoui M, Dang ZC, van den Brom CE, Vlasblom R, Rietdijk A, Boer C, Coort SL, Glatz JF, Luiken JJ (2007) Cardiac contractile dysfunction in insulin-resistant rats fed a high-fat diet is associated with elevated CD36-mediated fatty acid uptake and esterification. Diabetologia 50:1938–1948

Palm NW, Medzhitov R (2009) Pattern recognition receptors and control of adaptive immunity. Immunol Rev 227:221–233

Park BS, Song DH, Kim HM, Choi BS, Lee H, Lee JO (2009) The structural basis of lipopolysaccharide recognition by the TLR4-MD-2 complex. Nature 458:1191–1195

Pickup JC, Mattock MB, Chusney GD, Burt D (1997) NIDDM as a disease of the innate immune system: association of acute-phase reactants and interleukin-6 with metabolic syndrome X. Diabetologia 40:1286–1292

Pogozelski AR, Geng T, Li P, Yin X, Lira VA, Zhang M, Chi JT, Yan Z (2009) p38gamma mitogen-activated protein kinase is a key regulator in skeletal muscle metabolic adaptation in mice. PLoS One 4:e7934

Posey KA, Clegg DJ, Printz RL, Byun J, Morton GJ, Vivekanandan-Giri A, Pennathur S, Baskin DG, Heinecke JW, Woods SC, Schwartz MW, Niswender KD (2009) Hypothalamic proinflammatory lipid accumulation, inflammation, and insulin resistance in rats fed a high-fat diet. Am J Physiol Endocrinol Metab 296:E1003–E1012

Qiao L, Macdougald OA, Shao J (2006) CCAAT/enhancer-binding protein alpha mediates induction of hepatic phosphoenolpyruvate carboxykinase by p38 mitogen-activated protein kinase. J Biol Chem 281:24390–24397

Ramadori G, Armbrust T (2001) Cytokines in the liver. Eur J Gastroenterol Hepatol 13:777–784

Ramadori G, Christ B (1999) Cytokines and the hepatic acute-phase response. Semin Liver Dis 19:141–155

Reilly SM, Chiang SH, Decker SJ, Chang L, Uhm M, Larsen MJ, Rubin JR, Mowers J, White NM, Hochberg I, Downes M, Yu RT, Liddle C, Evans RM, Oh D, Li P, Olefsky JM, Saltiel AR (2013) An inhibitor of the protein kinases TBK1 and IKK-varepsilon improves obesity-related metabolic dysfunctions in mice. Nat Med 19:313–321

Roden M, Price TB, Perseghin G, Petersen KF, Rothman DL, Cline GW, Shulman GI (1996) Mechanism of free fatty acid-induced insulin resistance in humans. J Clin Invest 97:2859–2865

Romsicki Y, Reece M, Gauthier JY, Asante-Appiah E, Kennedy BP (2004) Protein tyrosine phosphatase-1B dephosphorylation of the insulin receptor occurs in a perinuclear endosome compartment in human embryonic kidney 293 cells. J Biol Chem 279:12868–12875

Roncon-Albuquerque R Jr, Moreira-Rodrigues M, Faria B, Ferreira AP, Cerqueira C, Lourenco AP, Pestana M, von Hafe P, Leite-Moreira AF (2008) Attenuation of the cardiovascular and metabolic complications of obesity in CD14 knockout mice. Life Sci 83:502–510

Rotter V, Nagaev I, Smith U (2003) Interleukin-6 (IL-6) induces insulin resistance in 3T3-L1 adipocytes and is, like IL-8 and tumor necrosis factor-alpha, overexpressed in human fat cells from insulin-resistant subjects. J Biol Chem 278:45777–45784

Ruderman NB, Keller C, Richard AM, Saha AK, Luo Z, Xiang X, Giralt M, Ritov VB, Menshikova EV, Kelley DE, Hidalgo J, Pedersen BK, Kelly M (2006) Interleukin-6 regulation of AMP-activated protein kinase. Potential role in the systemic response to exercise and prevention of the metabolic syndrome. Diabetes 55(Suppl 2):S48–S54

Saberi M, Woods NB, de Luca C, Schenk S, Lu JC, Bandyopadhyay G, Verma IM, Olefsky JM (2009) Hematopoietic cell-specific deletion of toll-like receptor 4 ameliorates hepatic and adipose tissue insulin resistance in high-fat-fed mice. Cell Metab 10:419–429

Sabio G, Das M, Mora A, Zhang Z, Jun JY, Ko HJ, Barrett T, Kim JK, Davis RJ (2008) A stress signaling pathway in adipose tissue regulates hepatic insulin resistance. Science 322:1539–1543

Sabio G, Cavanagh-Kyros J, Ko HJ, Jung DY, Gray S, Jun JY, Barrett T, Mora A, Kim JK, Davis RJ (2009) Prevention of steatosis by hepatic JNK1. Cell Metab 10:491–498

Sabio G, Cavanagh-Kyros J, Barrett T, Jung DY, Ko HJ, Ong H, Morel C, Mora A, Reilly J, Kim JK, Davis RJ (2010) Role of the hypothalamic-pituitary-thyroid axis in metabolic regulation by JNK1. Genes Dev 24:256–264

Sarvas JL, Khaper N, Lees SJ (2013) The IL-6 paradox: context dependent interplay of SOCS3 and AMPK. J Diabetes Metab Suppl 13. doi:10.4172/2155-6156.S13-003

Schaeffler A, Gross P, Buettner R, Bollheimer C, Buechler C, Neumeier M, Kopp A, Schoelmerich J, Falk W (2009) Fatty acid-induced induction of toll-like receptor-4/nuclear factor-kappaB pathway in adipocytes links nutritional signalling with innate immunity. Immunology 126:233–245

Schenk S, Saberi M, Olefsky JM (2008) Insulin sensitivity: modulation by nutrients and inflammation. J Clin Invest 118:2992–3002

Seimon TA, Nadolski MJ, Liao X, Magallon J, Nguyen M, Feric NT, Koschinsky ML, Harkewicz R, Witztum JL, Tsimikas S, Golenbock D, Moore KJ, Tabas I (2010) Atherogenic lipids and lipoproteins trigger CD36-TLR2-dependent apoptosis in macrophages undergoing endoplasmic reticulum stress. Cell Metab 12:467–482

Senftleben U, Karin M (2002) The IKK/NF-kappaB pathway. Crit Care Med 30:S18–S26

Senn JJ (2006) Toll-like receptor-2 is essential for the development of palmitate-induced insulin resistance in myotubes. J Biol Chem 281:26865–26875

Senn JJ, Klover PJ, Nowak IA, Mooney RA (2002) Interleukin-6 induces cellular insulin resistance in hepatocytes. Diabetes 51:3391–3399

Shi H, Kokoeva MV, Inouye K, Tzameli I, Yin H, Flier JS (2006) TLR4 links innate immunity and fatty acid-induced insulin resistance. J Clin Invest 116:3015–3025

Solinas G, Karin M (2010) JNK1 and IKKbeta: molecular links between obesity and metabolic dysfunction. FASEB J 24:2596–2611

Solinas G, Vilcu C, Neels JG, Bandyopadhyay GK, Luo JL, Naugler W, Grivennikov S, Wynshaw-Boris A, Scadeng M, Olefsky JM, Karin M (2007) JNK1 in hematopoietically derived cells contributes to diet-induced inflammation and insulin resistance without affecting obesity. Cell Metab 6:386–397

Spranger J, Kroke A, Mohlig M, Hoffmann K, Bergmann MM, Ristow M, Boeing H, Pfeiffer AF (2003) Inflammatory cytokines and the risk to develop type 2 diabetes: results of the prospective population-based European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study. Diabetes 52:812–817

Staehr P, Hother-Nielsen O, Landau BR, Chandramouli V, Holst JJ, Beck-Nielsen H (2003) Effects of free fatty acids per se on glucose production, gluconeogenesis, and glycogenolysis. Diabetes 52:260–267

Stewart CR, Stuart LM, Wilkinson K, van Gils JM, Deng J, Halle A, Rayner KJ, Boyer L, Zhong R, Frazier WA, Lacy-Hulbert A, El Khoury J, Golenbock DT, Moore KJ (2010) CD36 ligands promote sterile inflammation through assembly of a toll-like receptor 4 and 6 heterodimer. Nat Immunol 11:155–161

Stienstra R, Joosten LA, Koenen T, van Tits B, van Diepen JA, van den Berg SA, Rensen PC, Voshol PJ, Fantuzzi G, Hijmans A, Kersten S, Muller M, van den Berg WB, van Rooijen N, Wabitsch M, Kullberg BJ, van der Meer JW, Kanneganti T, Tack CJ, Netea MG (2010) The inflammasome-mediated caspase-1 activation controls adipocyte differentiation and insulin sensitivity. Cell Metab 12:593–605

Stienstra R, van Diepen JA, Tack CJ, Zaki MH, van de Veerdonk FL, Perera D, Neale GA, Hooiveld GJ, Hijmans A, Vroegrijk I, van den Berg S, Romijn J, Rensen PC, Joosten LA, Netea MG, Kanneganti TD (2011) Inflammasome is a central player in the induction of obesity and insulin resistance. Proc Natl Acad Sci U S A 108:15324–15329

Straub RH, Hense HW, Andus T, Scholmerich J, Riegger GA, Schunkert H (2000) Hormone replacement therapy and interrelation between serum interleukin-6 and body mass index in postmenopausal women: a population-based study. J Clin Endocrinol Metab 85:1340–1344

Sumara G, Formentini I, Collins S, Sumara I, Windak R, Bodenmiller B, Ramracheya R, Caille D, Jiang H, Platt KA, Meda P, Aebersold R, Rorsman P, Ricci R (2009) Regulation of PKD by the MAPK p38delta in insulin secretion and glucose homeostasis. Cell 136:235–248

Szendroedi J, Yoshimura T, Phielix E, Koliaki C, Marcucci M, Zhang D, Jelenik T, Muller J, Herder C, Nowotny P, Shulman GI, Roden M (2014) Role of diacylglycerol activation of PKCtheta in lipid-induced muscle insulin resistance in humans. Proc Natl Acad Sci U S A 111:9597–9602

Tilg H, Moschen AR (2006) Adipocytokines: mediators linking adipose tissue, inflammation and immunity. Nat Rev Immunol 6:772–783

Tishinsky JM, De Boer AA, Dyck DJ, Robinson LE (2014) Modulation of visceral fat adipokine secretion by dietary fatty acids and ensuing changes in skeletal muscle inflammation. Appl Physiol Nutr Metab 39:28–37

Tornatore L, Thotakura AK, Bennett J, Moretti M, Franzoso G (2012) The nuclear factor kappa B signaling pathway: integrating metabolism with inflammation. Trends Cell Biol 22:557–566
Tschopp J, Schroder K (2010) NLRP3 inflammasome activation: the convergence of multiple signalling pathways on ROS production? Nat Rev Immunol 10:210–215
Tsukumo DM, Carvalho-Filho MA, Carvalheira JB, Prada PO, Hirabara SM, Schenka AA, Araujo EP, Vassallo J, Curi R, Velloso LA, Saad MJ (2007) Loss-of-function mutation in toll-like receptor 4 prevents diet-induced obesity and insulin resistance. Diabetes 56:1986–1998
Ussher JR, Koves TR, Cadete VJ, Zhang L, Jaswal JS, Swyrd SJ, Lopaschuk DG, Proctor SD, Keung W, Muoio DM, Lopaschuk GD (2010) Inhibition of de novo ceramide synthesis reverses diet-induced insulin resistance and enhances whole-body oxygen consumption. Diabetes 59:2453–2464
Uysal KT, Wiesbrock SM, Marino MW, Hotamisligil GS (1997) Protection from obesity-induced insulin resistance in mice lacking TNF-alpha function. Nature 389:610–614
Vallerie SN, Furuhashi M, Fucho R, Hotamisligil GS (2008) A predominant role for parenchymal c-Jun amino terminal kinase (JNK) in the regulation of systemic insulin sensitivity. PLoS One 3:e3151
Vandanmagsar B, Youm YH, Ravussin A, Galgani JE, Stadler K, Mynatt RL, Ravussin E, Stephens JM, Dixit VD (2011) The NLRP3 inflammasome instigates obesity-induced inflammation and insulin resistance. Nat Med 17:179–188
Verhelst K, Verstrepen L, Carpentier I, Beyaert R (2013) IkappaB kinase epsilon (IKKepsilon): a therapeutic target in inflammation and cancer. Biochem Pharmacol 85:873–880
Wang XA, Zhang R, She ZG, Zhang XF, Jiang DS, Wang T, Gao L, Deng W, Zhang SM, Zhu LH, Guo S, Chen K, Zhang XD, Liu DP, Li H (2014) Interferon regulatory factor 3 constrains IKKbeta/NF-kappaB signaling to alleviate hepatic steatosis and insulin resistance. Hepatology 59:870–885
Weissmann L, Quaresma PG, Santos AC, de Matos AH, Pascoal VD, Zanotto TM, Castro G, Guadagnini D, da Silva JM, Velloso LA, Bittencourt JC, Lopes-Cendes I, Saad MJ, Prada PO (2014) IKKepsilon is key to induction of insulin resistance in the hypothalamus, and its inhibition reverses obesity. Diabetes 63:3334–3345
Wellen KE, Hotamisligil GS (2005) Inflammation, stress, and diabetes. J Clin Invest 115:1111–1119
Wen H, Gris D, Lei Y, Jha S, Zhang L, Huang MT, Brickey WJ, Ting JP (2011) Fatty acid-induced NLRP3-ASC inflammasome activation interferes with insulin signaling. Nat Immunol 12:408–415
Yang Z, Kahn BB, Shi H, Xue BZ (2010) Macrophage alpha1 AMP-activated protein kinase (alpha1AMPK) antagonizes fatty acid-induced inflammation through SIRT1. J Biol Chem 285:19051–19059
Yore MM, Syed I, Moraes-Vieira PM, Zhang T, Herman MA, Homan EA, Patel RT, Lee J, Chen S, Peroni OD, Dhaneshwar AS, Hammarstedt A, Smith U, Mcgraw TE, Saghatelian A, Kahn BB (2014) Discovery of a class of endogenous mammalian lipids with anti-diabetic and anti-inflammatory effects. Cell 159:318–332
Yuan M, Konstantopoulos N, Lee J, Hansen L, Li ZW, Karin M, Shoelson SE (2001) Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of Ikkbeta. Science 293:1673–1677
Zabolotny JM, Kim YB, Welsh LA, Kershaw EE, Neel BG, Kahn BB (2008) Protein-tyrosine phosphatase 1B expression is induced by inflammation in vivo. J Biol Chem 283:14230–14241
Zhang X, Zhang G, Zhang H, Karin M, Bai H, Cai D (2008) Hypothalamic IKKbeta/NF-kappaB and ER stress link overnutrition to energy imbalance and obesity. Cell 135:61–73
Zorrilla EP, Sanchez-Alavez M, Sugama S, Brennan M, Fernandez R, Bartfai T, Conti B (2007) Interleukin-18 controls energy homeostasis by suppressing appetite and feed efficiency. Proc Natl Acad Sci U S A 104:11097–11102

Immune Cells and Metabolism

Antonios Chatzigeorgiou and Triantafyllos Chavakis

Contents

Abstract

Low-grade inflammation in the obese AT (AT) and the liver is a critical player in the development of obesity-related metabolic dysregulation, including insulin resistance, type 2 diabetes and non-alcoholic steatohepatitis (NASH). Myeloid as well as lymphoid cells infiltrate the AT and the liver and expand within these metabolic organs as a result of excessive nutrient intake, thereby exacerbating tissue inflammation. Macrophages are the paramount cell population in the field of metabolism-related inflammation; as obesity progresses, a switch takes place within the AT environment from an M2-alternatively activated macrophage state to an M1-inflammatory macrophage-dominated milieu. M1-polarized

A. Chatzigeorgiou (✉) • T. Chavakis
Department of Clinical Pathobiochemistry, Technische Universität Dresden, Fetscherstrasse 74, 01307 Dresden, Germany

Institute for Clinical Chemistry and Laboratory Medicine, Technische Universität Dresden, Dresden, Germany

Paul Langerhans Institute Dresden, German Center for Diabetes Research, Dresden, Germany
e-mail: antonios.chatzigeorgiou@uniklinikum-dresden.de

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macrophages secrete inflammatory cytokines like TNF in the obese AT; such cytokines contribute to insulin resistance in adipocytes. Besides macrophages, also $CD8^+$ T cells promote inflammation in the AT and the liver and thereby the deterioration of the metabolic balance in adipocytes and hepatocytes. Other cells of the innate immunity, such as neutrophils or mast cells, interfere with metabolic homeostasis as well. On the other hand, eosinophils or T-regulatory cells, the number of which in the AT decreases in the course of obesity, function to maintain metabolic balance by ameliorating inflammatory processes. In addition, eosinophils and M2-polarized macrophages may contribute to "beige" adipogenesis under lean conditions; beige adipocytes are located predominantly in the subcutaneous AT and have thermogenic and optimal energy-dispensing properties like brown adipocytes. This chapter will summarize the different aspects of the regulation of homeostasis of metabolic tissues by immune cells.

Keywords
Adipose tissue · Inflammation · Insulin resistance · Liver · Lymphocytes · Macrophages · Non-alcoholic steatohepatitis (NASH) · T-regulatory cells

1 Introduction

Impaired insulin response, elevated fasting glucose and hypertriglyceridemia are principal signs of metabolic deterioration that occur in obese individuals (Kahn et al. 2006; Subramanian and Chait 2012). Obesity accounts for the development of diseases such as insulin resistance, type 2 diabetes, cardiovascular atherosclerotic disease and non-alcoholic steatohepatitis (NASH) (Cohen et al. 2011; Wong et al. 2010). Several studies, especially in the last decade, have shown that the low-grade inflammatory state present in obesity contributes to the development of metabolic dysregulation (Cildir et al. 2014; Osborn and Olefsky 2012; Seijkens et al. 2014). In the recent years, it has been recognized that different immune cell subpopulations present in metabolic organs are major regulators of the fine balance between metabolic homeostasis and dysfunction.

Under lean conditions, the AT is mainly predominated by T-regulatory cells (Tregs) and cells implicated in type 2 immune responses such as eosinophils, T-helper 2 (Th2) lymphocytes or M2-like macrophages (Kraakman et al. 2014; Lumeng et al. 2007, 2009; Molofsky et al. 2013; Osborn and Olefsky 2012). Additionally, type 2 innate lymphoid cells were recently identified in the lean AT as well (Molofsky et al. 2013). Cells of type 2 immunity produce cytokines such as IL-4 or IL-13, contributing to the maintenance of a balanced anti-inflammatory state in the AT microenvironment (Molofsky et al. 2013; Van Dyken and Locksley 2013; Wu et al. 2011). Moreover, the lean AT, apart from its classical function as energy storage organ, exerts an energy dissipation function through uncoupling protein 1 (UCP1)-mediated thermogenesis. The latter is primarily conferred by a

population of adipocytes with "brown-like" properties existing mainly in the subcutaneous AT depots; such adipocytes are designated "beige" or "brite" (Harms and Seale 2013; Rossato et al. 2014; Wu et al. 2012a). Notably, the brown adipose tissue depots in humans are mainly represented by cell populations with a rather beige-like phenotype and characteristics (Lidell et al. 2013; Virtanen et al. 2009). Recent evidence reveals that the presence of the aforementioned anti-inflammatory and immune-modulatory cell populations not only supports an increased level of adipocyte insulin sensitivity in the lean AT but also retains the beige adipogenesis-related energy dissipation (Brestoff et al. 2014; Lee et al. 2015; Qiu et al. 2014).

In the course of obesity due to excessive nutrient intake, white adipocytes accumulate lipids and become larger; at the same time, energy-dispensing thermogenic beige adipocytes are reduced; thus, in the obese AT, energy storage predominates over energy dissipation (Fromme and Klingenspor 2011; Saito et al. 2009). The enlargement of the adipocytes is accompanied by insufficient vascularization and oxygen supply, thereby leading to oxidative stress and hypoxia-induced inflammation in the AT (Ichiki and Sunagawa 2014). In the obese AT environment, dysfunctional adipocytes as well as cells of the stromal vascular fraction, including endothelial, immune and adipocyte precursors, contribute to a microenvironment that includes a broad spectrum of inflammatory cytokines, also designated as adipokines, including leptin or tumour necrosis factor (TNF) (Kwon and Pessin 2013; Ouchi et al. 2011). Moreover, chemokines, such as monocyte chemoattractant protein-1 (MCP-1), are increasingly present in the obese AT (Kanda et al. 2006). Inflammatory cytokines and chemokines in collaboration with fatty acids and/or products derived from adipocyte cell death promote the accumulation and activation of inflammatory cells in the AT and thereby the development of AT inflammation (Chmelar et al. 2013; Fantuzzi and Faggioni 2000; Kaminski and Randall 2010). Inflammatory cells, such as M1-polarized macrophages and $CD8^+$ T cells, exert detrimental effects on metabolic regulation of the AT and also of other metabolic tissues (Chatzigeorgiou et al. 2012; Nishimura et al. 2009).

Similarly to the AT, a plethora of cell–cell interactions amongst immune cells or between immune cells and parenchymal cells regulate obesity-related liver inflammation and contribute to the development of non-alcoholic steatohepatitis (NASH) (Chatzigeorgiou et al. 2014a; Sutti et al. 2014; Wree et al. 2013). NASH is characterized by exacerbated intrahepatic inflammation, intense steatosis and hepatocellular injury (Chatzigeorgiou et al. 2014a; Ma et al. 2007; Stienstra et al. 2010). Moreover, obesity-related inflammatory processes may also occur in further organs including pancreas and muscle; inflammation may thereby contribute to the occurrence of metabolic dysregulation in pancreas and muscle as well (Eguchi and Manabe 2013; Fink et al. 2014).

Cells of both myeloid and lymphoid lineages are therefore implicated in the changes that happen during the shift from the lean to the obese state. Immune cells

affect positively or negatively the development of insulin resistance and metabolism-related dysfunction (Chmelar et al. 2013; Cildir et al. 2014; Kraakman et al. 2014). In this chapter, we summarize and discuss the quantitative and functional obesity-associated changes of cellular components of the innate and adaptive immunity, and we focus on the role of immune cells in the inflammation-driven impairment of insulin sensitivity and derangement of metabolic homeostasis.

2 Cells of the Innate Immunity

2.1 Cellular Mediators of Type 1 and Type 2 Innate Immunity in Obesity-Related Inflammation

Cells of the innate and adaptive immune systems cooperate in the context of two major types of immune responses, namely, type 1 and type 2 immunity. Although T-helper 1 (Th1) and T-helper 2 (Th2) cells are considered the cellular initiators of type 1 and type 2 immune responses by secreting IFN-γ and IL-4/IL-13, respectively (Wan and Flavell 2009; Zhu et al. 2010), innate immune cells are indispensable players for the propagation of these divergent immune reactions (Annunziato et al. 2014; Pulendran and Artis 2012). In metabolism-related inflammatory responses, M1-like inflammatory macrophages are predominant innate cellular mediators of type 1 immunity, whereas eosinophils and M2-like anti-inflammatory macrophages are major innate cellular players of type 2 immunity (Kraakman et al. 2014; Lumeng et al. 2007; Wu et al. 2011). The predominance of type 1 or type 2 innate immune cells under obese and lean conditions, respectively, has been well established in the recent years (Lee and Lee 2014).

M1 macrophages are central players in low-grade inflammation associated with obesity (Chawla et al. 2011; Kraakman et al. 2014). The number of AT macrophages increases massively from the lean to the obese state. Resident (predominantly M2-polarized) macrophages comprise 5–10% of the stromal vascular fraction in the lean AT (Lumeng et al. 2008; Odegaard et al. 2007), while the macrophage percentage is elevated up to 40% of the stromal vascular fraction in the obese AT (Kraakman et al. 2014; Weisberg et al. 2003). In parallel, a shift towards M1-polarized macrophages is observed in obesity (Lumeng et al. 2007). Subsequently, as major secretors of inflammatory cytokines such as TNF, M1 macrophages can impair insulin signalling in AT or other tissues such as liver by activating pathways such as the IKK and JNK pathways (Arkan et al. 2005; Hirosumi et al. 2002). Inversely, weight loss leads to reduction in the numbers of AT macrophages (De Taeye et al. 2007; Surmi and Hasty 2008). The increase in AT macrophages in the course of obesity has been explained at least in parts by enhanced chemokine-mediated recruitment of monocytes to the AT (Bai and Sun 2015). For instance, the monocyte chemoattractant protein-1 and its receptor (C-C motif receptor type 2, CCR2) have been implicated in monocyte/macrophage recruitment to the obese AT; however, data from different studies assessing these

chemokine system yielded controversial results (Inouye et al. 2007; Kanda et al. 2006). Moreover, the chemokine (C-X-C motif) ligand 3/CX3C chemokine receptor 1 (CX3CL1/CX3CR1) as well as the leukotriene B4/leukotriene B4 receptor (LTB4/BTL1) pathways may control the recruitment of macrophages in the AT (Osborn and Olefsky 2012). Macrophage migration inhibitory factor has been recently implicated in the increased infiltration of macrophages to the obese AT, thereby also promoting insulin resistance (Finucane et al. 2014). In contrast to several studies that highlight the role of adipokines and chemokines in the recruitment of macrophages to the obese AT, only one recent study by Ramkhelawon et al. showed that netrin may function to promote macrophage retention within the obese AT (Ramkhelawon et al. 2014).

The polarization of macrophages into inflammatory or classically activated M1 and anti-inflammatory or alternatively activated M2 cells is of major importance for the regulation of inflammation in metabolic organs (Gordon 2003; Gordon and Martinez 2010; Lumeng et al. 2007; Mantovani et al. 2004; Martinez et al. 2009; Phieler et al. 2013). M1 macrophages secrete inflammatory cytokines such as TNF or IL-12 and can be generated in vitro upon stimulation with LPS or with T-helper cell (Th)1 cytokines, such as interferon (IFN)-γ. In contrast, Th2 cytokines, such as IL-4 and IL-13, stimulate generation and maintenance of M2 macrophages, by activating transcription factors such as the signal transducer and activator of transcription 6 (STAT6), the Krüppel-like factor 4 (KLF4) as well as the peroxisome proliferator-activated receptor g (PPARg) (Kang et al. 2008; Liao et al. 2011; Mantovani et al. 2004; Martinez et al. 2009; Ricciardi-Castagnoli and Granucci 2002; Stein et al. 1992). Under lean conditions, the AT macrophage population predominantly consists of M2-like macrophages that maintain the homeostasis of the tissue by secreting anti-inflammatory cytokines such as IL-10 and expressing molecules such as arginase, CD206 and macrophage galactose-type C-type lectin−1 (MGL1 or CD301) (Lumeng et al. 2007). Additionally, M2-like macrophages, in an IL-4-dependent manner, promote lipolysis in lean white AT, sustain brown AT thermogenesis by secreting catecholamines (Nguyen et al. 2011) and increase beige adipogenesis (Qiu et al. 2014), as will be outlined below.

M2-like macrophages cooperate with eosinophils in the context of type 2 immunity. Eosinophils are granulocytes primarily needed for the defence against parasites; concomitantly, they have a prominent role in the development of allergic reactions (Kita 2011). Eosinophils participate in the initiation of Th2-related responses by producing Th2 cytokines such as IL4 and IL-13 (Spencer and Weller 2010). This feature not only renders them important mediators of the polarization of M2-like macrophages but also contributes to their protective role in obesity-related inflammation and metabolic dysregulation. Indeed, eosinophils are the major cellular source of IL-4 in the AT, and their presence in the AT is indispensable for the maintenance of the alternatively activated M2 macrophages. Consistently, the numbers of eosinophils in the AT decrease in the course of obesity and are positively correlated with the numbers of M2 macrophages (Wu et al. 2011). The reduction in IL-4 levels upon a decrease in eosinophil numbers in AT is linked to worsened insulin sensitivity (Ricardo-Gonzalez et al. 2010). In accordance,

eosinophil-deficient mice exhibited increased body fat, impaired glucose tolerance and insulin resistance accompanied by exacerbated AT inflammation, while helminth-induced hypereosinophilic mice displayed improved metabolic phenotype, as compared to the normoeosinophilic ones (Wu et al. 2011).

Eosinophils in close cooperation with anti-inflammatory M2-like macrophages are crucial for the induction of beige adipogenesis in white AT in the course of cold adaptation. By producing IL-4/IL-13, eosinophils contribute to the alternative activation of AT macrophages that in turn secrete catecholamines inducing browning of white AT (Qiu et al. 2014), a phenomenon also designated as beige adipogenesis. Meteorin-like, a muscle-derived factor secreted upon exercise, stimulates eosinophil-dependent IL-4 expression, further promoting beige thermogenesis (Rao et al. 2014). Interestingly, type 2 innate lymphoid cells (ICL2) that reside in the lean white AT and secrete IL-5 and IL-13 in a IL-33-dependent manner play a homeostatic role in AT metabolism by promoting the presence of eosinophils and M2-like macrophages (Molofsky et al. 2013) and white AT beige thermogenesis (Brestoff et al. 2014; Lee et al. 2015). In these two recent papers, Lee et al. reported that ICL-2 and eosinophil-derived IL-4 promotes beige expansion of the PDGFRα^{+} bipotent adipocyte precursor cells, while Brestoff et al. demonstrated that IL-33-activated ICL2 cells can also promote beige adipogenesis independently of eosinophils by secreting methionine-enkephalin peptides that trigger direct UCP-1 expression in adipocytes (Brestoff et al. 2014; Lee et al. 2015) (Fig. 1).

In contrast, the obese AT features extensive accumulation of M1-like macrophages, often surrounding dead adipocytes in the so-called crown-like structures (Lee and Lee 2014). M1-like cells of the obese AT are defined as F4/80^{+}CD11b^{+}CD11c^{+} or F4/80^{+}CD11c^{+}MGL1^{-} when analysed by flow cytometry (Chatzigeorgiou et al. 2014b; Lumeng et al. 2007; Schenk et al. 2008; Westcott et al. 2009). Interestingly, AT inflammation and insulin resistance were reduced when CD11c^{+} cells were depleted in a mouse model of diet-induced obesity (Patsouris et al. 2008).

Many pathways and molecules have been implicated in the shift of the macrophage population into the proinflammatory M1-like phenotype. Toll-like receptor-4 (TLR-4) both on adipocytes and macrophages can serve as a receptor for saturated free fatty acids leading to inflammatory activation of these cells. Indeed, TLR-4 deficiency ameliorates AT inflammation and insulin resistance by inhibiting classical activation of macrophages (Orr et al. 2012; Shi et al. 2006). Moreover, fetuin-A, a liver-derived carrier protein of free fatty acids, serves as a molecule bridging TLR4 and free fatty acids and thereby promotes inflammatory signalling and insulin resistance in a TLR4-dependent manner (Pal et al. 2012). On the contrary, unsaturated omega-3 fatty acids could activate signalling via the G-protein-coupled receptor, GPR120, provoking anti-inflammatory effects in AT (Ichimura et al. 2012). Wnt5a-mediated, non-canonical Wnt signalling enhances the expression of proinflammatory cytokines, such as IL-6 by macrophages in a JNK-dependent manner, leading to deteriorated insulin signalling in adipocyte (Fuster et al. 2014). Concomitantly, the recently identified adipokine WISP1

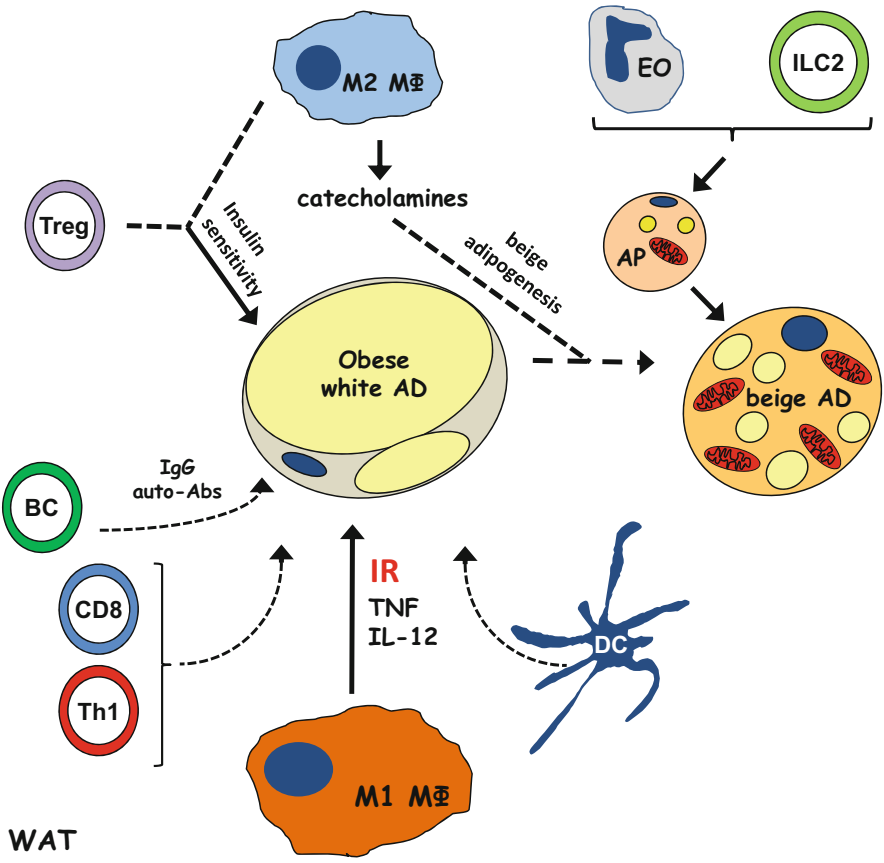

Fig. 1 Obesity-related inflammation in white adipose tissue (WAT): M1 macrophages in the AT play a central role in the exacerbation of obesity-related AT inflammation and dysregulation, by secreting inflammatory cytokines such as TNF, thereby promoting insulin resistance. Proinflammatory lymphocyte subpopulations such as $CD8^+$ or $CD4^+$ Th1 cells promote the activation of macrophages and contribute to insulin resistance. Dendritic cells also contribute to AT inflammation by promoting the accumulation of macrophages. Type 2 immunity cells, such as M2 macrophages and eosinophils, have a protective role against metabolic dysregulation. Eosinophils or ILC2 contribute to beige adipogenesis either directly by stimulating the differentiation of adipocyte progenitor cells into beige adipocytes or by stimulating the secretion of catecholamines by M2 macrophages in a paracrine way, thereby promoting the transformation of white to beige adipocytes. Notably, the production of pathogenic IgG autoantibodies by B cells during obesity likely promotes glucose intolerance. *AD* adipocyte, *AP* platelet-derived growth factor receptor alpha-positive ($PDGFR\alpha^+$) adipocyte progenitor cell, *BC* B cell, *DC* dendritic cell, *EO* eosinophil, *IL* interleukin, *ILC2* type 2 innate lymphoid cells, *IR* insulin resistance, *MΦ* macrophage, *Th* T-helper cell, *TNF* tumour necrosis factor, *Treg* regulatory T cell, *WAT* white adipose tissue

(Wnt1 inducible signalling pathway protein 1) triggers proinflammatory responses in macrophages, promoting thereby AT inflammation (Murahovschi et al. 2014). In addition, antigen MHC II-dependent interaction of macrophages with cells of the adaptive immunity contributes to dysregulation of AT inflammation (Cho et al. 2014; Morris et al. 2013). Surprisingly, the absence of CD40- or CD80/CD86-dependent costimulation, which represents the second signal in antigen-mediated T-cell activation, promotes the exacerbation of AT inflammation and M1-like macrophage accumulation (Chatzigeorgiou et al. 2014a, b; van den Berg et al. 2014; Zhong et al. 2014).

In the liver environment, Kupffer cells, the resident macrophages of the liver, are the primary immune cells regulating liver inflammation, insulin resistance and development of NASH (Lanthier et al. 2010; Stienstra et al. 2010). Depletion of liver Kupffer cells in rats and mice ameliorated obesity-related hepatic steatosis and prevented insulin resistance in a TNF-dependent manner (Huang et al. 2010; Lanthier et al. 2010). The activation of Kupffer cells and the subsequent secretion of TNF are followed by increased production of MCP-1 and IP-10 chemokines, which in turn promote blood-derived monocyte infiltration into the liver, thereby exacerbating liver inflammation (Miura et al. 2012; Tosello-Trampont et al. 2012). Additionally, the secretion of IL-1beta by Kupffer cells suppresses fatty acid oxidation genes such as carnitine palmitoyltransferase 1A (Cpt1a), peroxisome proliferator-activated receptor alpha (PPAR-a) and fatty acid transport protein 2 (Fatp2) in hepatocytes promoting further hepatic steatosis (Stienstra et al. 2010). The accumulation of oxidized LDL in the cytoplasm or lysosomes of Kupffer cells correlates to their shift into a more M1-like proinflammatory phenotype as well as to further propagation of inflammation in the steatotic liver (Bieghs et al. 2013; Leroux et al. 2012).

Similarly to the AT and the liver, beta-cell dysfunction in the pancreatic islets under obese conditions is associated with increased numbers of macrophages in the islets as shown in pancreas sections from patients with type 2 diabetes, db/db mice and Goto-Kakizaki (GK) rats (Ehses et al. 2007). Eguchi et al. (2012) reported that high free fatty acid conditions in vivo and in vitro induce a TLR4-dependent production of chemokines by pancreatic islets, leading to accumulation of M1-like macrophages. Monocyte/macrophage infiltration in the skeletal muscle has also been reported under obese conditions and is linked to insulin resistance, although the underlying molecular mechanisms are still unknown (Fink et al. 2014).

2.2 Other Cells of Innate Immunity

Neutrophil granulocytes are major players in the initial defence against microbial infections. Through an arsenal of antimicrobial peptides and enzymes, such as myeloperoxidase or elastase, as well as by secreting proinflammatory cytokines and chemokines, they promote acute inflammation and the subsequent infiltration of other immune cells (Mantovani et al. 2011). Circulating concentrations of the neutrophil-derived factors calprotectin and myeloperoxidase (MPO) have been

found elevated in obese individuals (Nijhuis et al. 2009; Zuniga et al. 2010), while the neutrophil/lymphocyte ratio seems to be predictive for the development of type 2 diabetes in morbidly obese individuals (Yilmaz et al. 2014). Similarly, hypercholesterolemia, diet-induced obesity and leptin receptor deficiency in mice provoke an increase of circulating neutrophil numbers (Drechsler et al. 2010; Kordonowy et al. 2012).

Elgazar-Carmon et al. (2008) found that myeloperoxidase expression was significantly increased in AT of mice already 3 days after the initiation of a high-fat diet, suggesting that neutrophils may accumulate in the AT very early in the course of obesity. In contrast to the aforementioned study that demonstrated a continuous decline in neutrophil numbers in AT starting at day 7 upon initiation of a high-fat diet (Elgazar-Carmon et al. 2008), Talukdar et al. showed that neutrophils also infiltrated the AT in the first week of a high-fat diet feeding, but their numbers remained constantly elevated after 90 days of feeding. This study also demonstrated that pharmacological or genetic inhibition of elastase decreased neutrophil and macrophage numbers in the AT, accompanied by improved insulin sensitivity. Additionally, under obese conditions, neutrophils infiltrate the liver and through elastase-mediated degradation of IRS-1 provoke insulin resistance (Talukdar et al. 2012). In addition, genetic ablation of myeloperoxidase in mice prevented the development of insulin resistance while increasing brown AT thermogenesis (Wang et al. 2014).

In humans, increased neutrophil numbers have been identified in the portal inflammatory infiltrate of patients with NASH (Gadd et al. 2014). Likewise, studies in humans and mice have shown that myeloperoxidase activity is elevated in steatotic livers, indicating that neutrophils may contribute to development of non-alcoholic steatohepatitis (Liang et al. 2014; Rensen et al. 2009, 2012). Supporting this notion, myeloperoxidase deficiency in mice ameliorated the development of NASH in low-density lipoprotein receptor (LDLR)-deficient mice (Rensen et al. 2012). Furthermore, transgenic expression of human neutrophil peptide-1 (HNP-1) promoted liver fibrosis by inducing hepatic stellate cell proliferation, in a choline-deficient diet-induced model of NASH (Ibusuki et al. 2013).

Dendritic cells link innate and adaptive immunity by performing antigen presentation to CD4 T cells through class II major histocompatibility complex molecules (MHC II), while they have a critical function in the activation of CD8 and natural killer (NK) cells (Hivroz et al. 2012). Accordingly, they may play a major role in the activation and proliferation of several T-cell subsets in the AT environment although current experimental evidence regarding antigens that can drive such a process is rather scarce (Merad and Manz 2009).

Obesity and type 2 diabetes mellitus (T2DM) are accompanied by increased numbers of circulating myeloid-derived dendritic cells in humans (Musilli et al. 2011). Recent studies revealed that dendritic cells accumulate in the AT of both mice and humans and are able to induce Th-17-related responses in vivo and Th-17 differentiation of T cells ex vivo (Bertola et al. 2012; Chen et al. 2014; Stefanovic-Racic et al. 2012). When analysed by flow cytometry, AT dendritic cells were defined in humans as $CD11c^{+}CD1c^{+}$ cells and they correlated positively to

body mass index (BMI), while in mice dendritic cells were defined as $CD11c^{high}F4/80^{low}$ (Bertola et al. 2012). Dendritic cell-deficient mice displayed lower numbers of recruited macrophages and reduced formation of crown-like structures in AT, reduced numbers of liver macrophages as well as improved insulin sensitivity and hepatic steatosis; these findings were associated however with resistance to weight gain (Stefanovic-Racic et al. 2012). Interestingly, targeting dendritic cells as well as AT macrophages by curcumin-containing liposomes improved insulin sensitivity and resulted in increased interleukin-4 and reduced TNF levels (Yekollu et al. 2011). On the other hand, $CD103^+$ dendritic cells are also present in the AT, and their numbers are decreased under obese conditions (Bertola et al. 2012; Jaensson et al. 2008). In addition, obesity diminishes the migration of dendritic cells into lymph nodes as well as their maturation ability, thereby leading to impaired systemic immune responses under obese conditions (O'Shea et al. 2013; Weitman et al. 2013).

Stefanovic-Racic et al. (2012) suggested that dendritic cells contribute to the accumulation of macrophages in the liver under obese conditions and thereby promote NASH. In contrast, another study showed that despite the expansion, maturation and activation of dendritic cells during NASH, dendritic cells might dampen the development of hepatic fibroinflammation by limiting cytokine production and TLR expression in Kupffer cells and neutrophils (Henning et al. 2013). The aforementioned controversies may rely on alterations in the subpopulation of $CD141^+$ dendritic cells that play a regulatory role in the healthy liver, while decrease in the diseased liver (Kelly et al. 2014). Consistently, the magnitude of antigen processing and presentation is diminished in splenic dendritic cells in a murine model of non-alcoholic fatty liver disease (Miyake et al. 2010).

Apart from dendritic cells and neutrophils, mast cells were found to contribute to the development of obesity-related inflammation. Tissue-resident mast cells colonize in barrier tissues, such as the skin and mucosa, serving as gatekeepers against invading microbes and as important mediators of allergic reactions (Gri et al. 2012; Sim et al. 2006). Their granules contain factors such as histamine, serotonin, heparin, lipid mediators (prostaglandins, leukotrienes, produced by phospholipase A2) as well as mast cell-specific enzymes such as chymases, tryptases and carboxypeptidase A (Gri et al. 2012). Additionally, several pro- and anti-inflammatory cytokines, like TNF, IFN-γ, IL-4, IL-6 and IL-10, can be released from mast cells (Gri et al. 2012). Recent studies have implicated mast cells in the development of metabolism-associated inflammation (Altintas et al. 2011a, b, 2012; Liu et al. 2009; Spencer and Weller 2010; Xu and Shi 2012). A chymase inhibitor was able to prevent NASH in hamsters fed a methionine- and choline-deficient diet (Tashiro et al. 2010). Likewise, mast cell-derived metalloproteinase-9 and phospholipase A2 contribute to metabolism-related inflammation by regulating macrophage activation (Iyer et al. 2012; Triggiani et al. 2006; Tsuruda et al. 2008). Mast cells are present in the AT of both mice and humans, as substantiated by tryptase staining, and their numbers increase in the course of obesity (Liu et al. 2009). Additionally, the mast cell factor 15-deoxy-delta12,14-prostaglandin-J2 is able to promote

adipogenesis through activation of PPAR-gamma (Tanaka et al. 2011). Mast cell-deficient mice exhibit reduced preadipocyte to adipocyte transition and thereby accumulation of preadipocytes in obese AT (Ishijima et al. 2014). In addition, mast cells might be responsible for promoting fibrosis in obese AT through mast cell protease 6-mediated production of collagen V and exacerbation of insulin resistance (Hirai et al. 2014). Mast cell-derived factors and enzymes are able to activate macrophages in the AT, via interacting with protease-activated receptors on their surface (Lim et al. 2013). Liu et al. have shown that mast cell-deficient mice exhibit improved insulin sensitivity and glucose tolerance, accompanied by reduced weight gain in diet-induced obesity. This phenotype was seemingly dependent on IL-6 and IFNγ production by mast cells. Mast cell-stabilizing agents such as disodium cromoglycate and ketotifen improved insulin sensitivity and AT inflammation (Liu et al. 2009).

3 Cells of the Adaptive Immunity

3.1 T Lymphocytes

T cells are the major players of adaptive immunity and mature in the thymus. They are divided into cytotoxic $CD8^+$ T cells that recognize MHC I-presented antigens, and $CD4^+$ T cells, that interact with MHC II-presented antigens (Mazza and Malissen 2007; Raphael et al. 2014). $CD4^+$ T cells comprise T-helper (Th) cells and regulatory T cells (Treg cells) (Raphael et al. 2014). T-helper cells include Th1 cells generated upon stimulation with IL-12, producing proinflammatory cytokines such as IFN-γ; Th2 cells, which are triggered by IL-4 and express IL-4, IL-5, IL-10 and IL-13; and Th17 subset that produces IL-17 and is differentiated by a complex interplay between IL-6, TGF-β and IL-23 (Stockinger and Veldhoen 2007). Maturation of Tregs from $CD4^+$ naïve T cells depends on the presence of IL-2 and TGF-beta and STAT5 signalling (Shan et al. 2014; Yoshimura et al. 2012).

3.1.1 $CD8^+$ T Cells

An increase of both $CD8^+$ and $CD4^+$ IFN-γ-producing T cells in the AT of obese subjects has been reported (Duffaut et al. 2009b). Parallel studies by Koenen et al. identified relatively increased $CD8^+$ cell presence in the visceral AT of overweight human subjects, as compared to their subcutaneous AT; in addition, a positive correlation of $CD8^+$ T cells with the levels of caspase-1 activity was observed (Koenen et al. 2011). The contribution of $CD8^+$ cells in human AT inflammation has been implicated by further studies, whereby $CD8^+$ T cells were found elevated not only in AT from obese individuals as compared to AT from lean ones but also in visceral AT depots as compared to the subcutaneous AT (McLaughlin et al. 2014). In liver biopsies from NASH patients, increased presence of $CD8^+$ T cells in portal inflammatory infiltrate analysis was shown, as compared to normal donor biopsies (Gadd et al. 2014), while the circulating numbers of both

IFN-γ-producing $CD4^+$ and $CD8^+$ T cells in patients with NASH were increased as compared to the ones from age-matched healthy controls (Inzaugarat et al. 2011).

In mice, an increased accumulation of $CD8^+$ T cells in the AT of diet-induced or genetically obese mice has been reported; $CD8^+$ T cells could potentially cooperate with macrophages and contribute to obese AT inflammation (Chatzigeorgiou et al. 2012, 2014b; Jiang et al. 2014; Khan et al. 2014a; Rausch et al. 2008; Rocha et al. 2008). $CD8^+$ T-cell numbers are elevated in the gonadal AT of mice fed a HFD, as opposed to $CD4^+$ cells and Tregs, which were decreased (Nishimura et al. 2009; Winer et al. 2009a). Nishimura et al. reported that the infiltration of $CD8^+$ T cells in the AT precedes macrophage infiltration in the course of a HFD feeding and that this subset of T cells facilitates the accumulation and activation of inflammatory $CD11c^+$ macrophages to the obese AT (Nishimura et al. 2009). Pharmacologic or genetic inactivation of $CD8^+$ T cells reduced macrophage infiltration and AT inflammation thereby improving insulin sensitivity in genetically or diet-induced obese mice, whereas adoptive transfer of $CD8^+$ T cells had detrimental actions in obesity-related AT inflammation (Nishimura et al. 2009). In murine models of NASH, oxidative stress in the liver was shown to promote increased numbers of hepatic $CD8^+CD57^+$ cells that further exacerbated intrahepatic inflammation (Seth et al. 2014; Sutti et al. 2014). In parallel, the crosstalk of activated $CD8^+$ T cells with hepatocytes is shown to promote inflammation and liver carcinogenesis in a murine model of choline-deficient high-fat diet-induced NASH (Wolf et al. 2014).

3.1.2 $CD4^+$ T Cells

The $CD4^+$ T-cell compartment comprises two main subpopulations, the T-helper (Th) cells and the T-regulatory cells (Stockinger and Veldhoen 2007). Th cells are an integral component of adaptive immunity and orchestrate several immune processes ranging from the activation of macrophages to activation of cytotoxic T cells. Th1 cells secrete IFN-γ and other proinflammatory cytokines, and Th2 cells produce the anti-inflammatory cytokines IL-4 and IL-10 (Stockinger and Veldhoen 2007; Winer et al. 2009a; Yoshimura et al. 2012), whereas the cardinal cytokine of Th17 cells is IL-17 (Korn et al. 2009).

In adipose tissue, MHC II on macrophages is required for the activation and proliferation of $CD4^+$ T cells and their differentiation into effector and memory cells (Cho et al. 2014). Effector T cells contribute in turn to the exacerbation of adipose tissue inflammation by interacting with macrophages in a MHC II-dependent manner (Cho et al. 2014; Khan et al. 2014a; Morris et al. 2013). IFN-γ, RANTES, TNF and IL-2 were found to be increased in AT lymphocytes from obese humans, as compared to the respective cytokine levels in their peripheral blood lymphocytes (Duffaut et al. 2009b; McLaughlin et al. 2014). In accordance, T cells from obese human and murine AT expressed higher levels of IFN-γ (Duffaut et al. 2009b; Kintscher et al. 2008; O'Rourke et al. 2009; Rocha et al. 2008). IFN-γ-deficient mice showed reduced TNF and CCL2 levels in their AT, fewer immune cell infiltration and improved insulin sensitivity, as compared to wild-type mice (Rocha et al. 2008). Th1, Th17 and $CD8^+$ numbers were elevated

and correlated positively to insulin resistance in visceral and subcutaneous AT of obese individuals as compared to the AT depots of lean ones, whereas the numbers of Th2 cells in their AT depots and blood were associated to insulin sensitivity (McLaughlin et al. 2014). Consistent with the human data, Th1 cell numbers increased in the AT with diet-induced obesity, whereas Th2 numbers remained relatively stable (Winer et al. 2009a), which may be linked to the observation that adipocyte-derived lipids promote the proliferation of Th1 cells (Ioan-Facsinay et al. 2013). Mice deficient in the Th1 cell-determining transcription factor T-bet had improved insulin sensitivity despite their increased visceral adiposity (Stolarczyk et al. 2013). Moreover, data from murine NASH models indicated that Th1 activation of $CD4^+$ T cells is related to the development and severity of steatosis and intrahepatic inflammation (Sutti et al. 2014).

T-regulatory cells (Treg), defined as $CD4^+CD25^+FOXP3^+$ cells, represent a subpopulation of T cells with suppressive function, thereby protecting from autoimmune disorders, such as colitis or rheumatoid arthritis (Piccirillo et al. 2008). They are divided into thymus-derived natural Tregs (nTreg) and peripherally induced Tregs (iTreg) and exert their anti-inflammatory activity by producing IL-10 and TGF-β (Chen et al. 2013; Wing and Sakaguchi 2012). Many studies have reported that Treg numbers in the AT and the liver are either reduced or remain stable under conditions of obesity-associated metabolic dysregulation, whereas the numbers of inflammatory Th1 cells are conversely increased (Chatzigeorgiou et al. 2014a; Cipolletta et al. 2015; Deiuliis et al. 2011; Feuerer et al. 2009; Winer et al. 2009a). Leptin, the levels of which increase in obesity (Myers et al. 2010), promotes the development of Th1 and Th17 subtypes in mice; in contrast, leptin deficiency stimulates the generation and proliferation of Tregs thereby protecting against atherosclerosis and autoimmune diseases (De Rosa et al. 2007; Matarese et al. 2001; Taleb et al. 2007; Yu et al. 2013). In two human studies, decreased expression of Foxp3 in visceral AT of obese subjects and reduction in circulating Treg numbers were demonstrated (Deiuliis et al. 2011; Wagner et al. 2013). However, Zeyda et al. (2011) showed that the number of Tregs in visceral AT of obese individuals were elevated, as compared to lean controls and correlated with BMI or markers of systemic or AT inflammation such as CRP and plasma IL-6. Similarly, $FOXP3^+$ cells were increased in liver biopsies from NASH patients, along with elevated numbers of $CD68^+$ macrophages (Soderberg et al. 2011).

The role of Tregs in metabolic dysregulation and NASH has been extensively studied in the murine system (Chen et al. 2013). In 2009, Feuerer et al. showed that the frequency of AT Tregs was reduced in obesity and that the AT Treg reduction contributed to the pathogenesis of AT inflammation (Chen et al. 2013; Feuerer et al. 2009). Treg depletion, by engaging mice expressing the diphtheria toxin receptor under the control of Foxp3, resulted in perturbation of metabolic function even in mice on a normal diet (Feuerer et al. 2009). Interestingly, AT Tregs have a unique transcript profile when compared to lymphoid organ Tregs that is featured by a different TCR profile and increased expression of PPARγ and PPARγ-related genes (Cipolletta et al. 2012; Feuerer et al. 2009). Accordingly, thiazolidinedione

treatment can improve the metabolic profile of obese mice by elevating Treg numbers in AT, as proven by using mice with a specific deletion of PPARγ in Tregs (Cipolletta et al. 2012). Obese AT Tregs have an altered gene expression profile, as compared to lean AT Tregs, mainly driven by the phosphorylation of PPARγ on the serine residue at position 273 (Cipolletta et al. 2015). Adoptive transfer or targeted activation and proliferation of Tregs improved insulin sensitivity and ameliorated inflammation in the AT of ob/ob mice (Ilan et al. 2010). IL-33 and its receptor ST2 are responsible for AT Treg development and their unique transcriptional signature (Vasanthakumar et al. 2015), while IL-21 is a negative regulator of AT Tregs (Fabrizi et al. 2014).

Tregs are also important in regulating NASH development. Ma et al. have shown that obesity-induced hepatosteatosis is linked to reduction of the hepatic Treg population and upregulation of TNF-related inflammation. Oxidative stress plays a major role in Treg apoptosis and promotes the transition of steatosis to steatohepatitis (Ma et al. 2007). CD80/CD86 double-deficient mice that are characterized by intrinsic developmental reduction in Treg numbers due to the absence of costimulatory signals displayed exacerbation of metabolic dysregulation and NASH that was associated with increased accumulation of macrophages and dendritic cells in the liver (Chatzigeorgiou et al. 2014a).

IL-17 contributes to NASH development (Zhao et al. 2010). A cellular source of IL-17 is Th17 cells, the differentiation of which is driven by the transcription factor ROR-γt (Ivanov et al. 2006). Increased numbers of Th17 cells as well as increased IL-17 levels have been detected in blood and AT from obese and T2DM humans (Cildir et al. 2014; Fabbrini et al. 2013; McLaughlin et al. 2014; Zhu et al. 2011). In the course of obesity, Th17 cell numbers are increased in AT of mice (Fabbrini et al. 2013; Winer et al. 2009b), and their activation is dependent on dendritic cells (Chen et al. 2014). On the other hand, reduced IL-17 levels were found in ob/ob and db/db mice, indicating that the activation of Th17 cells and/or of the IL-17 pathway is likely leptin dependent (Yu et al. 2013).

Intriguingly, Zuniga et al. reported that the presence of IL-17, which in the AT is expressed primarily by γδ T cells, inhibits adipogenesis in mouse 3T3-L1 preadipocytes. Furthermore, IL-17 reduced the uptake of glucose in adipocytes, whereas young IL-17-deficient mice demonstrated improved responses in glucose and insulin tolerance tests. In contrast, IL-17 deficiency gave rise to an increase in weight gain under high-fat feeding conditions (Zuniga et al. 2010). Consistent with these findings, Shin et al. showed that IL-17 inhibits adipogenesis from human mesenchymal stem cells and increases the expression of IL-6 and IL-8 by these cells (Shin et al. 2009). It should be emphasized that the function of IL-17 is not necessarily linked to Th17 cells activation, since additional types of immune cells, including neutrophils and γδ T cells, can also produce IL-17 besides Th17 cells (Eskan et al. 2012; Li et al. 2010; Sutton et al. 2012). Additionally, several cell populations bearing IL-17 receptors could be affected by IL-17, such as adipocytes, endothelial cells or neutrophils (Cua and Tato 2010; Fabbrini et al. 2013; Zuniga et al. 2010). Additionally, in NASH patients, the liver expression of Th17-related genes such as ROR-γt, IL-17, IL-21 and IL-23 is significantly elevated, as

compared to healthy controls (Harley et al. 2014; Tang et al. 2011). In murine models of NASH, IL-17 signalling promotes the development of hepatic steatosis, its transition to steatohepatitis and the progression to fibrosis via activation of Kupffer and hepatic stellate cells (Harley et al. 2014; Meng et al. 2012; Tang et al. 2011). Together, the aforementioned data suggest a complex role of IL-17 and Th17 cells in metabolism-related inflammation and obesity-related systemic complications (Ericksen et al. 2014; Monk et al. 2013).

3.2 Further Lymphocytes

Natural killer T cells (NKT) are a subpopulation of T cells with innate-like features. Via their T-cell receptor, NKT cells interact with non-peptidic, CD1d-related lipid and glycolipid antigens and are capable of producing both Th1 and Th2 cytokines (Borg et al. 2007; Getz et al. 2011; Mallevaey et al. 2011). Wu et al. (2012b) showed that NKT cells are activated under conditions of lipid excess and exacerbate insulin resistance, hepatic steatosis and metabolic dysregulation. HFD-fed NKT-deficient mice (β2-microglobulin knockout mice) display lower macrophage accumulation and better glucose tolerance, while no differences were observed in their body weights, as compared to control mice (Ohmura et al. 2010). However, Mantell et al. demonstrated that these effects of NKT cell deletion on metabolism occur solely in the absence of $CD8^+$ T cells (Mantell et al. 2011). Interestingly, the metabolic dysregulation of ob/ob mice may rather be ameliorated by adoptive transfer or agonistic activation of NKT cells (Elinav et al. 2006; Ilan et al. 2010). In further studies, NKT cells were shown to play a regulatory role under diet-induced obese conditions preventing obesity-related metabolic deterioration (Kondo et al. 2013; Martin-Murphy et al. 2014).

A clear role of NKT cells in the development of NASH has been proposed by numerous studies. First of all, liver and blood NKT cell numbers were found to be significantly elevated in patients with severe steatosis as compared to controls (Adler et al. 2011; Syn et al. 2010), while similar data have been obtained from mouse studies (Syn et al. 2010). CXCR6-dependent NKT cell accumulation to the liver takes place early during the development of NASH (Wehr et al. 2013). NKT-derived cytokines such as IFN-γ, as well as osteopontin and the foetal morphogen, sonic hedgehog, stimulate both the inflammation-driven progression of NASH and fibrosis through activation of hepatic stellate cells (Locatelli et al. 2013; Syn et al. 2012; Tajiri et al. 2009). NKT cell–hepatocyte interactions exacerbated NASH and contributed to the development of hepatocellular carcinoma in mice fed a choline-deficient high-fat diet (Wolf et al. 2014). Conversely, pioglitazone improved steatohepatitis in obese and diabetic KK-A(y) mice through restoration of hepatic NKT cells (Yamagata et al. 2013).

Few studies have reported that B cells infiltrate the AT earlier than T cells or macrophages and before the onset of insulin resistance (Duffaut et al. 2009a; Winer et al. 2011). B cells potently contribute to the induction of insulin resistance, since mice devoid of B cells as a result of anti-CD20 administration displayed lower

fasting glucose and improved glucose and insulin tolerance tests in the course of diet-induced obesity, as compared to control mice (Winer et al. 2011). Similarly, high-fat fed B-cell-deficient mice (IgM heavy chain knockouts) had reduced numbers of $CD11c^+$ macrophages and activated $CD8^+$ T cells, as well as decreased expression of proinflammatory cytokines and IL-17. However, reconstitution of T-cell- and B-cell-lacking Rag1−/− mice with B cells did not exacerbate insulin resistance, indicating that B-cell-mediated induction of metabolic dysregulation requires the presence of T cells (DeFuria et al. 2013; Winer et al. 2011). This hypothesis was further strengthened by experiments with B-cell-deficient mice that were reconstituted with B cells from MHC I- or II-deficient mice, which did not lead to deterioration of insulin resistance (DeFuria et al. 2013; Winer et al. 2011). Additionally, B-cell-activating factor (BAFF) is upregulated in sera and AT of obese mice. BAFF acts in an autocrine or paracrine manner to induce adipokine secretion and insulin resistance in adipocytes (Hamada et al. 2011); BAFF-deficient mice displayed reduced AT inflammation and thereby insulin sensitivity (Kim and Do 2015; Shen et al. 2014). Similarly, increased levels of BAFF were observed in patients with NASH (Kawasaki et al. 2013). Strikingly, obesity provokes the production of pathogenic IgG autoantibodies by B cells, which could promote glucose intolerance. Winer et al. compared the IgG autoantibody profiles between insulin-resistant and matched insulin-sensitive human subjects and thereby identified a relationship between autoantibodies to glial fibrillary acidic protein (GFAP), Golgi SNAP receptor complex member 1 (GOSR1) and Bruton agammaglobulinemia tyrosine kinase (BTK) with insulin resistance (Winer et al. 2014; Winer et al. 2011).

4 Concluding Remarks

Extensive experimental research of the last decade in the field of immunometabolism has implicated different immune cell subpopulations in the development of metabolism-related inflammation. However, several questions have to be addressed in the future. The overwhelming majority of existing data comes from mouse models. Apart from differences between the human and the mouse immune system, mouse strain differences may play a determinant role in the regulation of immune response. In line with this, the function of microbiota as integral players of systemic immunity and thereby of host metabolic profile becomes increasingly recognized (Khan et al. 2014b). Animals in different facilities may differ in their microbiomes, which may therefore cause discrepancies. Moreover, most of the studies have so far focused in AT inflammation. Other organs, such as the liver, the pancreas, the muscle or the brain, can contribute to metabolic dysregulation; the role of immune cells in the metabolic homeostasis of these organs requires intense attention. Additionally, the majority of the studies to date have engaged whole-body knockout mice, which bear several limitations in data interpretation. Undoubtedly, the field of immunometabolism is relatively new, and a lot of research has still

to be conducted so that immune pathways could be therapeutically targeted in metabolic diseases.

References

Adler M, Taylor S, Okebugwu K, Yee H, Fielding C, Fielding G, Poles M (2011) Intrahepatic natural killer T cell populations are increased in human hepatic steatosis. World J Gastroenterol 17:1725–1731

Altintas MM, Azad A, Nayer B, Contreras G, Zaias J, Faul C, Reiser J, Nayer A (2011a) Mast cells, macrophages, and crown-like structures distinguish subcutaneous from visceral fat in mice. J Lipid Res 52:480–488

Altintas MM, Rossetti MA, Nayer B, Puig A, Zagallo P, Ortega LM, Johnson KB, McNamara G, Reiser J, Mendez AJ, Nayer A (2011b) Apoptosis, mastocytosis, and diminished adipocytokine gene expression accompany reduced epididymal fat mass in long-standing diet-induced obese mice. Lipids Health Dis 10:198

Altintas MM, Nayer B, Walford EC, Johnson KB, Gaidosh G, Reiser J, De La Cruz-Munoz N, Ortega LM, Nayer A (2012) Leptin deficiency-induced obesity affects the density of mast cells in abdominal fat depots and lymph nodes in mice. Lipids Health Dis 11:21

Annunziato F, Romagnani C, Romagnani S (2014) The 3 major types of innate and adaptive cell-mediated effector immunity. J Allergy Clin Immunol 135:626–635

Arkan MC, Hevener AL, Greten FR, Maeda S, Li ZW, Long JM, Wynshaw-Boris A, Poli G, Olefsky J, Karin M (2005) IKK-beta links inflammation to obesity-induced insulin resistance. Nat Med 11:191–198

Bai Y, Sun Q (2015) Macrophage recruitment in obese adipose tissue. Obes Rev 16:127–136

Bertola A, Ciucci T, Rousseau D, Bourlier V, Duffaut C, Bonnafous S, Blin-Wakkach C, Anty R, Iannelli A, Gugenheim J, Tran A, Bouloumie A, Gual P, Wakkach A (2012) Identification of adipose tissue dendritic cells correlated with obesity-associated insulin-resistance and inducing Th17 responses in mice and patients. Diabetes 61:2238–2247

Bieghs V, Walenbergh SM, Hendrikx T, van Gorp PJ, Verheyen F, Olde Damink SW, Masclee AA, Koek GH, Hofker MH, Binder CJ, Shiri-Sverdlov R (2013) Trapping of oxidized LDL in lysosomes of Kupffer cells is a trigger for hepatic inflammation. Liver Int 33:1056–1061

Borg NA, Wun KS, Kjer-Nielsen L, Wilce MC, Pellicci DG, Koh R, Besra GS, Bharadwaj M, Godfrey DI, McCluskey J, Rossjohn J (2007) CD1d-lipid-antigen recognition by the semi-invariant NKT T-cell receptor. Nature 448:44–49

Brestoff JR, Kim BS, Saenz SA, Stine RR, Monticelli LA, Sonnenberg GF, Thome JJ, Farber DL, Lutfy K, Seale P, Artis D (2014) Group 2 innate lymphoid cells promote beiging of white adipose tissue and limit obesity. Nature 519:242–246

Chatzigeorgiou A, Karalis KP, Bornstein SR, Chavakis T (2012) Lymphocytes in obesity-related adipose tissue inflammation. Diabetologia 55:2583–2592

Chatzigeorgiou A, Chung KJ, Garcia-Martin R, Alexaki VI, Klotzsche-von Ameln A, Phieler J, Sprott D, Kanczkowski W, Tzanavari T, Bdeir M, Bergmann S, Cartellieri M, Bachmann M, Nikolakopoulou P, Androutsellis-Theotokis A, Siegert G, Bornstein SR, Muders MH, Boon L, Karalis KP, Lutgens E, Chavakis T (2014a) Dual role of B7 costimulation in obesity-related nonalcoholic steatohepatitis and metabolic dysregulation. Hepatology 60:1196–1210

Chatzigeorgiou A, Seijkens T, Zarzycka B, Engel D, Poggi M, van den Berg S, van den Berg S, Soehnlein O, Winkels H, Beckers L, Lievens D, Driessen A, Kusters P, Biessen E, Garcia-Martin R, Klotzsche-von Ameln A, Gijbels M, Noelle R, Boon L, Hackeng T, Schulte KM, Xu A, Vriend G, Nabuurs S, Chung KJ, Willems van Dijk K, Rensen PC, Gerdes N, de Winther M, Block NL, Schally AV, Weber C, Bornstein SR, Nicolaes G, Chavakis T, Lutgens E (2014b) Blocking CD40-TRAF6 signaling is a therapeutic target in obesity-associated insulin resistance. Proc Natl Acad Sci U S A 111:2686–2691

Chawla A, Nguyen KD, Goh YP (2011) Macrophage-mediated inflammation in metabolic disease. Nat Rev Immunol 11:738–749

Chen X, Wu Y, Wang L (2013) Fat-resident Tregs: an emerging guard protecting from obesity-associated metabolic disorders. Obes Rev 14:568–578

Chen Y, Tian J, Tian X, Tang X, Rui K, Tong J, Lu L, Xu H, Wang S (2014) Adipose tissue dendritic cells enhances inflammation by prompting the generation of Th17 cells. PLoS One 9: e92450

Chmelar J, Chung KJ, Chavakis T (2013) The role of innate immune cells in obese adipose tissue inflammation and development of insulin resistance. Thromb Haemost 109:399–406

Cho KW, Morris DL, DelProposto JL, Geletka L, Zamarron B, Martinez-Santibanez G, Meyer KA, Singer K, O'Rourke RW, Lumeng CN (2014) An MHC II-dependent activation loop between adipose tissue macrophages and CD4+ T cells controls obesity-induced inflammation. Cell Rep 9:605–617

Cildir G, Akincilar SC, Tergaonkar V (2014) Chronic adipose tissue inflammation: all immune cells on the stage. Trends Mol Med 19:487–500

Cipolletta D, Feuerer M, Li A, Kamei N, Lee J, Shoelson SE, Benoist C, Mathis D (2012) PPAR-gamma is a major driver of the accumulation and phenotype of adipose tissue Treg cells. Nature 486:549–553

Cipolletta D, Cohen P, Spiegelman BM, Benoist C, Mathis D (2015) Appearance and disappearance of the mRNA signature characteristic of Treg cells in visceral adipose tissue: age, diet, and PPARgamma effects. Proc Natl Acad Sci U S A 112:482–487

Cohen JC, Horton JD, Hobbs HH (2011) Human fatty liver disease: old questions and new insights. Science 332:1519–1523

Cua DJ, Tato CM (2010) Innate IL-17-producing cells: the sentinels of the immune system. Nat Rev Immunol 10:479–489

De Rosa V, Procaccini C, Cali G, Pirozzi G, Fontana S, Zappacosta S, La Cava A, Matarese G (2007) A key role of leptin in the control of regulatory T cell proliferation. Immunity 26:241–255

De Taeye BM, Novitskaya T, McGuinness OP, Gleaves L, Medda M, Covington JW, Vaughan DE (2007) Macrophage TNF-alpha contributes to insulin resistance and hepatic steatosis in diet-induced obesity. Am J Physiol Endocrinol Metab 293:E713–E725

DeFuria J, Belkina AC, Jagannathan-Bogdan M, Snyder-Cappione J, Carr JD, Nersesova YR, Markham D, Strissel KJ, Watkins AA, Zhu M, Allen J, Bouchard J, Toraldo G, Jasuja R, Obin MS, McDonnell ME, Apovian C, Denis GV, Nikolajczyk BS (2013) B cells promote inflammation in obesity and type 2 diabetes through regulation of T-cell function and an inflammatory cytokine profile. Proc Natl Acad Sci U S A 110:5133–5138

Deiuliis J, Shah Z, Shah N, Needleman B, Mikami D, Narula V, Perry K, Hazey J, Kampfrath T, Kollengode M, Sun Q, Satoskar AR, Lumeng C, Moffatt-Bruce S, Rajagopalan S (2011) Visceral adipose inflammation in obesity is associated with critical alterations in t regulatory cell numbers. PLoS One 6:e16376

Drechsler M, Megens RT, van Zandvoort M, Weber C, Soehnlein O (2010) Hyperlipidemia-triggered neutrophilia promotes early atherosclerosis. Circulation 122:1837–1845

Duffaut C, Galitzky J, Lafontan M, Bouloumie A (2009a) Unexpected trafficking of immune cells within the adipose tissue during the onset of obesity. Biochem Biophys Res Commun 384:482–485

Duffaut C, Zakaroff-Girard A, Bourlier V, Decaunes P, Maumus M, Chiotasso P, Sengenes C, Lafontan M, Galitzky J, Bouloumie A (2009b) Interplay between human adipocytes and T lymphocytes in obesity: CCL20 as an adipochemokine and T lymphocytes as lipogenic modulators. Arterioscler Thromb Vasc Biol 29:1608–1614

Eguchi K, Manabe I (2013) Macrophages and islet inflammation in type 2 diabetes. Diabetes Obes Metab 15(Suppl 3):152–158

Eguchi K, Manabe I, Oishi-Tanaka Y, Ohsugi M, Kono N, Ogata F, Yagi N, Ohto U, Kimoto M, Miyake K, Tobe K, Arai H, Kadowaki T, Nagai R (2012) Saturated fatty acid and TLR signaling link beta cell dysfunction and islet inflammation. Cell Metab 15:518–533

Ehses JA, Perren A, Eppler E, Ribaux P, Pospisilik JA, Maor-Cahn R, Gueripel X, Ellingsgaard H, Schneider MK, Biollaz G, Fontana A, Reinecke M, Homo-Delarche F, Donath MY (2007) Increased number of islet-associated macrophages in type 2 diabetes. Diabetes 56:2356–2370

Elgazar-Carmon V, Rudich A, Hadad N, Levy R (2008) Neutrophils transiently infiltrate intra-abdominal fat early in the course of high-fat feeding. J Lipid Res 49:1894–1903

Elinav E, Pappo O, Sklair-Levy M, Margalit M, Shibolet O, Gomori M, Alper R, Thalenfeld B, Engelhardt D, Rabbani E, Ilan Y (2006) Adoptive transfer of regulatory NKT lymphocytes ameliorates non-alcoholic steatohepatitis and glucose intolerance in ob/ob mice and is associated with intrahepatic CD8 trapping. J Pathol 209:121–128

Ericksen RE, Rose S, Westphalen CB, Shibata W, Muthupalani S, Tailor Y, Friedman RA, Han W, Fox JG, Ferrante AW Jr, Wang TC (2014) Obesity accelerates Helicobacter felis-induced gastric carcinogenesis by enhancing immature myeloid cell trafficking and TH17 response. Gut 63:385–394

Eskan MA, Jotwani R, Abe T, Chmelar J, Lim JH, Liang S, Ciero PA, Krauss JL, Li F, Rauner M, Hofbauer LC, Choi EY, Chung KJ, Hashim A, Curtis MA, Chavakis T, Hajishengallis G (2012) The leukocyte integrin antagonist Del-1 inhibits IL-17-mediated inflammatory bone loss. Nat Immunol 13:465–473

Fabbrini E, Cella M, McCartney SA, Fuchs A, Abumrad NA, Pietka TA, Chen Z, Finck BN, Han DH, Magkos F, Conte C, Bradley D, Fraterrigo G, Eagon JC, Patterson BW, Colonna M, Klein S (2013) Association between specific adipose tissue CD4+ T-cell populations and insulin resistance in obese individuals. Gastroenterology 145:366–74.e1-3

Fabrizi M, Marchetti V, Mavilio M, Marino A, Casagrande V, Cavalera M, Moreno-Navarrete JM, Mezza T, Sorice GP, Fiorentino L, Menghini R, Lauro R, Monteleone G, Giaccari A, Fernandez Real JM, Federici M (2014) IL-21 is a major negative regulator of IRF4-dependent lipolysis affecting Tregs in adipose tissue and systemic insulin sensitivity. Diabetes 63:2086–2096

Fantuzzi G, Faggioni R (2000) Leptin in the regulation of immunity, inflammation, and hematopoiesis. J Leukoc Biol 68:437–446

Feuerer M, Herrero L, Cipolletta D, Naaz A, Wong J, Nayer A, Lee J, Goldfine AB, Benoist C, Shoelson S, Mathis D (2009) Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. Nat Med 15:930–939

Fink LN, Costford SR, Lee YS, Jensen TE, Bilan PJ, Oberbach A, Bluher M, Olefsky JM, Sams A, Klip A (2014) Pro-inflammatory macrophages increase in skeletal muscle of high fat-Fed mice and correlate with metabolic risk markers in humans. Obesity (Silver Spring) 22:747–757

Finucane OM, Reynolds CM, McGillicuddy FC, Harford KA, Morrison M, Baugh J, Roche HM (2014) Macrophage migration inhibitory factor deficiency ameliorates high-fat diet induced insulin resistance in mice with reduced adipose inflammation and hepatic steatosis. PLoS One 9:e113369

Fromme T, Klingenspor M (2011) Uncoupling protein 1 expression and high-fat diets. Am J Physiol Regul Integr Comp Physiol 300:R1–R8

Fuster JJ, Zuriaga MA, Thi-Minh Ngo D, Farb MG, Aprahamian T, Yamaguchi TP, Gokce N, Walsh K (2014) Non-canonical Wnt signaling promotes obesity-induced adipose tissue inflammation and metabolic dysfunction independent of adipose tissue expansion. Diabetes 64: 1235–1248

Gadd VL, Skoien R, Powell EE, Fagan KJ, Winterford C, Horsfall L, Irvine K, Clouston AD (2014) The portal inflammatory infiltrate and ductular reaction in human nonalcoholic fatty liver disease. Hepatology 59:1393–1405

Getz GS, Vanderlaan PA, Reardon CA (2011) Natural killer T cells in lipoprotein metabolism and atherosclerosis. Thromb Haemost 106:814–819

Gordon S (2003) Alternative activation of macrophages. Nat Rev Immunol 3:23–35

Gordon S, Martinez FO (2010) Alternative activation of macrophages: mechanism and functions. Immunity 32:593–604

Gri G, Frossi B, D'Inca F, Danelli L, Betto E, Mion F, Sibilano R, Pucillo C (2012) Mast cell: an emerging partner in immune interaction. Front Immunol 3:120

Hamada M, Abe M, Miyake T, Kawasaki K, Tada F, Furukawa S, Matsuura B, Hiasa Y, Onji M (2011) B cell-activating factor controls the production of adipokines and induces insulin resistance. Obesity (Silver Spring) 19:1915–1922

Harley IT, Stankiewicz TE, Giles DA, Softic S, Flick LM, Cappelletti M, Sheridan R, Xanthakos SA, Steinbrecher KA, Sartor RB, Kohli R, Karp CL, Divanovic S (2014) IL-17 signaling accelerates the progression of nonalcoholic fatty liver disease in mice. Hepatology 59:1830–1839

Harms M, Seale P (2013) Brown and beige fat: development, function and therapeutic potential. Nat Med 19:1252–1263

Henning JR, Graffeo CS, Rehman A, Fallon NC, Zambirinis CP, Ochi A, Barilla R, Jamal M, Deutsch M, Greco S, Ego-Osuala M, Bin-Saeed U, Rao RS, Badar S, Quesada JP, Acehan D, Miller G (2013) Dendritic cells limit fibroinflammatory injury in nonalcoholic steatohepatitis in mice. Hepatology 58:589–602

Hirai S, Ohyane C, Kim YI, Lin S, Goto T, Takahashi N, Kim CS, Kang J, Yu R, Kawada T (2014) Involvement of mast cells in adipose tissue fibrosis. Am J Physiol Endocrinol Metab 306: E247–E255

Hirosumi J, Tuncman G, Chang L, Gorgun CZ, Uysal KT, Maeda K, Karin M, Hotamisligil GS (2002) A central role for JNK in obesity and insulin resistance. Nature 420:333–336

Hivroz C, Chemin K, Tourret M, Bohineust A (2012) Crosstalk between T lymphocytes and dendritic cells. Crit Rev Immunol 32:139–155

Huang W, Metlakunta A, Dedousis N, Zhang P, Sipula I, Dube JJ, Scott DK, O'Doherty RM (2010) Depletion of liver Kupffer cells prevents the development of diet-induced hepatic steatosis and insulin resistance. Diabetes 59:347–357

Ibusuki R, Uto H, Arima S, Mawatari S, Setoguchi Y, Iwashita Y, Hashimoto S, Maeda T, Tanoue S, Kanmura S, Oketani M, Ido A, Tsubouchi H (2013) Transgenic expression of human neutrophil peptide-1 enhances hepatic fibrosis in mice fed a choline-deficient, L-amino acid-defined diet. Liver Int 33:1549–1556

Ichiki T, Sunagawa K (2014) Novel roles of hypoxia response system in glucose metabolism and obesity. Trends Cardiovasc Med 24:197–201

Ichimura A, Hirasawa A, Poulain-Godefroy O, Bonnefond A, Hara T, Yengo L, Kimura I, Leloire A, Liu N, Iida K, Choquet H, Besnard P, Lecoeur C, Vivequin S, Ayukawa K, Takeuchi M, Ozawa K, Tauber M, Maffeis C, Morandi A, Buzzetti R, Elliott P, Pouta A, Jarvelin MR, Korner A, Kiess W, Pigeyre M, Caiazzo R, Van Hul W, Van Gaal L, Horber F, Balkau B, Levy-Marchal C, Rouskas K, Kouvatsi A, Hebebrand J, Hinney A, Scherag A, Pattou F, Meyre D, Koshimizu TA, Wolowczuk I, Tsujimoto G, Froguel P (2012) Dysfunction of lipid sensor GPR120 leads to obesity in both mouse and human. Nature 483:350–354

Ilan Y, Maron R, Tukpah AM, Maioli TU, Murugaiyan G, Yang K, Wu HY, Weiner HL (2010) Induction of regulatory T cells decreases adipose inflammation and alleviates insulin resistance in ob/ob mice. Proc Natl Acad Sci U S A 107:9765–9770

Inouye KE, Shi H, Howard JK, Daly CH, Lord GM, Rollins BJ, Flier JS (2007) Absence of CC chemokine ligand 2 does not limit obesity-associated infiltration of macrophages into adipose tissue. Diabetes 56:2242–2250

Inzaugarat ME, Ferreyra Solari NE, Billordo LA, Abecasis R, Gadano AC, Chernavsky AC (2011) Altered phenotype and functionality of circulating immune cells characterize adult patients with nonalcoholic steatohepatitis. J Clin Immunol 31:1120–1130

Ioan-Facsinay A, Kwekkeboom JC, Westhoff S, Giera M, Rombouts Y, van Harmelen V, Huizinga TW, Deelder A, Kloppenburg M, Toes RE (2013) Adipocyte-derived lipids modulate CD4+ T-cell function. Eur J Immunol 43:1578–1587

Ishijima Y, Ohmori S, Ohneda K (2014) Mast cell deficiency results in the accumulation of preadipocytes in adipose tissue in both obese and non-obese mice. FEBS Open Bio 4:18–24
Ivanov II, McKenzie BS, Zhou L, Tadokoro CE, Lepelley A, Lafaille JJ, Cua DJ, Littman DR (2006) The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells. Cell 126:1121–1133
Iyer A, Lim J, Poudyal H, Reid RC, Suen JY, Webster J, Prins JB, Whitehead JP, Fairlie DP, Brown L (2012) An inhibitor of phospholipase A2 group IIA modulates adipocyte signaling and protects against diet-induced metabolic syndrome in rats. Diabetes 61:2320–2329
Jaensson E, Uronen-Hansson H, Pabst O, Eksteen B, Tian J, Coombes JL, Berg PL, Davidsson T, Powrie F, Johansson-Lindbom B, Agace WW (2008) Small intestinal CD103+ dendritic cells display unique functional properties that are conserved between mice and humans. J Exp Med 205:2139–2149
Jiang E, Perrard XD, Yang D, Khan IM, Perrard JL, Smith CW, Ballantyne CM, Wu H (2014) Essential role of CD11a in CD8+ T-cell accumulation and activation in adipose tissue. Arterioscler Thromb Vasc Biol 34:34–43
Kahn SE, Hull RL, Utzschneider KM (2006) Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature 444:840–846
Kaminski DA, Randall TD (2010) Adaptive immunity and adipose tissue biology. Trends Immunol 31:384–390
Kanda H, Tateya S, Tamori Y, Kotani K, Hiasa K, Kitazawa R, Kitazawa S, Miyachi H, Maeda S, Egashira K, Kasuga M (2006) MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. J Clin Invest 116:1494–1505
Kang K, Reilly SM, Karabacak V, Gangl MR, Fitzgerald K, Hatano B, Lee CH (2008) Adipocyte-derived Th2 cytokines and myeloid PPARdelta regulate macrophage polarization and insulin sensitivity. Cell Metab 7:485–495
Kawasaki K, Abe M, Tada F, Tokumoto Y, Chen S, Miyake T, Furukawa S, Matsuura B, Hiasa Y, Onji M (2013) Blockade of B-cell-activating factor signaling enhances hepatic steatosis induced by a high-fat diet and improves insulin sensitivity. Lab Invest 93:311–321
Kelly A, Fahey R, Fletcher JM, Keogh C, Carroll AG, Siddachari R, Geoghegan J, Hegarty JE, Ryan EJ, O'Farrelly C (2014) CD141(+) myeloid dendritic cells are enriched in healthy human liver. J Hepatol 60:135–142
Khan IM, Dai Perrard XY, Perrard JL, Mansoori A, Smith CW, Wu H, Ballantyne CM (2014a) Attenuated adipose tissue and skeletal muscle inflammation in obese mice with combined CD4 + and CD8+ T cell deficiency. Atherosclerosis 233:419–428
Khan MT, Nieuwdorp M, Backhed F (2014b) Microbial modulation of insulin sensitivity. Cell Metab 20:753–760
Kim DH, Do MS (2015) BAFF knockout improves systemic inflammation via regulating adipose tissue distribution in high-fat diet-induced obesity. Exp Mol Med 47:e129
Kintscher U, Hartge M, Hess K, Foryst-Ludwig A, Clemenz M, Wabitsch M, Fischer-Posovszky P, Barth TF, Dragun D, Skurk T, Hauner H, Bluher M, Unger T, Wolf AM, Knippschild U, Hombach V, Marx N (2008) T-lymphocyte infiltration in visceral adipose tissue: a primary event in adipose tissue inflammation and the development of obesity-mediated insulin resistance. Arterioscler Thromb Vasc Biol 28:1304–1310
Kita H (2011) Eosinophils: multifaceted biological properties and roles in health and disease. Immunol Rev 242:161–177
Koenen TB, Stienstra R, van Tits LJ, Joosten LA, van Velzen JF, Hijmans A, Pol JA, van der Vliet JA, Netea MG, Tack CJ, Stalenhoef AF, de Graaf J (2011) The inflammasome and caspase-1 activation: a new mechanism underlying increased inflammatory activity in human visceral adipose tissue. Endocrinology 152:3769–3778
Kondo T, Toyoshima Y, Ishii Y, Kyuwa S (2013) Natural killer T cells in adipose tissue are activated in lean mice. Exp Anim 62:319–328
Kordonowy LL, Burg E, Lenox CC, Gauthier LM, Petty JM, Antkowiak M, Palvinskaya T, Ubags N, Rincon M, Dixon AE, Vernooy JH, Fessler MB, Poynter ME, Suratt BT (2012)

Obesity is associated with neutrophil dysfunction and attenuation of murine acute lung injury. Am J Respir Cell Mol Biol 47:120–127
Korn T, Bettelli E, Oukka M, Kuchroo VK (2009) IL-17 and Th17 cells. Annu Rev Immunol 27:485–517
Kraakman MJ, Murphy AJ, Jandeleit-Dahm K, Kammoun HL (2014) Macrophage polarization in obesity and type 2 diabetes: weighing down our understanding of macrophage function? Front Immunol 5:470
Kwon H, Pessin JE (2013) Adipokines mediate inflammation and insulin resistance. Front Endocrinol (Lausanne) 4:71
Lanthier N, Molendi-Coste O, Horsmans Y, van Rooijen N, Cani PD, Leclercq IA (2010) Kupffer cell activation is a causal factor for hepatic insulin resistance. Am J Physiol Gastrointest Liver Physiol 298:G107–G116
Lee BC, Lee J (2014) Cellular and molecular players in adipose tissue inflammation in the development of obesity-induced insulin resistance. Biochim Biophys Acta 1842:446–462
Lee MW, Odegaard JI, Mukundan L, Qiu Y, Molofsky AB, Nussbaum JC, Yun K, Locksley RM, Chawla A (2015) Activated type 2 innate lymphoid cells regulate beige fat biogenesis. Cell 160:74–87
Leroux A, Ferrere G, Godie V, Cailleux F, Renoud ML, Gaudin F, Naveau S, Prevot S, Makhzami S, Perlemuter G, Cassard-Doulcier AM (2012) Toxic lipids stored by Kupffer cells correlates with their pro-inflammatory phenotype at an early stage of steatohepatitis. J Hepatol 57:141–149
Li L, Huang L, Vergis AL, Ye H, Bajwa A, Narayan V, Strieter RM, Rosin DL, Okusa MD (2010) IL-17 produced by neutrophils regulates IFN-gamma-mediated neutrophil migration in mouse kidney ischemia-reperfusion injury. J Clin Invest 120:331–342
Liang W, Lindeman JH, Menke AL, Koonen DP, Morrison M, Havekes LM, van den Hoek AM, Kleemann R (2014) Metabolically induced liver inflammation leads to NASH and differs from LPS- or IL-1beta-induced chronic inflammation. Lab Invest 94:491–502
Liao X, Sharma N, Kapadia F, Zhou G, Lu Y, Hong H, Paruchuri K, Mahabeleshwar GH, Dalmas E, Venteclef N, Flask CA, Kim J, Doreian BW, Lu KQ, Kaestner KH, Hamik A, Clement K, Jain MK (2011) Kruppel-like factor 4 regulates macrophage polarization. J Clin Invest 121:2736–2749
Lidell ME, Betz MJ, Dahlqvist Leinhard O, Heglind M, Elander L, Slawik M, Mussack T, Nilsson D, Romu T, Nuutila P, Virtanen KA, Beuschlein F, Persson A, Borga M, Enerback S (2013) Evidence for two types of brown adipose tissue in humans. Nat Med 19:631–634
Lim J, Iyer A, Liu L, Suen JY, Lohman RJ, Seow V, Yau MK, Brown L, Fairlie DP (2013) Diet-induced obesity, adipose inflammation, and metabolic dysfunction correlating with PAR2 expression are attenuated by PAR2 antagonism. FASEB J 27:4757–4767
Liu J, Divoux A, Sun J, Zhang J, Clement K, Glickman JN, Sukhova GK, Wolters PJ, Du J, Gorgun CZ, Doria A, Libby P, Blumberg RS, Kahn BB, Hotamisligil GS, Shi GP (2009) Genetic deficiency and pharmacological stabilization of mast cells reduce diet-induced obesity and diabetes in mice. Nat Med 15:940–945
Locatelli I, Sutti S, Vacchiano M, Bozzola C, Albano E (2013) NF-kappaB1 deficiency stimulates the progression of non-alcoholic steatohepatitis (NASH) in mice by promoting NKT-cell-mediated responses. Clin Sci (Lond) 124:279–287
Lumeng CN, Bodzin JL, Saltiel AR (2007) Obesity induces a phenotypic switch in adipose tissue macrophage polarization. J Clin Invest 117:175–184
Lumeng CN, DelProposto JB, Westcott DJ, Saltiel AR (2008) Phenotypic switching of adipose tissue macrophages with obesity is generated by spatiotemporal differences in macrophage subtypes. Diabetes 57:3239–3246
Lumeng CN, Maillard I, Saltiel AR (2009) T-ing up inflammation in fat. Nat Med 15:846–847
Ma X, Hua J, Mohamood AR, Hamad AR, Ravi R, Li Z (2007) A high-fat diet and regulatory T cells influence susceptibility to endotoxin-induced liver injury. Hepatology 46:1519–1529

Mallevaey T, Clarke AJ, Scott-Browne JP, Young MH, Roisman LC, Pellicci DG, Patel O, Vivian JP, Matsuda JL, McCluskey J, Godfrey DI, Marrack P, Rossjohn J, Gapin L (2011) A molecular basis for NKT cell recognition of CD1d-self-antigen. Immunity 34:315–326

Mantell BS, Stefanovic-Racic M, Yang X, Dedousis N, Sipula IJ, O'Doherty RM (2011) Mice lacking NKT cells but with a complete complement of CD8+ T-cells are not protected against the metabolic abnormalities of diet-induced obesity. PLoS One 6:e19831

Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M (2004) The chemokine system in diverse forms of macrophage activation and polarization. Trends Immunol 25:677–686

Mantovani A, Cassatella MA, Costantini C, Jaillon S (2011) Neutrophils in the activation and regulation of innate and adaptive immunity. Nat Rev Immunol 11:519–531

Martinez FO, Helming L, Gordon S (2009) Alternative activation of macrophages: an immunologic functional perspective. Annu Rev Immunol 27:451–483

Martin-Murphy BV, You Q, Wang H, De La Houssaye BA, Reilly TP, Friedman JE, Ju C (2014) Mice lacking natural killer T cells are more susceptible to metabolic alterations following high fat diet feeding. PLoS One 9:e80949

Matarese G, Di Giacomo A, Sanna V, Lord GM, Howard JK, Di Tuoro A, Bloom SR, Lechler RI, Zappacosta S, Fontana S (2001) Requirement for leptin in the induction and progression of autoimmune encephalomyelitis. J Immunol 166:5909–5916

Mazza C, Malissen B (2007) What guides MHC-restricted TCR recognition? Semin Immunol 19:225–235

McLaughlin T, Liu LF, Lamendola C, Shen L, Morton J, Rivas H, Winer D, Tolentino L, Choi O, Zhang H, Hui Yen Chng M, Engleman E (2014) T-cell profile in adipose tissue is associated with insulin resistance and systemic inflammation in humans. Arterioscler Thromb Vasc Biol 34:2637–2643

Meng F, Wang K, Aoyama T, Grivennikov SI, Paik Y, Scholten D, Cong M, Iwaisako K, Liu X, Zhang M, Osterreicher CH, Stickel F, Ley K, Brenner DA, Kisseleva T (2012) Interleukin-17 signaling in inflammatory, Kupffer cells, and hepatic stellate cells exacerbates liver fibrosis in mice. Gastroenterology 143:765–76.e1-3

Merad M, Manz MG (2009) Dendritic cell homeostasis. Blood 113:3418–3427

Miura K, Yang L, van Rooijen N, Ohnishi H, Seki E (2012) Hepatic recruitment of macrophages promotes nonalcoholic steatohepatitis through CCR2. Am J Physiol Gastrointest Liver Physiol 302:G1310–G1321

Miyake T, Akbar SM, Yoshida O, Chen S, Hiasa Y, Matsuura B, Abe M, Onji M (2010) Impaired dendritic cell functions disrupt antigen-specific adaptive immune responses in mice with nonalcoholic fatty liver disease. J Gastroenterol 45:859–867

Molofsky AB, Nussbaum JC, Liang HE, Van Dyken SJ, Cheng LE, Mohapatra A, Chawla A, Locksley RM (2013) Innate lymphoid type 2 cells sustain visceral adipose tissue eosinophils and alternatively activated macrophages. J Exp Med 210:535–549

Monk JM, Hou TY, Turk HF, Weeks B, Wu C, McMurray DN, Chapkin RS (2013) Dietary n-3 polyunsaturated fatty acids (PUFA) decrease obesity-associated Th17 cell-mediated inflammation during colitis. PLoS One 7:e49739

Morris DL, Cho KW, Delproposto JL, Oatmen KE, Geletka LM, Martinez-Santibanez G, Singer K, Lumeng CN (2013) Adipose tissue macrophages function as antigen-presenting cells and regulate adipose tissue CD4+ T cells in mice. Diabetes 62:2762–2772

Murahovschi V, Pivovarova O, Ilkavets I, Dmitrieva RM, Docke S, Keyhani-Nejad F, Gogebakan O, Osterhoff M, Kemper M, Hornemann S, Markova M, Kloting N, Stockmann M, Weickert MO, Lamounier-Zepter V, Neuhaus P, Konradi A, Dooley S, von Loeffelholz C, Bluher M, Pfeiffer AF, Rudovich N (2014) WISP1 is a novel adipokine linked to inflammation in obesity. Diabetes 64:856–866

Musilli C, Paccosi S, Pala L, Gerlini G, Ledda F, Mugelli A, Rotella CM, Parenti A (2011) Characterization of circulating and monocyte-derived dendritic cells in obese and diabetic patients. Mol Immunol 49:234–238

Myers MG Jr, Leibel RL, Seeley RJ, Schwartz MW (2010) Obesity and leptin resistance: distinguishing cause from effect. Trends Endocrinol Metab 21:643–651

Nguyen KD, Qiu Y, Cui X, Goh YP, Mwangi J, David T, Mukundan L, Brombacher F, Locksley RM, Chawla A (2011) Alternatively activated macrophages produce catecholamines to sustain adaptive thermogenesis. Nature 480:104–108

Nijhuis J, Rensen SS, Slaats Y, van Dielen FM, Buurman WA, Greve JW (2009) Neutrophil activation in morbid obesity, chronic activation of acute inflammation. Obesity (Silver Spring) 17:2014–2018

Nishimura S, Manabe I, Nagasaki M, Eto K, Yamashita H, Ohsugi M, Otsu M, Hara K, Ueki K, Sugiura S, Yoshimura K, Kadowaki T, Nagai R (2009) CD8+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. Nat Med 15:914–920

O'Rourke RW, Metcalf MD, White AE, Madala A, Winters BR, Maizlin II, Jobe BA, Roberts CT Jr, Slifka MK, Marks DL (2009) Depot-specific differences in inflammatory mediators and a role for NK cells and IFN-gamma in inflammation in human adipose tissue. Int J Obes (Lond) 33:978–990

O'Shea D, Corrigan M, Dunne MR, Jackson R, Woods C, Gaoatswe G, Moynagh PN, O'Connell J, Hogan AE (2013) Changes in human dendritic cell number and function in severe obesity may contribute to increased susceptibility to viral infection. Int J Obes (Lond) 37:1510–1513

Odegaard JI, Ricardo-Gonzalez RR, Goforth MH, Morel CR, Subramanian V, Mukundan L, Red Eagle A, Vats D, Brombacher F, Ferrante AW, Chawla A (2007) Macrophage-specific PPARgamma controls alternative activation and improves insulin resistance. Nature 447:1116–1120

Ohmura K, Ishimori N, Ohmura Y, Tokuhara S, Nozawa A, Horii S, Andoh Y, Fujii S, Iwabuchi K, Onoe K, Tsutsui H (2010) Natural killer T cells are involved in adipose tissues inflammation and glucose intolerance in diet-induced obese mice. Arterioscler Thromb Vasc Biol 30:193–199

Orr JS, Puglisi MJ, Ellacott KL, Lumeng CN, Wasserman DH, Hasty AH (2012) Toll-like receptor 4 deficiency promotes the alternative activation of adipose tissue macrophages. Diabetes 61:2718–2727

Osborn O, Olefsky JM (2012) The cellular and signaling networks linking the immune system and metabolism in disease. Nat Med 18:363–374

Ouchi N, Parker JL, Lugus JJ, Walsh K (2011) Adipokines in inflammation and metabolic disease. Nat Rev Immunol 11:85–97

Pal D, Dasgupta S, Kundu R, Maitra S, Das G, Mukhopadhyay S, Ray S, Majumdar SS, Bhattacharya S (2012) Fetuin-A acts as an endogenous ligand of TLR4 to promote lipid-induced insulin resistance. Nat Med 18:1279–1285

Patsouris D, Li PP, Thapar D, Chapman J, Olefsky JM, Neels JG (2008) Ablation of CD11c-positive cells normalizes insulin sensitivity in obese insulin resistant animals. Cell Metab 8:301–309

Phieler J, Chung KJ, Chatzigeorgiou A, Klotzsche-von Ameln A, Garcia-Martin R, Sprott D, Moisidou M, Tzanavari T, Ludwig B, Baraban E, Ehrhart-Bornstein M, Bornstein SR, Mziaut H, Solimena M, Karalis KP, Economopoulou M, Lambris JD, Chavakis T (2013) The complement anaphylatoxin C5a receptor contributes to obese adipose tissue inflammation and insulin resistance. J Immunol 191:4367–4374

Piccirillo CA, d'Hennezel E, Sgouroudis E, Yurchenko E (2008) CD4+Foxp3+ regulatory T cells in the control of autoimmunity: in vivo veritas. Curr Opin Immunol 20:655–662

Pulendran B, Artis D (2012) New paradigms in type 2 immunity. Science 337:431–435

Qiu Y, Nguyen KD, Odegaard JI, Cui X, Tian X, Locksley RM, Palmiter RD, Chawla A (2014) Eosinophils and type 2 cytokine signaling in macrophages orchestrate development of functional beige fat. Cell 157:1292–1308

Ramkhelawon B, Hennessy EJ, Menager M, Ray TD, Sheedy FJ, Hutchison S, Wanschel A, Oldebeken S, Geoffrion M, Spiro W, Miller G, McPherson R, Rayner KJ, Moore KJ (2014)

Netrin-1 promotes adipose tissue macrophage retention and insulin resistance in obesity. Nat Med 20:377–384
Rao RR, Long JZ, White JP, Svensson KJ, Lou J, Lokurkar I, Jedrychowski MP, Ruas JL, Wrann CD, Lo JC, Camera DM, Lachey J, Gygi S, Seehra J, Hawley JA, Spiegelman BM (2014) Meteorin-like is a hormone that regulates immune-adipose interactions to increase beige fat thermogenesis. Cell 157:1279–1291
Raphael I, Nalawade S, Eagar TN, Forsthuber TG (2014) T cell subsets and their signature cytokines in autoimmune and inflammatory diseases. Cytokine doi: 10.1016/j.cyto.2014.09.011 (Epub ahead of print)
Rausch ME, Weisberg S, Vardhana P, Tortoriello DV (2008) Obesity in C57BL/6J mice is characterized by adipose tissue hypoxia and cytotoxic T-cell infiltration. Int J Obes (Lond) 32:451–463
Rensen SS, Slaats Y, Nijhuis J, Jans A, Bieghs V, Driessen A, Malle E, Greve JW, Buurman WA (2009) Increased hepatic myeloperoxidase activity in obese subjects with nonalcoholic steatohepatitis. Am J Pathol 175:1473–1482
Rensen SS, Bieghs V, Xanthoulea S, Arfianti E, Bakker JA, Shiri-Sverdlov R, Hofker MH, Greve JW, Buurman WA (2012) Neutrophil-derived myeloperoxidase aggravates non-alcoholic steatohepatitis in low-density lipoprotein receptor-deficient mice. PLoS One 7:e52411
Ricardo-Gonzalez RR, Red Eagle A, Odegaard JI, Jouihan H, Morel CR, Heredia JE, Mukundan L, Wu D, Locksley RM, Chawla A (2010) IL-4/STAT6 immune axis regulates peripheral nutrient metabolism and insulin sensitivity. Proc Natl Acad Sci U S A 107:22617–22622
Ricciardi-Castagnoli P, Granucci F (2002) Opinion: interpretation of the complexity of innate immune responses by functional genomics. Nat Rev Immunol 2:881–889
Rocha VZ, Folco EJ, Sukhova G, Shimizu K, Gotsman I, Vernon AH, Libby P (2008) Interferon-gamma, a Th1 cytokine, regulates fat inflammation: a role for adaptive immunity in obesity. Circ Res 103:467–476
Rossato M, Granzotto M, Macchi V, Porzionato A, Petrelli L, Calcagno A, Vencato J, De Stefani D, Silvestrin V, Rizzuto R, Bassetto F, De Caro R, Vettor R (2014) Human white adipocytes express the cold receptor TRPM8 which activation induces UCP1 expression, mitochondrial activation and heat production. Mol Cell Endocrinol 383:137–146
Saito M, Okamatsu-Ogura Y, Matsushita M, Watanabe K, Yoneshiro T, Nio-Kobayashi J, Iwanaga T, Miyagawa M, Kameya T, Nakada K, Kawai Y, Tsujisaki M (2009) High incidence of metabolically active brown adipose tissue in healthy adult humans: effects of cold exposure and adiposity. Diabetes 58:1526–1531
Schenk S, Saberi M, Olefsky JM (2008) Insulin sensitivity: modulation by nutrients and inflammation. J Clin Invest 118:2992–3002
Seijkens T, Kusters P, Chatzigeorgiou A, Chavakis T, Lutgens E (2014) Immune cell crosstalk in obesity: a key role for costimulation? Diabetes 63:3982–3991
Seth RK, Das S, Kumar A, Chanda A, Kadiiska MB, Michelotti G, Manautou J, Diehl AM, Chatterjee S (2014) CYP2E1-dependent and leptin-mediated hepatic CD57 expression on CD8 + T cells aid progression of environment-linked nonalcoholic steatohepatitis. Toxicol Appl Pharmacol 274:42–54
Shan J, Feng L, Sun G, Chen P, Zhou Y, Xia M, Li H, Li Y (2014) Interplay between mTOR and STAT5 signaling modulates the balance between regulatory and effective T cells. Immunobiology 220:510–517
Shen L, Chng MH, Alonso MN, Yuan R, Winer DA, Engleman EG (2014) B-1a lymphocytes attenuate insulin resistance. Diabetes 64:593–603
Shi H, Kokoeva MV, Inouye K, Tzameli I, Yin H, Flier JS (2006) TLR4 links innate immunity and fatty acid-induced insulin resistance. J Clin Invest 116:3015–3025
Shin JH, Shin DW, Noh M (2009) Interleukin-17A inhibits adipocyte differentiation in human mesenchymal stem cells and regulates pro-inflammatory responses in adipocytes. Biochem Pharmacol 77:1835–1844

Sim AT, Ludowyke RI, Verrills NM (2006) Mast cell function: regulation of degranulation by serine/threonine phosphatases. Pharmacol Ther 112:425–439

Soderberg C, Marmur J, Eckes K, Glaumann H, Sallberg M, Frelin L, Rosenberg P, Stal P, Hultcrantz R (2011) Microvesicular fat, inter cellular adhesion molecule-1 and regulatory T-lymphocytes are of importance for the inflammatory process in livers with non-alcoholic steatohepatitis. APMIS 119:412–420

Spencer LA, Weller PF (2010) Eosinophils and Th2 immunity: contemporary insights. Immunol Cell Biol 88:250–256

Stefanovic-Racic M, Yang X, Turner MS, Mantell BS, Stolz DB, Sumpter TL, Sipula IJ, Dedousis N, Scott DK, Morel PA, Thomson AW, O'Doherty RM (2012) Dendritic cells promote macrophage infiltration and comprise a substantial proportion of obesity-associated increases in CD11c+ cells in adipose tissue and liver. Diabetes 61:2330–2339

Stein M, Keshav S, Harris N, Gordon S (1992) Interleukin 4 potently enhances murine macrophage mannose receptor activity: a marker of alternative immunologic macrophage activation. J Exp Med 176:287–292

Stienstra R, Saudale F, Duval C, Keshtkar S, Groener JE, van Rooijen N, Staels B, Kersten S, Muller M (2010) Kupffer cells promote hepatic steatosis via interleukin-1beta-dependent suppression of peroxisome proliferator-activated receptor alpha activity. Hepatology 51:511–522

Stockinger B, Veldhoen M (2007) Differentiation and function of Th17 T cells. Curr Opin Immunol 19:281–286

Stolarczyk E, Vong CT, Perucha E, Jackson I, Cawthorne MA, Wargent ET, Powell N, Canavan JB, Lord GM, Howard JK (2013) Improved insulin sensitivity despite increased visceral adiposity in mice deficient for the immune cell transcription factor T-bet. Cell Metab 17:520–533

Subramanian S, Chait A (2012) Hypertriglyceridemia secondary to obesity and diabetes. Biochim Biophys Acta 1821:819–825

Surmi BK, Hasty AH (2008) Macrophage infiltration into adipose tissue: initiation, propagation and remodeling. Futur Lipidol 3:545–556

Sutti S, Jindal A, Locatelli I, Vacchiano M, Gigliotti L, Bozzola C, Albano E (2014) Adaptive immune responses triggered by oxidative stress contribute to hepatic inflammation in NASH. Hepatology 59:886–897

Sutton CE, Mielke LA, Mills KH (2012) IL-17-producing gammadelta T cells and innate lymphoid cells. Eur J Immunol 42:2221–2231

Syn WK, Oo YH, Pereira TA, Karaca GF, Jung Y, Omenetti A, Witek RP, Choi SS, Guy CD, Fearing CM, Teaberry V, Pereira FE, Adams DH, Diehl AM (2010) Accumulation of natural killer T cells in progressive nonalcoholic fatty liver disease. Hepatology 51:1998–2007

Syn WK, Agboola KM, Swiderska M, Michelotti GA, Liaskou E, Pang H, Xie G, Philips G, Chan IS, Karaca GF, Pereira Tde A, Chen Y, Mi Z, Kuo PC, Choi SS, Guy CD, Abdelmalek MF, Diehl AM (2012) NKT-associated hedgehog and osteopontin drive fibrogenesis in non-alcoholic fatty liver disease. Gut 61:1323–1329

Tajiri K, Shimizu Y, Tsuneyama K, Sugiyama T (2009) Role of liver-infiltrating CD3+CD56+ natural killer T cells in the pathogenesis of nonalcoholic fatty liver disease. Eur J Gastroenterol Hepatol 21:673–680

Taleb S, Herbin O, Ait-Oufella H, Verreth W, Gourdy P, Barateau V, Merval R, Esposito B, Clement K, Holvoet P, Tedgui A, Mallat Z (2007) Defective leptin/leptin receptor signaling improves regulatory T cell immune response and protects mice from atherosclerosis. Arterioscler Thromb Vasc Biol 27:2691–2698

Talukdar S, da Oh Y, Bandyopadhyay G, Li D, Xu J, McNelis J, Lu M, Li P, Yan Q, Zhu Y, Ofrecio J, Lin M, Brenner MB, Olefsky JM (2012) Neutrophils mediate insulin resistance in mice fed a high-fat diet through secreted elastase. Nat Med 18:1407–1412

Tanaka A, Nomura Y, Matsuda A, Ohmori K, Matsuda H (2011) Mast cells function as an alternative modulator of adipogenesis through 15-deoxy-delta-12, 14-prostaglandin J2. Am J Physiol Cell Physiol 301:C1360–C1367

Tang Y, Bian Z, Zhao L, Liu Y, Liang S, Wang Q, Han X, Peng Y, Chen X, Shen L, Qiu D, Li Z, Ma X (2011) Interleukin-17 exacerbates hepatic steatosis and inflammation in non-alcoholic fatty liver disease. Clin Exp Immunol 166:281–290

Tashiro K, Takai S, Jin D, Yamamoto H, Komeda K, Hayashi M, Tanaka K, Tanigawa N, Miyazaki M (2010) Chymase inhibitor prevents the nonalcoholic steatohepatitis in hamsters fed a methionine- and choline-deficient diet. Hepatol Res 40:514–523

Tosello-Trampont AC, Landes SG, Nguyen V, Novobrantseva TI, Hahn YS (2012) Kupffer cells trigger nonalcoholic steatohepatitis development in diet-induced mouse model through tumor necrosis factor-alpha production. J Biol Chem 287:40161–40172

Triggiani M, Granata F, Frattini A, Marone G (2006) Activation of human inflammatory cells by secreted phospholipases A2. Biochim Biophys Acta 1761:1289–1300

Tsuruda T, Kato J, Hatakeyama K, Kojima K, Yano M, Yano Y, Nakamura K, Nakamura-Uchiyama F, Matsushima Y, Imamura T, Onitsuka T, Asada Y, Nawa Y, Eto T, Kitamura K (2008) Adventitial mast cells contribute to pathogenesis in the progression of abdominal aortic aneurysm. Circ Res 102:1368–1377

van den Berg SM, Seijkens TT, Kusters PJ, Zarzycka B, Beckers L, den Toom M, Gijbels MJ, Chatzigeorgiou A, Weber C, de Winther MP, Chavakis T, Nicolaes GA, Lutgens E (2014) Blocking CD40-TRAF6 interactions by small-molecule inhibitor 6860766 ameliorates the complications of diet-induced obesity in mice. Int J Obes (Lond) doi: 10.1038/ijo.2014.198 (Epub ahead of print)

Van Dyken SJ, Locksley RM (2013) Interleukin-4- and interleukin-13-mediated alternatively activated macrophages: roles in homeostasis and disease. Annu Rev Immunol 31:317–343

Vasanthakumar A, Moro K, Xin A, Liao Y, Gloury R, Kawamoto S, Fagarasan S, Mielke LA, Afshar-Sterle S, Masters SL, Nakae S, Saito H, Wentworth JM, Li P, Liao W, Leonard WJ, Smyth GK, Shi W, Nutt SL, Koyasu S, Kallies A (2015) The transcriptional regulators IRF4, BATF and IL-33 orchestrate development and maintenance of adipose tissue-resident regulatory T cells. Nat Immunol 16:276–285

Virtanen KA, Lidell ME, Orava J, Heglind M, Westergren R, Niemi T, Taittonen M, Laine J, Savisto NJ, Enerback S, Nuutila P (2009) Functional brown adipose tissue in healthy adults. N Engl J Med 360:1518–1525

Wagner NM, Brandhorst G, Czepluch F, Lankeit M, Eberle C, Herzberg S, Faustin V, Riggert J, Oellerich M, Hasenfuss G, Konstantinides S, Schafer K (2013) Circulating regulatory T cells are reduced in obesity and may identify subjects at increased metabolic and cardiovascular risk. Obesity (Silver Spring) 21:461–468

Wan YY, Flavell RA (2009) How diverse–CD4 effector T cells and their functions. J Mol Cell Biol 1:20–36

Wang Q, Xie Z, Zhang W, Zhou J, Wu Y, Zhang M, Zhu H, Zou MH (2014) Myeloperoxidase deletion prevents high-fat diet-induced obesity and insulin resistance. Diabetes 63:4172–4185

Wehr A, Baeck C, Heymann F, Niemietz PM, Hammerich L, Martin C, Zimmermann HW, Pack O, Gassler N, Hittatiya K, Ludwig A, Luedde T, Trautwein C, Tacke F (2013) Chemokine receptor CXCR6-dependent hepatic NK T cell accumulation promotes inflammation and liver fibrosis. J Immunol 190:5226–5236

Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr (2003) Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 112:1796–1808

Weitman ES, Aschen SZ, Farias-Eisner G, Albano N, Cuzzone DA, Ghanta S, Zampell JC, Thorek D, Mehrara BJ (2013) Obesity impairs lymphatic fluid transport and dendritic cell migration to lymph nodes. PLoS One 8:e70703

Westcott DJ, Delproposto JB, Geletka LM, Wang T, Singer K, Saltiel AR, Lumeng CN (2009) MGL1 promotes adipose tissue inflammation and insulin resistance by regulating 7/4hi monocytes in obesity. J Exp Med 206:3143–3156

Winer S, Chan Y, Paltser G, Truong D, Tsui H, Bahrami J, Dorfman R, Wang Y, Zielenski J, Mastronardi F, Maezawa Y, Drucker DJ, Engleman E, Winer D, Dosch HM (2009a) Normalization of obesity-associated insulin resistance through immunotherapy. Nat Med 15:921–929

Winer S, Paltser G, Chan Y, Tsui H, Engleman E, Winer D, Dosch HM (2009b) Obesity predisposes to Th17 bias. Eur J Immunol 39:2629–2635

Winer DA, Winer S, Shen L, Wadia PP, Yantha J, Paltser G, Tsui H, Wu P, Davidson MG, Alonso MN, Leong HX, Glassford A, Caimol M, Kenkel JA, Tedder TF, McLaughlin T, Miklos DB, Dosch HM, Engleman EG (2011) B cells promote insulin resistance through modulation of T cells and production of pathogenic IgG antibodies. Nat Med 17:610–617

Winer DA, Winer S, Chng MH, Shen L, Engleman EG (2014) B Lymphocytes in obesity-related adipose tissue inflammation and insulin resistance. Cell Mol Life Sci 71:1033–1043

Wing JB, Sakaguchi S (2012) Multiple treg suppressive modules and their adaptability. Front Immunol 3:178

Wolf MJ, Adili A, Piotrowitz K, Abdullah Z, Boege Y, Stemmer K, Ringelhan M, Simonavicius N, Egger M, Wohlleber D, Lorentzen A, Einer C, Schulz S, Clavel T, Protzer U, Thiele C, Zischka H, Moch H, Tschop M, Tumanov AV, Haller D, Unger K, Karin M, Kopf M, Knolle P, Weber A, Heikenwalder M (2014) Metabolic activation of intrahepatic CD8+ T cells and NKT cells causes nonalcoholic steatohepatitis and liver cancer via cross-talk with hepatocytes. Cancer Cell 26:549–564

Wong VW, Wong GL, Choi PC, Chan AW, Li MK, Chan HY, Chim AM, Yu J, Sung JJ, Chan HL (2010) Disease progression of non-alcoholic fatty liver disease: a prospective study with paired liver biopsies at 3 years. Gut 59:969–974

Wree A, Broderick L, Canbay A, Hoffman HM, Feldstein AE (2013) From NAFLD to NASH to cirrhosis-new insights into disease mechanisms. Nat Rev Gastroenterol Hepatol 10:627–636

Wu D, Molofsky AB, Liang HE, Ricardo-Gonzalez RR, Jouihan HA, Bando JK, Chawla A, Locksley RM (2011) Eosinophils sustain adipose alternatively activated macrophages associated with glucose homeostasis. Science 332:243–247

Wu J, Bostrom P, Sparks LM, Ye L, Choi JH, Giang AH, Khandekar M, Virtanen KA, Nuutila P, Schaart G, Huang K, Tu H, van Marken Lichtenbelt WD, Hoeks J, Enerback S, Schrauwen P, Spiegelman BM (2012a) Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. Cell 150:366–376

Wu L, Parekh VV, Gabriel CL, Bracy DP, Marks-Shulman PA, Tamboli RA, Kim S, Mendez-Fernandez YV, Besra GS, Lomenick JP, Williams B, Wasserman DH, Van Kaer L (2012b) Activation of invariant natural killer T cells by lipid excess promotes tissue inflammation, insulin resistance, and hepatic steatosis in obese mice. Proc Natl Acad Sci U S A 109:E1143–E1152

Xu JM, Shi GP (2012) Emerging role of mast cells and macrophages in cardiovascular and metabolic diseases. Endocr Rev 33:71–108

Yamagata H, Ikejima K, Takeda K, Aoyama T, Kon K, Okumura K, Watanabe S (2013) Altered expression and function of hepatic natural killer T cells in obese and diabetic KK-A(y) mice. Hepatol Res 43:276–288

Yekollu SK, Thomas R, O'Sullivan B (2011) Targeting curcusomes to inflammatory dendritic cells inhibits NF-kappaB and improves insulin resistance in obese mice. Diabetes 60:2928–2938

Yilmaz H, Ucan B, Sayki M, Unsal I, Sahin M, Ozbek M, Delibasi T (2014) Usefulness of the neutrophil-to-lymphocyte ratio to prediction of type 2 diabetes mellitus in morbid obesity. Diabetes Metab Syndr doi: 10.1016/j.dsx.2014.04.009 (Epub ahead of print)

Yoshimura A, Suzuki M, Sakaguchi R, Hanada T, Yasukawa H (2012) SOCS, inflammation, and autoimmunity. Front Immunol 3:20

Yu Y, Liu Y, Shi FD, Zou H, Matarese G, La Cava A (2013) Cutting edge: leptin-induced RORgammat expression in CD4+ T cells promotes Th17 responses in systemic lupus erythematosus. J Immunol 190:3054–3058

Zeyda M, Huber J, Prager G, Stulnig TM (2011) Inflammation correlates with markers of T-cell subsets including regulatory T cells in adipose tissue from obese patients. Obesity (Silver Spring) 19:743–748

Zhao L, de Qiu K, Ma X (2010) Th17 cells: the emerging reciprocal partner of regulatory T cells in the liver. J Dig Dis 11:126–133

Zhong J, Rao X, Braunstein Z, Taylor A, Narula V, Hazey J, Mikami D, Needleman B, Rutsky J, Sun Q, Deiuliis JA, Satoskar AR, Rajagopalan S (2014) T-cell costimulation protects obesity-induced adipose inflammation and insulin resistance. Diabetes 63:1289–1302

Zhu J, Yamane H, Paul WE (2010) Differentiation of effector CD4 T cell populations (*). Annu Rev Immunol 28:445–489

Zhu L, Wu Y, Wei H, Xing X, Zhan N, Xiong H, Peng B (2011) IL-17R activation of human periodontal ligament fibroblasts induces IL-23 p19 production: differential involvement of NF-kappaB versus JNK/AP-1 pathways. Mol Immunol 48:647–656

Zuniga LA, Shen WJ, Joyce-Shaikh B, Pyatnova EA, Richards AG, Thom C, Andrade SM, Cua DJ, Kraemer FB, Butcher EC (2010) IL-17 regulates adipogenesis, glucose homeostasis, and obesity. J Immunol 185:6947–6959

Adipose Tissue Stem Cells

Sebastian Müller, Elisabeth Kulenkampff, and Christian Wolfrum

Contents

Keywords

Adipocyte differentiation · Adipocyte inter-conversion · Adipogenesis · Adipose tissue · Brown adipocytes · Obesity · Stem cells · Type 2 Diabetes · White adipocytes

1 Introduction

Adipose tissue is the major storage sites of energy deposition which can be recruited in times of need to provide fuel for other organs (reviewed in Gunawardana 2014). When normalized to volume, adipose tissue is mainly composed of so-called mature adipocytes which are cells that have the capacity to store energy in the form of triacylglycerols (TAGs) in lipid droplets. When normalized to cell number, only 20–30% of the adipose tissue is made up from mature adipocytes; the other 70–80% are composed of the so-called stromal vascular fraction (SVF), which consists of fibroblasts, adipocyte precursors, endothelial cells, and immune cells (Rosenwald et al. 2013; Wang et al. 2013). This cell heterogeneity clearly demonstrates that adipose tissue is a complex organ with various different functions

S. Müller • E. Kulenkampff • C. Wolfrum (✉)
ETH Zürich, Schorenstrasse 16, 8603 Schwerzenbach, Switzerland
e-mail: christian-wolfrum@ethz.ch

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in the regulation of whole body metabolism. In line with this, over the past several years, our understanding of adipose tissue has changed. Only 20 years ago adipose tissue was considered to be an inert energy storage organ, while nowadays it is accepted that besides its role in energy storage and dissipation, adipose tissue serves as a key organ for the regulation of whole body energy metabolism by cross talk with other organs through the secretion of adipokines, such as tumor necrosis factor α, (TNF-α), interleukin-6 (IL-6), adiponectin, leptin, and resistin, just to mention a few (Bluher and Mantzoros 2015).

The adipose tissue organ is nowadays divided into two distinct forms, white adipose tissue (WAT) and brown adipose tissue (BAT). While both adipose tissues share the features mentioned above with respect to endocrine signaling, they are very different with regard to metabolic homeostasis. WAT is important for energy storage and release, and its adipocytes are defined by a unilocular lipid droplet structure, while BAT is important for energy dissipation in the form of heat and is defined by a multilocular lipid droplet structure. In this overview, we will first highlight the formation of WAT and the current hypothesis regarding the precursor cells that lead to the formation of WAT. In the second part of this overview, we will disseminate the current knowledge and hypothesis underlying the formation of BAT.

Why is adipose tissue so important and why has this tissue generated so much interest in recent years? Clearly the massive increase in the prevalence of obesity in the last 30 years which is due to an uncontrolled expansion of adipose tissue has increased the interest in research in that particular field. Obesity is associated with a multiple comorbidities which severely impact the quality of life. Most important are the obesity-associated developments of type II diabetes and cardiovascular complications; however, even the development of cancer is nowadays to be considered to be secondary to the development of obesity in certain cases (Kwan et al. 2014). While adipose tissue growths per se is just a reflection of the skewed energy balance, i.e., increased energy intake versus energy expenditure, already more than 50 years ago the composition of adipose tissue has been suggested to influence the metabolic outcomes of obesity.

In mammals several adipose tissue depots have been described. They all differentially impact on whole body energy homeostasis and also show different developmental patterns (Frontini and Cinti 2010). The two major WATs are considered to be the subcutaneous and the intra-abdominal/visceral compartment; however, around specific organs like the blood vessels (perivascular), the kidney (renal), and the heart (epicardial), adipose tissue depots can be found. BAT is mainly found in the intra-scapular region (newborns and mammals), and brown adipocytes are detected in the subcutaneous adipose tissue interspersed with the white adipocytes, while there are also smaller patches of periaortic and perirenal BAT (Sanchez-Gurmaches and Guertin 2014b). In addition to the abovementioned depots, several intra-organ adipose tissues have been described (i.e., intramuscular fat).

2 White Adipose Tissue Formation

In mammals, adipose tissue is formed in utero, in the perinatal stage as well as throughout life (Chau et al. 2014; Green and Kehinde 1975; Gregoire et al. 1998; Poissonnet et al. 1983; Spalding et al. 2008; Wang et al. 2013). Early work analyzing human adipose tissue formation was done by Bookstein and colleagues in 1983 by morphological analysis of embryos and fetuses. The authors could show that fat tissue forms between week 14 and 16 of gestation and that they are five morphogenic phases that are strongly associated with the formation of blood vessels, suggesting the importance of vascularization for adipose tissue formation (Poissonnet et al. 1983). The authors furthermore could demonstrate that at approximately week 23 of gestation the total number of adipose tissue lobules remains constant and increase is mainly achieved by growth of the respective lobules. Shortly after birth white adipose tissue expansion is induced as a result of increased fat cell size as well as an increase in fat cell number. This data was recently corroborated by work from Scherer and colleagues that used a novel adipocyte-specific marker mouse line to label white adipocytes and trace their development (Wang et al. 2013). The authors could show that in mice subcutaneous adipose tissue develops between embryonic day 14 and 18, while visceral adipose tissue develops mostly in the postnatal period, suggesting a different developmental origin for these two depots. This hypothesis was further supported by a recent report from Hastie and colleagues who could show that visceral adipose tissue is derived from Wt1-expressing cells, while subcutaneous adipose tissue is not (Chau et al. 2014). In addition to the development of the various adipose tissue depots, adipose tissue mass can be expanded later in life, especially in the context of obesity development. In a set of very elegant experiments using radioisotope tracing, Arner and colleagues could show that adipocyte number in adults is a major determinant of fat mass which is set during childhood and adolescence (Spalding et al. 2008). Furthermore, the authors could demonstrate that a marked amount of adipocytes is renewed in adult humans at approximately 10% every year.

The increase in adipose tissue mass can be achieved by two different strategies, which significantly differ with regard to their metabolic outcome and the development of obesity-associated comorbidities. On the one hand adipose tissue mass can be increased by increasing the size of the existing adipocytes, a process called adipocyte hypertrophy. In that context it has to be noted that adipocytes can change approximately 100-fold in volume in response to lipolytic and lipogenic stimuli (Kaplan et al. 1976). In contrast, adipose tissue mass can be increased by enhancing the number of adipocytes formed de novo from adipocyte precursor cells by a process termed adipocyte hyperplasia. In 1971 this process was studied in detail by Matthew and coworkers, who could show that visceral adipocyte hyperplasia in rats is completed between 9 and 14 weeks of age and that during that time adipose tissue expands mainly through adipocyte hypertrophy (Hubbard and Matthew 1971). In 1972 this work was expanded by Lemonnier who could show that obesity in rats and mice led to alterations in both hypertrophy and hyperplasia of adipose tissue (Lemonnier 1972). These studies were translated to the human system in 1976,

when Batchelor and colleagues showed that in the context of obesity these two processes play an important role (Hirsch and Batchelor 1976). The authors could demonstrate that in patients whose body weight exceeded 170% of the ideal weight, a maximum of cell size is approximately double that of a lean individual. In morbidly obese patients who accumulate weight past the abovementioned threshold, hyperplasia becomes an important process to regulate adipose tissue mass. Recent work has again corroborated these findings as it was shown by Scherer and colleagues that initially high-fat diet feeding in mice leads to adipocyte hypertrophy and only after long-term exposure to a high-fat diet adipocyte hyperplasia is observed. Interestingly this adipocyte hyperplasia is mainly seen in visceral adipose tissue and not in subcutaneous adipose tissue, suggesting again depot-specific differences, regarding the origin of adipocytes (Wang et al. 2013).

In the late 1970s and early 1980s, several studies suggested that adipocyte tissue hypertrophy and hyperplasia can actually influence food intake and thus modulate the development of obesity. Evidence for this hypothesis came from the findings that individuals with a hypercellular adipose tissue show a later onset of obesity than their counterparts. In addition to the relationship between adipocyte size and energy intake, several studies have suggested a link between adipocyte size and the development of obesity-associated comorbidities. In 1972 the group of Haller showed that adipocyte size correlated with the degree of type II diabetes development (Leonhardt et al. 1972). These findings were corroborated by Kipnis and colleagues in 1975 which showed that hyperinsulinemia in patients which are not obese or type II diabetic is directly associated with adipocyte size (Bernstein et al. 1975). In 1978 Hannefeld and colleagues linked adipose tissue hypertrophy and hyperplasia to the development of type II diabetes (Leonhardt et al. 1978). They could show that adipocyte hypertrophy, exceeding the degree predicted by the normal adipocyte growth curves, was observed in patients with subclinical type II diabetes, patients with type II diabetes, suggesting that enlarged adipocytes contribute to the pathology of obesity-associated comorbidities. Given the fact that very few animal models exist that can differentiate between adipose tissue accumulation and adipocyte size, the degree by which adipocyte size influences these processes is still not clear. One line of evidence that underscores the importance of adipose tissue formation in metabolic control is the drug class of glitazones which has been used in the past 20 years as the main treatment option for type II diabetes and which also influences de novo adipocyte formation as evidenced by increased weight gain in patients that take these medications (Desvergne et al. 2004). Given the fact that PPARγ activation controls multiple other pathways that might act on adipocyte size, such as de novo lipid and triglyceride synthesis, the contribution of adipocyte hyperplasia to the improved metabolic control is difficult to quantify. We have recently shown in our laboratory that in a model of RORγ deficiency, mice develop obesity on a high-fat diet associated with a massive increase in adipocyte hyperplasia. These mice are protected from diet-induced development of type II diabetes suggesting again that adipocyte size place an important role in regulating whole body metabolism (Meissburger et al. 2011). Taken together these data suggest that adipocyte tissue hyperplasia is an important process that might improve metabolic

control and thus prevent the development of obesity-associated comorbidities. A lot of research in recent years has focused on elucidating the adipose tissue stem cells that give rise to new adipocytes. Knowledge of these cells could help us to devise strategies to induce adipocyte hyperplasia and to reduce adipocyte hypertrophy.

3 White Adipose Tissue Precursors

Already in 1960s, it was noted that fibroblast-like cells change morphology and acquire unilocular lipid droplets suggesting that adipocytes are derived from a fibroblastic precursor (Napolitano 1963). Since the late 1970s when adipocyte hyperplasia was discovered, a lot of effort has focused on the identification of adipose tissue stem cells that could lead to the formation of new adipocytes. Most of the work performed relied on ^{3}H-thymidine incorporation assays to demonstrate the de novo formation of adipocytes from proliferating precursors. By combining these tracing techniques with activity measurements of the resulting cells, several groups aimed at distinguishing precursors from mature adipocytes. For example, Swierczewski and coworkers could show that the proliferative capacity is highest in precursor cells, while mature adipocytes lose their capacity for adipocyte proliferation (Gaben-Cogneville and Swierczewski 1979). Based on these findings a lot of studies have aimed at elucidating the regulation of adipocyte formation; however, until a few years ago, the identification of the adipocyte precursor has remained elusive and is still under debate today. This is due to the fact that stromal vascular fraction from adipose tissue can be differentiated into mature adipocytes using a specific differentiation cocktail containing insulin, glucocorticoids, and glitazones. This treatment gives rise to a large number of adipocytes and has led to the notion that approximately 20–30% of the stromal vascular fraction of adipose tissue actually is comprised of adipocyte precursors. Given the strong induction potential of the differentiation cocktail used in cell culture systems, it can be envisaged that several cells would never form an adipocyte under physiological conditions, but can be pushed toward the adipocyte fate in vitro, a point that is well illustrated by more recent findings (Rodeheffer et al. 2008). Due to these problems it might be necessary to rely on in vivo experiments to elucidate the physiological role and function as well as origin of an adipocyte precursor. The first work that really described adipocyte precursor in an in vivo setting was presented in 2008 by Friedman and colleagues (Rodeheffer et al. 2008). Using a Fluorescent Activated Cell Sorting (FACS) strategy, the authors demonstrated that combination of different mesenchymal markers as well as the exclusion of lineage-positive cells (endothelial cells and dendritic cells) defined a specific cell population which comprised approximately 53% of the adipose tissue stromal vascular fraction. The authors could thereby define a lineage ($Lin^{-}CD34^{+}CD29^{+}Sca1^{+}$) which could undergo differentiation into bona fide adipocytes in vitro; however, only the lineage comprising $Lin^{-}CD34^{+}CD29^{+-}Sca1^{+}CD24^{+}$ cells (0.08% of the SVF) was able to replenish adipose tissue in lipoatrophic A-Zip mice. A different approach was employed by Graff and colleagues (Tang et al. 2008) which used PPARγ as a surrogate marker to define the adipogenic

lineage. By generating a reporter mouse line which labeled PPARγ-expressing cells, they could isolate fibroblast-like cells from the SVF which had the capacity for forming adipocytes in vitro and in vivo. Furthermore, the authors could demonstrate that these cells could proliferate and self-renew and thus form a stem cell pool of adipocyte precursors. The finding that adipocyte precursors reside close to the arterial wall led to the hypothesis that adipose tissue stem cells are present in the vascular region especially during early development. Whether this holds true for adult adipocyte precursors remains to be seen. Several studies have since then reported a contribution of the endothelial lineage to adipocyte formation using a wide variety of markers (Gupta et al. 2012; Tran et al. 2012). Nevertheless, several lines of in vivo evidence point against such a contribution, as it was shown that deletion of PPARγ from endothelial cells did not affect fat formation. Furthermore, it was shown in 2013 by Rodeheffer and colleagues that the endothelial lineage even under massive induction of adipose tissue formation by high-fat diet does not contribute to the formation of adipose tissue (Berry and Rodeheffer 2013). A more recent report used Pref-1, which has been viewed as a factor important for the maintenance of the preadipocyte state for several decades now, to mark the adipocyte lineage (Hudak et al. 2014). In a report by Sul and colleagues it was shown that Pref-1-marked cells have a high proliferative capacity and seem to comprise early adipose precursors. Similar to the abovementioned findings, the authors could demonstrate Pref-1-marked cells with a mesenchymal, but not with an endothelial origin. Combining genetic lineage tracing and imaging, Granneman and colleagues described a precursor population which stains positive for PDGFRα, Sca1, and CD34. When these cells are genetically labeled and high fat is fed for 8 weeks, up to 25% of the mature visceral adipocytes derive from this lineage (Lee et al. 2012). Interestingly, these cells have the capacity in vitro to form white as well as brown adipocytes, but in vivo specifically only form white or brown adipocytes, depending on the anatomical location (Lee et al. 2015). These findings highlight once more the complex composition of the SVF in the different depots, so that cell fate is not only genetically determined, but also by direct queues from the microenvironment.

As a completely different concept to the resident stem cell theory, it has been shown that adipocytes can also be formed from bone-marrow-derived circulating precursor cells (Crossno et al. 2006; Sera et al. 2009). Yet, the physiologic role of this pathway remains under investigation.

4 Brown Adipose Tissue Precursors

As opposed to white adipocytes, brown adipocytes contain various small lipid droplets (multilocular) and a larger number of mitochondria. Furthermore, their mitochondria have the unique ability to dissipate energy in the form of heat instead of producing ATP (Cannon et al. 1982). Brown adipose tissue (BAT) is, therefore, responsible for the maintenance of body temperature by a mechanism called non-shivering thermogenesis. For long time it was believed that among humans BAT only exists in newborns and is absent in adults (Cypess et al. 2009). Only in

recent years it was shown by positron emission tomography (PET) that adult humans also have BAT and that it might even play a role in adult human metabolism (Cypess et al. 2009; Virtanen et al. 2009).

Similar to the white counterpart, brown adipose tissue also develops from mesenchymal precursors. The exact similarity between WAT and BAT and how closely they are related is still poorly understood and also whether WAT and BAT have a common precursor is still under debate. Various findings indicate that brown adipose tissue is developmentally more similar to skeletal muscle than to white adipose tissue, as brown adipocytes show a myogenic transcriptional signature and originate from precursor cells, which express myogenic transcription factor 5 (Myf5) (Sanchez-Gurmaches et al. 2012; Timmons et al. 2007). For long time, it was believed that WAT develops from myf5-negative precursors. However, in a recent paper, the exclusive expression of myf5 in BAT and not in WAT precursors was challenged as it could be shown that myf5 is also expressed in some extent in white adipose tissue (Sanchez-Gurmaches et al. 2012). More recently Guertin and coworkers combined several different tracer mouse models to elucidate the lineage background of brown adipocytes in the different adipose tissue depots. The results show that brown adipocytes are not in general derived from the Myf5 or the Pax3 lineage, because they stained positive for different lineages in different depots and under different conditions (Sanchez-Gurmaches and Guertin 2014b). Thus, the adipocyte lineage has a certain plasticity and a clear distinction between the brown and white adipocyte precursor remains elusive.

Adipogenesis is regulated by a complex transcription cascade. The activation of the adipose program in brown adipose tissue requires on the one side the activation of various genes and on the other side the release of suppressive markers (Rosen and MacDougald 2006). Various factors were proposed which have different influence on the preadipocytes of BAT and WAT. Similar to the development of white adipose tissue, the development of BAT is controlled by a transcriptional cascade involving the transcription factors C/EBPs and PPARγ (Rosen and MacDougald 2006). The similarity in regulation of the development by these factors in BAT and WAT is still under debate, and contradictory theories were proposed as to which effect PPARγ has on each of these organs (He et al. 2003; Linhart et al. 2001). PPARγ-coactivator 1a (PGC-1a) is important in several aspects of mitochondrial biogenesis and is early expressed in brown fat differentiation (Uldry et al. 2006). Brown preadipocytes lacking PGC-1a are able to develop normally and accumulate fat, but specific brown adipocyte genes, such as UCP1, which are important for the thermogenic function, are poorly expressed (Lin et al. 2004; Uldry et al. 2006). Another factor promoting differentiation to brown adipocytes is BMP7 (Tseng et al. 2008). It activates the program required for the brown adipogenesis even in the absence of normally required induction cocktails and triggers commitment of mesenchymal progenitor cells to a brown adipose lineage (Tseng et al. 2008). Knockdown of BMP7 on the other side results in a deficiency of brown adipose tissue and the absence of UCP1 (Tseng et al. 2008).

Complicating the story is the fact that in addition to brown cells, in response to chronic cold or beta-adrenergic stimulation, brown-like cells are formed in

predominantly white adipose tissues (brown-in-white, brite adipocytes; also known as beige adipocytes). The appearance of brite cells is very heterogeneous within various types of WAT and also within one white adipose fat pad (Sanchez-Gurmaches and Guertin 2014a). Brite cells exist among classical white adipocytes and they cannot be morphologically distinguished within the surrounding WAT in an unstimulated state. Upon cold or beta-adrenergic stimulation, they become multilocular, express UCP-1, and thus help to maintain the body temperature (Frontini and Cinti 2010; Lee et al. 2015; Rosenwald et al. 2013).

The question whether brite cells are closer related to brown or white adipocytes is at present highly discussed. Due to their expression of UCP1, they seem to be more similar to brown adipocytes. On the other side it was shown that brite cells do not develop from myf-5 expressing cells and that brown and brite cell formation is differentially regulated (Sanchez-Gurmaches et al. 2012; Wu et al. 2012). It was shown that brite cells have a unique expression signature, with a very low level of UCP1 in the basal level, but the ability to activate UCP1 to a similar level to brown adipose tissue upon cold induction (Wu et al. 2012). Various genes were shown to promote browning in WAT when overexpressed. For instance PRDM16 has been found to be specifically expressed in brown adipose tissue and to be important for the development and induction of brite adipose tissue (Cohen et al. 2014). Its overexpression results in the development of a robust brown phenotype in subcutaneous white adipose tissue, but not in other white depots (Seale et al. 2007, 2011). Depletion of PRDM16 on the other side results in a near total loss of the brown characteristics (Seale et al. 2007). Another factor which was shown to cause browning in WAT is Zfp516. It activates the expression of UCP1 and other BAT genes and therefore promotes browning and BAT development (Dempersmier et al. 2015).

The presence of brite adipocytes is generally accepted for decades, but it is still highly debated if they are formed by de novo differentiation of stem cells or by direct transdifferentiation of mature white adipocytes. Several reports propose a distinct mesenchymal stem cell population, which differentiates upon stimulus and forms part of the pool of mature brite adipocytes (Gesta et al. 2007; Vegiopoulos et al. 2010). Based on cell surface marker expression, subsets of stromal cells of the white adipose depot have been identified and isolated, which are able to differentiate into phenotypic brown adipocytes in vitro and in vivo after transplantation (Petrovic et al. 2010; Schulz et al. 2011; Lee et al. 2012). In addition polyclonal immortalization of the whole stem cell pool of the subcutaneous adipocyte depot can give rise to several different cell lines, some which preferentially differentiate into brite and some which can form white mature adipocytes in vitro (Wu et al. 2012). This concept of de novo differentiation is also supported by a tracer mouse study, which employs the adiponectin promoter to label all mature adipocytes at a given time. Subsequent treatment with cold or beta-adrenergic agonists led to the emerging of brite adipocytes, which are in part unlabeled and should therefore derive directly from differentiating stem cells, after the stimulus was applied (Wang et al. 2013). In 2014 Long and colleagues described that at least

a subset of brite adipocytes is derived from Myh11-positive cells and has a smooth muscle cell-like origin and transcriptional signature (Long et al. 2014).

A direct inter-conversion of mature white to brite adipocytes, and vice versa, is another long-standing hypothesis since the existence of brite was experimentally verified (Cousin et al. 1992). Initially, this concept was driven by gross microscopic observations, showing that there is no clear anatomical separation between white and brite adipocytes (Young et al. 1984) and that the whole inguinal subcutaneous fat pad can switch between a white and brite phenotype, if extreme conditions, i.e., thermo-neutrality and long-term cold stimulation, are compared (Cinti 2002). Another hallmark of transdifferentiation is the existence of in-between cell types, which exhibit characteristic features of both white and brown adipocytes. These cells of a mixed morphology were found after beta-adrenergic stimulus and termed paucilocular adipocytes (Barbatelli et al. 2010). In addition, only very little proliferation is measurable during the browning of white adipose tissue, and the total number of adipocytes does not change considerably, which is in contrast to differentiation from a stem cell, which would require at least one round of cell division (Himms-Hagen et al. 2000; Vitali et al. 2012). Recently, tracer mouse studies, which employ the UCP1 promoter to follow the appearance and fate of brite adipocytes, have proven the direct transdifferentiation. In mature mice, the brite adipocytes after 1 week of cold stimulation were labeled and followed for weeks after the stimulus was removed. Not only was it shown that the mature cells completely reverted from a brite to a white phenotype, but also that these cells convert to brite adipocytes again, if a subsequent cold induction is applied (Rosenwald et al. 2013). Another study used a different tracing model, which labels all adipocytes via adiponectin expression and showed the emerging adipocytes after cold stimulus, derived from preexisting mature cells (Lee et al. 2015). In neonatal mice, brite adipocytes are present in subcutaneous depot until weaning at 3 weeks of age. By labeling these cells via their UCP1 expression, it was shown that these cells convert to white phenotype but can reform a part of the brite adipocyte pool, if beta-adrenergically stimulated later in life (Contreras et al. 2014).

The relevance of adipocyte phenotype plasticity in humans was shown by the human multipotent adipose-derived stem cell line in an in vitro model. These cells can differentiate into fully functional white adipocytes, but upon chronic PPARγ stimulation transdifferentiate to a brite phenotype thereafter (Elabd et al. 2009). Moreover, white to brite adipocyte conversion has been shown in vivo on a morphological level in adult human pheochromocytoma patients, which are in a state of chronic adrenergic hyperstimulation (Frontini et al. 2013).

In conclusion, the diversity of reports suggests that both processes, de novo recruitment and direct transdifferentiation, contribute to the formation of brite adipocytes, but the extent of the two processes remains to be conclusively studied.

References

Barbatelli G, Murano I, Madsen L, Hao Q, Jimenez M, Kristiansen K, Giacobino JP, De Matteis R, Cinti S (2010) The emergence of cold-induced brown adipocytes in mouse white fat depots is determined predominantly by white to brown adipocyte transdifferentiation. Am J Physiol Endocrinol Metab 298:E1244–E1253

Bernstein RS, Grant N, Kipnis DM (1975) Hyperinsulinemia and enlarged adipocytes in patients with endogenous hyperlipoproteinemia without obesity or diabetes mellitus. Diabetes 24: 207–213

Berry R, Rodeheffer MS (2013) Characterization of the adipocyte cellular lineage in vivo. Nat Cell Biol 15:302–308

Bluher M, Mantzoros CS (2015) From leptin to other adipokines in health and disease: facts and expectations at the beginning of the 21st century. Metab Clin Expl 64:131–145

Cannon B, Hedin A, Nedergaard J (1982) Exclusive occurrence of thermogenin antigen in brown adipose tissue. FEBS Lett 150:129–132

Chau YY, Bandiera R, Serrels A, Martinez-Estrada OM, Qing W, Lee M, Slight J, Thornburn A, Berry R, McHaffie S et al (2014) Visceral and subcutaneous fat have different origins and evidence supports a mesothelial source. Nat Cell Biol 16:367–375

Cinti S (2002) Adipocyte differentiation and transdifferentiation: plasticity of the adipose organ. J Endocrinol Invest 25:823–835

Cohen P, Levy JD, Zhang Y, Frontini A, Kolodin DP, Svensson KJ, Lo JC, Zeng X, Ye L, Khandekar MJ et al (2014) Ablation of PRDM16 and beige adipose causes metabolic dysfunction and a subcutaneous to visceral fat switch. Cell 156:304–316

Contreras GA, Lee YH, Mottillo EP, Granneman JG (2014) Inducible brown adipocytes in subcutaneous inguinal white fat: the role of continuous sympathetic stimulation. Am J Physiol Endocrinol Metab 307:E793–799

Cousin B, Cinti S, Morroni M, Raimbault S, Ricquier D, Penicaud L, Casteilla L (1992) Occurrence of brown adipocytes in rat white adipose tissue: molecular and morphological characterization. J Cell Sci 103(Pt 4):931–942

Crossno JT Jr, Majka SM, Grazia T, Gill RG, Klemm DJ (2006) Rosiglitazone promotes development of a novel adipocyte population from bone marrow-derived circulating progenitor cells. J Clin Invest 116:3220–3228

Cypess AML, Lehman S, Williams G, Tal I, Rodman D, Goldfine AB, Kuo FC, Palmer EL, Tseng YH, Doria A, Kolodny GM, Kahn CR (2009) Identification and importance of brown adipose tissue in adult humans. N Engl J Med 360:1509–1517

Dempersmier J, Sambeat A, Gulyaeva O, Paul SM, Hudak CS, Raposo HF, Kwan HY, Kang C, Wong RH, Sul HS (2015) Cold-inducible Zfp516 activates UCP1 transcription to promote browning of white Fat and development of brown Fat. Mol Cell 57:235–246

Desvergne B, Michalik L, Wahli W (2004) Be fit or be sick: peroxisome proliferator-activated receptors are down the road. Mol Endocrinol 18:1321–1332

Elabd C, Chiellini C, Carmona M, Galitzky J, Cochet O, Petersen R, Penicaud L, Kristiansen K, Bouloumie A, Casteilla L et al (2009) Human multipotent adipose-derived stem cells differentiate into functional brown adipocytes. Stem Cells 27:2753–2760

Frontini A, Cinti S (2010) Distribution and development of brown adipocytes in the murine and human adipose organ. Cell Metab 11:253–256

Frontini A, Vitali A, Perugini J, Murano I, Romiti C, Ricquier D, Guerrieri M, Cinti S (2013) White-to-brown transdifferentiation of omental adipocytes in patients affected by pheochromocytoma. Biochim Biophys Acta 1831:950–959

Gaben-Cogneville AM, Swierczewski E (1979) Studies on cell proliferation in inguinal adipose tissue during early development in the rat. Lipids 14:669–675

Gesta S, Tseng YH, Kahn CR (2007) Developmental origin of fat: tracking obesity to its source. Cell 131:242–256

Green H, Kehinde O (1975) An established pre-adipose cell line and its differentiation in culture. II. Factors affecting adipose conversion. Cell 5:19–27

Gregoire FM, Smas CM, Sul HS (1998) Understanding adipocyte differentiation. Physiol Rev 78:783–809

Gunawardana SC (2014) Benefits of healthy adipose tissue in the treatment of diabetes. World J Diabetes 5:420–430

Gupta RK, Mepani RJ, Kleiner S, Lo JC, Khandekar MJ, Cohen P, Frontini A, Bhowmick DC, Ye L, Cinti S et al (2012) Zfp423 expression identifies committed preadipocytes and localizes to adipose endothelial and perivascular cells. Cell Metab 15:230–239

He W, Barak Y, Hevener A, Olson P, Liao D, Le J, Nelson M, Ong E, Olefsky JM, Evans RM (2003) Adipose-specific peroxisome proliferator-activated receptor gamma knockout causes insulin resistance in fat and liver but not in muscle. Proc Natl Acad Sci U S A 100: 15712–15717

Himms-Hagen J, Melnyk A, Zingaretti MC, Ceresi E, Barbatelli G, Cinti S (2000) Multilocular fat cells in WAT of CL-316243-treated rats derive directly from white adipocytes. Am J Physiol Cell Physiol 279:C670–C681

Hirsch J, Batchelor B (1976) Adipose tissue cellularity in human obesity. Clin Endocrinol Metab 5:299–311

Hubbard RW, Matthew WT (1971) Growth and lipolysis of rat adipose tissue: effect of age, body weight, and food intake. J Lipid Res 12:286–293

Hudak CS, Gulyaeva O, Wang Y, Park SM, Lee L, Kang C, Sul HS (2014) Pref-1 marks very early mesenchymal precursors required for adipose tissue development and expansion. Cell Rep 8: 678–687

Kaplan ML, Trout JR, Leveille GA (1976) Adipocyte size distribution in ob/ob mice during preobese and obese phases of development. Proc Soc Exp Biol Med 153:476–482

Kwan HY, Chao X, Su T, Fu X, Liu B, Tse AK, Fong WF, Yu Z (2014) Dietary lipids and adipocytes: potential therapeutic targets in cancers. J Nutr Biochem 26:303–311

Lee YH, Petkova AP, Mottillo EP, Granneman JG (2012) In vivo identification of bipotential adipocyte progenitors recruited by beta3-adrenoceptor activation and high-fat feeding. Cell Metab 15:480–491

Lee YH, Petkova AP, Konkar AA, Granneman JG (2015) Cellular origins of cold-induced brown adipocytes in adult mice. FASEB J 29:286–299

Lemonnier D (1972) Effect of age, sex, and sites on the cellularity of the adipose tissue in mice and rats rendered obese by a high-fat diet. J Clin Invest 51:2907–2915

Leonhardt W, Hanefeld M, Schneider H, Haller H (1972) Human adipocyte volumes: maximum size, and correlation to weight index in maturity onset-diabetes. Diabetologia 8:287–291

Leonhardt W, Haller H, Hanefeld M (1978) The adipocyte volume in human adipose tissue: II. Observations in diabetes mellitus, primary hyperlipoproteinemia and weight reduction. Int J Obes (Lond) 2:429–439

Lin J, Wu PH, Tarr PT, Lindenberg KS, St-Pierre J, Zhang CY, Mootha VK, Jager S, Vianna CR, Reznick RM et al (2004) Defects in adaptive energy metabolism with CNS-linked hyperactivity in PGC-1alpha null mice. Cell 119:121–135

Linhart HG, Ishimura-Oka K, DeMayo F, Kibe T, Repka D, Poindexter B, Bick RJ, Darlington GJ (2001) C/EBPalpha is required for differentiation of white, but not brown, adipose tissue. Proc Natl Acad Sci U S A 98:12532–12537

Long JZ, Svensson KJ, Tsai L, Zeng X, Roh HC, Kong X, Rao RR, Lou J, Lokurkar I, Baur W et al (2014) A smooth muscle-like origin for beige adipocytes. Cell Metab 19:810–820

Meissburger B, Ukropec J, Roeder E, Beaton N, Geiger M, Teupser D, Civan B, Langhans W, Nawroth PP, Gasperikova D et al (2011) Adipogenesis and insulin sensitivity in obesity are regulated by retinoid-related orphan receptor gamma. EMBO Mol Med 3:637–651

Napolitano L (1963) The differentiation of white adipose cells. An electron microscope study. J Cell Biol 18:663–679

Petrovic N, Walden TB, Shabalina IG, Timmons JA, Cannon B, Nedergaard J (2010) Chronic peroxisome proliferator-activated receptor gamma (PPARgamma) activation of epididymally derived white adipocyte cultures reveals a population of thermogenically competent, UCP1-containing adipocytes molecularly distinct from classic brown adipocytes. J Biol Chem 285: 7153–7164

Poissonnet CM, Burdi AR, Bookstein FL (1983) Growth and development of human adipose tissue during early gestation. Early Hum Dev 8:1–11

Rodeheffer MS, Birsoy K, Friedman JM (2008) Identification of white adipocyte progenitor cells in vivo. Cell 135:240–249

Rosen ED, MacDougald OA (2006) Adipocyte differentiation from the inside out. Nat Rev Mol Cell Biol 7:885–896

Rosenwald M, Perdikari A, Rulicke T, Wolfrum C (2013) Bi-directional interconversion of brite and white adipocytes. Nat Cell Biol 15:659–667

Sanchez-Gurmaches J, Guertin DA (2014a) Adipocyte lineages: tracing back the origins of fat. Biochim Biophys Acta 1842:340–351

Sanchez-Gurmaches J, Guertin DA (2014b) Adipocytes arise from multiple lineages that are heterogeneously and dynamically distributed. Nat Commun 5:4099

Sanchez-Gurmaches J, Hung CM, Sparks CA, Tang Y, Li H, Guertin DA (2012) PTEN loss in the Myf5 lineage redistributes body fat and reveals subsets of white adipocytes that arise from Myf5 precursors. Cell Metab 16:348–362

Schulz TJ, Huang TL, Tran TT, Zhang H, Townsend KL, Shadrach JL, Cerletti M, McDougall LE, Giorgadze N, Tchkonia T et al (2011) Identification of inducible brown adipocyte progenitors residing in skeletal muscle and white fat. Proc Natl Acad Sci U S A 108:143–148

Seale P, Kajimura S, Yang W, Chin S, Rohas LM, Uldry M, Tavernier G, Langin D, Spiegelman BM (2007) Transcriptional control of brown fat determination by PRDM16. Cell Metab 6:38–54

Seale P, Conroe HM, Estall J, Kajimura S, Frontini A, Ishibashi J, Cohen P, Cinti S, Spiegelman BM (2011) Prdm16 determines the thermogenic program of subcutaneous white adipose tissue in mice. J Clin Invest 121:96–105

Sera Y, LaRue AC, Moussa O, Mehrotra M, Duncan JD, Williams CR, Nishimoto E, Schulte BA, Watson PM, Watson DK et al (2009) Hematopoietic stem cell origin of adipocytes. Exp Hematol 37: 1108–1120, 1120 e1101-1104

Spalding KL, Arner E, Westermark PO, Bernard S, Buchholz BA, Bergmann O, Blomqvist L, Hoffstedt J, Naslund E, Britton T et al (2008) Dynamics of fat cell turnover in humans. Nature 453:783–787

Tang W, Zeve D, Suh JM, Bosnakovski D, Kyba M, Hammer RE, Tallquist MD, Graff JM (2008) White fat progenitor cells reside in the adipose vasculature. Science 322:583–586

Timmons JA, Wennmalm K, Larsson O, Walden TB, Lassmann T, Petrovic N, Hamilton DL, Gimeno RE, Wahlestedt C, Baar K et al (2007) Myogenic gene expression signature establishes that brown and white adipocytes originate from distinct cell lineages. Proc Natl Acad Sci U S A 104:4401–4406

Tran KV, Gealekman O, Frontini A, Zingaretti MC, Morroni M, Giordano A, Smorlesi A, Perugini J, De Matteis R, Sbarbati A et al (2012) The vascular endothelium of the adipose tissue gives rise to both white and brown fat cells. Cell Metab 15:222–229

Tseng YH, Kokkotou E, Schulz TJ, Huang TL, Winnay JN, Taniguchi CM, Tran TT, Suzuki R, Espinoza DO, Yamamoto Y et al (2008) New role of bone morphogenetic protein 7 in brown adipogenesis and energy expenditure. Nature 454:1000–1004

Uldry M, Yang W, St-Pierre J, Lin J, Seale P, Spiegelman BM (2006) Complementary action of the PGC-1 coactivators in mitochondrial biogenesis and brown fat differentiation. Cell Metab 3:333–341

Vegiopoulos A, Muller-Decker K, Strzoda D, Schmitt I, Chichelnitskiy E, Ostertag A, Berriel Diaz M, Rozman J, Hrabe de Angelis M, Nusing RM et al (2010) Cyclooxygenase-2 controls energy homeostasis in mice by de novo recruitment of brown adipocytes. Science 328: 1158–1161

Virtanen KNL, Lidell ME, Orava J, Heglind M, Westergren R, Niemi T, Taittonen M, Laine J, Savisto NJ, Enerbäck S, Nuutila P (2009) Functional brown adipose tissue in healthy adults. N Engl J Med 360:1518–1525

Vitali A, Murano I, Zingaretti MC, Frontini A, Ricquier D, Cinti S (2012) The adipose organ of obesity-prone C57BL/6J mice is composed of mixed white and brown adipocytes. J Lipid Res 53:619–629

Wang QA, Tao C, Gupta RK, Scherer PE (2013) Tracking adipogenesis during white adipose tissue development, expansion and regeneration. Nat Med 19:1338–1344

Wu J, Bostrom P, Sparks LM, Ye L, Choi JH, Giang AH, Khandekar M, Virtanen KA, Nuutila P, Schaart G et al (2012) Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. Cell 150:366–376

Young P, Wilson S, Arch JR (1984) Prolonged beta-adrenoceptor stimulation increases the amount of GDP-binding protein in brown adipose tissue mitochondria. Life Sci 34(12):1111–1117

Adipokines and the Endocrine Role of Adipose Tissues

Marta Giralt, Rubén Cereijo, and Francesc Villarroya

Contents

Abstract

The last two decades have witnessed a shift in the consideration of white adipose tissue as a mere repository of fat to be used when food becomes scarce to a true endocrine tissue releasing regulatory signals, the so-called adipokines, to the whole body. The control of eating behavior, the peripheral insulin sensitivity, and even the development of the female reproductive system are among the physiological events controlled by adipokines. Recently, the role of brown adipose tissue in human physiology has been recognized. The metabolic role of brown adipose tissue is opposite to white fat; instead of storing fat, brown

M. Giralt • R. Cereijo • F. Villarroya (✉)
Departament de Bioquímica i Biologia Molecular, Institute of Biomedicine (IBUB), University of Barcelona, Barcelona, Spain

CIBER Fisiopatología de la Obesidad y Nutrición, Barcelona, Spain
e-mail: fvillarroya@ub.edu

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adipose tissue is a site of energy expenditure via adaptive thermogenesis. There is growing evidence that brown adipose tissue may have its own pattern of secreted hormonal factors, the so-called brown adipokines, having distinctive biological actions on the overall physiological adaptations to enhance energy expenditure.

Keywords
Adipokine · Brown adipocyte · Endocrine · Inflammation · White adipocyte

1 Introduction: Adipose Tissues and the Concept of "Adipokine"

"Adipokine" is a term that first appeared in the biological literature a few decades ago with the recognition of a secretory role for adipose tissue. By default, the term "adipose tissue" is generally interpreted to mean "white" adipose tissue (WAT). The predominant cell type in this tissue is the white adipocyte, a specialized fat storage cell containing a single triglyceride vacuole that occupies more than 90% of the cell volume. WAT is the main type of adipose tissue in the organism and is responsible for storing fat for use when food availability is limited. A second distinct type of adipose tissue, termed "brown" adipose tissue (BAT), serves a totally different – in fact, opposite – role: consumption of metabolic energy to produce heat. The brown adipocyte contains adipose depots in the form of multiple triglyceride vacuoles, but they are not used to fuel other tissues but instead sustain uncoupled intracellular mitochondrial oxidation to produce heat. In contrast to white adipocytes, brown adipocytes contain large amounts of mitochondria. Almost everything we currently know about adipokines relates to the secretory role of WAT. One of the most recently emerging issues in relation to adipokines is the potential secretory function of BAT. The specific secretory properties of BAT are just beginning to be recognized and will be discussed separately at the end of this chapter. Finally, there is the bone marrow adipose tissue, a type of adipose tissue present in the interior of bones whose relative abundance is known to increase with aging (Justesen et al. 2001). Its biological role is poorly understood, and little information about its secretory properties is available, as cited below.

Just over 50 years ago, Kennedy postulated the existence of some kind of circulating "lipostatic" factor that exerts inhibitory functions capable of acting at central level to control energy expenditure and food intake (Kennedy 1953). In subsequent years, studies in which mice of the genetically obese *ob*/*ob* strain and control mice were surgically linked (parabiosis) allowed to confirm the presence of such a factor, as in animals linked by parabiosis, a normalization of weight was observed in obese mice due to the transfer of some molecule/s from control mice (Coleman 1973). However, it was not until the cloning of the gene for leptin in 1994 that an identity could be assigned to this mysterious entity (Zhang et al. 1994). The

finding that leptin, as a signaling molecule, was produced mainly by adipose tissue led to a major paradigm shift in thinking about the biological role of WAT, establishing this lipid reservoir as an endocrine organ capable of actively controlling overall energy homeostasis. This finding was followed by the discovery of additional molecules secreted mainly by adipose tissue. These studies, which are still ongoing today, showed that these factors act on the cell that excretes them (autocrine function), act on nearby cells (paracrine function), or enter the circulation to act on distant cells (endocrine function) – actions that led to the designation of these factors as adipokines (from adipose tissue + cytokines) (Ouchi et al. 2011).

An important issue relating to the use of the term "adipokine" concerns whether it refers to factors released specifically by adipocytes, the predominant cell type present in adipose depots, or applies more generally to factors released by any type of cell present in adipose tissue. The existing literature tends to use the term indistinctly, but to understand the endocrine role of adipose tissue, it is important to be aware that, whereas some of the currently known adipokines are expressed mainly by adipocytes – and in some cases, even by specific types of adipocyte – others are secreted by non-adipocytes, such as immune cells, vascular cells, and preadipocytes, present in adipose tissue depots.

2 Adipokines and the Secretory Function of White Adipose Tissue

The extensive body of research developed in recent years regarding major adipokines with signaling properties secreted by adipose tissue, together with the myriad novel molecules that are being continuously identified, makes it unrealistic to comprehensively describe every reported factor in a single book chapter. Hereby we provide an updated summary of the main adipokines, the basics of their biological roles, and their involvement in metabolic physiopathology.

2.1 Leptin

As noted above, leptin was identified as the product of the gene associated with obesity in mice homozygous for the recessive *ob* allele (Zhang et al. 1994). Loss of function for the *ob* gene, encoding leptin, results in hyperphagia, obesity, and insulin resistance, which can be reversed by administering leptin (Friedman and Halaas 1998). Leptin is a peptide hormone with a helical structure similar to that of cytokines such as interleukin (IL)-2 (Lord et al. 1998). Leptin is produced mainly by adipocytes, especially in subcutaneous fat depots, and, to a lesser extent, by other tissues such as the gastric epithelium and brain (Harwood 2012). Leptin acts mainly on the hypothalamus, which expresses the long, plasma membrane form of the leptin receptor, which mediates intracellular signals in response to leptin binding. The action of leptin on the hypothalamus signals ultimately the central nervous system, informing the nutritional status of the adipocytes and controlling

food intake, acting basically as a satiety signal (Ahima et al. 1996). In response to leptin-mediated signaling, the brain commands the peripheral tissues to increase the oxidation of fatty acids, reduce plasma glucose, induce thermogenesis, and, ultimately, reduce adipocyte mass. Moreover, in a fasting state, leptin levels decrease in coordination with insulin, stimulating appetite, inhibiting sympathetic activity-induced lipolysis and thermogenesis, and suppressing the action of anabolic hormones (Chan et al. 2003). The identification of a rare form of a morbid obesity disease in children carrying homozygosis-null mutations of the leptin gene and the decrease in hyperphagia and normalization of body weight produced by therapeutic administration of leptin provide confirmation in humans of the experimentally based concepts of leptin biological actions (Montague et al. 1997). The actions of leptin are not limited to the central nervous system; many peripheral tissues express some forms of leptin receptor and therefore respond to catabolic stimulation of leptin (Harwood 2012). Actions other than metabolic regulation are also recognized, e.g., the control of female reproductive system development (Friedman 2014).

In obese individuals, circulating leptin levels are high and correlate positively with adiposity. The fact that these high levels of leptin are unable to elicit an anorectic response is indicative of leptin resistance. It is known that leptin resistance in obesity involves the impairment of both central and peripheral responsiveness; however, it is not the variability of the leptin receptor molecule but alterations in intracellular signaling that determine impaired responsiveness (Steinberg et al. 2002). Extensive information has been generated on the subject of leptin – the molecule that inspired enormous research efforts in recent decades in adipokine identification and characterization – and a number of excellent reviews are currently available describing the multiple facets of leptin biology (Friedman 2014).

2.2 Adiponectin

Adiponectin (also known as AdipoQ or ACRP30) is another peptide hormone that originates in adipose tissues. Among the currently known adipokines, adiponectin, which is present at high concentrations in plasma (3–30 μg/ml), is possibly the one that is most exclusively secreted by adipocytes (Scherer et al. 1995; Hu et al. 1996; Maeda et al. 1996). This molecule has a remarkable structure, consisting of an N-terminal collagen-like sequence and a C-terminal globular domain homologous to the globular complement factor C1q. The crystal structure of the adiponectin globular domain reveals a striking resemblance to the structure of tumor necrosis factor-α (TNFα) (Shapiro and Scherer 1998). Adiponectin forms a wide range of multimeric species from trimers and hexamers to high-molecular-weight (HMW) multimers. All forms are detectable in the blood, but it is believed that only the HMW form has biological activity (Schraw et al. 2008). The synthesis and secretion of this adipokine is complex. Transcription of the adipokine gene is controlled by the adipogenic transcription factor PPARγ (peroxisome proliferator-activated

receptor-γ). Moreover, secretion is regulated at the level of the endoplasmic reticulum (ER), where the chaperone ERp44 and oxidoreductase Ero-Lα together retain HMW forms, which are released only in response to the appropriate stimuli (Halberg et al. 2008).

Adiponectin acts in peripheral tissues through interaction with its membrane receptors, AdipoR1 and AdipoR. Adiponectin increases insulin sensitivity, in part by promoting the action of AMP-activated kinase (AMPK) (Yamauchi et al. 2002). This kinase acts as a sensor of nutritional status and can activate fatty acid oxidation in skeletal muscle and inhibits hepatic gluconeogenesis and adipocyte lipolysis. In skeletal muscle, adiponectin also activates PGC-1α (PPARγ coactivator 1α), promoting mitochondrial biogenesis and oxidative metabolism (Iwabu et al. 2010). The actions of adiponectin on brown adipocytes are unclear, with some reports indicating that adiponectin promotes thermogenesis and others reporting that adiponectin blunts BAT recruitment by decreasing the expression and activation of the β-adrenergic receptor (Qiao et al. 2014).

In contrast to leptin levels, which are increased in obese individuals with type 2 diabetes, adiponectin levels are decreased in obese individuals and correlate negatively with adiposity. Adiponectin is one of the few adipokines that correlate negatively with insulin resistance; its release is increased under the conditions of leanness and is inhibited by pro-inflammatory molecules, the latter of which are known to worsen the metabolic status of obese patients (Li et al. 2009).

2.3 Resistin

Resistin (also known as FIZZ3) is a protein secreted by WAT that is closely associated with activation of inflammatory processes. In mice, resistin is produced exclusively by white adipocytes; however in humans, resistin is mostly produced by macrophages and monocytes (Steppan et al. 2001; Patel et al. 2003). Resistin and adiponectin show some structural similarities in that both form a circular homohexamer. In mice, resistin induces insulin resistance through a mechanism involving the activation of SOCS3 (suppressor of cytokine signaling 3), an inhibitor of insulin signaling, and the suppression of AMPK activation in peripheral tissues (Steppan et al. 2005). Resistin effects in humans are not entirely clear, but the prediction is that resistin would participate in promoting insulin resistance as well (Harwood 2012).

2.4 Other Adipokines

In addition to the abovementioned adipokines, WAT is capable of producing a variety of other signal molecules:

- *Adipsin*: Produced mainly by resident macrophages as well as by adipocytes, it is a protein that activates the complement alternative pathway and stimulates the

accumulation of triglycerides in adipocytes (Cook et al. 1987). It has been recently shown to be involved in maintaining pancreatic β-cell function (Lo et al. 2014).

- *Apelin*: Produced by adipocytes and other stromal stem cells, it has been proposed to play a role in the regulation of glucose homeostasis. Circulating apelin levels have been found to be increased in obese and insulin-resistant patients (Castan-Laurell et al. 2011).
- *Omentin*: Produced by non-adipocyte cells in adipose depots, it is mainly found in visceral adipose tissue rather than in subcutaneous adipose tissue (Yang et al. 2006). Plasma omentin levels are reduced in obesity, insulin resistance, and type 2 diabetes. Omentin has insulin-sensitizing effects and also has been reported to have anti-inflammatory, anti-atherogenic, and anti-cardiovascular disease properties (Tan et al. 2010).
- *RBP4 (retinol-binding protein 4)*: Secreted by adipocytes, its primary action is as a transporter of retinol; however, signaling properties that appear unrelated to vitamin A-related transport have been reported, mainly the induction of hepatic glucose production and insulin resistance (Yang et al. 2005). Its levels are increased in obesity (Ouchi et al. 2011).
- *Vaspin (visceral adipose tissue-derived serpin)*: Predominantly secreted by visceral fat, it is a serine protease inhibitor that reduces the levels of leptin, resistin, and pro-inflammatory cytokines, thereby improving insulin sensitivity (Hida et al. 2005). Its levels are increased in obese patients (Youn et al. 2008).
- *Visfatin*: Produced by adipocytes and lymphocytes, it is mainly produced in visceral adipose depots. Also known as the cytokine PBEF (pre-B-cell colony-enhancing factor), it is an extracellular form of the enzyme Nampt (nicotinamide phosphoribosyltransferase), an essential enzyme in the NAD biosynthetic pathway starting from nicotinamide. It has been proposed to act as an insulinomimetic capable of increasing glucose uptake and improving insulin resistance (Fukuhara et al. 2005).

In addition to these molecules, there are numerous other hormonal factors that are mainly released by tissues and cell types other than adipose tissue and adipocytes that have increasingly been found to be released by adipose tissue as well. Among these factors are plasminogen activator inhibitor-1 (PAI-1), hepatocyte growth factor (HGF), nerve growth factor (NGF), and fibroblast growth factor-21 (FGF21), as described below.

3 Pro-inflammatory Cytokines as Adipokines

In addition to these "conventional" adipokines, adipose tissue and adipocytes themselves secrete a large number of pro-inflammatory molecules and chemokines that are often involved in insulin resistance associated with obesity or lipodystrophy through the induction of local inflammation in adipose tissue.

Table 1 Summary of cytokines and chemokines released by white adipose tissue

Cytokine or chemokine	Function	Primary source(s)
Pro-inflammatory		
TNFα	Promotion of inflammatory innate and T_H1 immune responses, inhibition of PPARγ	Adipocytes, macrophages
IL-1β	Activation of macrophages and T cells	Adipocytes, macrophages
IL-6	Pleiotropic, promotion of T- and B-cell differentiation and synthesis of acute-phase proteins, enhancement of lipolysis, sensitization to IL-4 actions	Adipocytes, SVF cells
IL-7	Homeostatic preservation of lymphoid precursors, modulation of adipose tissue mass and insulin signaling	SVF cells
IL-18	Induction of a broad range of inflammatory responses	SVF cells
MCP1/CCL2	$CCR2^+$ monocyte chemotaxis	Adipocytes, SVF cells
RANTES/CCL5	Lymphocyte chemotaxis	Adipocytes, SVF cells
ENA-78/CXCL5	Neutrophil chemotaxis, antagonism of insulin signaling through the JAK-STAT pathway	SVF cells
IL-8/CXCL8	Neutrophil chemotaxis	Adipocytes, macrophages
IP-10/CXCL10	$CXCR3^+$ leukocyte chemotaxis	Adipocytes, SVF cells
SDF-1/CXCL12	$CXCR4^+$ macrophage chemotaxis	Adipocytes, SVF cells
Oncostatin M	Regulation of immune responses, inhibition of adipocyte differentiation	Macrophages, SVF cells
CSF-1	Promotion of macrophage and monocyte proliferation and differentiation	SVF cells
MIF	Activation of T_H1 cellular responses	Adipocytes, SVF cells
Anti-inflammatory		
IL-4 IL-13	Promotion of a T_H2 response and alternative macrophage activation	Eosinophils
IL-5	Eosinophil activation	Type 2 innate lymphoid cells
IL-10	Attenuation of inflammatory responses	Macrophages, SVF cells
TGFβ	Regulation of preadipocyte differentiation, inhibition of immune cell proliferation	SVF cells

CSF colony-stimulating factor, *IL* interleukin, *SVF* stromal vascular fraction, *TGF* tumor growth factor

It is evident that many pro-inflammatory cytokines are secreted in adipose tissue by infiltrating immune cells, such as macrophages and lymphocytes. The first evidence for a connection between inflammatory processes and obesity-related insulin resistance was provided by an observation in the nineteenth century that

treatment with high doses of the anti-inflammatory drug, sodium salicylate, attenuated hyperglycemia in diabetic patients. By the end of the twentieth century, a clear correlation between circulating levels and local production of pro-inflammatory cytokines and obesity had been fully established (Shoelson et al. 2006). In a time when WAT was just beginning to be recognized as a secretory organ, the revelation that TNFα was overproduced by WAT during obesity and evoked insulin resistance was an unexpected turn of events in research on this disease (Feinstein et al. 1993; Hotamisligil et al. 1993). These studies laid the foundation for WAT as a site of cytokine production, and since that time, a myriad of WAT-derived cytokines have been identified, as summarized in Table 1. Although mainly associated with infiltrating immune cells, the secretion of many of these factors by adipocytes (to a greater or lesser extent) has become increasingly evident, although it is difficult to precisely evaluate the relative contribution of adipocytes and non-adipocyte cells to total release.

In addition to cytokines, factors that fail to be classified as such, including acute-phase proteins and other inflammatory mediators, are also produced by adipose tissue. Serum amyloid A3, haptoglobin, lipocalin-2, and PAI-1 are among the acute-phase reactants produced by adipose tissue (Halberg et al. 2008). PAI-1 is a potent antagonist of fibrinolysis produced by adipocytes. In obesity, type 2 diabetes mellitus, and lipodystrophies, PAI-1 is greatly overexpressed by WAT, and it is well documented that its circulating levels rise dramatically, further contributing to insulin resistance (Alessi et al. 2007). The pro-inflammatory milieu of adipokines is completed by angiopoietin-like-2 (ANGPTL2), which acts in a paracrine way and promotes inflammatory immune cell extravasation by inducing the endothelial integrin expression, chemerin (also known as retinoic acid receptor responder-2), a secreted chemoattractant protein associated with systemic inflammation, recruitment of immune cells, and visceral fat accumulation, and progranulin, a small protein produced by stromal vascular fraction cells within WAT depots that is involved in cell proliferation and enhancement of IL-6 expression (Ouchi et al. 2011; Bluher 2014).

4 Free Fatty Acids: Nonprotein Signaling Molecules Released by White Adipose Tissue

Finally, we should be aware that, well before an endocrine role for adipose tissue was recognized, white adipocytes were known as sources of non-esterified fatty acids resulting from activation of lipolysis. In addition to their role as a metabolic fuel, free fatty acids have been recently recognized to function as signaling molecules, capable of influencing intracellular signaling in multiple cell types as well as modulate gene transcription of target cells and organs. The mechanisms are complex and involve membrane-associated G protein-coupled receptors sensitive to activation by fatty acids. Free fatty acids are thought to be responsible for insulin resistance in peripheral tissues, impaired insulin secretion by pancreatic β-cells, and enhancement of inflammatory processes. It is claimed that high rates of lipolysis

and the release of fatty acids by adipose tissue are important factors in the signaling associated with these metabolic disturbances. However, the signaling actions of fatty acids are complicated by their dependence on the type of fatty acid. It is well known that deleterious effects on metabolism are primarily attributable to saturated fatty acids (e.g., palmitate) rather than unsaturated fatty acids (e.g., oleic acid), both of which are present as adipose tissue triglycerides and therefore capable of being released into the circulation as a consequence of adipose tissue lipolysis. Recent rodent studies have shown that adipose-derived palmitoleic acid may serve as a "lipokine" that contributes to resistance to diet-induced obesity. Whether adipose tissue palmitoleic acid behaves as a lipokine to reduce the occurrence of obesity in humans remains to be established (Cao et al. 2008; Gong et al. 2011).

5 Regional Differences in Adipokine Secretion Between Subcutaneous and Visceral White Adipose Depots

WAT is distributed anatomically into different depots in the body, mainly under the epidermis, known as the subcutaneous adipose tissue, and in intra-abdominal depots, commonly referred to as the visceral adipose tissue. About 80% of the total fat in healthy humans is located in the subcutaneous fat depots, whereas visceral fat accounts for up to 10–20% in men and 5–8% in women (Wajchenberg 2000). In addition to sex-based differences, fat distribution also varies with age, genetic background, and disease state. Indeed, an elevated risk for systemic metabolic dysfunction is not simply associated with the amount of body fat but instead is closely linked to central (visceral) obesity as opposed to peripheral (subcutaneous) obesity.

It is well recognized that there are structural and functional differences between visceral and subcutaneous fat depots (recently reviewed in Lafontan 2013). Among these differences is their secretory activity. For example, subcutaneous fat depots are the major source of leptin, whereas adiponectin secretion is higher in visceral than in subcutaneous adipose tissue (Motoshima et al. 2002; Kovacova et al. 2012). Visceral adipose tissue also exhibits greater infiltration by macrophages and other immune cells than does subcutaneous depots, especially in obese individuals (Cancello et al. 2006). Accordingly, pro-inflammatory cytokines such as IL-6, IL-8, TNFα, or MCP-1 (monocyte chemoattractant protein-1, also known as CCL2) are generally considered to be more highly expressed in visceral fat depots than in subcutaneous depots (Fontana et al. 2007; Pou et al. 2007).

In humans, subcutaneous fat depots account for up to 60% of systemic free fatty acids under basal conditions, reflecting their greater mass, but the contribution of visceral depots increases greatly during hyperinsulinemia. Visceral fat is also more prone to catecholamine activation but less susceptible to the antilipolytic action of insulin, in effect making its lipolytic activity higher than that of subcutaneous fat (Arner 1995, 2005). Owing to their anatomical positions, visceral depots partly drain into the liver via the portal vein. Therefore, free fatty acids and pro-inflammatory cytokines released by visceral fat may directly impact liver

function, thus providing a potential mechanistic link between visceral obesity and hepatic and systemic disturbances (Fontana et al. 2007).

6 Secretory Function of Bone Marrow Adipose Tissue

The secretory functions of adipocytes within the bone are largely unknown, as are other basic aspects of their biology and physiological role. Until recently, it was thought that, in any case, the molecules secreted might be the same as those by WAT and that they could act locally. However, a recent report suggests that bone marrow adipocytes may act at the systemic level by serving as a relevant source of adiponectin under conditions such as caloric restriction or anorexia nervosa (Cawthorn et al. 2014).

7 Brown Adipokines: The Emerging Secretory Function of Brown Adipose Tissue

Analogously to WAT and its energy storage function, BAT was hitherto considered just a site of metabolic consumption that happened to be able to dissipate energy in the form of heat. Systemic metabolic adaptations, namely, glycemia reduction and insulin sensitization, arise upon the activation of BAT in response to thermogenic stimuli (i.e., cold), and local glucose uptake and insulin action in BAT are thought to be major contributors to these effects. In other organs, however, catabolic processes are upregulated in association with BAT activation, like an enhancement of WAT lipolysis to provide oxidative fuels for non-shivering thermogenesis (Bukowiecki 1989). It is thus logical to hypothesize the existence of circulating molecules that allow BAT to communicate with other cells and peripheral organs in order to coordinate the adaptive thermogenic response.

The first hint of a secretory role for BAT emerged from the observation that genetic loss of BAT had a more profound impact on general metabolism than blocking BAT energy expenditure functions by specifically disrupting uncoupling protein-1 (UCP1) (Lowell et al. 1993; Hamann et al. 1996; Enerback et al. 1997). Moreover, the expression of WAT adipokines, such as leptin and adiponectin, is lower in BAT, especially after thermogenic activation (Cannon and Nedergaard 2004). Therefore, the existence of different factors preferentially secreted by BAT – brown adipokines or "batokines" – was postulated. Brown adipokines may thus serve their own functions and behave differently from white adipokines, be upregulated in thermogenically recruited BAT, and act locally or in distant tissues.

Several BAT-derived molecules that act either in a paracrine or autocrine fashion have already been identified, and most of them are produced by brown adipocytes and induced in situations of BAT recruitment. Vascular endothelial factor-A (VEGF-A), nitric oxide (NO), NGF, insulin-like growth factor I (IGF-1), and fibroblast growth factor-2 (FGF2) are all examples of this. VEGF-A promotes angiogenesis in response to the sympathetic recruitment of BAT, and NO is thought

to be involved in endothelium-dependent increases in blood flow to BAT and the induction of the master regulator of mitochondrial biogenesis, PGC-1α. NGF released by brown adipocytes would serve to enhance sympathetic innervation of BAT, and both IGF-I and FGF2 have roles in increasing the number of brown adipocyte precursors (Cannon and Nedergaard 2004; Villarroya et al. 2013). Collectively, these molecules would act to enhance BAT expansion in situations of thermogenic need.

In contrast to other bone morphogenetic proteins (BMPs), BMP8b has recently been demonstrated to have no role in white or brown adipogenesis but instead is involved in BAT activation. This protein is secreted by, and in turn acts on, brown adipocytes, sensitizing these cells to adrenergic stimuli required for their activation. Moreover, BMP8b has an effect on hypothalamic neurons and enhances neural signaling to favor norepinephrine release in BAT (Whittle et al. 2012).

Lipocalin prostaglandin-D synthase (L-PGDS) is a prostaglandin-synthesizing enzyme that also acts as a carrier of lipophilic molecules, such as thyroid hormones and retinoic acid, which are well-known BAT activators. Thus, L-PGDS may exert its function in an autocrine or paracrine manner, as confirmed by loss-of-function studies in which the lack of L-PGDS was shown to result in an impaired activation of BAT (Virtue et al. 2012).

Until recently, immune infiltration in BAT had been overlooked. However, the tables were turned with the discovery that less inflammatory, alternatively activated macrophages are recruited upon BAT activation and actively produce norepinephrine to sustain adaptive thermogenesis. Accordingly, pro-inflammatory cytokines are in general less expressed in BAT than in WAT due to a reduced and more homeostatic immune infiltration (Nguyen et al. 2011). Some of these cytokines, such as IL-1β and IL-6, are nonetheless overexpressed and released by brown adipocytes and immune cells in the presence of thermogenic stimuli. Whereas IL-1β may act by modulating IL-1 receptor signaling and diminishing inflammation (Cannon and Nedergaard 2004), IL-6 confers insulin sensitization to BAT (discussed in detail below). It has recently been described that MTRNL (meteorin-like) is produced by brown-like adipocytes – termed "beige/brite" adipocytes – that occur in WAT depots upon thermogenic activation but not by "classical" brown adipocytes. MTRNL is responsible for eosinophil infiltration and activation. Since these cells are the main source of IL-4 and IL-13 in adipose tissue and both cytokines promote the alternative activation of macrophages, MTRNL would act as a browning agent for WAT and further exemplifies the contribution of the adipose-immune crosstalk to thermogenesis (Rao et al. 2014; Qiu et al. 2014).

Although the endocrine function of BAT is still in the process of being fully elucidated, there are a number of different factors that have already been recognized as having such a role, starting with triiodothyronine (T3). Type II iodothyronine 5′-deiodinase (DIO2) is highly expressed in BAT and catalyzes the conversion of thyroxine to T3. DIO2 is induced and T3 production is subsequently increased during thermogenic activation in brown adipocytes. T3 binds intracellularly to the

thyroid hormone receptor, which is involved in the activation of thermogenic genes, including UCP1. It is known, however, that cold-induced T3 release is not limited to a local function: BAT-derived T3 enters the bloodstream in substantial amounts to further mediate distal actions required for thermogenesis (e.g., in the hypothalamus). Given the role of thyroid hormones in the promotion of energy expenditure, it is reasonable to infer that their release by BAT might play a role in this context (Silva and Larsen 1985; Fernandez et al. 1987).

RBP4 offers further clues in the search for BAT adipokines. Though RBP4 was initially reported to be a WAT adipokine, its expression and release are strongly induced by cold in brown adipocytes (Rosell et al. 2012). Although the release of RBP4 by WAT is positively correlated with insulin resistance, as noted above, BAT-derived RBP4 may provide a mechanism for delivering retinol – a precursor of retinoic acid, which is a known activator of BAT – to adjacent brown adipocytes or even to distant WAT depots in order to promote beige/brite thermogenesis therein (Villarroya et al. 2013).

One of the most robust links of BAT with an endocrine function is arguably FGF21. In the absence of specific stimuli, the liver is the main site of FGF21 synthesis. However, FGF21 expression is greatly induced in brown adipocytes upon thermogenic activation and BAT is capable of releasing this hormone into the circulation (Hondares et al. 2011; Fisher et al. 2012). Locally, FGF21 promotes the expression of PGC-1α and UCP1, thereby enhancing thermogenic activation (Hondares et al. 2010; Fisher et al. 2012). Systemically, FGF21 promotes glucose oxidation in many different tissues (e.g., liver, WAT, pancreas), and studies using animal models have demonstrated that FGF21 confers protection against obesity and type 2 diabetes (Coskun et al. 2008). BAT transplantation studies, discussed below, have revealed enhanced FGF21 production and an improved metabolic profile in animals on a high-fat diet, consistent with the idea that BAT-derived, circulating FGF21 acts systemically to exert beneficial effects. Though expressed in WAT as well, thermogenic stimulation yields no upregulation of FGF21 in this tissue, hence implying a more autocrine role (Dutchak et al. 2012). Since FGF21 is highly expressed in and released by beige/brite adipocytes in both mice and humans, it may be particularly relevant in the browning processes (Lee et al. 2014). On the other hand, FGF21 appears to be expressed more specifically in BAT versus WAT in humans; in contrast with rodents, FGF21 expression is practically negligible in human WAT (Hondares et al. 2014).

Recently, several groups almost simultaneously discovered a role for the gene product of Gm6484 (in mice) and C19orf80 (in humans) in lipid metabolism and trafficking. The protein encoded by these genes was revealed to be a member of the ANGPTL family and has since been variously named TD26, RIFL (refeeding-induced in fat and liver), lipasin, ANGPTL8, and betatrophin (Quagliarini et al. 2012; Zhang 2012; Ren et al. 2012; Yi et al. 2013). Although each study focused on different questions, all concurred that this protein is expressed at the highest levels in liver and BAT, followed by WAT. Expression of its transcript is

induced during adipogenesis in both white and brown adipocytes and is upregulated by insulin and cold, resulting in elevated circulating levels. However, it is downregulated by lipolytic effectors such as cAMP, norepinephrine, and fasting, which decrease its circulating levels. Disruption of this gene in animal models results in reduced triglyceridemia and a mild lipodystrophic phenotype. Collectively, these puzzle pieces form a picture of TD26/RIFL/ANGPTL8/betatrophin as a lipoprotein lipase inhibitor involved in replenishing WAT and BAT lipid stores. Moreover, this protein is thought to promote the proliferation of β-cells and subsequently increase pancreatic insulin production, which illustrates the existence of interactions between BAT and the pancreas (Yi et al. 2013).

Recent studies have used BAT transplantation as a powerful, albeit complex, tool to study the potential roles of BAT-derived endocrine molecules. Several of these studies identified the systemic effects of BAT transplantation that could not be accounted for by the increase in energy expenditure and metabolite oxidation attributable to the additional mass of transplanted BAT. One study demonstrated that subcutaneous BAT transplantation normalized glucose levels and ameliorated the symptoms of type 1 diabetes in rodent models. IGF-1 was proposed as a candidate for this behavior, since its actions would mimic those of insulin and reverse the diabetic phenotype (Gunawardana and Piston 2012). Other independent studies showed that BAT transplantation improved the whole-body energy metabolism and protected mice from high-fat-diet-induced obesity (Liu et al. 2013; Stanford et al. 2013). Stanford et al. reported a decrease in body weight and improved glucose metabolism and insulin sensitivity in high-fat-diet-fed mice upon BAT transplantation. Intriguingly, these effects were absent in IL-6-null mice and, in fact, the FGF21 increase associated with BAT transplantation is lost when IL-6-null BAT was transplanted (Stanford et al. 2013). IL-6 expression is induced in BAT upon thermogenic activation and it has a well-established function in the promotion of WAT lipolysis, indeed suggesting a role in adaptive thermogenesis. Mauer et al. shed some light on this issue by demonstrating that IL-6 receptor-mediated pathways were able to sensitize adipose-resident macrophages to IL-4 action, thus promoting alternative activation, concomitant catecholamine production, and BAT activity (Mauer et al. 2014).

Taken together, accumulating evidence suggests that BAT, like WAT, indeed has an endocrine role (see Fig. 1 for summary). The old designation of BAT as a hibernating "gland" might prove to be unintentionally less misleading than once thought, since all of the findings discussed above are probably just the beginning of an era of discovery for BAT-released factors. Given the renewed interest in BAT since its rediscovery in adult humans (Cereijo et al. 2014), BAT activation and brown adipokines have risen to the fore as promising tools for clinical treatment of obesity and diabetes.

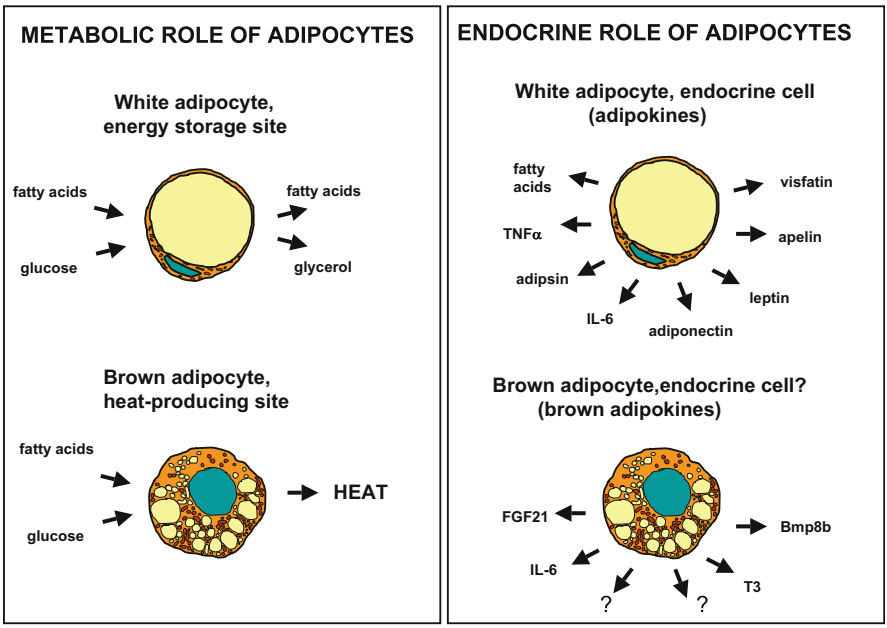

Fig. 1 Schematic representation of evolving concepts on the endocrine role of adipose cells. The classical metabolic role of white adipocytes and brown adipocytes is depicted (*left*). Examples of adipokines released by white adipocytes and of currently recognized "brown adipokines" are shown (*right*)

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References

Ahima RS, Prabakaran D, Mantzoros C, Qu D, Lowell B, Maratos-Flier E, Flier JS (1996) Role of leptin in the neuroendocrine response to fasting. Nature 382:250–252

Alessi MC, Poggi M, Juhan-Vague I (2007) Plasminogen activator inhibitor-1, adipose tissue and insulin resistance. Curr Opin Lipidol 18:240–245

Arner P (1995) Differences in lipolysis between subcutaneous and omental adipose tissues. Ann Med 27:435–438

Arner P (2005) Human fat cell lipolysis: biochemistry, regulation and clinical role. Best Pract Res Clin Endocrinol Metab 19:471–482

Bluher M (2014) Adipokines – removing road blocks to obesity and diabetes therapy. Mol Metab 3:230–240

Bukowiecki LJ (1989) Energy balance and diabetes. The effects of cold exposure, exercise training, and diet composition on glucose tolerance and glucose metabolism in rat peripheral tissues. Can J Physiol Pharmacol 67:382–393

Cancello R, Tordjman J, Poitou C, Guilhem G, Bouillot JL, Hugol D, Coussieu C, Basdevant A, Bar Hen A, Bedossa P, Guerre-Millo M, Clement K (2006) Increased infiltration of

macrophages in omental adipose tissue is associated with marked hepatic lesions in morbid human obesity. Diabetes 55:1554–1561
Cannon B, Nedergaard J (2004) Brown adipose tissue: function and physiological significance. Physiol Rev 84:277–359
Cao H, Gerhold K, Mayers JR, Wiest MM, Watkins SM, Hotamisligil GS (2008) Identification of a lipokine, a lipid hormone linking adipose tissue to systemic metabolism. Cell 134:933–944
Castan-Laurell I, Dray C, Attane C, Duparc T, Knauf C, Valet P (2011) Apelin, diabetes, and obesity. Endocrine 40:1–9
Cawthorn WP, Scheller EL, Learman BS, Parlee SD, Simon BR, Mori H, Ning X, Bree AJ, Schell B, Broome DT, Soliman SS, DelProposto JL, Lumeng CN, Mitra A, Pandit SV, Gallagher KA, Miller JD, Krishnan V, Hui SK, Bredella MA, Fazeli PK, Klibanski A, Horowitz MC, Rosen CJ, MacDougald OA (2014) Bone marrow adipose tissue is an endocrine organ that contributes to increased circulating adiponectin during caloric restriction. Cell Metab 20:368–375
Cereijo R, Giralt M, Villarroya F (2014) Thermogenic brown and beige/brite adipogenesis in humans. Ann Med 18:1–9
Chan JL, Heist K, DePaoli AM, Veldhuis JD, Mantzoros CS (2003) The role of falling leptin levels in the neuroendocrine and metabolic adaptation to short-term starvation in healthy men. J Clin Invest 111:1409–1421
Coleman DL (1973) Effects of parabiosis of obese with diabetes and normal mice. Diabetologia 9:294–298
Cook KS, Min HY, Johnson D, Chaplinsky RJ, Flier JS, Hunt CR, Spiegelman BM (1987) Adipsin: a circulating serine protease homolog secreted by adipose tissue and sciatic nerve. Science 237:402–405
Coskun T, Bina HA, Schneider MA, Dunbar JD, Hu CC, Chen Y, Moller DE, Kharitonenkov A (2008) Fibroblast growth factor 21 corrects obesity in mice. Endocrinology149::6018–6027
Dutchak PA, Katafuchi T, Bookout AL, Choi JH, Yu RT, Mangelsdorf DJ, Kliewer SA (2012) Fibroblast growth factor-21 regulates PPARgamma activity and the antidiabetic actions of thiazolidinediones. Cell 148:556–567
Enerback S, Jacobsson A, Simpson EM, Guerra C, Yamashita H, Harper ME, Kozak LP (1997) Mice lacking mitochondrial uncoupling protein are cold-sensitive but not obese. Nature 387:90–94
Feinstein R, Kanety H, Papa MZ, Lunenfeld B, Karasik A (1993) Tumor necrosis factor-alpha suppresses insulin-induced tyrosine phosphorylation of insulin receptor and its substrates. J Biol Chem 268:26055–26058
Fernandez JA, Mampel T, Villarroya F, Iglesias R (1987) Direct assessment of brown adipose tissue as a site of systemic tri-iodothyronine production in the rat. Biochem J 243:281–284
Fisher FM, Kleiner S, Douris N, Fox EC, Mepani RJ, Verdeguer F, Wu J, Kharitonenkov A, Flier JS, Maratos-Flier E, Spiegelman BM (2012) FGF21 regulates PGC-1alpha and browning of white adipose tissues in adaptive thermogenesis. Genes Dev 26:271–281
Fontana L, Eagon JC, Trujillo ME, Scherer PE, Klein S (2007) Visceral fat adipokine secretion is associated with systemic inflammation in obese humans. Diabetes 56:1010–1013
Friedman J (2014) 20 Years of Leptin: Leptin at 20: an overview. J Endocrinol 223:T1–T8
Friedman JM, Halaas JL (1998) Leptin and the regulation of body weight in mammals. Nature 395:763–770
Fukuhara A, Matsuda M, Nishizawa M, Segawa K, Tanaka M, Kishimoto K, Matsuki Y, Murakami M, Ichisaka T, Murakami H, Watanabe E, Takagi T, Akiyoshi M, Ohtsubo T, Kihara S, Yamashita S, Makishima M, Funahashi T, Yamanaka S, Hiramatsu R, Matsuzawa Y, Shimomura I (2005) Visfatin: a protein secreted by visceral fat that mimics the effects of insulin. Science 307:426–430
Gong J, Campos H, McGarvey S, Wu Z, Goldberg R, Baylin A (2011) Adipose tissue palmitoleic acid and obesity in humans: does it behave as a lipokine? Am J Clin Nutr 93:186–191

Gunawardana SC, Piston DW (2012) Reversal of type 1 diabetes in mice by brown adipose tissue transplant. Diabetes 61:674–682

Halberg N, Wernstedt-Asterholm I, Scherer PE (2008) The adipocyte as an endocrine cell. Endocrinol Metab Clin North Am 37:753–768

Hamann A, Flier JS, Lowell BB (1996) Decreased brown fat markedly enhances susceptibility to diet-induced obesity, diabetes, and hyperlipidemia. Endocrinology 137:21–29

Harwood HJ Jr (2012) The adipocyte as an endocrine organ in the regulation of metabolic homeostasis. Neuropharmacol 63:57–75

Hida K, Wada J, Eguchi J, Zhang H, Baba M, Seida A, Hashimoto I, Okada T, Yasuhara A, Nakatsuka A, Shikata K, Hourai S, Futami J, Watanabe E, Matsuki Y, Hiramatsu R, Akagi S, Makino H, Kanwar YS (2005) Visceral adipose tissue-derived serine protease inhibitor: a unique insulin-sensitizing adipocytokine in obesity. Proc Natl Acad Sci U S A 102:10610–10615

Hondares E, Rosell M, Gonzalez FJ, Giralt M, Iglesias R, Villarroya F (2010) Hepatic FGF21 expression is induced at birth via PPARalpha in response to milk intake and contributes to thermogenic activation of neonatal brown fat. Cell Metab 11:206–212

Hondares E, Iglesias R, Giralt A, Gonzalez FJ, Giralt M, Mampel T, Villarroya F (2011) Thermogenic activation induces FGF21 expression and release in brown adipose tissue. J Biol Chem 286:12983–12990

Hondares E, Gallego-Escuredo JM, Flachs P, Frontini A, Cereijo R, Goday A, Perugini J, Kopecky P, Giralt M, Cinti S, Kopecky J, Villarroya F (2014) Fibroblast growth factor-21 is expressed in neonatal and pheochromocytoma-induced adult human brown adipose tissue. Metabolism 63:312–317

Hotamisligil GS, Shargill NS, Spiegelman BM (1993) Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 259:87–91

Hu E, Liang P, Spiegelman BM (1996) AdipoQ is a novel adipose-specific gene dysregulated in obesity. J Biol Chem 271:10697–10703

Iwabu M, Yamauchi T, Okada-Iwabu M, Sato K, Nakagawa T, Funata M, Yamaguchi M, Namiki S, Nakayama R, Tabata M, Ogata H, Kubota N, Takamoto I, Hayashi YK, Yamauchi N, Waki H, Fukayama M, Nishino I, Tokuyama K, Ueki K, Oike Y, Ishii S, Hirose K, Shimizu T, Touhara K, Kadowaki T (2010) Adiponectin and AdipoR1 regulate PGC-1alpha and mitochondria by Ca(2+) and AMPK/SIRT1. Nature 464:1313–1319

Justesen J, Stenderup K, Ebbesen EN, Mosekilde L, Steiniche T, Kassem M (2001) Adipocyte tissue volume in bone marrow is increased with aging and in patients with osteoporosis. Biogerontology 2:165–171

Kennedy GC (1953) The role of depot fat in the hypothalamic control of food intake in the rat. Proc R Soc Lond B Biol Sci 140:578–596

Kovacova Z, Tencerova M, Roussel B, Wedellova Z, Rossmeislova L, Langin D, Polak J, Stich V (2012) The impact of obesity on secretion of adiponectin multimeric isoforms differs in visceral and subcutaneous adipose tissue. Int J Obes (Lond) 36:1360–1365

Lafontan M (2013) Differences between subcutaneous and visceral adipose tissues. In: Bastard JP, Fève B (eds) Physiology and physiopathology of adipose tissue. Springer-Verlag, France, pp 329–350

Lee P, Werner CD, Kebebew E, Celi FS (2014) Functional thermogenic beige adipogenesis is inducible in human neck fat. Int J Obes (Lond) 38:170–176

Li S, Shin HJ, Ding EL, van Dam RM (2009) Adiponectin levels and risk of type 2 diabetes: a systematic review and meta-analysis. JAMA 302:179–188

Liu X, Zheng Z, Zhu X, Meng M, Li L, Shen Y, Chi Q, Wang D, Zhang Z, Li C, Li Y, Xue Y (2013) Brown adipose tissue transplantation improves whole-body energy metabolism. Cell Res 23:851–854

Lo JC, Ljubicic S, Leibiger B, Kern M, Leibiger IB, Moede T, Kelly ME, Chatterjee Bhowmick D, Murano I, Cohen P, Banks AS, Khandekar MJ, Dietrich A, Flier JS, Cinti S, Bluher M, Danial NN, Berggren PO, Spiegelman BM (2014) Adipsin is an adipokine that improves beta cell function in diabetes. Cell 158:41–53

Lord GM, Matarese G, Howard JK, Baker RJ, Bloom SR, Lechler RI (1998) Leptin modulates the T-cell immune response and reverses starvation-induced immunosuppression. Nature 394:897–901

Lowell, B.B., V, S.S., Hamann, A., Lawitts, J.A., Himms-Hagen, J., Boyer, B.B., Kozak, L.P., and Flier, J.S. (1993). Development of obesity in transgenic mice after genetic ablation of brown adipose tissue. Nature 366, 740–742.

Maeda K, Okubo K, Shimomura I, Funahashi T, Matsuzawa Y, Matsubara K (1996) cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1 (AdiPose Most abundant Gene transcript 1). Biochem Biophys Res Commun 221:286–289

Mauer J, Chaurasia B, Goldau J, Vogt MC, Ruud J, Nguyen KD, Theurich S, Hausen AC, Schmitz J, Bronneke HS, Estevez E, Allen TL, Mesaros A, Partridge L, Febbraio MA, Chawla A, Wunderlich FT, Bruning JC (2014) Signaling by IL-6 promotes alternative activation of macrophages to limit endotoxemia and obesity-associated resistance to insulin. Nat Immunol 15:423–430

Montague CT, Farooqi IS, Whitehead JP, Soos MA, Rau H, Wareham NJ, Sewter CP, Digby JE, Mohammed SN, Hurst JA, Cheetham CH, Earley AR, Barnett AH, Prins JB, O'Rahilly S (1997) Congenital leptin deficiency is associated with severe early-onset obesity in humans. Nature 387:903–908

Motoshima H, Wu X, Sinha MK, Hardy VE, Rosato EL, Barbot DJ, Rosato FE, Goldstein BJ (2002) Differential regulation of adiponectin secretion from cultured human omental and subcutaneous adipocytes: effects of insulin and rosiglitazone. J Clin Endocrinol Metab 87:5662–5667

Nguyen KD, Qiu Y, Cui X, Goh YP, Mwangi J, David T, Mukundan L, Brombacher F, Locksley RM, Chawla A (2011) Alternatively activated macrophages produce catecholamines to sustain adaptive thermogenesis. Nature 480:104–108

Ouchi N, Parker JL, Lugus JJ, Walsh K (2011) Adipokines in inflammation and metabolic disease. Nat Rev Immunol 11:85–97

Patel L, Buckels AC, Kinghorn IJ, Murdock PR, Holbrook JD, Plumpton C, Macphee CH, Smith SA (2003) Resistin is expressed in human macrophages and directly regulated by PPAR gamma activators. Biochem Biophys Res Commun 300:472–476

Pou KM, Massaro JM, Hoffmann U, Vasan RS, Maurovich-Horvat P, Larson MG, Keaney JF Jr, Meigs JB, Lipinska I, Kathiresan S, Murabito JM, O'Donnell CJ, Benjamin EJ, Fox CS (2007) Visceral and subcutaneous adipose tissue volumes are cross-sectionally related to markers of inflammation and oxidative stress: the Framingham Heart Study. Circulation 116:1234–1241

Qiao L, Yoo H, Bosco C, Lee B, Feng GS, Schaack J, Chi NW, Shao J (2014) Adiponectin reduces thermogenesis by inhibiting brown adipose tissue activation in mice. Diabetologia 57:1027–1036

Qiu Y, Nguyen KD, Odegaard JI, Cui X, Tian X, Locksley RM, Palmiter RD, Chawla A (2014) Eosinophils and type 2 cytokine signaling in macrophages orchestrate development of functional beige fat. Cell 157:1292–1308

Quagliarini F, Wang Y, Kozlitina J, Grishin NV, Hyde R, Boerwinkle E, Valenzuela DM, Murphy AJ, Cohen JC, Hobbs HH (2012) Atypical angiopoietin-like protein that regulates ANGPTL3. Proc Natl Acad Sci U S A 109:19751–19756

Rao RR, Long JZ, White JP, Svensson KJ, Lou J, Lokurkar I, Jedrychowski MP, Ruas JL, Wrann CD, Lo JC, Camera DM, Lachey J, Gygi S, Seehra J, Hawley JA, Spiegelman BM (2014) Meteorin-like is a hormone that regulates immune-adipose interactions to increase beige fat thermogenesis. Cell 157:1279–1291

Ren G, Kim JY, Smas CM (2012) Identification of RIFL, a novel adipocyte-enriched insulin target gene with a role in lipid metabolism. Am J Physiol Endocrinol Metab 303:E334–E351

Rosell M, Hondares E, Iwamoto S, Gonzalez FJ, Wabitsch M, Staels B, Olmos Y, Monsalve M, Giralt M, Iglesias R, Villarroya F (2012) Peroxisome proliferator-activated receptors-alpha and -gamma, and cAMP-mediated pathways, control retinol-binding protein-4 gene expression in brown adipose tissue. Endocrinology 153:1162–1173

Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HF (1995) A novel serum protein similar to C1q, produced exclusively in adipocytes. J Biol Chem 270:26746–26749
Schraw T, Wang ZV, Halberg N, Hawkins M, Scherer PE (2008) Plasma adiponectin complexes have distinct biochemical characteristics. Endocrinology 149:2270–2282
Shapiro L, Scherer PE (1998) The crystal structure of a complement-1q family protein suggests an evolutionary link to tumor necrosis factor. Curr Biol 8:335–338
Shoelson SE, Lee J, Goldfine AB (2006) Inflammation and insulin resistance. J Clin Invest 116:1793–1801
Silva JE, Larsen PR (1985) Potential of brown adipose tissue type II thyroxine 5′-deiodinase as a local and systemic source of triiodothyronine in rats. J Clin Invest 76:2296–2305
Stanford KI, Middelbeek RJ, Townsend KL, An D, Nygaard EB, Hitchcox KM, Markan KR, Nakano K, Hirshman MF, Tseng YH, Goodyear LJ (2013) Brown adipose tissue regulates glucose homeostasis and insulin sensitivity. J Clin Invest 123:215–223
Steinberg GR, Parolin ML, Heigenhauser GJ, Dyck DJ (2002) Leptin increases FA oxidation in lean but not obese human skeletal muscle: evidence of peripheral leptin resistance. Am J Physiol Endocrinol Metab 283:E187–E192
Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, Patel HR, Ahima RS, Lazar MA (2001) The hormone resistin links obesity to diabetes. Nature 409:307–312
Steppan CM, Wang J, Whiteman EL, Birnbaum MJ, Lazar MA (2005) Activation of SOCS-3 by resistin. Mol Cell Biol 25:1569–1575
Tan BK, Adya R, Randeva HS (2010) Omentin: a novel link between inflammation, diabesity, and cardiovascular disease. Trends Cardiovasc Med 20:143–148
Villarroya J, Cereijo R, Villarroya F (2013) An endocrine role for brown adipose tissue? Am J Physiol Endocrinol Metab 305:E567–E572
Virtue S, Feldmann H, Christian M, Tan CY, Masoodi M, Dale M, Lelliott C, Burling K, Campbell M, Eguchi N, Voshol P, Sethi JK, Parker M, Urade Y, Griffin JL, Cannon B, Vidal-Puig A (2012) A new role for lipocalin prostaglandin d synthase in the regulation of brown adipose tissue substrate utilization. Diabetes 61:3139–3147
Wajchenberg BL (2000) Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. Endocr Rev 21:697–738
Whittle AJ, Carobbio S, Martins L, Slawik M, Hondares E, Vazquez MJ, Morgan D, Csikasz RI, Gallego R, Rodriguez-Cuenca S, Dale M, Virtue S, Villarroya F, Cannon B, Rahmouni K, Lopez M, Vidal-Puig A (2012) BMP8B increases brown adipose tissue thermogenesis through both central and peripheral actions. Cell 149:871–885
Yamauchi T, Kamon J, Minokoshi Y, Ito Y, Waki H, Uchida S, Yamashita S, Noda M, Kita S, Ueki K, Eto K, Akanuma Y, Froguel P, Foufelle F, Ferre P, Carling D, Kimura S, Nagai R, Kahn BB, Kadowaki T (2002) Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nat Med 8:1288–1295
Yang Q, Graham TE, Mody N, Preitner F, Peroni OD, Zabolotny JM, Kotani K, Quadro L, Kahn BB (2005) Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. Nature 436:356–362
Yang RZ, Lee MJ, Hu H, Pray J, Wu HB, Hansen BC, Shuldiner AR, Fried SK, McLenithan JC, Gong DW (2006) Identification of omentin as a novel depot-specific adipokine in human adipose tissue: possible role in modulating insulin action. Am J Physiol Endocrinol Metab 290: E1253–E1261
Yi P, Park JS, Melton DA (2013) Betatrophin: a hormone that controls pancreatic beta cell proliferation. Cell 153:747–758
Youn BS, Kloting N, Kratzsch J, Lee N, Park JW, Song ES, Ruschke K, Oberbach A, Fasshauer M, Stumvoll M, Bluher M (2008) Serum vaspin concentrations in human obesity and type 2 diabetes. Diabetes 57:372–377
Zhang R (2012) Lipasin, a novel nutritionally-regulated liver-enriched factor that regulates serum triglyceride levels. Biochem Biophysical Res Commun 424:786–792
Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM (1994) Positional cloning of the mouse obese gene and its human homologue. Nature 372:425–432

cGMP and Brown Adipose Tissue

Linda S. Hoffmann, Christopher J. Larson, and Alexander Pfeifer

Contents

L.S. Hoffmann
Institute of Pharmacology and Toxicology, University Hospital, University of Bonn, 53127 Bonn, Germany

C.J. Larson
Cardiovascular and Metabolic Diseases, Drug Discovery Unit, Takeda Pharmaceuticals, San Diego, CA 92121, USA

A. Pfeifer (✉)
Institute of Pharmacology and Toxicology, University Hospital, University of Bonn, 53127 Bonn, Germany

NRW International Graduate Research School Biotech PHARMA, Bonn, Germany

PharmaCenter, University of Bonn, 53127 Bonn, Germany
e-mail: alexander.pfeifer@uni-bonn.de

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Abstract

The second messenger cyclic guanosine monophosphate (cGMP) is a key mediator in physiological processes such as vascular tone, and its essential involvement in pathways regulating metabolism has been recognized in recent years. Here, we focus on the fundamental role of cGMP in brown adipose tissue (BAT) differentiation and function. In contrast to white adipose tissue (WAT), which stores energy in the form of lipids, BAT consumes energy stored in lipids to generate heat. This so-called non-shivering thermogenesis takes place in BAT mitochondria, which express the specific uncoupling protein 1 (UCP1). The energy combusting properties of BAT render it a promising target in antiobesity strategies in which BAT could burn the surplus energy that has accumulated in obese and overweight individuals. cGMP is generated by guanylyl cyclases upon activation by nitric oxide or natriuretic peptides. It affects several downstream molecules including cGMP-receptor proteins such as cGMP-dependent protein kinase and is degraded by phosphodiesterases. The cGMP pathway contains several signaling molecules that can increase cGMP signaling, resulting in activation and recruitment of brown adipocytes, and hence can enhance the energy combusting features of BAT. In this review we highlight recent results showing the physiological significance of cGMP signaling in BAT, as well as pharmacological options targeting cGMP signaling that bear a high potential to become BAT-centered therapies for the treatment of obesity.

Keywords

Brown adipose tissue · cGMP · Inducible brown adipocytes · PDE5 inhibitors · PKGI · sGC · Thermogenesis · UCP1 · White adipose tissue

1 cGMP Signaling

The second messenger cyclic guanosine monophosphate (cGMP) is synthesized from guanosine triphosphate by guanylyl cyclases (GC), which exist as membrane bound (pGC) or soluble forms (sGC). pGCs are activated by the natriuretic peptides (NP) atrial NP (ANP), brain NP (BNP), and C-type NP (CNP) (Potter 2011). The clearance receptor NPR-C also binds NPs but lacks a cyclase domain. Nevertheless, recent evidence suggests that it also contributes to signaling pathways (Anand-Srivastava 2005; Azer et al. 2012). GC-A and GC-B are the two cGMP-synthesizing isoforms expressed in mammals. sGC is a heterodimeric hemeprotein consisting of an α-subunit (α_1 or α_2) and a heme-containing β-subunit (Derbyshire and Marletta 2012). In the reduced Fe^{2+} state, sGC is activated by its endogenous ligand nitric oxide (NO). Upon oxidation, which happens during increased oxidative stress, the heme group is oxidized and unresponsive to NO. The gaseous molecule NO is endogenously produced by NO synthases (NOS) of which three isoforms have been identified: endothelial NOS (eNOS), neuronal NOS (nNOS), and inducible NOS (iNOS) (Murad 2006).

The downstream effects of cGMP are mediated by cGMP-dependent protein kinases (PKGI or PKGII), cyclic nucleotide-gated channels (CNG), and phosphodiesterases (PDE) (Francis et al. 2010). PDEs break down cGMP but can also be activated or inhibited by cGMP or its sibling cyclic adenosine phosphate (cAMP), which allows crosstalk between cGMP and cAMP signaling pathways (Francis et al. 2011). PKG is a major transmitter of cGMP signaling in many cells and phosphorylates a variety of substrates, thereby regulating important physiological processes such as blood pressure, learning, platelet aggregation (Schlossmann and Desch 2009), and metabolic control. Here, we review the role of the cGMP pathway in brown adipose tissue (BAT).

2 BAT and the Brown-Beige Debate

2.1 Brown Adipose Tissue

BAT can produce heat from energy stored in lipids by a process termed non-shivering thermogenesis (NST). The main function of BAT is to maintain the body temperature of mammals; hence, it is highly abundant in newborns yet remains active in adults (Cypess et al. 2009, 2013; Saito et al. 2009; van Marken Lichtenbelt et al. 2009; Virtanen et al. 2009). Mitochondria are the powerhouses for NST and are highly abundant in brown adipocytes (BA). Together with the high density of vessels, mitochondria give BAT its brownish color. Uncoupling protein 1 (UCP1) is a special protein in BAT mitochondria that drives NST. It is expressed in the inner mitochondrial membrane and can be activated by free fatty acids (FFA), which are released from lipids stored in multilocular droplets within BA (Cannon and Nedergaard 2004). Lipolysis is initiated by cold exposure leading to activation of sympathetic nerves innervating BAT. Sympathetic nerves release norepinephrine (NE) which activates β-adrenoceptors (AR) on BA. Recent evidence shows sympathetic cotransmission of NE and adenosine, which activates A_{2A} receptors on human and murine BA (Gnad et al. 2014). Activation of G_s-coupled receptors induces cAMP production, which in turn drives FFA liberation (Robidoux et al. 2004). Once activated, UCP1 disrupts the mitochondrial proton gradient resulting in production of heat instead of ATP. Besides cold stimulation, BAT might also be activated by food ingestion resulting in diet-induced thermogenesis (DIT), but this concept is still a matter of debate (Kozak 2010; Cannon and Nedergaard 2011; Vosselman et al. 2013). In addition to β-adrenergic and adenosine receptor signaling pathways, BAT is regulated by hormones such as melanocortin or by thyroid hormones (Vaughan et al. 2011; Lahesmaa et al. 2014).

Activation of BAT results in depletion of the intracellular BA lipid stores, and lipids from other peripheral tissues are transported to BAT. It has been shown that activated BAT takes up 50% of nutrient lipids (Bartelt and Heeren 2012). Thus, BAT functions as a major sink for lipids and dissipates the contained energy. BAT

contributes significantly to whole-body energy expenditure (EE), i.e., the amount of energy needed for food digestion, breathing, heat production, and physical activity.

2.2 White Adipose Tissue

In contrast to BAT, the major task of white adipose tissue (WAT) is to store energy in the triacylglycerol form of lipids. WAT takes up lipids through specialized transporters such as CD36 or fatty acid transport proteins or synthesizes lipids through de novo lipogenesis (Lobo et al. 2007; Bartelt et al. 2013). Lipids are stored in big unilocular droplets that fill up white adipocytes (WA). When energy intake persistently exceeds EE, as in the positive energy balance state underlying overweight and obesity, surplus energy will be stored in WAT, from where it can be released in times of energy shortage or negative energy balance. Lipolysis makes FFA available and is driven by hormone-sensitive lipase and perilipins (Large et al. 2004). In addition to its energy-storing properties, WAT functions as an endocrine organ and secretes adipokines such as leptin, TNFα, or interleukins, which can affect metabolism and can increase systemic inflammation. WAT also secretes adipokines with beneficial effects on whole-body metabolism such as adiponectin (Kwon and Pessin 2013; Turer and Scherer 2012). This topic is covered in another chapter of this volume. In humans it was estimated that activation of BAT could result in EE equivalent to approximately 4 kg fat tissue during a year (Virtanen et al. 2009).

2.3 Inducible Brown Adipocytes and Beige/Brite Adipocytes

BAT consists of constitutive classical BA, which are present at birth (Pfeifer and Hoffmann 2014), along with a second type of BA discovered in humans and mice in the recent years: inducible BA (iBA) or beige/brite (brown in white) cells that appear within WAT depots. iBA share many features with classical BA: they have multilocular lipid droplets, are rich in mitochondria, and express UCP1 highly, allowing them to combust significant amounts of energy in a nonproductive manner (Rosen and Spiegelman 2014; Frontini and Cinti 2010; Wu et al. 2013). iBA are induced by several physiological stimuli such as cold exposure or through treatment with pharmacological compounds such as β_3AR and peroxisome proliferator-activated receptor gamma (PPARγ) agonists (Vegiopoulos et al. 2012; Petrovic et al. 2010). Two mechanisms of browning have been identified (Rosenwald and Wolfrum 2014): iBA are formed through a classic differentiation cascade of iBA precursors residing in WAT or through transdifferentiation of WA (Lee et al. 2012; Barbatelli et al. 2010 and references in Rosenwald and Wolfrum 2014). The potential for browning is higher in inguinal WAT than in epididymal WAT and occurs in a mottled pattern in areas of particular adipose depots (Frontini and Cinti 2010; Rosenwald et al. 2013). iBA contribute to whole-body EE and protect mice from diet-induced obesity (Seale et al. 2011; Vegiopoulos et al. 2012).

Importantly, iBA also have been discovered in humans, where they were found in close vicinity to areas that contain classical BAT or within BAT depots (Wu et al. 2012; Jespersen et al. 2013).

iBA can revert to WA (Rosenwald et al. 2013). This process is known as "whitening" and can be observed for iBA and also in the classical BAT of obese mice. Whitening in BAT is characterized by an increase in lipid droplet size, a reduction in vascularization, and a decrease in VEGF expression compared to nonobese mice (Shimizu et al. 2014).

Pink adipocytes add another color to adipose tissue. These special adipocytes arise during pregnancy and lactation in subcutaneous WA of the mammary gland (Giordano et al. 2014). They not only produce milk but they also secrete leptin which might play a role in the regulation of obesity in the offspring (Giordano et al. 2014; Morroni et al. 2004).

In summary, adipose tissue is a highly dynamic tissue, with a white compartment that can expand and contract to match the energy supply and demand of the organism and a brown compartment comprised of both classical and inducible cell types, which shows high plasticity in number and activation of BA. Understanding the signaling pathways that regulate adipose tissue might reveal new targets for antiobesity therapies, which are highly needed in times of the obesity pandemic.

3 Central Nervous System Control of BAT

As with other organ systems in the body, BAT is under both neural and endocrine control. Autonomic regulation of BAT has been recently reviewed (Tupone et al. 2014), and its role in health and disease suggests potential clinical applications through altering BAT activity.

BAT sympathetic nerve system (SNS) activity controlling BAT thermogenic function is modulated by central nervous system neural networks that react to thermal afferent signaling from cutaneous and body core thermal receptor systems and also to changes in the firing of central neurons with intrinsic thermal reactivity. In addition to this basal network providing thermal regulation of BAT thermogenesis, a second system of central networks controlling metabolic aspects of energy homeostasis provides centrally derived neural inputs into peripheral BAT thermogenic action. The role of cGMP signaling in central control of energy balance has been most recently demonstrated through intraventricular application of CNP, which alters food intake by activation of the melanocortin system in mice (Yamada-Goto et al. 2013). In addition, intravenously applied BNP modulated ghrelin, hunger, and satiety in humans (Vila et al. 2012).

Central control of energy balance through activation of BAT also has been demonstrated by several recent cases. First, induction of UCP1 by central delivery of IL-6 requires autonomic sympathetic innervation of BAT and causes body weight reduction in rodents (Li et al. 2002). Second, central signaling originating from the calcitonin gene-related peptide (CGRP) and amylin receptors, which are

expressed in areas of the brain regulating energy balance, has been shown to regulate peripheral activity of BAT. Overexpressing RAMP1, one of several accessory proteins controlling the activity of these receptors through modulation of the ligand-binding specificity, decreases body weight, lowers fat mass and circulating leptin, elevates total body EE as measured by increased oxygen consumption, and raises body temperature and sympathetic tone into BAT. As well, the elevated BAT RNA levels of PPARγ coactivator 1 alpha (*PGC1α*), *UCP1*, and *UCP3* in these mice can be reversed by chronic blockade of sympathetic nervous system signaling (Zhang et al. 2011). Finally, blocking K_{ATP}-channel-dependent modulation of catecholaminergic neurons in the locus coeruleus (LC), which alter their firing frequency according to their external glycemic environment, by expressing a variant K_{ATP} channel enhances diet-induced obesity in mice through lower EE and BAT sympathetic tone and a decreased ability of centrally administered glucose to stimulate BAT sympathetic nerve activity (Tovar et al. 2013).

4 cGMP and Metabolic Control

The cGMP pathway is involved in the regulation of various physiological functions ranging from regulation of vascular tone, memory and hearing processes, and metabolic control. The crucial role of cyclic nucleotides in metabolism has long been appreciated with a special focus on cAMP which is essential for sympathetic activation of BAT. But the other cyclic nucleotide cGMP also plays a key role in adipose tissue. Here, we focus on the role of cGMP signaling in adipose tissue especially in brown adipose tissue.

4.1 Adipose Tissue

4.1.1 cGMP Downstream Signaling

Initially, though, the expression of PKGI in adipose tissue was questioned (Lutz et al. 2011). In contrast, several other papers described PKGI to be expressed in both BAT and WAT (Haas et al. 2009; Mitschke et al. 2013; Leiss et al. 2014). Importantly, in vivo studies during the last several years have proven the functional importance of cGMP/PKGI signaling for adipose tissue. As the major downstream target of cGMP, PKGI plays a central role in BAT, most obviously in mice lacking PKGI. These mice show dramatically reduced thermogenesis compared to WT littermates. BAT of $PKGI^{-/-}$ mice has dramatically reduced protein levels of the thermogenic key protein UCP1 and shows significantly decreased amounts of mitochondrial DNA (Haas et al. 2009). Molecular analysis of BA differentiated from the stromal vascular fraction of these $PKGI^{-/-}$ mice revealed that BA differentiation is dependent on functional cGMP signaling. Furthermore, abrogation of the cGMP pathway leads to downregulation of BA key features such as expression of thermogenic markers *UCP1* and *PGC1α* and abundance of

mitochondria. The adipogenic pathway in PKGI-deficient BA is impaired as well: lipid incorporation and adipogenic marker expression are significantly reduced in PKGI$^{-/-}$ cells compared to WT cells. Importantly, with the use of PKGI$^{-/-}$ BA, a crosstalk between cGMP and insulin signaling was revealed that explained the great impact of PKGI deficiency on BA differentiation and function. In BA, Ras homolog gene family member A (RhoA), activates Rho-associated protein kinase (ROCK) which in turn inhibits the insulin receptor substrate 1 (IRS-1). IRS-1 initiates a signaling cascade that transmits insulin binding to its receptor into mitochondrial biogenesis and adipogenic differentiation. PKGI inhibits RhoA and thereby releases the inhibitory effect of RhoA/ROCK on insulin signaling. Hence, insulin signaling in PKGI$^{-/-}$ BA is hampered resulting in the observed defects in differentiation and function (Haas et al. 2009).

In contrast, global overexpression of PKGI results in increased mitochondrial DNA content and upregulation of *PGC1α* and *UCP1* in BAT (Miyashita et al. 2009). This seems to result in increased thermogenesis as PKGI transgenic mice display a higher body temperature. Importantly, PKGI transgenic mice are leaner than WT littermates and protected against diet-induced obesity which is accompanied by improved insulin sensitivity (Miyashita et al. 2009; Nikolic et al. 2009).

In addition to BA, PKGI also plays an important role in WA. Ablation of PKGI in primary murine adipocytes represses adipogenesis resulting in decreased lipid storage and reduced expression of the adipogenic markers PPARγ and adipocyte protein 2 (aP2) (Mitschke et al. 2013). Similar to the situation in BA, mitochondrial markers *UCP1* and cytochrome c are also reduced in PKGI$^{-/-}$ WA. In addition to its role in adipogenesis and mitochondrial biogenesis, the cGMP pathway also influences the endocrine function of WA. Overexpression of PKG in WA results in a reduction of pro-inflammatory cytokines such as monocyte chemoattractant protein 1 (*MCP1*) and *IL-6* and increases the antidiabetic and anti-inflammatory adipokine adiponectin (*Adipoq*) (Caselli 2014; Mitschke et al. 2013). Taken together, the studies on PKGI$^{-/-}$ mice and cells clearly show that the cGMP pathway is an essential enhancer of BA differentiation. Moreover, the elegant studies on PKG transgenic mice (Miyashita et al. 2009) and on adipocytes incubated with cGMP (Haas et al. 2009) show that cGMP activation results in "super" BAT.

Vasodilator-stimulated phosphoprotein (VASP) is phosphorylated by PKGI and plays an important and unexpected role in BAT through regulation of cGMP signaling. As PKGI$^{-/-}$ BA show dysfunctional differentiation and function, the same was anticipated for BA lacking VASP. Surprisingly, VASP$^{-/-}$ BA show increased adipogenesis and have an upregulated thermogenic program resulting in enhanced BA function: Cellular respiration and lipolysis are significantly higher in VASP$^{-/-}$ cells than in WT cells (Jennissen et al. 2011). In WT cells, VASP blocks Rac-mediated activation of sGC-gene transcription in a negative feedback loop (Jennissen et al. 2011). In VASP$^{-/-}$ cells, sGC promoter activity is upregulated resulting in increased cGMP signaling. This pathway is also active in WA. VASP$^{-/-}$ mice are leaner and have increased EE. Importantly, activation of the cGMP pathway through ablation of the inhibitory effect of VASP on sGC expression

results in browning of WAT in VASP$^{-/-}$. Taken together, the results obtained from VASP$^{-/-}$ cells and mice demonstrate the central role of cGMP in regulating BA differentiation and function and in inducing and recruiting BA.

Apart from PKGI and VASP, PDEs that break down cGMP levels also have been shown to regulate BAT function. PDE5 is the major PDE for the breakdown of cGMP and is expressed in adipose tissue (Moro et al. 2007). Inhibition of PDE5 by sildenafil in WA resulted in upregulation of the BA markers UCP1 and PGC1α, indicating browning of WA (Mitschke et al. 2013). In addition, an antidiabetic potential was observed in a WA cell line that showed increased basal and insulin-stimulated glucose uptake after incubation with sildenafil (Zhang et al. 2010).

4.1.2 cGMP Upstream Signaling

Concerning the upstream regulation of cGMP signaling, a major focus was on NOS and NO. Exogenously applied NO results in the upregulation of *PGC1α* and *UCP1*, mitochondrial DNA, and the mitochondrial markers nuclear respiratory factor 1 (*Nrf1*) and mitochondrial transcription factor A (*mtTFA*) (Nisoli et al. 1998, 2003). Inhibition of the NO receptor sGC with ODQ abrogates these effects (Nisoli et al. 1998, 2003). In BAT of mice deficient for eNOS, the major NOS in BAT (Haas et al. 2009), mitochondrial content is reduced and lipid droplets are enlarged pointing to whitening (Nisoli et al. 2003). eNOS$^{-/-}$ mice gain more weight than WT littermates and have reduced EE (Nisoli et al. 2003). Overexpression of sGC results in improved BAT function and WAT browning (Jennissen et al. 2011). Apart from NO/sGC, also NPs induce cGMP production in BAT and have been shown to be involved in the induction of iBA and increased BA function. In a human BA cell line, ANP and BNP increased thermogenic marker expression and cellular respiration (Bordicchia et al. 2012). Enhancing NP signaling by deletion of the clearance receptor NPR-C results in reduction of adipose tissue mass in mice (Bordicchia et al. 2012).

5 cGMP and Mitochondrial Biology

The relationship between cGMP signaling and mitochondrial biology is complex. The stimulatory role of NO/sGC/cGMP signaling toward mitochondrial biogenesis can be measured in cellular lineages beyond BA, such as 3T3-L1, U937, and HeLa cells, is mediated by the transcriptional coactivator PGC1α (Nisoli et al. 2003), and can be exploited to protect and restore the function of highly respiring tissues such as the kidney (Whitaker et al. 2013). The NO/sGMP pathway stimulation of mitochondria extends even outside the animal kingdom to enhance mitochondrial respiration in plants such as *Arabidopsis* (Wang et al. 2010). Beyond the NO/sGC/cGMP input to mitochondrial biogenesis, NO inhibits mitochondrial respiration by competing with O_2 at cytochrome oxidase, and reactive nitrogen derivatives of NO irreversibly inhibit multiple sites on cytochrome oxidase. As well, NO/sGC/cGMP-mediated vasodilation drives mitochondria respiration through increased circulatory delivery of substrate and oxygen, and reactive nitrogen derivatives of NO can

activate the mitochondrial permeability transition pore, leading to apoptosis or necrosis (Brown 2007).

As noted above, cGMP plays an important role in the biology of classical and inducible BAT, stimulating BA differentiation and mitochondrial biogenesis as well as healthy expansion and browning of WAT (Mitschke et al. 2013; Haas et al. 2009). These data have been extended to human WAT (De Toni et al. 2011) upon the observation that human adipose tissue contains an intact signaling system of NO/sGC/cGMP/PKGI, as well as PDE5, the main cGMP catabolizing enzyme. Treating cultured human omental adipose tissue explants with the PDE5 inhibitor vardenafil increased PPARγ, PGC1α, and mitochondrial DNA levels, suggesting a beneficial effect on energy metabolism in human white adipose tissue (De Toni et al. 2011).

Given the ancestral relationship between BAT and the skeletal muscle (Seale et al. 2008), it is not surprising that cGMP signaling promotes mitochondrial biogenesis in the skeletal muscle in a manner analogous to that in BAT. In vitro, cGMP rescues mitochondrial dysfunction induced by glucose and insulin in myocytes (Mitsuishi et al. 2008). In C2C12 myotubular cells with mitochondrial dysfunction generated by high-glucose and high-insulin treatment, cGMP treatment elevated genes involved in oxidative phosphorylation and ROS reduction, resulting in increased mitochondrial biogenesis and ATP production with no increase in ROS levels. Likewise, in cultured rat L6 myotubes, NO/cGMP-dependent mitochondrial biogenesis correlates with coupled respiration and content of ATP, with the latter not generated from glycolysis (Nisoli et al. 2004). In the same report, the gastrocnemius muscle from $eNOS^{-/-}$ mice exhibited reduced mitochondrial content, smaller mitochondria, and a decrease in the number of mitochondria in the subsarcolemmal region of the gastrocnemius muscle. These results are consistent with findings from other investigators demonstrating NOS expression in the sarcolemma, sarcoplasmic reticulum, and mitochondria regions, suggesting a paracrine nature of NO/cGMP signaling in the skeletal muscle that contributes to increased cGMP-dependent mitochondrial functioning (Buchwalow et al. 2005). Lastly, studies with transgenic mice have demonstrated that chronic in vivo activation of natriuretic peptides/cGMP/cGMP-dependent protein kinase cascades promotes increased muscle mitochondrial content and fat oxidation through elevated PPARδ and PGC1α content, which work to lower glucose intolerance and body weight (Miyashita et al. 2009).

As BAT and skeletal muscle together comprise a large fraction of the total EE in lean individuals, augmenting the metabolic actions of these tissues, particularly those of BAT, represents a promising therapeutic strategy for the treatment of obesity and its associated comorbidities.

6 BAT-Centered Antiobesity Therapies

The global obesity epidemic continues to expand, with prevalence of disease and its comorbidities now increasing in both developed and developing nations. The comorbidities and associated economic burdens bring substantial and growing healthcare and financial consequences throughout the globe (Imes and Burke 2014). Weight loss strategies for the treatment of obesity escalate from lifestyle approaches including diet, physical activity, and behavior change therapies to pharmacological agents approved as adjuncts to lifestyle modification to bariatric surgery, demonstrated to be the most effective and persistent treatment for individuals with severe obesity or moderate obesity complicated by comorbid conditions refractory to other approaches (Kushner 2014). Current pharmacotherapy for obesity has been recently reviewed and will not be detailed here (Gadde 2014). With increases in obesity prevalence in most of the developed world continuing, and the largest increases now being seen in developing countries, new antiobesity therapies – including pharmacotherapies – are needed.

Activation and recruitment of BAT represents a promising strategy for obesity treatment in humans, as BAT is the primary source of sympathetically activated adaptive thermogenesis during cold exposure thus modulating whole-body EE and adiposity, with an inverse relationship between BAT activity and adiposity implying that the energy-dissipating activity of BAT defends against body fat accumulation and potentially glucose intolerance (Contreras et al. 2014). As noted earlier, both autonomic sympathetic nervous system activity and endocrine factors have been demonstrated to affect BAT activity and thus provide guidance toward novel therapeutic strategies (Lidell et al. 2014).

A number of BAT-centered antiobesity potential strategies have proven unsuccessful, for a variety of reasons. β_3-adrenoceptor agonists are efficacious antiobesity and insulin-sensitizing agents acting in WAT, BAT, and muscles. However, the selectivity needed to avoid $\beta_{1/2}$-adrenoceptor-mediated side effects has been shown to be challenging (Arch 2002). Analogs of fibroblast growth factor 21 (FGF21), which act in part through BAT activation, have been advanced into early clinical trials recently but have provided only modest metabolic improvements in patients (Gaich et al. 2013). A PGC1α-dependent myokine irisin, cleaved from the membrane protein FNDC5, generated significant excitement due to its ability to stimulate browning of WAT and thermogenesis (Bostrom et al. 2012), though recently a number of published and unpublished reports suggest a more complicated biology with less clear therapeutic implications that had been originally hoped. Finally, increased T3 production through elevating peripheral deiodinase 2 (Dio2) levels, such as can be achieved by agonism of the bile acid receptor TGR5, would be predicted to increase EE through increased thermogenesis and thus decrease body weight (Mullur et al. 2014). However, few pharmacological TGR5 programs have advanced into clinical development due to frequent observations of gallbladder fibrosis in nonclinical species.

Given its importance in classical and inducible BA and the potential relevance of these cells in weight management, modulating cGMP signaling may represent an

important goal for an antiobesity pharmacotherapy. Drugs that target the cGMP pathway have already proven to be safe and are approved for clinical use.

Among these are PDE5 inhibitors that have been used to treat erectile dysfunction and pulmonary hypertension for more than 15 years (Huang and Lie 2013). PDE5 inhibitors have been shown to induce browning in mice. A short-term treatment with the PDE5 inhibitor sildenafil for only 7 days significantly increased UCP1 and PGC1α expression and induced iBA in WATi of WT mice (Mitschke et al. 2013). Importantly, this short time of treatment shows that molecular changes take place relatively early and indicated that a long-term treatment might have an influence on whole-body energy metabolism. Interestingly, when mice on a high-fat diet were treated with sildenafil, weight gain was significantly lower and EE was significantly higher than in untreated obese mice (Ayala et al. 2007). Unfortunately, WAT of these mice was not analyzed in this study. Taken together, the two studies point to a potential of PDE5 inhibitors to induce browning and thereby induce weight loss in mice. Nevertheless, data from humans are still missing.

The potential for browning also extends to PDE3 and PDE4 which regulate cAMP inhibition. After addition of the PDE3 inhibitor cilostamide and the PDE4 inhibitor rolipram, UCP1 expression increased in BAT of mice and in in vitro differentiated BA (Kraynik et al. 2013).

cGMP levels can be elevated not only by PDE5 inhibition but also via activation of pGC by NP. Transgenic overexpression of BNP in mice challenged with a high-fat diet results in decreased WAT mass; increases body temperature, mitochondrial biogenesis, and *UCP1* and *PGC1α* expression in BAT; and induces mitochondrial biogenesis in the muscle (Miyashita et al. 2009). Furthermore, short-term infusion of BNP for 7 days in mice results in significantly upregulated EE and increased thermogenic marker gene expression in BAT and WAT (Bordicchia et al. 2012). In humans and isolated human WA, NPs induce lipolysis which can provide fuel in the form of FFA for oxidative tissues such as BAT and the muscle (see references in Schlueter et al. 2014). Interestingly, a connection between weight loss and ANP and BNP has been shown in type 2 diabetes patients that were treated with the antidiabetic drug liraglutide (Li et al. 2014). Weight loss in this study was positively correlated with an increase in plasma ANP levels. Furthermore, BNP levels were increased after liraglutide-induced weight loss. However, the significant renal and cardiovascular actions of natriuretic peptides in reducing arterial blood pressure as well as sodium reabsorption could potentially limit their application to modulating biological processes beyond hemodynamic regulation.

Recently, bone morphogenic protein 7 (BMP7) and BMP8B have been shown to have effects on BAT development and energy balance and thus may represent a novel approach to BAT-mediated antiobesity therapy (Saini et al. 2014; Whittle et al. 2012).

Finally, the canonical ventromedial nucleus of the hypothalamus (VMH) AMP-activated protein kinase (AMPK)-SNS-BAT axis has long been regarded as an attractive hypothalamic and peripheral obesity target, particularly through its direct and indirect actions in BAT (Klaus et al. 2012), but the challenges of

developing a drug-like, selective kinase activator have precluded advancement into significant clinical development.

7 BAT-Centered Antidiabetic Therapies

Obesity is accompanied by many comorbidities such as type 2 diabetes, certain types of cancer, and cardiovascular diseases (Cao 2010). The prevalence of type 2 diabetes is increasing in the recent years and, just like obesity, is a major burden to the global health system (Hu 2011). BAT has been shown to have antidiabetic functions. Recently it has been shown in humans that activated BAT not only increases EE but importantly improves plasma glucose oxidation and insulin sensitivity (Chondronikola et al. 2014). As well, increases in BAT activity through acute cold exposure and increases in BAT volume through long-term exposure can modulate insulin sensitivity in humans (Lee et al. 2014), an important observation given the persistent need for insulin sensitizers as disease-modifying antidiabetic drugs (Larson 2014). This suggests the aforementioned BAT-mediated antiobesity strategies may also be indicated to address the global diabetes epidemic, a critical point since modern drug discovery has not resulted in agents that substantially reverse the molecular or cellular origins of either insulin resistance or loss of functional beta-cell mass. Diabetic pathophysiology can become disabling and even life threatening because it is concentrated in the circulatory and nervous systems, thereby resulting in heart disease; chronic kidney disease, and kidney failure; peripheral artery disease; neuropathies leading to ulcerations, infections, and amputation; and retinopathies triggering loss of vision. A recent encouraging report suggests that diabetic nephropathy might be beneficially addressed with PDE5 inhibitors that reduce glomerular hypertension and other hemodynamic aspects of diabetic nephropathy, as well as metabolic and inflammatory aspects of disease (Thompson 2013).

8 Conclusions

The cGMP second messenger system plays a crucial role in the functions of BAT and WAT, in the induction of iBA/beige adipocytes, and in the interplay between these cell types and tissue depots important for maintaining energetic balance in mammals, including humans. As mitochondria are the major source of energy in the mammalian cells, it is not surprising that the cGMP pathway plays a critical role in the activities of this organelle, though the relationship between cGMP signaling and mitochondrial biology is surprisingly complex. The cGMP pathway contributes to brain pathways underlying central metabolic control, as well as functioning downstream of the neural and endocrine inputs into these tissues. Modulating the cGMP pathway could lead to increased EE through BAT activation and recruitment. Hence, the cGMP pathway has a high therapeutic potential to be used in antiobesity therapies as both classical and inducible BAT can increase EE and thereby might be

protective against obesity and could possibly induce weight loss. Furthermore, cGMP signaling could be used to induce antidiabetic effects. Pharmacological or genetic enhancement of cGMP pathways has shown beneficial effects in models of obesity and diabetes. These effects might be translated into the clinics with the use of already available drugs targeting the cGMP pathway such as PDE5 inhibitors.

References

Anand-Srivastava MB (2005) Natriuretic peptide receptor-C signaling and regulation. Peptides 26:1044–1059, Epub 2005 Apr 8

Arch JR (2002) beta(3)-Adrenoceptor agonists: potential, pitfalls and progress. Eur J Pharmacol 440:99–107

Ayala JE, Bracy DP, Julien BM, Rottman JN, Fueger PT, Wasserman DH (2007) Chronic treatment with sildenafil improves energy balance and insulin action in high fat-fed conscious mice. Diabetes 56:1025–1033, Epub 2007 Jan 17

Azer J, Hua R, Vella K, Rose RA (2012) Natriuretic peptides regulate heart rate and sinoatrial node function by activating multiple natriuretic peptide receptors. J Mol Cell Cardiol 53:715–724

Barbatelli G, Murano I, Madsen L, Hao Q, Jimenez M, Kristiansen K, Giacobino JP, De Matteis R, Cinti S (2010) The emergence of cold-induced brown adipocytes in mouse white fat depots is determined predominantly by white to brown adipocyte transdifferentiation. Am J Physiol Endocrinol Metab 298:30

Bartelt A, Heeren J (2012) The holy grail of metabolic disease: brown adipose tissue. Curr Opin Lipidol 23:190–195

Bartelt A, Weigelt C, Cherradi ML, Niemeier A, Todter K, Heeren J, Scheja L (2013) Effects of adipocyte lipoprotein lipase on de novo lipogenesis and white adipose tissue browning. Biochim Biophys Acta 1831:934–942

Bordicchia M, Liu D, Amri EZ, Ailhaud G, Dessi-Fulgheri P, Zhang C, Takahashi N, Sarzani R, Collins S (2012) Cardiac natriuretic peptides act via p38 MAPK to induce the brown fat thermogenic program in mouse and human adipocytes. J Clin Invest 122:1022–1036

Bostrom P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC, Rasbach KA, Bostrom EA, Choi JH, Long JZ, Kajimura S, Zingaretti MC, Vind BF, Tu H, Cinti S, Hojlund K, Gygi SP, Spiegelman BM (2012) A PGC1-alpha-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. Nature 481:463–468

Brown GC (2007) Nitric oxide and mitochondria. Front Biosci 12:1024–1033

Buchwalow IB, Minin EA, Samoilova VE, Boecker W, Wellner M, Schmitz W, Neumann J, Punkt K (2005) Compartmentalization of NO signaling cascade in skeletal muscles. Biochem Biophys Res Commun 330:615–621

Cannon B, Nedergaard J (2004) Brown adipose tissue: function and physiological significance. Physiol Rev 84:277–359

Cannon B, Nedergaard J (2011) Nonshivering thermogenesis and its adequate measurement in metabolic studies. J Exp Biol 214:242–253

Cao Y (2010) Adipose tissue angiogenesis as a therapeutic target for obesity and metabolic diseases. Nat Rev Drug Discov 9:107–115

Caselli C (2014) Role of adiponectin system in insulin resistance. Mol Genet Metab 7192:00292–00293

Chondronikola M, Volpi E, Borsheim E, Porter C, Annamalai P, Enerback S, Lidell ME, Saraf MK, Labbe SM, Hurren NM, Yfanti C, Chao T, Andersen CR, Cesani F, Hawkins H, Sidossis LS (2014) Brown adipose tissue improves whole body glucose homeostasis and insulin sensitivity in humans. Diabetes 63:4089–4099

Contreras C, Gonzalez F, Ferno J, Dieguez C, Rahmouni K, Nogueiras R, Lopez M (2014) The brain and brown fat. Ann Med 10:1–19

Cypess AM, Lehman S, Williams G, Tal I, Rodman D, Goldfine AB, Kuo FC, Palmer EL, Tseng YH, Doria A, Kolodny GM, Kahn CR (2009) Identification and importance of brown adipose tissue in adult humans. N Engl J Med 360:1509–1517

Cypess AM, White AP, Vernochet C, Schulz TJ, Xue R, Sass CA, Huang TL, Roberts-Toler C, Weiner LS, Sze C, Chacko AT, Deschamps LN, Herder LM, Truchan N, Glasgow AL, Holman AR, Gavrila A, Hasselgren PO, Mori MA, Molla M, Tseng YH (2013) Anatomical localization, gene expression profiling and functional characterization of adult human neck brown fat. Nat Med 19:635–639

De Toni L, Strapazzon G, Gianesello L, Caretta N, Pilon C, Bruttocao A, Foresta C (2011) Effects of type 5-phosphodiesterase inhibition on energy metabolism and mitochondrial biogenesis in human adipose tissue ex vivo. J Endocrinol Invest 34:738–741

Derbyshire ER, Marletta MA (2012) Structure and regulation of soluble guanylate cyclase. Annu Rev Biochem 81:533–559

Francis SH, Busch JL, Corbin JD, Sibley D (2010) cGMP-dependent protein kinases and cGMP phosphodiesterases in nitric oxide and cGMP action. Pharmacol Rev 62:525–563

Francis SH, Blount MA, Corbin JD (2011) Mammalian cyclic nucleotide phosphodiesterases: molecular mechanisms and physiological functions. Physiol Rev 91:651–690

Frontini A, Cinti S (2010) Distribution and development of brown adipocytes in the murine and human adipose organ. Cell 11:253–256

Gadde KM (2014) Current pharmacotherapy for obesity: extrapolation of clinical trials data to practice. Expert Opin Pharmacother 15:809–822

Gaich G, Chien JY, Fu H, Glass LC, Deeg MA, Holland WL, Kharitonenkov A, Bumol T, Schilske HK, Moller DE (2013) The effects of LY2405319, an FGF21 analog, in obese human subjects with type 2 diabetes. Cell Metab 18:333–340

Giordano A, Smorlesi A, Frontini A, Barbatelli G, Cinti S (2014) White, brown and pink adipocytes: the extraordinary plasticity of the adipose organ. Eur J Endocrinol 170:13–0945

Gnad T, Scheibler S, von Kugelgen I, Scheele C, Kilic A, Glode A, Hoffmann LS, Reverte-Salisa L, Horn P, Mutlu S, El-Tayeb A, Kranz M, Deuther-Conrad W, Brust P, Lidell ME, Betz MJ, Enerback S, Schrader J, Yegutkin GG, Muller CE, Pfeifer A (2014) Adenosine activates brown adipose tissue and recruits beige adipocytes via A receptors. Nature 516:395–399

Haas B, Mayer P, Jennissen K, Scholz D, Diaz MB, Bloch W, Herzig S, Fassler R, Pfeifer A (2009) Protein kinase G controls brown fat cell differentiation and mitochondrial biogenesis. Sci Signal 2:ra78

Hu FB (2011) Globalization of diabetes: the role of diet, lifestyle, and genes. Diabetes Care 34:1249–1257

Huang SA, Lie JD (2013) Phosphodiesterase-5 (PDE5) inhibitors in the management of erectile dysfunction. P T 38:407–419

Imes CC, Burke LE (2014) The obesity epidemic: the United States as a cautionary tale for the rest of the world. Curr Epidemiol Rep 1:82–88

Jennissen K, Haas B, Kunz W, Pfeifer A (2011) cGMP and cAMP differentially regulate differentiation and function of brown adipocytes. BMC Pharmacol 11:P37

Jespersen NZ, Larsen TJ, Peijs L, Daugaard S, Homoe P, Loft A, de Jong J, Mathur N, Cannon B, Nedergaard J, Pedersen BK, Moller K, Scheele C (2013) A classical brown adipose tissue mRNA signature partly overlaps with brite in the supraclavicular region of adult humans. Cell Metab 17:798–805

Klaus S, Keipert S, Rossmeisl M, Kopecky J (2012) Augmenting energy expenditure by mitochondrial uncoupling: a role of AMP-activated protein kinase. Genes Nutr 7:369–386

Kozak LP (2010) Brown fat and the myth of diet-induced thermogenesis. Cell Metab 11:263–267

Kraynik SM, Miyaoka RS, Beavo JA (2013) PDE3 and PDE4 isozyme-selective inhibitors are both required for synergistic activation of brown adipose tissue. Mol Pharmacol 83:1155–1165

Kushner RF (2014) Weight loss strategies for treatment of obesity. Prog Cardiovasc Dis 56:465–472

Kwon H, Pessin JE (2013) Adipokines mediate inflammation and insulin resistance. Front Endocrinol 4:71

Lahesmaa M, Orava J, Schalin-Jantti C, Soinio M, Hannukainen JC, Noponen T, Kirjavainen A, Iida H, Kudomi N, Enerback S, Virtanen KA, Nuutila P (2014) Hyperthyroidism increases brown fat metabolism in humans. J Clin Endocrinol Metab 99:2013–2312

Large V, Peroni O, Letexier D, Ray H, Beylot M (2004) Metabolism of lipids in human white adipocyte. Diabetes Metab 30:294–309

Larson CJ (2014) The persistent need for insulin sensitizers and other disease-modifying anti-diabetic drugs. Expert Rev Endocrinol Metab 9:1–3

Lee YH, Petkova AP, Mottillo EP, Granneman JG (2012) In vivo identification of bipotential adipocyte progenitors recruited by beta3-adrenoceptor activation and high-fat feeding. Cell Metab 15:480–491

Lee P, Smith S, Linderman J, Courville AB, Brychta RJ, Dieckmann W, Werner CD, Chen KY, Celi FS (2014) Temperature-acclimated brown adipose tissue modulates insulin sensitivity in humans. Diabetes 63:3686–3698

Leiss V, Illison J, Domes K, Hofmann F, Lukowski R (2014) Expression of cGMP-dependent protein kinase type I in mature white adipocytes. Biochem Biophys Res Commun 452:151–156

Li G, Klein RL, Matheny M, King MA, Meyer EM, Scarpace PJ (2002) Induction of uncoupling protein 1 by central interleukin-6 gene delivery is dependent on sympathetic innervation of brown adipose tissue and underlies one mechanism of body weight reduction in rats. Neuroscience 115:879–889

Li CJ, Yu Q, Yu P, Yu TL, Zhang QM, Lu S, Yu DM (2014) Changes in liraglutide-induced body composition are related to modifications in plasma cardiac natriuretic peptides levels in obese type 2 diabetic patients. Cardiovasc Diabetol 13:1475–2840

Lidell ME, Betz MJ, Enerback S (2014) Brown adipose tissue and its therapeutic potential. J Intern Med 276:364–377

Lobo S, Wiczer BM, Smith AJ, Hall AM, Bernlohr DA (2007) Fatty acid metabolism in adipocytes: functional analysis of fatty acid transport proteins 1 and 4. J Lipid Res 48:609–620, Epub 2006 Dec 11

Lutz SZ, Hennige AM, Feil S, Peter A, Gerling A, Machann J, Krober SM, Rath M, Schurmann A, Weigert C, Haring HU, Feil R (2011) Genetic ablation of cGMP-dependent protein kinase type I causes liver inflammation and fasting hyperglycemia. Diabetes 60:1566–1576

Mitschke MM, Hoffmann LS, Gnad T, Scholz D, Kruithoff K, Mayer P, Haas B, Sassmann A, Pfeifer A, Kilic A (2013) Increased cGMP promotes healthy expansion and browning of white adipose tissue. FASEB J 27:1621–1630

Mitsuishi M, Miyashita K, Itoh H (2008) cGMP rescues mitochondrial dysfunction induced by glucose and insulin in myocytes. Biochem Biophys Res Commun 367:840–845

Miyashita K, Itoh H, Tsujimoto H, Tamura N, Fukunaga Y, Sone M, Yamahara K, Taura D, Inuzuka M, Sonoyama T, Nakao K (2009) Natriuretic peptides/cGMP/cGMP-dependent protein kinase cascades promote muscle mitochondrial biogenesis and prevent obesity. Diabetes 58:2880–2892

Moro C, Klimcakova E, Lafontan M, Berlan M, Galitzky J (2007) Phosphodiesterase-5A and neutral endopeptidase activities in human adipocytes do not control atrial natriuretic peptide-mediated lipolysis. Br J Pharmacol 152:1102–1110, Epub 2007 Oct 1

Morroni M, Giordano A, Zingaretti MC, Boiani R, De Matteis R, Kahn BB, Nisoli E, Tonello C, Pisoschi C, Luchetti MM, Marelli M, Cinti S (2004) Reversible transdifferentiation of secretory epithelial cells into adipocytes in the mammary gland. Proc Natl Acad Sci U S A 101:16801–16806, Epub 2004 Nov 19

Mullur R, Liu YY, Brent GA (2014) Thyroid hormone regulation of metabolism. Physiol Rev 94:355–382

Murad F (2006) Shattuck Lecture. Nitric oxide and cyclic GMP in cell signaling and drug development. N Engl J Med 355:2003–2011

Nikolic DM, Li Y, Liu S, Wang S (2009) Overexpression of constitutively active PKG-I protects female, but not male mice from diet-induced obesity. Obesity 19:784–791

Nisoli E, Clementi E, Tonello C, Sciorati C, Briscini L, Carruba MO (1998) Effects of nitric oxide on proliferation and differentiation of rat brown adipocytes in primary cultures. Br J Pharmacol 125:888–894

Nisoli E, Clementi E, Paolucci C, Cozzi V, Tonello C, Sciorati C, Bracale R, Valerio A, Francolini M, Moncada S, Carruba MO (2003) Mitochondrial biogenesis in mammals: the role of endogenous nitric oxide. Science 299:896–899

Nisoli E, Falcone S, Tonello C, Cozzi V, Palomba L, Fiorani M, Pisconti A, Brunelli S, Cardile A, Francolini M, Cantoni O, Carruba MO, Moncada S, Clementi E (2004) Mitochondrial biogenesis by NO yields functionally active mitochondria in mammals. Proc Natl Acad Sci U S A 101:16507–16512

Petrovic N, Walden TB, Shabalina IG, Timmons JA, Cannon B, Nedergaard J (2010) Chronic peroxisome proliferator-activated receptor gamma (PPARgamma) activation of epididymally derived white adipocyte cultures reveals a population of thermogenically competent, UCP1-containing adipocytes molecularly distinct from classic brown adipocytes. J Biol Chem 285:7153–7164

Pfeifer A, Hoffmann LS (2014) Brown, beige, and white: the new color code of fat and its pharmacological implications. Annu Rev Pharmacol Toxicol 55:207–227

Potter LR (2011) Guanylyl cyclase structure, function and regulation. Cell Signal 23:1921–1926

Robidoux J, Martin TL, Collins S (2004) Beta-adrenergic receptors and regulation of energy expenditure: a family affair. Annu Rev Pharmacol Toxicol 44:297–323

Rosen ED, Spiegelman BM (2014) What we talk about when we talk about fat. Cell 156:20–44

Rosenwald M, Wolfrum C (2014) The origin and definition of brite versus white and classical brown adipocytes. Adipocyte 3:4–9

Rosenwald M, Perdikari A, Rulicke T, Wolfrum C (2013) Bi-directional interconversion of brite and white adipocytes. Nat Cell Biol 15:659–667

Saini S, Duraisamy AJ, Bayen S, Vats P, Singh SB (2014) Role of BMP7 in appetite regulation, adipogenesis, and energy expenditure. Endocrine 48(2):405–409

Saito M, Okamatsu-Ogura Y, Matsushita M, Watanabe K, Yoneshiro T, Nio-Kobayashi J, Iwanaga T, Miyagawa M, Kameya T, Nakada K, Kawai Y, Tsujisaki M (2009) High incidence of metabolically active brown adipose tissue in healthy adult humans: effects of cold exposure and adiposity. Diabetes 58:1526–1531

Schlossmann J, Desch M (2009) cGK substrates. Handb Exp Pharmacol 163–193. doi:10.1007/978-3-540-68964-5_9

Schlueter N, de Sterke A, Willmes DM, Spranger J, Jordan J, Birkenfeld AL (2014) Metabolic actions of natriuretic peptides and therapeutic potential in the metabolic syndrome. Pharmacol Ther 144:12–27

Seale P, Bjork B, Yang W, Kajimura S, Chin S, Kuang S, Scime A, Devarakonda S, Conroe HM, Erdjument-Bromage H, Tempst P, Rudnicki MA, Beier DR, Spiegelman BM (2008) PRDM16 controls a brown fat/skeletal muscle switch. Nature 454:961–967

Seale P, Conroe HM, Estall J, Kajimura S, Frontini A, Ishibashi J, Cohen P, Cinti S, Spiegelman BM (2011) Prdm16 determines the thermogenic program of subcutaneous white adipose tissue in mice. J Clin Invest 121:96–105

Shimizu I, Aprahamian T, Kikuchi R, Shimizu A, Papanicolaou KN, MacLauchlan S, Maruyama S, Walsh K (2014) Vascular rarefaction mediates whitening of brown fat in obesity. J Clin Invest 124:2099–2112

Thompson CS (2013) Diabetic nephropathy: treatment with phosphodiesterase type 5 inhibitors. World J Diabetes 4:124–129. doi:10.4239/wjd.v4.i4.124

Tovar S, Paeger L, Hess S, Morgan DA, Hausen AC, Bronneke HS, Hampel B, Ackermann PJ, Evers N, Buning H, Wunderlich FT, Rahmouni K, Kloppenburg P, Bruning JC (2013) K (ATP)-channel-dependent regulation of catecholaminergic neurons controls BAT sympathetic nerve activity and energy homeostasis. Cell Metab 18:445–455

Tupone D, Madden CJ, Morrison SF (2014) Autonomic regulation of brown adipose tissue thermogenesis in health and disease: potential clinical applications for altering BAT thermogenesis. Front Neurosci 8:14. doi:10.3389/fnins.2014.00014, eCollection 2014

Turer AT, Scherer PE (2012) Adiponectin: mechanistic insights and clinical implications. Diabetologia 55:2319–2326

van Marken Lichtenbelt WD, Vanhommerig JW, Smulders NM, Drossaerts JM, Kemerink GJ, Bouvy ND, Schrauwen P, Teule GJ (2009) Cold-activated brown adipose tissue in healthy men. N Engl J Med 360:1500–1508

Vaughan CH, Shrestha YB, Bartness TJ (2011) Characterization of a novel melanocortin receptor-containing node in the SNS outflow circuitry to brown adipose tissue involved in thermogenesis. Brain Res 1411:17–27

Vegiopoulos A, Muller-Decker K, Strzoda D, Schmitt I, Chichelnitskiy E, Ostertag A, Berriel Diaz M, Rozman J, Hrabe de Angelis M, Nusing RM, Meyer CW, Wahli W, Klingenspor M, Herzig S (2012) Cyclooxygenase-2 controls energy homeostasis in mice by de novo recruitment of brown adipocytes. Science 328:1158–1161

Vila G, Grimm G, Resl M, Heinisch B, Einwallner E, Esterbauer H, Dieplinger B, Mueller T, Luger A, Clodi M (2012) B-type natriuretic peptide modulates ghrelin, hunger, and satiety in healthy men. Diabetes 61:2592–2596

Virtanen KA, Lidell ME, Orava J, Heglind M, Westergren R, Niemi T, Taittonen M, Laine J, Savisto NJ, Enerback S, Nuutila P (2009) Functional brown adipose tissue in healthy adults. N Engl J Med 360:1518–1525

Vosselman MJ, Brans B, van der Lans AA, Wierts R, van Baak MA, Mottaghy FM, Schrauwen P, van Marken Lichtenbelt WD (2013) Brown adipose tissue activity after a high-calorie meal in humans. Am J Clin Nutr 98:57–64

Wang X, Li J, Liu J, He W, Bi Y (2010) Nitric oxide increases mitochondrial respiration in a cGMP-dependent manner in the callus from *Arabidopsis thaliana*. Nitric Oxide 23:242–250

Whitaker RM, Wills LP, Stallons LJ, Schnellmann RG (2013) cGMP-selective phosphodiesterase inhibitors stimulate mitochondrial biogenesis and promote recovery from acute kidney injury. J Pharmacol Exp Ther 347:626–634

Whittle AJ, Carobbio S, Martins L, Slawik M, Hondares E, Vazquez MJ, Morgan D, Csikasz RI, Gallego R, Rodriguez-Cuenca S, Dale M, Virtue S, Villarroya F, Cannon B, Rahmouni K, Lopez M, Vidal-Puig A (2012) BMP8B increases brown adipose tissue thermogenesis through both central and peripheral actions. Cell 149:871–885

Wu J, Bostrom P, Sparks LM, Ye L, Choi JH, Giang AH, Khandekar M, Virtanen KA, Nuutila P, Schaart G, Huang K, Tu H, van Marken Lichtenbelt WD, Hoeks J, Enerback S, Schrauwen P, Spiegelman BM (2012) Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. Cell 150:366–376

Wu J, Cohen P, Spiegelman BM (2013) Adaptive thermogenesis in adipocytes: is beige the new brown? Genes Dev 27:234–250

Yamada-Goto N, Katsuura G, Ebihara K, Inuzuka M, Ochi Y, Yamashita Y, Kusakabe T, Yasoda A, Satoh-Asahara N, Ariyasu H, Hosoda K, Nakao K (2013) Intracerebroventricular administration of C-type natriuretic peptide suppresses food intake via activation of the melanocortin system in mice. Diabetes 62:1500–1504

Zhang X, Ji J, Yan G, Wu J, Sun X, Shen J, Jiang H, Wang H (2010) Sildenafil promotes adipogenesis through a PKG pathway. Biochem Biophys Res Commun 396:1054–1059

Zhang Z, Liu X, Morgan DA, Kuburas A, Thedens DR, Russo AF, Rahmouni K (2011) Neuronal receptor activity-modifying protein 1 promotes energy expenditure in mice. Diabetes 60:1063–1071

Brown Adipose Tissue: A Human Perspective

Mariëtte R. Boon and Wouter D. van Marken Lichtenbelt

Contents

Abstract

Since 2009, the presence of brown adipose tissue (BAT) in adult humans has been irrefutably proven. It is estimated that active BAT can contribute up to 2.5–5% of

M.R. Boon • W.D. van Marken Lichtenbelt (✉)
Maastricht University, Maastricht, The Netherlands
e-mail: markenlichtenbelt@maastrichtuniversity.nl

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resting metabolic rate in humans, suggesting that sustained activation of BAT may alleviate obesity and associated disorders. In the current chapter, the discovery of BAT in adult humans will be discussed. Furthermore, the characteristics of human BAT, methods to visualize the tissue as well as physiological and pharmacological methods to enhance its activity will be stressed.

Keywords
Beige adipocytes · Brown adipocytes · Cold acclimation · Energy expenditure · PET-CT scans · Pharmacology

1 Human Thermoregulation

1.1 Components of Thermogenesis in Humans

Body temperature is the net result of heat production (thermogenesis) and heat loss. Thermogenesis is the consequence of the body's energy metabolism, while heat loss, i.e., heat transport from the body to the environment, depends on conduction, convection, radiation, and evaporation (insensible and sweat). Body temperature can thus be regulated on the level of thermogenesis, e.g., shivering and nonshivering thermogenesis, and heat loss, e.g., sweat production in the heat and during exercise. Crucial in effective thermoregulation is the amount of heat

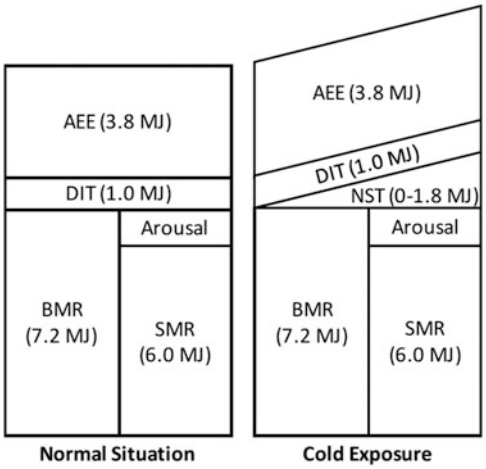

Fig. 1 Building blocks of total daily energy expenditure. Subdivision of daily energy expenditure in an individual with a usual energy expenditure of 12 MJ/day in a normal situation (*left pane*). During mild cold exposure (*right pane*), when no shivering occurs, large interindividual differences in NST exist, ranging from 0 to 30% (normal situation adapted from vMLAJP). *BMR* basal metabolic rate, *SMR* sleeping metabolic rate, *DIT* diet-induced thermogenesis, *AEE* activity-induced energy expenditure, *NST* nonshivering thermogenesis

transport from the core to the skin by blood perfusion, regulated by peripheral vasoconstriction and vasodilation. This also determines the heat distribution of the body. There are significant individual differences in the amount of insulation (due to relative low skin and peripheral tissue temperatures), which may affect energy metabolism. Indeed, an increase in insulation is negatively related to the amount of cold-induced thermogenesis (van Marken Lichtenbelt et al. 2002).

In this chapter on brown fat, we will mainly focus on the thermogenesis side of temperature regulation. Thermogenesis (or total daily energy expenditure, ADMR) can be divided in different components (see Fig. 1): basal metabolic rate (BMR, roughly 55–65% of ADMR), diet-induced thermogenesis (DIT, about 10% of ADMR), and energy expenditure for physical activity (AEE). BMR is measured under strictly defined conditions in the morning in fasted state in a (semi-)supine position and in a thermoneutral environment (see for more details (van Marken Lichtenbelt and Schrauwen 2011)). Resting metabolic rate (RMR) is often used instead of BMR, as this is measured under less strict conditions, for instance, in the afternoon, after food intake, or even sitting in a chair. In many human brown fat studies, RMR is used. Therefore, a clear description of the actual measurement conditions is needed in order to be able to compare different studies.

An alternative division in components of total daily energy expenditure is the use of obligatory and facultative thermogenesis. Obligatory thermogenesis refers to the energy expenditure needed for daily body functions, i.e., needed for the cells and organs to maintain the daily living functions. This also includes parts of DIT and AEE that are not needed for extra heat production. Facultative thermogenesis, on the other hand, is highly variable and consists of extra heat production in response to cold and diet, e.g., cold-induced thermogenesis (CIT) and DIT, respectively. DIT therefore can consist of both an obligatory part and a facultative part. In rodents, it has been shown that unbalanced diets can increase the facultative thermogenesis in order to be able to eat more to obtain enough valuable nutrients without gaining too much weight. Whether facultative DIT exists in humans is still under debate (Kozak 2010), although ingestion of a large meal increased DIT together with BAT activity (Vosselman et al. 2013). Most important in relation to human brown fat is the CIT. This consists of shivering thermogenesis (ST) and nonshivering thermogenesis (NST). ST can increase human energy expenditure by as much as 3–5 times BMR. Shivering, however, is generally experienced uncomfortable, leads to fatigue, and negatively affects the coordination of our movements. NST is more modest, ranges from 0 to 30% of RMR, but can be sustained without appreciable discomfort. Therefore, NST seems to be a way to increase energy expenditure with the potential to create a negative energy balance. This may have large health implications, especially for targeting disorders that are linked to overweight and obesity.

1.2 Adaptation to Long-Term Cold Acclimation

There is a large individual variation in NST in response to mild cold (Celi et al. 2010; van Ooijen et al. 2004; Warwick and Busby 1990). Some subjects

may increase NST by more than 30%, while others even drop their energy expenditure. The latter occurs most likely because of no or very small amounts of NST combined with a reduction of the energy expenditure in the cool peripheral tissue (Arrhenius law). Van Ooijen, however, also noted that NST is subjected to seasonal variation (van Ooijen et al. 2004). Twenty subjects were measured in both summer and winter season and under the same test conditions; NST was significantly higher in winter. Moreover, those individuals showing high NST in summer also did so in winter, indicating that individual differences persisted. In conclusion, there is individual variation in NST, but the level of NST is not fixed and can be increased. The latter was elegantly shown already in 1961 in a study by Davis (1961) who demonstrated that daily frequent cold exposure over time reduced shivering in humans without giving in on total energy expenditure, pointing to increased NST capacity.

In animals like rodents, it is well established that the tissue responsible for NST is brown adipose tissue (BAT), which combusts fatty acids toward heat in a process called mitochondrial uncoupling (Cannon and Nedergaard 2004). This uncoupling process is executed by uncoupling protein 1 (UCP1), a unique inner membrane mitochondrial protein for BAT. Briefly, UCP1 uncouples the electron transport chain, causing an increased proton leakage over the mitochondrial inner membrane. This results in production of extra heat instead of ATP. Indeed, in rodents, intermittent cold exposure results in both enhanced NST and enhanced uncoupling protein 1 (UCP1) content and BAT mass, underscoring that BAT contributes to the enhanced NST (Davis 1961). However, in humans, the tissue responsible for NST remained an enigma until 2009. Though early anatomical studies identified the presence of BAT in humans (Heaton et al. 1978; Huttunen et al. 1981), physiological experiments could not identify a functional role of BAT (Astrup et al. 1984). Therefore, it was generally agreed that, although present and functional in newborns, during maturing, the amount of BAT decreases to become physiologically insignificant in adults.

2 The Discovery of BAT in Adult Humans

2.1 FDG PET-CT Scans Lead to Discovery of Human BAT

It was not until the clinical application of the FDG PET-CT scans by nuclear medicine that functional BAT was identified in humans. This technique makes use of a labeled glucose analog (^{18}F-FDG) that is taken up by metabolically active tissues without being metabolized and therefore becomes trapped in cells. While the PET scan can visualize and quantify uptake of this radioactive tracer, the CT is needed for anatomical information. The ^{18}F-FDG PET-CT scan is used in oncology for localization of metabolically active tumors that exhibit relatively high glucose uptake (e.g., for glycolysis). Since active BAT also takes up high amounts of glucose (i.e., for de novo lipogenesis, see below), the FDG PET-CT can be used to visualize BAT. One of the earliest reported images of BAT in a patient was by Barrington and Maisey (1996) in 1996 who by the way classified the tissue as tense muscle. After this, several other

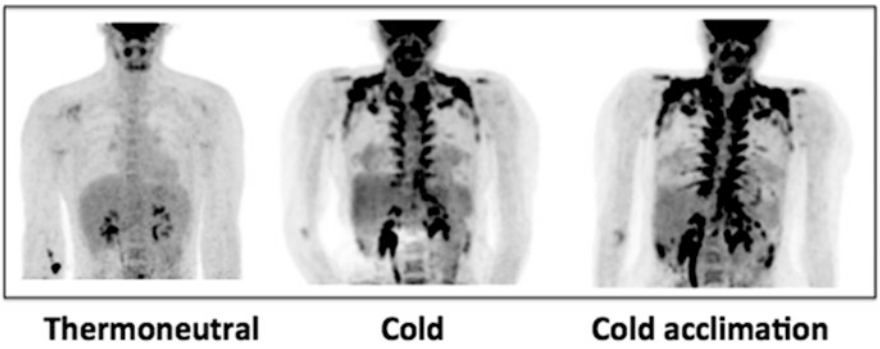

Fig. 2 ^{18}F-Fluorodeoxyglucose (FDG) PET-CT scan visualizing brown adipose tissue in adult humans under thermoneutral conditions after short-term cold exposure and after 10 days of cold acclimation. Brown adipose tissue (BAT) can be visualized by the use of an FDG PET-CT scan. To this end, the subjects (middle and right pane) are exposed to cold (16°C) for 2 h in order to activate BAT. After 1 h of cold induction, the radioactive tracer ^{18}F-FDG is injected intravenously. ^{18}F-FDG, a glucose analog, is taken up by organs which have a high glucose metabolism, especially the brain, heart, and BAT. After 2 h of cold induction, the uptake of ^{18}F-FDG is visualized by means of a low-dose CT scan, immediately followed by a PET scan. The CT scan is used for localization of the uptake areas. The activity and volume of the BAT are quantified by autocontouring the areas of FDG uptake, by the use of a previously set threshold. The left pane shows FDG uptake after 2 h of exposure to thermoneutral temperature (22°C). The right pane shows FDG uptake after 2 h of cold exposure (16°C) in a subject that has been cold acclimated (15–16°C, 6 h/day) for 10 consecutive days

studies from the field of nuclear medicine reported BAT in cancer patients, where it was regarded an artifact potentially obscuring the image in search for tumors. It was Nedergaard et al. (2007) in 2007 who reviewed these nuclear medicine retrospective studies in the context of metabolic implications for human brown fat physiology. In 2009, several research groups independently identified functional brown fat in adult humans after performance of dedicated cold exposure experiments (Saito et al. 2009; van Marken Lichtenbelt et al. 2009; Virtanen et al. 2009) together with retrospective patient studies (Cypess et al. 2009).

More specifically, the FDG PET-CT scans revealed increased glucose uptake upon cold exposure in fat tissue located in the supraclavicular, neck, paravertebral, mediastinal, para-aortic, and suprarenal areas (see Fig. 2). However, direct evidence that the tissue was indeed BAT came from biopsy material obtained from the supraclavicular and neck region, in which the presence of UCP1 was shown (van Marken Lichtenbelt et al. 2009; Virtanen et al. 2009; Zingaretti et al. 2009). Since UCP1 is the bona fide marker of BAT (Cannon and Nedergaard 2004), this information together with the imaging proved the presence of functional BAT in adult humans.

2.2 Other Methods of BAT Visualization

Strictly, FDG PET-CT scans only visualize tissue glucose uptake (static scan) or glucose uptake rate (dynamic scan) (van der Lans et al. 2014). However, high

glucose uptake does not necessarily mean that the tissue is metabolically active. A first indication that BAT is indeed metabolically active comes from the study of Orava et al. (2011), who used [^{15}O]H_2O to show that BAT activation results in enhanced blood flow in the tissue. However, even increased blood flow is not the final proof of metabolic activity because blood flow may also increase in tissues that are not metabolically active. The final proof came from Ouellet et al. (2012) who determined oxidative metabolism in human BAT indirectly using ^{11}C-acetate PET imaging. ^{11}C-acetate is rapidly taken up by BAT and other tissues (i.e., myocardium, muscle) and metabolized to $^{11}CO_2$ and H_2O after intravenous injection. The rate of clearance of ^{11}C-acetate from the tissue reflects oxidative metabolism, with higher oxidative metabolism resulting in faster clearance from the tissue. Indeed, based on ^{11}C-acetate tissue kinetics, cold exposure markedly increased oxidative metabolism in BAT in all subjects ($n = 6$), which has recently been confirmed (Blondin et al. 2014). In a recent study, oxygen consumption was measured in BAT during cold exposure by means of dynamic oxygen ($^{15}O_2$) PET imaging, a possibly very adequate marker for BAT metabolism (Muzik et al. 2013).

It is well known from animal studies that not glucose but fatty acids (FA) are the main source of fuel in BAT (see below). Therefore, Ouellet also used the fatty acid tracer ^{18}F-fluoro-thiaheptadecanoic acid (FTHA) in their cold exposure tests, next to ^{18}F-FDG and ^{11}C-acetate (Ouellet et al. 2012). They showed both substantial FA and glucose uptake upon cold exposure. However, a drawback of the use of a FA tracer as compared to a glucose tracer is that the uptake is rather nonspecific since it is also largely taken up by other organs such as the liver and intestine. Thus, the FA tracer needs to be optimized more before it can be used on a large scale.

Though these tracer studies reveal important new information on BAT metabolism and fuel utilization, an important aspect is not yet sufficiently covered. That is the fact that BAT, when activated, also uses its own internal triglyceride (TG) stores. In this respect, CT scans can provide useful information, since the radiodensity expressed in Hounsfield units reveals the water fat ratio of the tissue. Indeed, cold-exposed subjects show an increase in BAT radiodensity indicating reduced BAT TG after cold exposure (Ouellet et al. 2012).

3 Human BAT: Brown or Beige?

As described in Chapter 4a, from rodent studies, it is evident that besides brown adipocytes, also beige adipocytes exist (Seale et al. 2008; Wu et al. 2012). In rodents, these beige adipocytes lie mainly dispersed between WAT but can also be found in muscle. A recent topic of debate is on how “brown” human BAT actually is. Distinction between classical brown and beige adipocytes cannot be simply made on the basis of an FDG PET-CT scan, as both types of brown adipocytes take up high amounts of glucose when stimulated (Bartelt and Heeren 2013). Recent genotyping of human BAT biopsies, obtained from the supraclavicular area from subjects who showed FDG uptake in this area, demonstrated that human BAT more closely resembles the beige fat found in WAT depots in mice rather than the classical murine BAT (Wu et al. 2012).

Therefore, a former vision was that human BAT solely consists of "beige" adipocytes. A recent study by Cypess et al. (2013) refuted this vision. Different depots of neck adipose tissue were isolated from adult human volunteers, and gene expression, differentiation capacity, and basal oxygen consumption were compared to different mouse adipose depots. Although the variation in the properties of human neck adipose tissue was substantial between subjects, they showed that some human samples have many similarities with the classical BAT found in rodents. Intriguingly, it appeared that the unstimulated energy expenditure of the human BAT samples is similar to that of mouse interscapular BAT, underscoring the energy-combusting potential of this adipose tissue in humans (Okamatsu-Ogura et al. 2013). Moreover, we (Nascimento et al., unpublished) and others (Wu et al. 2012) have recently shown that human brown adipocytes respond to noradrenalin by markedly enhancing uncoupled respiration.

4 Contribution of BAT to Energy Expenditure and Lipid and Glucose Metabolism in Humans

4.1 Estimation: What Is the Potential Contribution of BAT Thermogenesis to Whole-Body Energy Expenditure in Humans?

As shown above, the combined information of different scanning techniques, together with tissue characterization including UCP1 staining, revealed metabolically active BAT in adult humans. However, the actual contribution of BAT (and beige) metabolism to whole-body NST is not yet determined. In rodents, it is very likely that BAT is the only tissue responsible for the classical nonshivering thermogenesis and that upon cold exposure, BAT can contribute to up to 60% of RMR (van Marken Lichtenbelt and Schrauwen 2011; Cannon and Nedergaard 2004; Feldmann et al. 2009). In humans, a large body of evidence points toward BAT as a contributor to nonshivering thermogenesis, possibly in conjunction with mitochondrial uncoupling in other tissues (Wijers et al. 2008). With proper standardized cold exposure tests, it has been shown that cold-induced BAT activity (based on glucose uptake) is significantly related to NST (Bakker et al. 2014; van der Lans et al. 2013). Unfortunately, from correlation studies, quantification is not possible. The abovementioned study that used the ^{15}O tracer technique could show oxygen consumption by BAT, but the used cooling protocol prevented calculation of the actual (maximal) BAT contribution during cold (Muzik et al. 2013). Alternatively, from estimates of the amount of BAT present in the body and potential tissue-specific metabolism, one could estimate the total oxidative capacity. However, several problems arise. The first is the volume quantification. PET volume can easily be overestimated, because of the partial volume effect (see for more details (van der Lans et al. 2014)). On the other hand, estimation based on CT may reveal an underestimation. Moreover, both scanning techniques may miss small more dispersed brown and beige fat depots because of the limited resolution of the scans. Nevertheless, a volume estimate can be made and has been made: 50–

100 g (van Marken Lichtenbelt and Schrauwen 2011; Virtanen et al. 2009). The second problem is that the tissue is very inhomogeneous. As described above, BAT in humans is very likely a mix of both white and brown (beige) adipocytes in contrast to BAT in rodents. This means that estimates of tissue-specific metabolic rate from animals may not apply to humans. Third is from which animal can tissue-specific metabolism be calculated. From limited animal data, it is revealed that the maximal heat-producing capacity of BAT is 300 W/kg (Rothwell and Stock 1983). However, it is well known that small mammals have higher tissue-specific metabolic rates than larger animals. Therefore, based on allometric corrections and a volume of BAT of 50 g, a careful estimation revealed a contribution of BAT of 2.5–5% of RMR. Virtanen et al. (2009) also came to approximately the same results. It should be noted that these are just estimations and in future studies this quantification requires much more attention.

4.2 Differences in BAT Volume/Activity

There is a striking individual variation in brown fat activity, which appears to be related to the level of NST. In addition, there are group differences. For instance, BAT presence and activity are reduced in obesity compared to lean adults (van Marken Lichtenbelt et al. 2009; Wang et al. 2011) and almost absent in morbid obesity (Vijgen et al. 2011). Also the elderly have reduced cold-induced BAT activity (Yoneshiro et al. 2011a), and from retrospective studies, it appears that diabetes is characterized by a diminished amount of BAT (Ouellet et al. 2011), but dedicated studies are needed to confirm this. Retrospective studies also hinted toward higher BAT presence in women compared to men. However, dedicated cold exposure studies showed no gender-specific BAT activity (van der Lans et al. 2013).

Though BAT studies have been carried out in many different ethnic groups (Caucasian, Chinese, Japanese), hardly any comparative studies have been carried out. In fact, only two studies have been carried out so far on South Asian and Caucasian populations. Relative to Caucasians, the South Asian population is characterized by a high risk of developing type 2 diabetes. Moreover, type 2 diabetes occurs at a younger age and lower BMI in South Asians than Caucasians (Mukhopadhyay et al. 2006; Razak et al. 2007), and the risk of complications related to diabetes is increased in this group (Chandie Shaw et al. 2002). The first study did not find differences in BAT activity between the two groups (Admiraal et al. 2013), while the second study did observe a significant difference (Bakker et al. 2014). The main difference between the two studies was the cooling protocol: while it was “fixed” in study one (e.g., constant environmental temperature of 16–18°C) and cooling was performed by air cooling, a standard individual attuned water cooling protocol that maximizes NST was used in study two. Interestingly, in the latter study, the lower BAT activity in South Asians did go hand in hand with differences in NST. RMR was significantly lower in SA, and NST only significantly increased in Caucasians. In conclusion, it appears that South Asians do have reduced BAT availability. Whether this is mainly due to an actual ethnic difference

that affects BAT availability or whether the lifestyle (culture, thermal behavior) is also involved remains to be investigated.

4.3 Involvement of BAT in Lipid Metabolism

As mentioned above, animal studies have shown that triglyceride-derived FA are the main fuel for BAT thermogenesis. The activation of BAT results in a fast induction of intracellular lipolysis, induced by the activation of adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL), resulting in the release of FA from TG-filled lipid droplets. FA are directed to the mitochondria where they either allosterically activate uncoupling protein 1 (UCP1) present in the inner membrane of the mitochondrion or can undergo oxidation (Cannon and Nedergaard 2004). Accordingly, after intracellular lipolysis, the intracellular TG stores of the brown adipocyte need to be replenished. This is mediated via three mechanisms: (1) uptake of glucose followed by de novo lipogenesis (see below), (2) uptake of albumin-bound free FA, and (3) uptake of TG-derived FA from very-low-density lipoproteins and chylomicrons in the plasma.

The magnitude of the TG clearance capacity of BAT became clear only recently when Bartelt et al. (Bartelt et al. 2011) demonstrated that mice that are housed at 4°C for 24 h, a major trigger for BAT activation, show a massive lowering of plasma TG levels. Furthermore, BAT activation by means of cold exposure is able to correct hyperlipidemia in hyperlipidemic ApoA5 knockout mice (Bartelt et al. 2011). In contrast, animals in which BAT is surgically denervated become rapidly obese and hypertriglyceridemic (Dulloo and Miller 1984). These findings underscore the involvement of BAT in total energy expenditure and TG clearance, at least in mice.

Also in humans, BAT likely contributes to TG metabolism. As mentioned above, exposure of humans to cold for 2 h resulted in enhanced FA uptake by BAT as compared to muscle and WAT. It is likely that human BAT also utilizes FA from circulating lipoproteins, though this has not been investigated yet. Furthermore, 2 h of cold exposure results in a rapid increase in BAT CT radiodensity, suggestive of lowering of intracellular TG stores in BAT. Indeed, a depletion of intracellular lipid in BAT was found at necropsy in newborn infants and adults who died from hypothermia (Aherne and Hull 1966). Thus, a fast initial combustion of intracellular TG upon acute BAT activation may well explain why short-term cold exposure does not result in acute lowering of plasma TG in human subjects, but does result in increased fat oxidation, while glucose oxidation is not changed (Bakker et al. 2014). Likely, prolonged BAT activation will result in lowering of plasma TG levels in human subjects as a consequence of increased clearance from the plasma toward BAT.

4.4 Involvement of BAT in Glucose Metabolism

Murine studies have shown that BAT expresses both GLUT-4 and GLUT-1, suggesting both insulin-dependent and insulin-independent uptake of glucose by the tissue (Cannon and Nedergaard 2004). As mentioned, while fatty acids are the main substrate used for oxidation and uncoupling, glucose is mainly used for de novo lipogenesis (e.g., to replenish intracellular lipid droplets) and ATP generation (e.g., via glycolysis) rather than oxidation. Still, murine studies suggest that plasma clearance of glucose by BAT can substantially contribute to whole-body glucose metabolism. For instance, transplantation of extra BAT in mice results in improved glucose tolerance due to higher uptake of glucose by the tissue (Stanford et al. 2013). Furthermore, long-term BAT activation by means of a β3 adrenergic agonist lowered plasma glucose levels (Wang and Li 2013). Of note, also induction of white adipose tissue "browning" by means of the recently identified hormone irisin improved glucose tolerance suggesting that also beige adipocytes have the capacity to contribute to whole-body glucose metabolism, at least in mice (Bostrom et al. 2012).

In humans, it is well established that upon cold exposure, BAT takes up high amounts of FDG, underscoring the large glucose clearance capacity of the tissue. Whether uptake of glucose is mainly mediated via the GLUT-1 or GLUT-4 transporter remains to be determined. However, Orava et al. (2011) showed that insulin stimulation markedly enhances FDG uptake by BAT to an extent comparable to muscle, suggesting that the GLUT-4 transporter is at least in part involved in glucose uptake by human BAT and that the tissue is insulin sensitive. Despite the large glucose uptake capacity of human BAT, the question remains whether active BAT is sufficient to impact on whole-body glucose metabolism in humans. An association study in which different plasma parameters were measured in healthy humans with and without BAT (BAT status was determined via FDG PET-CT scans) showed that BAT was a significantly independent determinant of glucose and HbA1c levels, suggesting that BAT could impact on glucose metabolism (Matsushita et al. 2014). However, whether long-term BAT activation indeed results in improvement of glucose metabolism in obese subjects with impaired glucose tolerance remains to be determined and is an interesting and relevant topic for future studies.

5 Involvement of BAT in Human Pathology

5.1 Pheochromocytomas

Pheochromocytomas are neuroendocrine tumors that secrete excessive amounts of noradrenalin, an important activator of BAT. Indeed, on ^{18}F-FDG PET-CT scans in patients with this tumor, an increased mass and activity of BAT are seen, accompanied by increased energy expenditure (Dong et al. 2013; Petrak et al. 2013). Moreover, after resection of the tumor, FDG uptake and energy

expenditure decrease dramatically (Kuji et al. 2008), supporting that the increased energy expenditure typical for this condition is likely due to increased BAT-mediated NST. Furthermore, pheochromocytoma patients exhibit increased browning of visceral WAT (Frontini et al. 2013), which may also contribute to the enhanced energy expenditure. Whether this browning is due to increased transdifferentiation of white into brown adipocytes or differentiation of brown preadipocytes present in the visceral WAT toward mature brown adipocytes remains to be determined.

5.2 Hyperthyroidism/Hypothyroidism

Mouse studies have shown that, in addition to cold, thyroid hormone is also involved in the activation of BAT. After uptake of T3 and T4 by the brown adipocyte and intracellular conversion of T4 into T3 by the enzyme type 2 deiodinase (D2), the active thyroid hormone T3 is translocated into the nucleus and binds to a thyroid hormone-responsive element located on the promoter of the UCP1 gene (Branco et al. 1999). This leads to increased transcription of UCP1 and ultimately to increased uncoupling. Furthermore, T3 is able to stabilize the UCP1 mRNA, thereby reducing its degradation in the cell (Cannon and Nedergaard 2004). During cold induction, the activity of D2 is increased in BAT, leading to locally increased amounts of T3 (Bianco and McAninch 2013). This is an additional and necessary mechanism to stimulate thermogenesis by BAT. In addition, thyroid hormone also activates BAT indirectly by enhancing sympathetic nervous system outflow toward the tissue (Lopez et al. 2010).

Interestingly, energy expenditure is increased in patients with hyperthyroidism and decreased in patients with hypothyroidism. A recent study showed that hyperthyroidism in human patients increases glucose uptake in BAT independently of BAT perfusion (Lahesmaa et al. 2013). Therefore, the weight loss and excessive transpiration in hyperthyroidism, and the weight gain and reduced cold tolerance in hypothyroidism, can at least be partly attributed to an increased and decreased activity of BAT, respectively. Furthermore, recent studies have shown that treatment of human stem cells with T3 results in the development of UCP1-positive cells within white adipose tissue, pointing to "browning" of white adipose tissue (Lee et al. 2012). This may also contribute to the enhanced energy expenditure seen in hyperthyroidism.

5.3 Obesity, Dyslipidemia, and Type 2 Diabetes

When energy intake exceeds energy expenditure (i.e., positive energy balance), TG is stored in WAT. In addition, TG may be stored ectopically in organs such as the skeletal muscle and liver, resulting in malfunction of these organs. A prolonged positive energy balance may result in development of overweight and obesity. Currently, in the USA, over 69% of the adult population is overweight

($25 < \text{BMI} < 30$ kg/m^2), and more than 35% is already obese (BMI > 30 kg/m^2) (CDC 2014). Obesity is strongly associated with the development of other disorders and diseases, such as dyslipidemia, type 2 diabetes, cardiovascular disease, and cancer (World Health Organ 2000).

Interestingly, recent studies point toward a role of disturbed BAT function in the development of obesity and related disorders. In human adults, the amount of BAT is inversely correlated with BMI and percentage of body fat (van Marken Lichtenbelt et al. 2009). More specifically, BAT volume is inversely correlated with parameters of central obesity, such as visceral fat volume on CT scan and waist circumference (Wang et al. 2011). These findings suggest that obesity is associated with a low level of BAT activity. Indeed, excision of BAT or sympathetic denervation of BAT in mice results in hypertriglyceridemia and obesity (Dulloo and Miller 1984). Thus, in humans, a reduced activity of BAT may predispose to obesity and obesity-related diseases such as dyslipidemia and type 2 diabetes by accumulation of TG in the blood and subsequent storage in WAT as well as in ectopic fat depots such as skeletal muscle and the liver. This is associated with reduced insulin sensitivity of these organs and eventually type 2 diabetes. Furthermore, since BAT is also involved in clearance of plasma glucose (i.e., for de novo lipogenesis) (Stanford et al. 2013), BAT could also contribute to glucose homeostasis, particularly in resting conditions when glucose utilization by skeletal muscle is minimal. A low activity of BAT might thus predispose to T2DM not only via the above described relation to obesity but also via reduced glucose uptake at rest (Nedergaard and Cannon 2010). However, the lower BAT activity found in overweight and obese human subjects may also at least in part be a consequence of their increased subcutaneous white fat layer, which may substantially contribute to the maintenance of body temperature, making active BAT redundant (Vijgen et al. 2011).

6 BAT as a Novel Target to Combat Obesity and Associated Disorders

When considering BAT as a novel therapeutic tool to enhance energy expenditure thereby lowering obesity and related diseases in humans, it is highly relevant to assess the actual contribution of human BAT to total daily energy expenditure. As mentioned above, BAT may contribute to 2.5–5% of RMR in humans when the tissue is maximally stimulated. BAT has the potential to contribute even more to total daily energy expenditure when its mass and activity are enhanced due to catecholamine excess. This appears from patients with pheochromocytomas, as discussed above. ^{18}F-FDG PET-CT scans in patients with such tumors show an increased mass and activity of BAT (Dong et al. 2013; Yoneshiro et al. 2011b), accompanied by increased energy expenditure up to twofold in a recent case report (Sondergaard et al., unpublished). Thus, when further stimulated due to endogenous or exogenous factors, BAT has the potential to even more substantially contribute to total daily energy expenditure.

All together, these data unequivocally demonstrate that BAT contributes to NST in humans and that BAT activation is a promising novel therapeutic modality to combat obesity. Therefore, identification of novel therapeutic targets that may activate BAT is highly warranted.

6.1 Physiological Activation of BAT

6.1.1 Cold Exposure

The most well-known stimulus to activate BAT is cold exposure. Cold exposure results in activation of transient receptor potential (TRP) channels in the skin, mediating signaling in the hypothalamic temperature center and subsequent sympathetic outflow toward BAT (Whittle et al. 2013). Short-term cold exposure results in a massive induction of FDG uptake by BAT and a concomitant increase in nonshivering thermogenesis of up to 70%. But does long-term cold acclimation impact on BAT activity and NST and even metabolic parameters? Several recent studies have investigated this issue. Acclimation of healthy young adults to 15–16°C for 10 consecutive days for 6 h/day resulted in recruitment and enhanced activity of BAT as well as enhanced NST (van der Lans et al. 2013). However, resting energy expenditure was not affected after the cold acclimation period, nor were effects found on body weight and plasma glucose and lipid levels. In contrast, another study in which healthy young adults were acclimated to 17°C for 6 weeks for 2 h/day did find that fat mass was significantly reduced in addition to positive effects on BAT (Yoneshiro et al. 2013). Whether cold acclimation is indeed a novel tool to improve obesity and associated disorders (e.g., dyslipidemia and insulin resistance) remains to be determined, for instance, by investigating the effect of cold acclimation in obese subjects.

6.1.2 Food Ingredients

The effect of cold exposure on BAT activation and recruitment relies on its potential to enhance sympathetic outflow toward the tissue. Of note, also bioactive food ingredients such as methylxanthines (caffeine or theophylline), ephedrine, and polyphenols (catechins, resveratrol, quercetin, kaempferol) are effective at increasing energy expenditure and lowering body weight (Dulloo 2011; Finer et al. 2000; Hansen et al. 1998), likely by enhancing activation of BAT. Of special interest are the capsinoids (nonpungent capsaicin analogs), the active compound found in chili pepper. Capsinoids can bind to and activate TRP 1 channels located in the upper digestive tract, leading to increased sympathetic nerve activity to BAT (Ono et al. 1985). Capsinoids have been shown to increase BAT activity in rodents (Kawabata et al. 2009). Of note, capsinoids could be effective in activating BAT in humans, without inducing unwanted side effects. Treatment of healthy young adults with daily capsinoid tablets increased energy expenditure more in subjects with BAT (as based on PET-CT) compared to subjects without BAT. Moreover, in subjects with BAT, capsinoids tended to reduce fat mass (Yoneshiro et al. 2013).

Direct evidence for the effect of capsinoids on BAT in humans is needed to draw definite conclusions.

6.2 Pharmacological Activation of BAT

6.2.1 β3-Agonism

Cold exposure stimulates murine BAT via activation of the β3-AR located on the membrane of the brown adipocytes (Cannon and Nedergaard 2004). Although β3-AR agonists have been shown to be potent inducers of BAT activation in mice, resulting in lowering of fat mass, plasma triglyceride, cholesterol, and glucose levels (Wang and Li 2013), the role of the β3-AR for human BAT remains elusive. Although both human and rodent isoforms of the β3-AR respond to specific agonists in adipocytes in vitro (Soeder et al. 1999), in vivo studies with β3 agonists in humans showed disappointing results. Whereas some agonists such as L796568 were able to enhance energy expenditure after a single dose (van Baak et al. 2002), none had beneficial effects on metabolic parameters (Whittle et al. 2013). This has been suggested to be due to the small amount of β3-expressing tissues in humans, downregulation of the receptor following stimulation, or perhaps due to the fact that the β3-AR is not responsible for BAT activation in humans (Larsen et al. 2002). Possibly, the β1-AR and/or β2-AR could be responsible for BAT activation in humans. Indeed, propranolol, a β-AR antagonist with low β3-AR efficacy compared to β1-AR and β2-AR, decreases 18F-FDG uptake by BAT visualized by PET-CT scans (Agrawal et al. 2009). In this respect, it is surprising that broad β-adrenergic receptor agonism with isoprenaline or the sympathomimetic ephedrine did not simulate BAT glucose uptake similar to cold exposure (Cypess et al. 2012; Vosselman et al. 2012). This may be due to the fact that local (e.g., within BAT) availability of both compounds did not reach the same extent as noradrenalin at nerve endings does in the case of cold exposure. Recently a study showed that a specific B3 agonist, Mirabegron, activates brown adipose tissue (Cypess et al. 2015).

All in all, future studies are evidently needed to illuminate the role of β-AR in human BAT activation. More recent studies have focused on the identification of cytokines and hormones that enhance BAT activity, either via enhancing sympathetic outflow of the tissue (i.e., central mechanism) or via direct activation of the brown adipocyte.

6.2.2 FGF21

Fibroblast growth factor 21 (FGF21) is a hormone that is excreted upon fasting, feeding a ketogenic diet (high fat, low carbohydrate), or after amino acid deprivation (Gimeno and Moller 2014). It is predominantly secreted by the liver, but also by other tissues such as WAT, BAT, skeletal muscle, and pancreatic β cells. FGF21 regulates both carbohydrate and lipid metabolism by impacting on different tissues. Interestingly, FGF21 administration results in increased energy expenditure (EE), enhanced thermogenesis in BAT, and lowering of plasma lipid levels and obesity in mice (Emanuelli et al. 2014). Furthermore, stimulation of isolated human preadipocytes with FGF21 induces the formation of beige cells (Lee et al. 2014a,

b), and administration of FGF21 in obese humans with type 2 diabetes lowers fat mass and dyslipidemia (Gaich et al. 2013). Although it is not known whether these effects in humans are due to BAT activation, FGF21 is considered a promising new therapy to activate BAT thereby lowering obesity and associated disorders.

6.3 Promising Novel BAT Activators

Animal studies have identified various other promising compounds that activate BAT in vivo. Although a thorough description of these compounds is beyond the scope of this chapter, a few will be shortly mentioned. The anti-diabetes drug metformin was recently shown to exhibit its triglyceride-lowering effect by enhancing BAT activation through a mechanism involving activation of AMP-activated protein kinase (AMPK) in the tissue (Geerling et al. 2014). As metformin is also associated with slight weight loss in type 2 diabetes patients (Golay 2008), this may be – at least in part – attributed to activation of BAT. Furthermore, irisin has been identified as a signaling peptide that is released by muscle upon exercise (Bostrom et al. 2012), providing a possible mechanism between the well-established links between exercise and enhanced energy expenditure. In mice, irisin treatment resulted in massive browning of WAT and improved insulin sensitivity. Whether irisin also exhibits beneficial metabolic effects in humans remains to be investigated. However, a recent study by Lee et al. (2014a) did show that upon shivering, irisin levels increased in human subjects and that the induction of irisin secretion was proportional to shivering intensity. Thus, during cold acclimation, release of irisin following shivering may be involved in BAT recruitment.

References

Admiraal WM, Holleman F, Bahler L, Soeters MR, Hoekstra JB, Verberne HJ (2013) Combining 123I-metaiodobenzylguanidine SPECT/CT and 18F-FDG PET/CT for the assessment of brown adipose tissue activity in humans during cold exposure. J Nucl Med 54:208–212

Agrawal A, Nair N, Baghel NS (2009) A novel approach for reduction of brown fat uptake on FDG PET. Br J Radiol 82:626–631

Aherne W, Hull D (1966) Brown adipose tissue and heat production in the newborn infant. J Pathol Bacteriol 91:223–234

Astrup A, Bulow J, Christensen NJ, Madsen J (1984) Ephedrine-induced thermogenesis in man: no role for interscapular brown adipose tissue. Clin Sci (Lond) 66:179–186

Bakker LE, Boon MR, van der Linden RA, Arias-Bouda LP, van Klinken JB, Smit F, Verberne HJ, Jukema JW, Tamsma JT, Havekes LM, van Marken Lichtenbelt WD, Jazet IM, Rensen PC (2014) Brown adipose tissue volume in healthy lean south Asian adults compared with white Caucasians: a prospective, case-controlled observational study. Lancet Diabetes Endocrinol 2:210–217

Barrington SF, Maisey MN (1996) Skeletal muscle uptake of fluorine-18-FDG: effect of oral diazepam. J Nucl Med 37:1127–1129

Bartelt A, Heeren J (2013) Adipose tissue browning and metabolic health. Nat Rev Endocrinol 10 (1):24–36

Bartelt A, Bruns OT, Reimer R, Hohenberg H, Ittrich H, Peldschus K, Kaul MG, Tromsdorf UI, Weller H, Waurisch C, Eychmuller A, Gordts PL, Rinninger F, Bruegelmann K, Freund B,

Nielsen P, Merkel M, Heeren J (2011) Brown adipose tissue activity controls triglyceride clearance. Nat Med 17:200–205

Bianco AC, McAninch EA (2013) The role of thyroid hormone and brown adipose tissue in energy homoeostasis. Lancet Diabetes Endocrinol 1:250–258

Blondin DP, Labbe SM, Tingelstad HC, Noll C, Kunach M, Phoenix S, Guerin B, Turcotte EE, Carpentier AC, Richard D, Haman F (2014) Increased brown adipose tissue oxidative capacity in cold-acclimated humans. J Clin Endocrinol Metab 99:E438–E446

Bostrom P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC, Rasbach KA, Bostrom EA, Choi JH, Long JZ, Kajimura S, Zingaretti MC, Vind BF, Tu H, Cinti S, Hojlund K, Gygi SP, Spiegelman BM: A PGC1-alpha-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature* 481:463-468, 2012

Branco M, Ribeiro M, Negrao N, Bianco AC (1999) 3,5,3′-Triiodothyronine actively stimulates UCP in brown fat under minimal sympathetic activity. Am J Physiol 276:E179–E187

Cannon B, Nedergaard J (2004) Brown adipose tissue: function and physiological significance. Physiol Rev 84:277–359

Celi FS, Brychta RJ, Linderman JD, Butler PW, Alberobello AT, Smith S, Courville AB, Lai EW, Costello R, Skarulis MC, Csako G, Remaley A, Pacak K, Chen KY (2010) Minimal changes in environmental temperature result in a significant increase in energy expenditure and changes in the hormonal homeostasis in healthy adults. Eur J Endocrinol 163:863–872

Chandie Shaw PK, Vandenbroucke JP, Tjandra YI, Rosendaal FR, Rosman JB, Geerlings W, de Charro FT, van Es LA (2002) Increased end-stage diabetic nephropathy in Indo-Asian immigrants living in the Netherlands. Diabetologia 45:337–341

Cypess AM, Lehman S, Williams G, Tal I, Rodman D, Goldfine AB, Kuo FC, Palmer EL, Tseng YH, Doria A, Kolodny GM, Kahn CR (2009) Identification and importance of brown adipose tissue in adult humans. N Engl J Med 360:1509–1517

Cypess AM, Chen YC, Sze C, Wang K, English J, Chan O, Holman AR, Tal I, Palmer MR, Kolodny GM, Kahn CR (2012) Cold but not sympathomimetics activates human brown adipose tissue in vivo. Proc Natl Acad Sci U S A 109:10001–10005

Cypess AM, White AP, Vernochet C, Schulz TJ, Xue R, Sass CA, Huang TL, Roberts-Toler C, Weiner LS, Sze C, Chacko AT, Deschamps LN, Herder LM, Truchan N, Glasgow AL, Holman AR, Gavrila A, Hasselgren PO, Mori MA, Molla M, Tseng YH (2013) Anatomical localization, gene expression profiling and functional characterization of adult human neck brown fat. Nat Med 19:635–639

Cypess AM, Weiner LS, Roberts-Toler C et al (2015) Activation of human brown adipose tissue by a beta3-adrenergic receptor agonist. Cell Metab 21:33–38

Davis TR (1961) Chamber cold acclimatization in man. J Appl Physiol 16:1011–1015

Dong A, Wang Y, Lu J, Zuo C (2013) Hypermetabolic mesenteric brown adipose tissue on dual-time point FDG PET/CT in a patient with benign retroperitoneal pheochromocytoma. Clin Nucl Med 39(3):e229–32

Dulloo AG (2011) The search for compounds that stimulate thermogenesis in obesity management: from pharmaceuticals to functional food ingredients. Obes Rev 12:866–883

Dulloo AG, Miller DS (1984) Energy balance following sympathetic denervation of brown adipose tissue. Can J Physiol Pharmacol 62:235–240

Emanuelli B, Vienberg SG, Smyth G, Cheng C, Stanford KI, Arumugam M, Michael MD, Adams AC, Kharitonenkov A, Kahn CR (2014) Interplay between FGF21 and insulin action in the liver regulates metabolism. J Clin Invest 124:515–527

Feldmann HM, Golozoubova V, Cannon B, Nedergaard J (2009) UCP1 ablation induces obesity and abolishes diet-induced thermogenesis in mice exempt from thermal stress by living at thermoneutrality. Cell Metab 9:203–209

Finer N, Bloom SR, Frost GS, Banks LM, Griffiths J (2000) Sibutramine is effective for weight loss and diabetic control in obesity with type 2 diabetes: a randomised, double-blind, placebo-controlled study. Diabetes Obes Metab 2:105–112

Frontini A, Vitali A, Perugini J, Murano I, Romiti C, Ricquier D, Guerrieri M, Cinti S (2013) White-to-brown transdifferentiation of omental adipocytes in patients affected by pheochromocytoma. Biochim Biophys Acta 1831:950–959

Gaich G, Chien JY, Fu H, Glass LC, Deeg MA, Holland WL, Kharitonenkov A, Bumol T, Schilske HK, Moller DE (2013) The effects of LY2405319, an FGF21 analog, in obese human subjects with type 2 diabetes. Cell Metab 18:333–340

Geerling JJ, Boon MR, van der Zon GC, van den Berg SA, van den Hoek AM, Lombes M, Princen HM, Havekes LM, Rensen PC, Guigas B (2014) Metformin lowers plasma triglycerides by promoting VLDL-triglyceride clearance by brown adipose tissue in mice. Diabetes 63:880–891

Gimeno RE, Moller DE (2014) FGF21-based pharmacotherapy–potential utility for metabolic disorders. Trends Endocrinol Metab 25:303–311

Golay A (2008) Metformin and body weight. Int J Obes (Lond) 32:61–72

Hansen DL, Toubro S, Stock MJ, Macdonald IA, Astrup A (1998) Thermogenic effects of sibutramine in humans. Am J Clin Nutr 68:1180–1186

Heaton GM, Wagenvoord RJ, Kemp A Jr, Nicholls DG (1978) Brown-adipose-tissue mitochondria: photoaffinity labelling of the regulatory site of energy dissipation. Eur J Biochem 82:515–521

Huttunen P, Hirvonen J, Kinnula V (1981) The occurrence of brown adipose tissue in outdoor workers. Eur J Appl Physiol Occup Physiol 46:339–345

Kawabata F, Inoue N, Masamoto Y, Matsumura S, Kimura W, Kadowaki M, Higashi T, Tominaga M, Inoue K, Fushiki T (2009) Non-pungent capsaicin analogs (capsinoids) increase metabolic rate and enhance thermogenesis via gastrointestinal TRPV1 in mice. Biosci Biotechnol Biochem 73:2690–2697

Kozak LP (2010) Brown fat and the myth of diet-induced thermogenesis. Cell Metab 11:263–267

Kuji I, Imabayashi E, Minagawa A, Matsuda H, Miyauchi T (2008) Brown adipose tissue demonstrating intense FDG uptake in a patient with mediastinal pheochromocytoma. Ann Nucl Med 22:231–235

Lahesmaa M, Orava J, Schalin-Jantti C, Soinio M, Hannukainen JC, Noponen T, Kirjavainen A, Iida H, Kudomi N, Enerback S, Virtanen KA, Nuutila P (2013) Hyperthyroidism increases brown fat metabolism in humans. J Clin Endocrinol Metab 99(1):E28–35

Larsen TM, Toubro S, van Baak MA, Gottesdiener KM, Larson P, Saris WH, Astrup A (2002) Effect of a 28-d treatment with L-796568, a novel beta(3)-adrenergic receptor agonist, on energy expenditure and body composition in obese men. Am J Clin Nutr 76:780–788

Lee JY, Takahashi N, Yasubuchi M, Kim YI, Hashizaki H, Kim MJ, Sakamoto T, Goto T, Kawada T (2012) Triiodothyronine induces UCP-1 expression and mitochondrial biogenesis in human adipocytes. Am J Physiol Cell Physiol 302:C463–C472

Lee P, Linderman JD, Smith S, Brychta RJ, Wang J, Idelson C, Perron RM, Werner CD, Phan GQ, Kammula US, Kebebew E, Pacak K, Chen KY, Celi FS (2014a) Irisin and FGF21 are cold-induced endocrine activators of brown fat function in humans. Cell Metab 19:302–309

Lee P, Werner CD, Kebebew E, Celi FS (2014b) Functional thermogenic beige adipogenesis is inducible in human neck fat. Int J Obes (Lond) 38:170–176

Lopez M, Varela L, Vazquez MJ, Rodriguez-Cuenca S, Gonzalez CR, Velagapudi VR, Morgan DA, Schoenmakers E, Agassandian K, Lage R, Martinez de Morentin PB, Tovar S, Nogueiras R, Carling D, Lelliott C, Gallego R, Oresic M, Chatterjee K, Saha AK, Rahmouni K, Dieguez C, Vidal-Puig A (2010) Hypothalamic AMPK and fatty acid metabolism mediate thyroid regulation of energy balance. Nat Med 16:1001–1008

Matsushita M, Yoneshiro T, Aita S, Kameya T, Sugie H, Saito M (2014) Impact of brown adipose tissue on body fatness and glucose metabolism in healthy humans. Int J Obes (Lond) 38:812–817

Mukhopadhyay B, Forouhi NG, Fisher BM, Kesson CM, Sattar N (2006) A comparison of glycaemic and metabolic control over time among South Asian and European patients with Type 2 diabetes: results from follow-up in a routine diabetes clinic. Diabet Med 23:94–98

Muzik O, Mangner TJ, Leonard WR, Kumar A, Janisse J, Granneman JG (2013) 15O PET measurement of blood flow and oxygen consumption in cold-activated human brown fat. J Nucl Med 54:523–531

Nedergaard J, Cannon B (2010) The changed metabolic world with human brown adipose tissue: therapeutic visions. Cell Metab 11:268–272

Nedergaard J, Bengtsson T, Cannon B (2007) Unexpected evidence for active brown adipose tissue in adult humans. Am J Physiol Endocrinol Metab 293:E444–E452

Okamatsu-Ogura Y, Fukano K, Tsubota A, Uozumi A, Terao A, Kimura K, Saito M (2013) Thermogenic ability of uncoupling protein 1 in beige adipocytes in mice. PLoS One 8:e84229

Ono K, Tsukamoto-Yasui M, Hara-Kimura Y, Inoue N, Nogusa Y, Okabe Y, Nagashima K, Kato F (2011) Intragastric administration of capsiate, a transient receptor potential channel agonist, triggers thermogenic sympathetic responses. J Appl Physiol (1985) 110:789–798

Orava J, Nuutila P, Lidell ME, Oikonen V, Noponen T, Viljanen T, Scheinin M, Taittonen M, Niemi T, Enerback S, Virtanen KA (2011) Different metabolic responses of human brown adipose tissue to activation by cold and insulin. Cell Metab 14:272–279

Ouellet V, Routhier-Labadie A, Bellemare W, Lakhal-Chaieb L, Turcotte E, Carpentier AC, Richard D (2011) Outdoor temperature, age, sex, body mass index, and diabetic status determine the prevalence, mass, and glucose-uptake activity of 18F-FDG-detected BAT in humans. J Clin Endocrinol Metab 96:192–199

Ouellet V, Labbe SM, Blondin DP, Phoenix S, Guerin B, Haman F, Turcotte EE, Richard D, Carpentier AC (2012) Brown adipose tissue oxidative metabolism contributes to energy expenditure during acute cold exposure in humans. J Clin Invest 122:545–552

Petrak O, Haluzikova D, Kavalkova P, Strauch B, Rosa J, Holaj R, Brabcova VA, Michalsky D, Haluzik M, Zelinka T, Widimsky J Jr (2013) Changes in energy metabolism in pheochromocytoma. J Clin Endocrinol Metab 98:1651–1658

Razak F, Anand SS, Shannon H, Vuksan V, Davis B, Jacobs R, Teo KK, McQueen M, Yusuf S (2007) Defining obesity cut points in a multiethnic population. Circulation 115:2111–2118

Rothwell NJ, Stock MJ (1983) Luxuskonsumption, diet-induced thermogenesis and brown fat: the case in favour. Clin Sci (Lond) 64:19–23

Saito M, Okamatsu-Ogura Y, Matsushita M, Watanabe K, Yoneshiro T, Nio-Kobayashi J, Iwanaga T, Miyagawa M, Kameya T, Nakada K, Kawai Y, Tsujisaki M (2009) High incidence of metabolically active brown adipose tissue in healthy adult humans: effects of cold exposure and adiposity. Diabetes 58:1526–1531

Seale P, Bjork B, Yang W, Kajimura S, Chin S, Kuang S, Scime A, Devarakonda S, Conroe HM, Erdjument-Bromage H, Tempst P, Rudnicki MA, Beier DR, Spiegelman BM (2008) PRDM16 controls a brown fat/skeletal muscle switch. Nature 454:961–967

Soeder KJ, Snedden SK, Cao W, Della Rocca GJ, Daniel KW, Luttrell LM, Collins S (1999) The beta3-adrenergic receptor activates mitogen-activated protein kinase in adipocytes through a Gi-dependent mechanism. J Biol Chem 274:12017–12022

Stanford KI, Middelbeek RJ, Townsend KL, An D, Nygaard EB, Hitchcox KM, Markan KR, Nakano K, Hirshman MF, Tseng YH, Goodyear LJ (2013) Brown adipose tissue regulates glucose homeostasis and insulin sensitivity. J Clin Invest 123:215–223

van Baak MA, Hul GB, Toubro S, Astrup A, Gottesdiener KM, DeSmet M, Saris WH (2002) Acute effect of L-796568, a novel beta 3-adrenergic receptor agonist, on energy expenditure in obese men. Clin Pharmacol Ther 71:272–279

van der Lans AA, Wierts R, Vosselman MJ, Schrauwen P, Brans B, van Marken Lichtenbelt WD (2014) Cold-activated brown adipose tissue in human ADU. Am J Physiol Regul Integr Comp Physiol 307(2):R103–13

van der Lans AA, Hoeks J, Brans B, Vijgen GH, Visser MG, Vosselman MJ, Hansen J, Jorgensen JA, Wu J, Mottaghy FM, Schrauwen P, van Marken Lichtenbelt WD (2014b) Cold acclimation recruits human brown fat and increases nonshivering thermogenesis. J Clin Invest 123:3395–3403

van Marken Lichtenbelt WD, Schrauwen P (2011) Implications of nonshivering thermogenesis for energy balance regulation in humans. Am J Physiol Regul Integr Comp Physiol 301:R285–R296

van Marken Lichtenbelt WD, Schrauwen P, van De KS, Westerterp-Plantenga MS (2002) Individual variation in body temperature and energy expenditure in response to mild cold. Am J Physiol Endocrinol Metab 282:E1077–E1083

van Marken Lichtenbelt WD, Vanhommerig JW, Smulders NM, Drossaerts JM, Kemerink GJ, Bouvy ND, Schrauwen P, Teule GJ (2009) Cold-activated brown adipose tissue in healthy men. N Engl J Med 360:1500–1508

van Ooijen AM, van Marken Lichtenbelt WD, van Steenhoven AA, Westerterp KR (2004) Seasonal changes in metabolic and temperature responses to cold air in humans. Physiol Behav 82:545–553

Vijgen GH, Bouvy ND, Teule GJ, Brans B, Schrauwen P, van Marken Lichtenbelt WD (2011) Brown adipose tissue in morbidly obese subjects. PLoS One 6:e17247

Virtanen KA, Lidell ME, Orava J, Heglind M, Westergren R, Niemi T, Taittonen M, Laine J, Savisto NJ, Enerback S, Nuutila P (2009) Functional brown adipose tissue in healthy adults. N Engl J Med 360:1518–1525

Vosselman MJ, van der Lans AA, Brans B, Wierts R, van Baak MA, Schrauwen P, van Marken Lichtenbelt WD (2012) Systemic beta-adrenergic stimulation of thermogenesis is not accompanied by brown adipose tissue activity in humans. Diabetes 61:3106–3113

Vosselman MJ, Brans B, van der Lans AA, Wierts R, van Baak MA, Mottaghy FM, Schrauwen P, van Marken Lichtenbelt WD (2013) Brown adipose tissue activity after a high-calorie meal in humans. Am J Clin Nutr 98:57–64

Wang ZH, Li YF (2013) Guo YQ: beta3-Adrenoceptor activation attenuates atherosclerotic plaque formation in ApoE(-/-) mice through lowering blood lipids and glucose. Acta Pharmacol Sin 34:1156–1163

Wang Q, Zhang M, Ning G, Gu W, Su T, Xu M, Li B, Wang W (2011) Brown adipose tissue in humans is activated by elevated plasma catecholamines levels and is inversely related to central obesity. PLoS One 6:e21006

Warwick PM, Busby R (1990) Influence of mild cold on 24 h energy expenditure in 'normally' clothed adults. Br J Nutr 63:481–488

Whittle A, Relat-Pardo J, Vidal-Puig A (2013) Pharmacological strategies for targeting BAT thermogenesis. Trends Pharmacol Sci 34:347–355

Wijers SL, Schrauwen P, Saris WH, van Marken Lichtenbelt WD (2008) Human skeletal muscle mitochondrial uncoupling is associated with cold induced adaptive thermogenesis. PLoS One 3:e1777

World Health Organ (2000) Obesity: preventing and managing the global epidemic. Report of a WHO consultation. Tech Rep Ser 894:i–xii, 1–253

Wu J, Bostrom P, Sparks LM, Ye L, Choi JH, Giang AH, Khandekar M, Virtanen KA, Nuutila P, Schaart G, Huang K, Tu H, van Marken Lichtenbelt WD, Hoeks J, Enerback S, Schrauwen P, Spiegelman BM (2012) Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. Cell 150:366–376

Yoneshiro T, Aita S, Matsushita M, Okamatsu-Ogura Y, Kameya T, Kawai Y, Miyagawa M, Tsujisaki M, Saito M (2011a) Age-related decrease in cold-activated brown adipose tissue and accumulation of body fat in healthy humans. Obesity (Silver Spring) 19:1755–1760

Yoneshiro T, Aita S, Matsushita M, Kameya T, Nakada K, Kawai Y, Saito M (2011) Brown adipose tissue, whole-body energy expenditure, and thermogenesis in healthy adult men. Obesity (Silver Spring) 19:13–16

Yoneshiro T, Aita S, Matsushita M, Kayahara T, Kameya T, Kawai Y, Iwanaga T, Saito M (2013) Recruited brown adipose tissue as an antiobesity agent in humans. J Clin Invest 123:3404–3408

Zingaretti MC, Crosta F, Vitali A, Guerrieri M, Frontini A, Cannon B, Nedergaard J, Cinti S (2009) The presence of UCP1 demonstrates that metabolically active adipose tissue in the neck of adult humans truly represents brown adipose tissue. FASEB J 23:3113–3120

Organ-Specific Cancer Metabolism and Its Potential for Therapy

Ilaria Elia*, Roberta Schmieder*, Stefan Christen*, and Sarah-Maria Fendt

Contents

Abstract

Targeting cancer metabolism has the potential to lead to major advances in tumor therapy. Numerous promising metabolic drug targets have been identified. Yet, it has emerged that there is no singular metabolism that defines the

*Equal contributing authors.

I. Elia • R. Schmieder • S. Christen • S.-M. Fendt (✉)
Vesalius Research Center, VIB, 3000 Leuven, Belgium

Department of Oncology, KU Leuven, 3000 Leuven, Belgium
e-mail: sarah-maria.fendt@vib-kuleuven.be

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oncogenic state of the cell. Rather, the metabolism of cancer cells is a function of the requirements of a tumor. Hence, the tissue of origin, the (epi)genetic drivers, the aberrant signaling, and the microenvironment all together define these metabolic requirements. In this chapter we discuss in light of (epi)genetic, signaling, and environmental factors the diversity in cancer metabolism based on triple-negative and estrogen receptor-positive breast cancer, early- and late-stage prostate cancer, and liver cancer. These types of cancer all display distinct and partially opposing metabolic behaviors (e.g., *Warburg* versus *reverse Warburg* metabolism). Yet, for each of the cancers, their distinct metabolism supports the oncogenic phenotype. Finally, we will assess the therapeutic potential of metabolism based on the concepts of metabolic *normalization* and metabolic *depletion*.

Keywords
Cancer metabolism · Epigenetic drivers · Estrogen receptor-positive breast cancer · Fatty acid metabolism · Genetic drivers · Gluconeogenesis · Glucose metabolism · Glutamine metabolism · Liver cancer · Metabolic depletion · Metabolic normalization · Metabolic therapy · Microenvironment · Mixed Warburg effect · Prostate cancer · Reverse Warburg effect · Serine metabolism · Tissue-specific metabolism · Triple-negative breast cancer · Warburg effect

1 Introduction

Cellular metabolism describes a network of biochemical reactions that convert nutrients taken up from the environment into small molecules called metabolites. These metabolites serve as energy equivalents, redox cofactors, biomass building blocks, and substrates for DNA/RNA and protein modifications. In this way metabolism is involved in virtually any cellular process: e.g., proliferation and growth signaling, maintenance of ion gradients across membranes, or epigenetic remodeling via DNA/protein modifications. Hence, metabolism is highly tissue specific, because it is optimized to the function and cellular processes of the different organs. Moreover, metabolism is tightly interconnected with the upstream signaling network to directly link it with the regulation of dependent cellular processes.

Cancer cells induce a major reprogramming of essential cellular processes, which leads to novel abilities such as the evasion of growth control and cell death, the induction of motility and invasion, and the promotion of angiogenesis and to avoidance of immune destruction (Hanahan and Weinberg 2011). Since these processes are directly or indirectly linked to metabolism, the oncogenic transformation of cells requires metabolic changes. Specifically, metabolism fuels the altered cellular requirements of oncogenesis such as energy, redox cofactors, biomass building blocks, or metabolites for DNA/protein modification. In this sense metabolic changes in cancer cells compared to non-malignant cells are a consequence of the cancer needs. Yet, there is also evidence that metabolic changes

can be a cause of cellular transformation. It has been shown that the overexpression of certain enzymes such as 3-phosphoglycerate dehydrogenase leads to oncogenic transformation of non-malignant cells (Locasale 2013). Also, epidemiological studies provide evidence that certain metabolic preconditions such as mutations in the enzyme fumarate hydratase, diabetes, or obesity are correlated with a significantly increased cancer risk (Bianchini et al. 2002; Badrick and Renehan 2014; Tomlinson et al. 2002).

Thus, targeting the altered metabolism of cancer cells in the context of the connected (epi)genetics, signaling, microenvironments, and tumor heterogeneity has the potential to identify novel therapeutic strategies (Galluzzi et al. 2013; Vander Heiden 2011; Cairns et al. 2011; Fulda et al. 2010; Hamanaka and Chandel 2012; Carracedo et al. 2013; DeBerardinis and Thompson 2012; Hensley et al. 2013; Tennant et al. 2010; Keibler et al. 2012; Metallo and Vander Heiden 2013). Besides these factors, another essential parameter in the successful development of metabolism-based anticancer therapies is the organ in which the cancer arises. The organ of origin is important, because its tissue is optimized for a different cellular function with specific metabolic needs. Therefore, oncogenic transformation does not lead to one common set of metabolic changes but multiple metabolic changes that overlap only partially and inconsistently.

Here, we will review based on the examples of breast, liver, and prostate cancer the metabolic diversity of tumors. Subsequently, we will link the organ-specific tumor metabolism to causal (epi)genetic and signaling changes.

2 Breast Cancer Metabolism

2.1 Breast Cancer Facts and Current Treatment

Breast cancer is the leading cause of cancer death in women worldwide (Jemal et al. 2011). Thereby, 90% of all breast cancer deaths are caused by distant metastases to the lung, brain, or bone (Kennecke et al. 2010; Petrut et al. 2008). Up to 30% of all patients diagnosed with early-stage breast cancer develop distant metastases and relapse (Kennecke et al. 2010).

Breast tumors are very heterogeneous at the morphological, molecular, and genetic level (Eroles et al. 2012). With respect to the treatment of breast cancer, three main therapeutic classes can be distinguished. Patients with estrogen receptor-positive tumors (which include luminal A and B tumors) receive endocrine therapy (Goldhirsch et al. 2011; Cancer Genome Atlas Network 2012). Endocrine therapeutics such as tamoxifen block the estrogen receptor and prevent estrogen-induced growth signaling in the tumor. Patients whose tumors show human epidermal growth factor receptor *HER2* (also known as *ERBB2*) amplification receive anti-*HER2* therapy. Triple-negative breast cancers (lacking expression of estrogen receptor, progesterone receptor, and *HER2*), which include primarily basal-like tumors, are currently treated with chemotherapy (Goldhirsch et al. 2011; Cancer Genome Atlas Network 2012; Voduc et al. 2010).

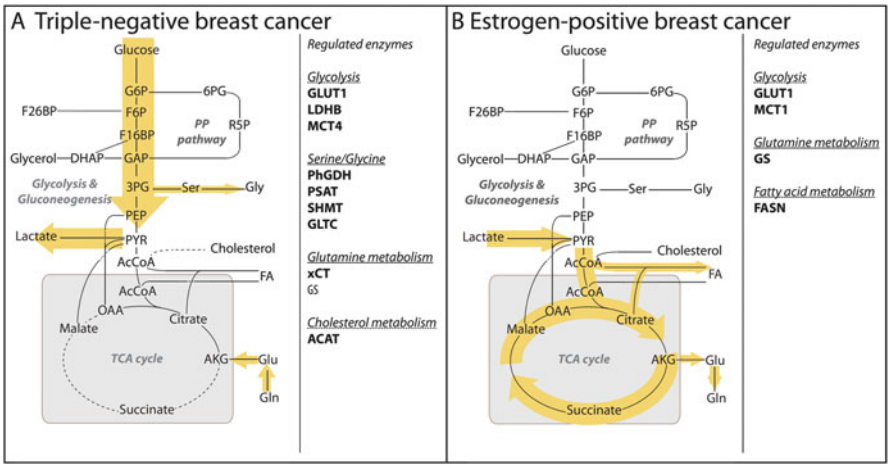

Fig. 1 *Breast cancer metabolism*. (**a**) Metabolism of triple-negative breast cancer. (**b**) Metabolism of estrogen receptor-positive breast cancer. *Yellow arrows* depict the main fluxes within central metabolism, and the *dashed lines* indicate a downregulation of the according metabolic pathway. The reportedly altered enzyme activities are described on the *right of each panel*, where *bold names* indicate an upregulation and *condensed* names a downregulation of the according enzymes. *G6P* glucose-6-phosphate, *F6P* fructose-6-phosphate, *F26BP* fructose-2,6-bisphosphate, *F16BP* fructose-1,6-bisphosphate, *GAP* glyceraldehyde-phosphate, *DHAP* dihydroxyacetone-phosphate, *3PG* 3-phosphosglycerate, *PEP* phosphoenolpyruvate, *6PG* 6-phosphogluconate, *R5P* ribose-5-phosphate, *Pyr* pyruvate, *AcCoA* acetyl-CoA, *FA* fatty acids, *AKG* α-ketoglutarate, *OAA* oxaloacetate

In this section we will discuss the metabolic features of triple-negative and estrogen receptor-positive breast cancers (Fig. 1). We focus on these two subtypes, because triple-negative breast cancers have been studied most intensively due to the lack of specific therapeutic approaches, their prevalence for distant metastasis (Kennecke et al. 2010), and their responsibility for overall high mortality rates (Foulkes et al. 2010). Estrogen receptor-positive (luminal) breast cancer, on the contrary, has led to a lower death incidence rate than triple-negative breast cancer, and specific treatment strategies exist, but it is the most frequently occurring subtype (about 70% of all breast tumors are estrogen receptor positive) (Howlader et al. 2014). Generally, the metabolism of these two subtypes differs dramatically: triple-negative breast cancers mostly display a classical *Warburg* metabolism, while estrogen receptor-positive breast cancers are enriched for a *reverse Warburg* metabolism. This further shows the need to link genetic, molecular, and environmental specificities of cancer cells to their metabolic needs, to enable the development of targeted metabolism-based therapies.

2.2 Triple-Negative Breast Cancer

Many triple-negative breast cancers display a classical *Warburg* metabolism with high glucose uptake and increased lactate secretion even in the presence of oxygen. In vivo measurements of glucose uptake rates using fluorine-18 fluorodeoxyglucose positron emission tomography (FDG-PET) demonstrated that the highly glycolytic phenotype of triple-negative breast cancers is not an artifact from cell culture conditions (Tchou et al. 2010; Groheux et al. 2011; Koo et al. 2014). In line with the increased glucose uptake, several studies have shown an increased expression of glucose and lactate transporters, as well as lactate dehydrogenase, which interconverts pyruvate and lactate (Doyen et al. 2014; Choi et al. 2013; Jeon et al. 2013; McCleland et al. 2012).

There is also evidence that the glycolytic rate of triple-negative breast cancers correlates with tumor aggressiveness. A retrospective analysis of the Ki-67 nuclear stain, which is a measure for the proliferation index of tumors, with the maximal glucose uptake rates measured by FDG-PET, showed a strong correlation of both parameters in triple-negative breast cancers (Tchou et al. 2010). In line with this finding, a correlation between the expression of monocarboxylate transporter 4 (needed for lactate secretion) and clinical outcome has been discovered (Doyen et al. 2014). Interestingly, while lactate dehydrogenase A is ubiquitously highly expressed in many breast tumors, it has been shown that lactate dehydrogenase B is essential for triple-negative breast cancers and co-expressed with monocarboxylate transporter 1 (McCleland et al. 2012). This is surprising, since lactate dehydrogenase B is believed to preferentially convert lactate to pyruvate (Adeva et al. 2013; Porporato et al. 2011). Yet, another study showed that the knockdown of lactate dehydrogenase B similar to the knockdown of lactate dehydrogenase A increased oxygen consumption (Dennison et al. 2013), which is unexpected since these enzymes preferentially use either pyruvate or lactate as a substrate. Thus, the metabolic role of lactate dehydrogenase B in triple-negative breast cancer remains to be elucidated.

The glycolytic phenotype of triple-negative breast cancer correlates with the proliferation index. This implies that glycolysis is important to fuel the energy, redox, or biosynthetic needs of fast-growing tumors. Yet, it is currently not clear which of the three metabolic needs is most important for triple-negative breast cancers. Interestingly, activation of oxidative metabolism in triple-negative breast cancers led to decreased metastasis and decreased tumor growth (Santidrian et al. 2013). This activation was triggered by increasing the activity of complex I of the respiratory chain, which results in an increased NAD^+/NADH ratio (Santidrian et al. 2013; Fendt et al. 2013a). Santidrian et al. correlated the increased NAD^+/NADH ratio with decreased mTORC1 activity (Santidrian et al. 2013) and subsequently increased autophagy (Santidrian et al. 2013). These data argue for a small dynamic range of redox metabolism in triple-negative breast cancer, implying that any imbalance between reduced and oxidized redox cofactors might hamper tumor growth and that only the combination of decreased oxidative

phosphorylation with increased glycolysis allows fast proliferation in certain types of triple-negative breast cancers.

Triple-negative breast cancers have been shown to display an increased uptake of glutamine and cholesterol, while the de novo synthesis of these metabolites was decreased (Antalis et al. 2010; Timmerman et al. 2013; Kung et al. 2011). Timmerman et al. demonstrated in a subset of breast cancer cells – which was highly enriched for the triple-negative subtype – that targeting the activity of the xCT glutamate-cystine antiporter (which mediates the exchange of extracellular L-cysteine and intracellular L-glutamate across the cellular plasma membrane) reduced tumor growth in vivo and in vitro (Timmerman et al. 2013). Recent studies also found that estrogen receptor-negative breast cancers (which include triple-negative breast cancers) have a low glutamine synthetase activity (Kung et al. 2011), resulting in a significantly increased glutamate-to-glutamine ratios compared to normal tissue (Budczies et al. 2014). Contrary to triple-negative breast cancer, estrogen receptor-positive breast cancers have a high glutamine synthetase activity and increased glutamine secretion (Kung et al. 2011). Furthermore, cholesterol storage in the form of cholesterol esters produced by acyl-CoA:cholesterol acyltransferase and cholesterol trafficking was increased in triple-negative breast cancer (Antalis et al. 2010).

In contrast to the decreased de novo synthesis of glutamine and cholesterol, many triple-negative breast cancers activate the serine-glycine biosynthesis pathway. This is mainly caused by gene amplification or expression increase in the enzymes of the serine-glycine pathway such as 3-phosphoglycerate dehydrogenase (Possemato et al. 2011; Locasale et al. 2011; Pollari et al. 2011). Interestingly, in non-transformed breast cancer cell lines 3-phosphoglycerate dehydrogenase is rate limiting for serine biosynthesis (Possemato et al. 2011). This is opposite to the liver where it has been found that phosphoserine phosphatase is rate limiting (Lund et al. 1985). This difference could be explained by low intracellular serine levels in the breast compared to the liver and thus full activity of phosphoserine phosphatase (which is feedback inhibited by serine) (Possemato et al. 2011). Corroborating the relevance of alterations in the serine pathway, increased expression of 3-phosphoglycerate dehydrogenase and phosphoserine aminotransferase 1 is correlated with a decreased relapse-free time and reduced overall survival of breast cancer patients (Pollari et al. 2011). Moreover, the overexpression of 3-phosphoglycerate dehydrogenase, phosphoserine aminotransferase 1, serine hydroxymethyltransferase, or glycine dehydrogenase provoked an oncogenic transformation of non-malignant cells in vitro (Locasale et al. 2011; Zhang et al. 2012; Lee et al. 2014a).

Currently, it is not well understood how alterations in serine-glycine metabolism support tumor proliferation. On one hand, serine hydroxymethyltransferase and glycine dehydrogenase both provide 5,10-methylene-THF, which is an important cofactor precursor for nucleotide biosynthesis and DNA methylation, and both processes are increased in many tumors (Locasale 2013; Xu and Chen 2009). Moreover, the first chemotherapeutic agents (which are chemical derivatives that are still used today) were antifolates and inhibit enzymes in the folate pathway, such as dihydrofolate reductase (Walling 2006). Dihydrofolate reductase provides

THF, which is a precursor to 5,10-methylene-THF. On the other hand, the proliferation defect induced by the knockdown of amplified 3-phosphoglycerate dehydrogenase in triple-negative breast cancers could not be rescued with the supplementation of extracellular serine (Possemato et al. 2011; Locasale et al. 2011), despite the fact that extracellular serine can fuel folate metabolism (Labuschagne et al. 2014). Thus, increased supply of serine does not seem to be the critical function of 3-phosphoglycerate dehydrogenase amplification. Interestingly, Possemato et al. suggested that the coupling of serine biosynthesis to α-ketoglutarate production could be important in 3-phosphoglycerate dehydrogenase-amplified cells (Possemato et al. 2011). Thus, multiple roles of serine-glycine metabolism for breast cancer proliferation are possible, and the identification of precise mechanisms requires further study.

In contrast to the success of an antifolate therapy, several epidemiological studies showed that the risk of breast cancers (and maybe particularly estrogen receptor-negative breast cancer (Harris et al. 2012)) is reduced with increased dietary levels of folate (Harris et al. 2012; Zhang et al. 2003, 2011; Gong et al. 2014; Shrubsole et al. 2011; Chen et al. 2014). This controversial finding of intracellular serine-glycine-folate metabolism versus extracellular, nutrient-derived folate clearly demonstrates the need to not only identify metabolic differences in diseased versus healthy cells but also to understand the regulation of metabolism based on nutrient availability. This important link between nutrient availability and intracellular metabolism is further highlighted through epidemiological studies that associate obesity with the prevalence of triple-negative breast cancer (Pierobon and Frankenfeld 2013).

2.3 Estrogen Receptor-Positive Breast Cancer

In contrast to triple-negative breast cancers, many estrogen receptor-positive breast cancers display the so-called *reverse Warburg* effect. The *reverse Warburg* effect in estrogen receptor-positive breast cancers includes the metabolic interaction between tumor cells and stromal cells. Thereby, cancer cells promote the metabolic reprogramming of stromal cells such as fibroblasts into cancer-associated fibroblasts (Martinez-Outschoorn et al. 2010a). Specifically, fibroblasts co-cultured with breast cancer cells displayed decreased caveolin-1 expression and induction of HIF1α and NFκB (Martinez-Outschoorn et al. 2010b). Notably, HIF1α stabilization can lead to aerobic glycolysis and subsequent increase in lactate and pyruvate secretion via the overexpression of the monocarboxylate transporter 4 (Sotgia et al. 2011). In turn, the cancer cells use the secreted lactate and pyruvate to fuel their tricarboxylic acid cycle (Sotgia et al. 2011). Thus, in this complex interaction, the cancer-associated fibroblasts display a glycolytic metabolism, while the cancer cells rely on oxidative metabolism. The in vivo occurrence of *Warburg* versus *reverse Warburg* effect was shown based on immunostaining of several indicative proteins such as glucose transporter 1, monocarboxylate transporter 4, and ATP synthase in tumoral and stromal tissue microsections (Choi et al. 2013). Interestingly,

this symbiotic behavior of lactate catabolizing and anabolizing cells does not only exist between tumor cells and cancer-associated fibroblasts but also between tumor cells residing in hypoxic regions and tumor cells residing in oxygenated regions (Sonveaux et al. 2008).

In conclusion, triple-negative and estrogen receptor-positive breast cancers show very diverse metabolic phenotypes with rapidly proliferating triple-negative breast cancers displaying a pronounced glycolytic phenotype. This raises the question whether fast proliferation requires a glycolytic metabolism. In line with this notion is the fact that luminal B estrogen receptor-positive breast cancers, which are defined by a high proliferative index, display less often a *reverse Warburg* metabolism compared to slow proliferating luminal A estrogen receptor-positive breast cancers (Choi et al. 2013). Thus, a glycolytic metabolism might promote fast proliferation, yet we still need to understand which metabolic requirement it fulfills.

2.4 Genetic Alterations in Breast Cancer and Their Connection to Metabolism

In addition to the proliferative index of tumors, genetic alterations define metabolic needs of breast cancer cells. The most commonly mutated gene in breast cancer is *p53* (Banerji et al. 2012). p53 is a major regulator of apoptosis, senescence, and cell cycle arrest. However, p53 also regulates various enzymes in metabolism and can promote an oxidative metabolism over a glycolytic metabolism (Berkers et al. 2013). Yet, there is also evidence that certain metabolic targets of p53 such as hexokinase rather promote than inhibit glycolysis (Berkers et al. 2013). Thus, alterations in p53 status might be able to contribute to both the *Warburg* metabolism of triple-negative breast cancer and the *reverse Warburg* metabolism of estrogen receptor-positive breast cancer.

The oncogene MYC is a major regulator of glutamine and glucose metabolism (Dang 2012a). Specifically, MYC promotes catabolism of glutamine (e.g., through glutaminase 1 regulation) (Dang 2012b). Since many triple-negative breast cancers have elevated MYC expression (Horiuchi et al. 2012), this could explain their shift from glutamine synthesis to glutamine uptake. Additionally, MYC induces glycolysis by upregulating lactate dehydrogenase (Dang 2012b) and glucose uptake (Alvarez et al. 2014). Yet, glucose metabolism is not only under the control of MYC and p53. Alvarez et al. demonstrated the importance of the genetic drivers by determining glucose uptake in breast cancer tumors by FDG-PET. Specifically, Akt- and MYC-driven tumors exhibited higher FDG uptake than Wnt1-, HER2-, or RAS-driven tumors, although without a difference in tumor growth. At molecular level, FDG uptake and the activity of the above-mentioned pathways were generally associated with hexokinase-2 and HIF1α stabilization, whereas an association with glucose transporter 1 was only observed in Akt- and HER2-driven tumors (Alvarez et al. 2014).

Additionally, the amplification/overexpression of the genetic driver *HER2* defines fatty acid metabolism in one subtype of breast cancers: it has recently been suggested that HER2 directly phosphorylates and thereby activates fatty acid synthase (Jin et al. 2010). Thus, fatty acid synthase inhibitors might be potent drugs in *HER2*-amplified breast tumors (Jin et al. 2010).

3 Liver Cancer Metabolism

3.1 Liver Cancer Facts and Current Treatment

Under normal physiological conditions, the liver performs essential functions such as processing nutrients, degradation and storage of body fuels, and clearance of toxins. To fulfill these functions, hepatocytes are highly specialized and organized in periportal or perivenous hepatocytes, this is known as functional zonation. Mostly affected by this zonation are processes like ammonia detoxification, glucose/energy metabolism, and xenobiotic metabolism.

Hepatocellular carcinoma (HCC) is one of the deadliest and most common cancers worldwide (El-Serag 2011). In the development of HCC, nutrition- and metabolic-related factors like alcohol consumption, aflatoxin contamination in food (Dohnal et al. 2014), the gut microbiota (Yoshimoto et al. 2013), diabetes (Giovannucci et al. 2010), and bodyweight (Larsson and Wolk 2007) influence initiation and progression of HCC. To treat HCC is difficult, because its onset often occurs on top of an underlying liver disease such as hepatitis B or C virus infection, alcoholic liver disease, or non-alcoholic fatty liver disease. Depending on the stage of the cancer and the liver function, surgical resection, liver transplantation, and radiofrequency ablation are options for patients with early-stage disease. For advanced liver cancer, the multi-kinase inhibitor sorafenib is to date the only approved agent (El-Serag 2011; Bruix et al. 2011). In the following sections, we focus on the metabolic features of healthy versus cancerous liver tissue and the connected (epi)genetic and signaling drivers (Fig. 2).

3.2 Metabolic Alterations in Liver Cancer

The most fundamental change in HCC metabolism is the switch from glucose production (gluconeogenesis) to glucose usage. For more than 50 years, it has been described that in liver cancer compared to normal liver cells the activity of glucose-6-phosphatase, phosphoenolpyruvate carboxykinase, as well as fructose-diphosphatase and hence gluconeogenesis is decreased or absent (Weber and Cantero 1955; Weber and Ashmore 1958; Sweeney et al. 1963; Shonk et al. 1965; Wang et al. 2012). Ma et al. showed in 2013 that this lack of gluconeogenesis might occur due to the decreased expression of 11-β-hydroxysteroid dehydrogenase type 1 and an increased expression of 11-β-hydroxysteroid dehydrogenase type 2. These enzymes control the activity of

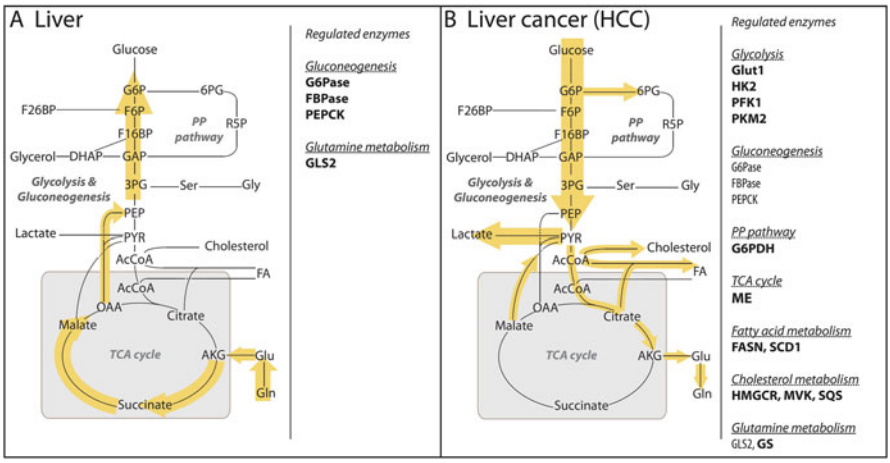

Fig. 2 *Liver cancer metabolism*. (**a**) Metabolism of normal hepatocytes. (**b**) Metabolism of liver cancer. *Yellow arrows* depict the main fluxes within central metabolism, and the *dashed lines* indicate a downregulation of the according metabolic pathway. The reportedly altered enzyme activities are described on the *right of each panel*, where *bold names* indicate an upregulation and *condensed* names a downregulation of the according enzymes. *G6P* glucose-6-phosphate, *F6P* fructose-6-phosphate, *F26BP* fructose-2,6-bisphosphate, *F16BP* fructose-1,6-bisphosphate, *GAP* glyceraldehyde-phosphate, *DHAP* dihydroxyacetone-phosphate, *3PG* 3-phosphosglycerate *PEP* phosphoenolpyruvate, *6PG* 6-phosphogluconate, *R5P* ribose-5-phosphate, *Pyr* pyruvate, *AcCoA* acetyl-CoA, *FA* fatty acids, *AKG* α-ketoglutarate, *OAA* oxaloacetate

glucocorticoids (Ma et al. 2013). Glucocorticoids promote gluconeogenesis, but in liver cancer cells, the altered expression of 11β-hydroxysteroid dehydrogenase 1/2 results in the insensitiveness of liver cancer cells to endogenous glucocorticoids (Ma et al. 2013). Moreover, it was shown that Stat3-mediated activation of the microRNA23a suppresses gluconeogenesis by targeting glucose-6-phosphatase expression (Wang et al. 2012). Consistently, restoring gluconeogenesis by dexamethasone treatment showed significant inhibition of in vivo tumor growth (Ma et al. 2013). A block in gluconeogenesis might contribute to the survival of HCC cells by increased usage of glycolysis and pentose phosphate pathway due to accumulation of glucose-6-phosphate (Wang et al. 2012). In addition to the fundamental loss of gluconeogenesis, it was demonstrated that glycogenesis also decreases during the oncogenic transformation of the liver (Weber and Cantero 1955; Shonk et al. 1965; Weber and Morris 1963). Both phenomena indicate that the catabolism of glucose, compared to its anabolism and storage in the form of glycogen, is essential for liver cancers.

Further evidence for the importance of glucose catabolism in liver cancer has been described since the 1960s. Rapid proliferation was correlated with increased activity of fetal-type liver enzymes like hexokinase-2, glucose-6-phosphate dehydrogenase, and pyruvate kinase-M2 (Shonk et al. 1965). In accordance a decreased activity for adult-type liver enzymes like hexokinase-4 or pyruvate kinase-L was measured (Taketa et al. 1988). To enable a high glycolytic capacity, the liver cancer

tissue must attain an enhanced uptake of glucose. While glucose transporter 2 is most important in healthy liver, the fetal glucose transporter 1 is upregulated in HCC, and its expression is correlated with HCC proliferation and invasiveness (Levitsky et al. 1994; Zheng et al. 1995; Amann et al. 2009; Kitamura et al. 2011). Moreover, patients with a high glucose transporter 1 expression showed higher α-fetoprotein (HCC tumor marker) and poorer differentiation compared to the glucose transporter 1 low-expression cohort (Mano et al. 2014). Finally, FDG-PET imaging confirmed that the described alteration in the expression of glycolytic enzymes and glucose transporter results in an increased glucose uptake rate (Mano et al. 2014; Torizuka et al. 1995; Khan et al. 2000).

Besides glucose metabolism, glutamine metabolism is also significantly altered in liver cancer. The liver-type glutaminase 2, which catalyzes the conversion of glutamine to glutamate, is almost absent or significantly decreased in human HCC (Hu et al. 2010). Consistently, the expression of glutamine synthetase, which catalyzes the opposite reaction, is increased in HCC patients with β-catenin mutations (Audard et al. 2007; Long et al. 2011; Lee et al. 2014b). Glutamine synthetase is a target gene of β-catenin, and overexpression of glutamine synthetase is highly correlated with β-catenin mutations (Audard et al. 2007), which in turn is related to early-stage HCC (Thorgeirsson and Grisham 2002). Moreover, glutamine synthetase expression is correlated with HCC progression (Long et al. 2011; Di Tommaso et al. 2009). Supporting the importance of glutamine metabolism in liver cancer, increased concentrations of glutamate and glutamine have been detected in human HCC in comparison to adjacent normal tissue (Yang et al. 2007). Interestingly, glutamine synthetase expression is related to liver regeneration and therefore is observed during cirrhosis (a pre-state of liver cancer) (Long et al. 2011; Niva et al. 2008). Thus, whether glutamine synthetase expression is a drug target or only a biomarker remains to be determined.

Differential lipid metabolism is an important risk factor in liver cancer (Wu et al. 2010; Calvisi et al. 2011; Budhu et al. 2013). Accordingly, the expression of stearoyl-CoA desaturase, a membrane protein of the endoplasmic reticulum that catalyzes the formation of monounsaturated fatty acids from saturated fatty acids, was found to be associated with aggressiveness of HCC (Calvisi et al. 2011; Budhu et al. 2013; Falvella et al. 2002). Moreover, suppression of stearoyl-CoA desaturase could reduce proliferation in HCC cell lines in an Akt-dependent fashion (Calvisi et al. 2011). One proposed mechanism for the correlation between stearoyl-CoA desaturase expression and HCC aggressiveness might be the systemic link to insulin signaling. Specifically, monounsaturated fatty acids could have an insulin-sensitizing function and thus affect glucose uptake, leading to enhanced capacity for cell proliferation (Cao et al. 2008). In addition to desaturation of lipids, also de novo lipid synthesis was increased in liver cancer. Calvisi et al. showed a progressive induction of mRNA and protein expression of fatty acid synthase, adenosine triphosphate citrate lyase, acetyl-CoA carboxylase, malic enzyme, stearoyl-CoA desaturase 1, 3-hydroxy-3-methylglutaryl-CoA reductase, mevalonate kinase, and squalene synthetase, sterol regulatory element-binding protein 1 and 2, liver X receptors α and β, and carbohydrate-responsive element-binding protein. The induction

of these enzymes was most pronounced in a patient cohort with poor survival outcome (Calvisi et al. 2011).

In conclusion, the loss of gluconeogenesis and potentially glycogenesis is a major factor in the metabolic reprogramming of transformed liver cells. Consequently, glycolysis and fatty acid synthesis are activated. This major switch implies that reactivation of gluconeogenesis has the potential to counteract liver cancer progression (see therapy section). Moreover, it is striking that many of the embryonic isoforms of enzymes such as hexokinase-2 are activated, while the adult isoforms are downregulated. This implies that the liver cancer cells use the embedded natural processes of embryonic proliferation to sustain their uncontrolled proliferation phenotype.

3.3 Genetic, Epigenetic, and Signaling Drivers of Liver Cancer and Their Connection to Metabolism

The most frequently mutated genes in liver cancer are *p53* and *β-catenin* (Moeini et al. 2012). Also signaling pathways like EGFR, VEGFR, Met, and intracellular mediators such as Ras and Akt/mTORC1 may play a role in HCC development and progression (Moeini et al. 2012; Farazi and DePinho 2006). With no common mutations in coding genes that can account for all cases of HCC, it is likely that epigenetic changes are key driving mechanisms of HCC development (Puszyk et al. 2013). In line with this, aberrant DNA methylation patterns have been reported (Yang et al. 2003), and also histone deacetylase 2 is commonly upregulated in human HCC (Lee et al. 2014c). In this section we discuss how these genetic drivers are linked to metabolic changes in liver cancer.

The p53 status in liver cancer can contribute to the alterations in glucose and glutamine metabolism. Huang et al. identified CD147 as an important regulator of the *Warburg* metabolism in HCC cells via a p53 route (Huang et al. 2014). Additionally, Hu et al. demonstrated that the downregulation of glutaminase 2 is p53 dependent. Thus, the p53 status of liver cancer may contribute to the altered glucose and glutamine metabolism during oncogenesis (Hu et al. 2010). Another enzyme involved in glutamine metabolism is the β-catenin target gene glutamine synthetase. Overexpression of glutamine synthetase is highly correlated with *β-catenin* mutation (Audard et al. 2007).

Mutant *EGFR* has been shown in other cancer types to activate Stat3 pathway by means of IL-6 upregulation (Gao et al. 2007). This contributes to inhibition of gluconeogenesis through Stat3-mediated, glucose-6-phosphatase suppression in HCC (Wang et al. 2012).

Histone deacetylase 2 is commonly upregulated in HCC. Upon histone deacetylase 2 knockdown, glycolysis and lipid accumulation are decreased due to the inhibition of PPARγ, ChREBPα, FAS, and SREBP regulation (Lee et al. 2014c). Additionally, the knockdown of histone deacetylase 2 increased acetylation of p53 and in turn led to the expression of p53 target genes, which can counteract the metabolic requirements of liver cancer cells.

4 Prostate Cancer

4.1 Prostate Cancer Facts and Current Treatment

Prostate cancer is the most commonly diagnosed cancer in men in developed countries (Jemal et al. 2011). Whereas patients with well-differentiated tumors can mostly be cured (9.1% mortality after 10 years Lu-Yao et al. 2009), the outcome for patients with progressed and poorly differentiated tumors is detrimental (25.6% mortality after 10 years Lu-Yao et al. 2009).

Currently, there are several options to treat prostate cancer depending on its progression. At first, these therapies include androgen deprivation therapy, radical prostatectomy, and different radiation therapies (Adamis and Varkarakis 2014; Fung et al. 2014; Heidenreich et al. 2014a). Advanced, relapsing, and castration-insensitive prostate cancers are largely treated with therapies that target the androgen signaling pathway and immune therapy (reviewed in Carver 2014; Heidenreich et al. 2014b). In addition, more attempts to develop drugs that act on other prostate cancer targets, such as ETS fusions, or the PI3K signaling pathway, or fatty acid metabolism, are reported (Roychowdhury and Chinnaiyan 2013; Zadra et al. 2013).

Prostate cancer originates in the peripheral zone of the prostate where about 70% of the cancer emerges in a multifocal manner (Shen and Abate-Shen 2010). Upon an inflammatory event, reactive oxygen species accumulation and certain driver mutations, normal epithelial cells progress through different stages to build an adenocarcinoma. The latter might become castration insensitive and metastasizes, preferentially in the bone (reviewed in Shen and Abate-Shen 2010).

Initiation and progression of prostate cancer are highly coupled with metabolic rearrangements. In the following we divide these metabolic events into early changes (primary tumor metabolism) and late stages (undifferentiated and castration-resistant prostate cancer metabolism) (Fig. 3).

4.2 Early-Stage Prostate Cancer Metabolism

In contrast to other tissues, normal prostate epithelial cells rely on aerobic glycolysis, because the tricarboxylic acid cycle enzyme m-aconitase (and thus glucose oxidation) is blocked by high intracellular zinc levels (i.e., zinc levels are three- to tenfold higher than in other soft tissues; Costello et al. 2004, 2005; Franklin and Costello 2007). As a consequence, citrate accumulates and is excreted into the prostatic fluid. In an early step during the transition from healthy to malignant tissue, prostate cells lose their ability to accumulate zinc due to the downregulation of zinc transporters (mainly ZIP1) (Franklin and Costello 2007; Franz et al. 2013). Therefore, the block of the tricarboxylic acid cycle is relieved, and the cells switch their metabolism to generate energy via oxidation of citrate and coupled respiration. Concomitant low glucose uptake rates (i.e., FDG-PET cannot be used to detect

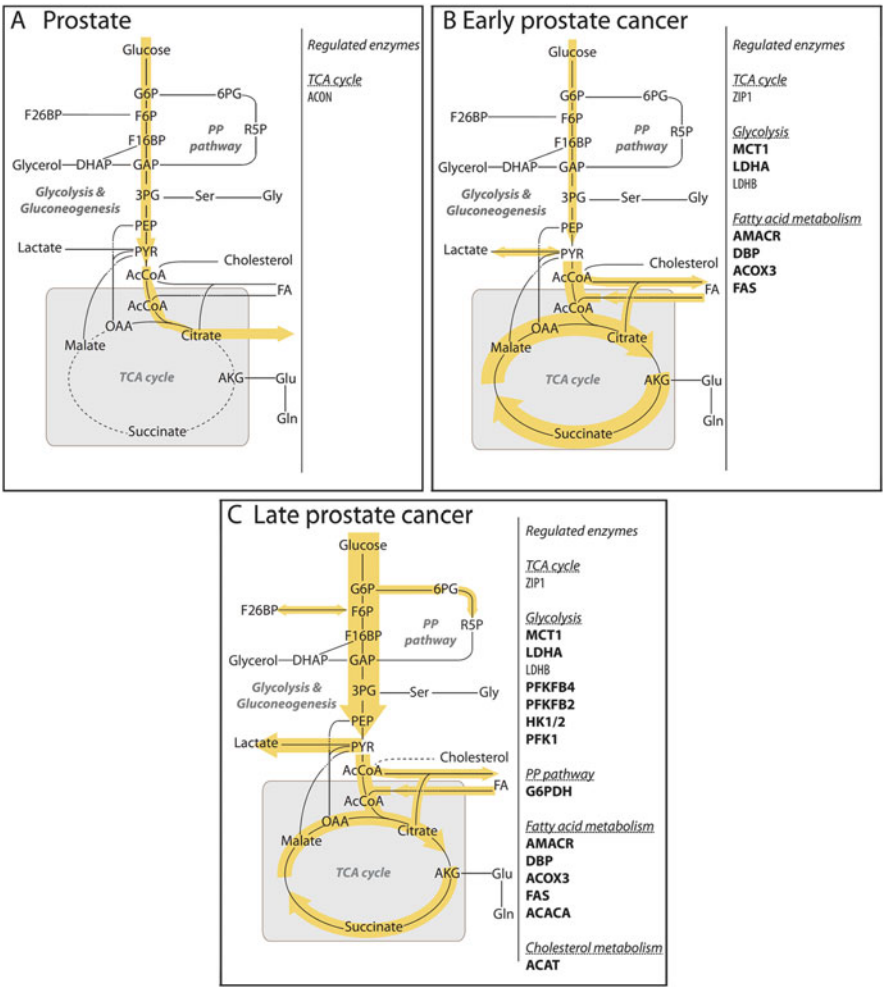

Fig. 3 *Prostate metabolism at different stages of carcinogenesis*. (**a**) Metabolism of healthy prostate fibroblasts. (**b**) Early prostate cancer metabolism. (**c**) Late-stage prostate cancer metabolism. *Yellow arrows* depict the main fluxes within central metabolism and the *dashed lines* indicate a downregulation of the according metabolic pathway. The reportedly altered enzyme activities are described on the *right of each panel*, where *bold names* indicate an upregulation and *condensed* names a downregulation of the according enzymes. *G6P* glucose-6-phosphate, *F6P* fructose-6-phosphate, *F26BP* fructose-2,6-bisphosphate, *F16BP* fructose-1,6-bisphosphate, *GAP* glyceraldehyde-phosphate, *DHAP* dihydroxyacetone-phosphate, *3PG* 3-phosphosglycerate *PEP* phosphoenolpyruvate, *6PG* 6-phosphogluconate, *R5P* ribose-5-phosphate, *Pyr* pyruvate, *AcCoA* acetyl-CoA, *FA* fatty acids, *AKG* α-ketoglutarate, *OAA* oxaloacetate

primary tumors; reviewed in Jadvar 2011; Jadvar et al. 2013) suggest that other substrates are fueling cancer growth. Specifically, a lactate shuttle between cancer-associated fibroblasts and tumor tissue was proposed (Draoui and Feron 2011), since cancer-associated fibroblasts express the monocarboxylate transporter 4 and

tumors overexpress the monocarboxylate transporter 1 (*reverse Warburg* effect) (Fiaschi et al. 2012; Giatromanolaki et al. 2012; Pértega-Gomes et al. 2014; Sanità et al. 2014). This relationship allows the tumor to take up lactate and to convert it to pyruvate, which is processed in the tricarboxylic acid cycle. However, opposing this model, prostate tumors overexpress lactate dehydrogenase A (Pértega-Gomes et al. 2014; Leiblich et al. 2006; Giatromanolaki et al. 2014; Koukourakis et al. 2014), which preferentially works in the direction of lactate production (Adeva et al. 2013; Porporato et al. 2011). Consistently, lactate dehydrogenase B, which catalyzes the lactate to pyruvate conversion, is suppressed (Leiblich et al. 2006; Glen et al. 2008). This is further functionally supported by experiments with hyperpolarized pyruvate, where in situ tumors convert pyruvate into lactate (Albers et al. 2008; Tessem et al. 2008; Keshari et al. 2013). Thus, it is questionable whether cells in vivo exchange lactate or rather other substrates (e.g., pyruvate, since the K_m of monocarboxylate transporter 1 for pyruvate is lower than for lactate Draoui and Feron 2011). Specifically, a more systematic analysis of the cancer environment and consumption and secretion rates might be helpful in resolving this intercell dependency.

However, the cell–cell interaction seems to support in any case oxidative phosphorylation, which is in line with a *reverse Warburg* metabolism. In addition, prostate cancer cells seem to induce peroxisomal branched-chain fatty acid oxidation (alpha-methylacyl-CoA racemase, D-bifunctional protein, acyl-CoA oxidase 3), which includes α-oxidation and a partial β-oxidation (Pértega-Gomes et al. 2014; Rubin et al. 2002; Kumar-Sinha et al. 2004; Zha et al. 2005; Hunt et al. 2014; Visser et al. 2007). Moreover, the short chain fatty acids, which then are released from the peroxisome, might further fuel the TCA cycle by their full β-oxidation in the mitochondria. Although enzymes of mitochondrial β-oxidation are not increased in prostate cancer (Zha et al. 2005), it has been shown that etomoxir-mediated inhibition of mitochondrial β-oxidation at the level of carnitine palmitoyl-transferase 1 induces cell death in prostate cancer cell lines (Schlaepfer et al. 2014). Interestingly, prostate cancer also induces fatty acid biosynthesis by overexpressing fatty acid synthase early during tumor progression without lipid accumulation (Swinnen et al. 2002; Rossi et al. 2003), which is further consistent with ^{11}C-acetate PET/CT experiments (Mena et al. 2012). Why a futile cycle of simultaneous fatty acid oxidation and fatty acid synthesis is beneficial to prostate cancer cells remains an open question.

4.3 Later-Stage Prostate Cancer Metabolism

Further dedifferentiation of the tumor is connected with a more pronounced expression of monocarboxylate transporter 1, lactate dehydrogenase A and altered cholesterol metabolism (Yue et al. 2014), as well as with a boost in fatty acid biosynthesis and associated enzymes (reviewed in Zadra et al. 2013). Moreover, inhibition of fatty acid biosynthesis and oxidation was reported to inhibit tumor growth (Schlaepfer et al. 2014; De Schrijver et al. 2003; Kridel et al. 2004). Yet,

cancer cell lines, such as DU-145, LNCaP, and PC-3, exhibit an elevated fatty acid uptake from the environment, which renders them less sensitive to inhibition of fatty acid biosynthesis (Liu 2006; Liu et al. 2010; Ros et al. 2012). This further underlines the importance of considering the microenvironment, which potentially modulates intracellular metabolism (Ros et al. 2012). Additionally, high-grade (Gleason > 7) and castration-resistant tumors seem to reactivate glycolysis, since FDG-PET and FDG-PET/CT studies indicate an increased glucose uptake compared to benign and low-stage prostate cancers (Yeh et al. 1996; Oyama et al. 1999; Sung et al. 2003; Jadvar et al. 2005; Minamimoto et al. 2011). Accordingly, prostate cancer is affected by 2-deoxyglucose (DiPaola et al. 2008; Ben Sahra et al. 2010; Stein et al. 2010). This is further in agreement with cell line studies, which show that the metastatic androgen-sensitive cell line LNCaP and the castration-resistant low-differentiation cell lines DU-145 and PC-3 are sensitive to glucose starvation (Ros et al. 2012) and that androgen signaling enhances the expression of glycolytic enzymes (Massie et al. 2011; Moon et al. 2011; Tennakoon et al. 2013). However, whether or not an increased expression of the glucose transporter 1 or other hexose transporters is causal for this phenotype is still a matter of debate, since histological data are inconclusive (Chandler et al. 2003; Jans et al. 2010; Reinicke et al. 2012; Vaz et al. 2012).

Interestingly, it was shown in vitro and in xenograft models that prostate cancer cell lines are highly dependent on the phosphofructokinase-fructose-bisphosphatase 2 isoform PFKFB4 and on glucose-6-phosphate dehydrogenase (Ros et al. 2012; Tsouko et al. 2014). Specifically, PFKFB4 drives the balance of glucose-6-phosphate and fructose-1,6-bisphosphate towards glucose-6-phosphate. Latter is used as a substrate in the glucose-6-phosphate dehydrogenase reaction, the initial step of the oxidative pentose phosphate pathway. This suggests that a substantial portion of the glucose taken up is channeled through the pentose phosphate pathway for reduction of $NADP^+$ and consequently glutathione disulfide, as well as for de novo nucleotide biosynthesis (Tsouko et al. 2014; Ros and Schulze 2013). Besides, prostate cancer still relies on oxidative phosphorylation, which is enforced by the interaction with cancer-associated fibroblasts as shown for PC-3 cells (Fiaschi et al. 2012). Consistently metformin, an inhibitor of mitochondrial complex I, provokes a decrease of proliferation with a subsequent activation of reductive glutamine metabolism in vitro and in a TRAMP mouse model (Ben Sahra et al. 2010; Fendt et al. 2013b). Moreover, a combinatorial therapy of metformin and an inhibitor of glutamine metabolism might be promising for therapeutics, since PC-3 and DU-145 cell lines are reportedly glutamine addicted (Liu et al. 2011; Canape et al. 2014), and respiration and fatty acid biosynthesis could thus be inhibited simultaneously (Fendt et al. 2013b).

In sum, so far published data indicate that the first step of an oncogenic transformation of the prostate is a switch to oxidative metabolism. During prostate cancer progression to castrate resistance, this oxidative or *reversed Warburg* metabolism changes to a *mixed Warburg* metabolism, where both glycolysis and respiration serve as sites for energy and biomass precursor generation.

4.4 Genetic Drivers, Signaling, and Microenvironment in Prostate Cancer and Their Connection to Metabolism

In the past decades, several drivers for prostate cancer and progression were identified at the genomic, transcriptional, and protein level (Shen and Abate-Shen 2010; Feldman and Feldman 2001; El Gammal et al. 2010; Gurel et al. 2010; Taylor et al. 2010; Berger et al. 2011; Rubin et al. 2011; Grasso et al. 2012; Karantanos et al. 2013; Weischenfeldt et al. 2013; Walsh et al. 2014). The acquisition of these driver mutations and subsequent tumor progression could either follow a linear pathway or a molecular diversity model as suggested by Rubin et al. (2011). Early drivers are commonly thought to be loss of the transcription factor NKX3.1 protein expression, activation of the MYC transcription factor, and TMPRSS2-ERG gene fusions. These events are often followed by alterations in the PI3K signaling, RB signaling, and RAS/Raf signaling, which render the tumor more aggressive (Taylor et al. 2010). Finally, androgen resistance occurs, which is consistent with the fact that most metastatic tumors have an alteration in the androgen signaling pathway (Feldman and Feldman 2001; Taylor et al. 2010; Karantanos et al. 2013). While there was much effort in mechanistically unraveling these signaling pathways and their interplay, there is less knowledge about their specific impact on prostate cancer metabolism.

As mentioned above, a key event in prostate cancer initiation is the downregulation of zinc transporters, which then allows the cancer to fully oxidize citrate. The regulation of zinc transporters in prostate cancer was shown to depend on the expression of the microRNA cluster miR-183-96-182, which correlates with Gleason score (Mihelich et al. 2011; Tsuchiyama et al. 2013). Another mechanism involves the RAS-responsive element-binding protein 1 (Zou et al. 2011). Specifically, it was found that RAS-responsive element-binding protein 1 is overexpressed early in prostate carcinogenesis and that it downregulates zinc transporter 1. Although zinc transporter 1 might be a target for therapy, currently no drug exists to activate this transporter (Franz et al. 2013). Additionally, a low zinc level might be maintained in later stages by HoxB13-mediated induction of the zinc output transporter ZnT4 (Kim et al. 2013).

The progressive switch of *reverse Warburg* metabolism to a *mixed Warburg* metabolism is mediated by a multitude of signaling pathways, which reportedly include p53 loss, PI3K/AKT activation, MYC overexpression, and androgen signaling. The links to metabolism for p53 loss, PI3K/AKT signaling, and MYC were investigated with the focus on specific metabolic pathways; i.e., MYC induces glutaminase and proline synthesis via miR-23a/miR-23b (Gao et al. 2009; Liu et al. 2012a), the PI3K/AKT pathway upregulates FASN expression (Van de Sande et al. 2002, 2005), and p53 loss induces mitochondrial aconitase expression (Tsui et al. 2011). The role of the androgen receptor in the induction of a *mixed Warburg* phenotype was more globally assessed. Increased androgen-receptor signaling might directly or with the aid of other signaling pathways increase overall metabolic activity (Massie et al. 2011; Moon et al. 2011; Vaz et al. 2012). Specifically, AMPK and consequently PGC-1α could be activated by androgen-receptor-

dependent CAMKK induction, which is connected to increased glycolysis and lactate excretion but also mitochondrial biogenesis (Massie et al. 2011; Tennakoon et al. 2013). Increased glycolytic metabolism is further supported by higher hexokinase-2 expression, which is at least partly triggered by androgen-receptor-dependent activation of PKA/CREB (Moon et al. 2011). Interestingly, AMPK activation seems not to antagonize mTOR signaling status (Massie et al. 2011) and thus allows a mTOR-dependent induction of glucose-6-phosphate dehydrogenase and the oxidative pentose phosphate pathway (Tsouko et al. 2014), which allow an increased $NADP^+$ reduction and nucleotide biosynthesis.

Besides, hypoxic environments and pseudo-hypoxia might play a pivotal role in the modulation of prostate cancer metabolism via HIF1α stabilization. It was shown that HIF1α generally upregulates lactate dehydrogenase A, mitochondrial aconitase and more specifically fatty acid synthase in high-grade tumors (Tsui et al. 2013). Corroborating this result β-arrestin 1 is upregulated in high-grade prostate cancer and stabilizes HIF1α, which is connected to a reduction of succinate dehydrogenase A, fumarate hydratase, dihydrolipoamide dehydrogenase, dihydrolipoyl transacetylase, and pyruvate dehydrogenase (Zecchini et al. 2014). This was further accompanied with an upregulation of glucose uptake and lactate secretion (Zecchini et al. 2014). Overall this indicates that prostate cancer might partly promote anaerobic glycolysis and reductive carboxylation from glutamine in a HIF1α-dependent manner.

In summary, the comparison between breast, liver, and prostate cancer clearly shows that the tissue of origin has a substantial contribution to the definition of a transformed versus healthy metabolism. For example, the reactivation of respiratory metabolism in the prostate is of oncogenic potential, while as well the highly glycolytic phenotype of triple-negative breast cancers sustains tumor proliferation. Beyond the tissue of origin, it seems equally important to consider the (epi)genetic drivers, aberrant signaling, and the microenvironment, since different subtypes of tumors from the same tissue result in a differential metabolism as described for breast cancer. Moreover, the metabolism within one tumor is not static, but – as highlighted for the prostate – it is a dynamic parameter that adapts to the requirements during tumor progression.

5 Therapeutic Opportunities of Cancer Metabolism

The central role of metabolism for all cellular processes in the cell defines it as a promising target for cancer therapy (Table 1), specifically because catalytic functions of metabolic enzymes are generally considered to be easily druggable by small molecules (Keibler et al. 2012). Metabolism is the downstream converging point of the highly interconnected signaling pathways, and thus side effects are less likely and resistance mechanisms harder to employ (Vander Heiden 2011). Moreover, metabolic changes are necessary to enable a certain carcinogenic phenotype. There are two concepts for metabolic drugs, which are given by *normalization* and *depletion*. In the concept of *normalization*, metabolic drugs enforce the redirection

Table 1 Metabolic targets in tumor therapy. Updated and extended summary based on Galluzzi et al. (2013). *PFKFB3* 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3, *PFKFB4* 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 4, *PKM2* pyruvate kinase muscle isozyme 2, *HK* hexokinase, *PGAM* phosphoglycerate mutase family, *G6Pase* glucose-6-phosphatase, *PEPCK* phosphoenolpyruvate carboxykinase, *CK* choline kinase, *ACLY* ATP citrate lyase, *FASN* fatty acid synthase, *MGLL* monoacylglycerol lipase, *CPT1C* carnitine palmitoyltransferase 1C, *HMGCR* 3-hydroxy-3-methylglutaryl-CoA reductase, *PDK1* pyruvate dehydrogenase kinase-1, *GLS1* glutaminase 1, *GDH* glutamate dehydrogenase, *IDH1*/*IDH2* isocitrate dehydrogenase 1/isocitrate dehydrogenase 2, *MCT1* monocarboxylate transporter 1, *MCT4* monocarboxylate transporter 4, *MAE2* malic enzyme 2, *PhGDH* phosphoglycerate dehydrogenase, *ODC* ornithine decarboxylase, *DHFR* dihydrofolate reductase, *RNR* ribonucleotide reductase

Target	Mode of action	Agent	Stage of development	Indication	Refs.
Glycolysis					
Glucose transporters	Depletion	Ritonavir (off-target inhibitory effects on GLUT4)	Preclinical	Multiple myeloma	McBrayer et al. (2012), Liu et al. (2012b)
		WZB117	Preclinical	Lung cancer	
PFKFB3	Normalization	3PO	Preclinical	Lung, breast, leukemic tumors	Klarer et al. (2014), Clem et al. (2008), Telang et al. (2014)
		PFK158	Phase I	Advanced malignancies	
PFKFB4	Normalization	RNAi	Preclinical	Multiple cancer cell lines	Goidts et al. (2012), Chesney et al. (2014)
PKM2	Normalization	Shikonin	Preclinical	Various cancers	Chen et al. (2011), Walsh et al. (2010), Vander Heiden et al. (2010)
		TLN-232	Discontinued clinical trials	Metastatic melanoma	
HK	Depletion	2-DG, 3-BP, lonidamine	Discontinued clinical trials	Advanced solid tumors	Yeruva et al. (2010)
		Methyl jasmonate	Preclinical	Bladder and breast cancer	
PGAM	Depletion	Preclinical compounds	Preclinical	Breast cancer	Evans et al. (2005)
MCT4	Depletion	RNAi	Preclinical	Glioblastoma	Lim et al. (2014)
Gluconeogenesis					
G6Pase, PEPCK	Normalization	Dexamethasone	Preclinical	Liver cancer	Ma et al. (2013)

(continued)

Table 1 (continued)

Target	Mode of action	Agent	Stage of development	Indication	Refs.
Fatty acid metabolism					
CK	Depletion	TCD-717	Approved phase I	Advanced solid tumors	Yalcin et al. (2010)
ACLY	Depletion	RNAi, preclinical compounds	Preclinical	Various cancers	Bauer et al. (2005), Migita et al. (2014), Hatzivassiliou et al. (2005)
FASN	Depletion	TVB-2640	Phase I	Advanced solid tumors	Flavin et al. (2010)
MGLL	Depletion	CK37	Preclinical	Various cancer cells	Nomura et al. (2010), Kapanda et al. (2012)
		JZL184			
CPT1C	Depletion	RNAi	Preclinical	Lung tumor	Zaugg et al. (2011)
HMGCR	Depletion	Statins	Approved	Solid tumors	Nielsen et al. (2012)
TCA cycle and mitochondrial metabolism					
PDK1	Normalization	DCA	Phase II	Glioblastoma; melanoma; non-small cell lung cancer	Dunbar et al. (2014), Zheng et al. (2013)
Complex I	Depletion	Metformin	Approved (not for cancer)	Metformin treatment improves outcomes in cancer patients	Rizos and Elisaf (2013), Oppong et al. (2014), Fendt et al. (2013b)
GLS1	Normalization	RNAi, preclinical compounds	Preclinical		Wang et al. (2010), Gross et al. (2014)
		CB-839	Phase I	Advanced hematologic malignancies and solid tumors	
GDH	Depletion	RNAi, preclinical compounds	Preclinical	Glioblastoma cells	Yang et al. (2009), Li et al. (2011), Friday et al. (2012)
		EGCG			

Mutant IDH1/ IDH2	Normalization	RNAi, preclinical compounds	Preclinical	Glioma, leukemia	Zhang et al. (2013), Dang et al. (2009), Rohle et al. (2013), Stein et al. (2014), Dimitrov et al. (2015)
		AG-221	Phase I	Acute myeloid leukemia	
		AG-120	Phase I	Advanced hematologic tumor and solid tumors	
MCT1	Depletion	RNAi, preclinical compounds (AR-C155858, AR-117977)			Birsoy et al. (2013), Polanski et al. (2014)
		AZD3965 inhibitor	Phase I	Advanced cancers	
MAE2	Normalization	RNAi	Preclinical	Leukemia; solid tumors	Ren et al. (2010, 2014)
Amino acid metabolism					
Asparagine	Depletion	L-asparaginase	Approved	Leukemia	Muller and Boos (1998)
PhGDH	Depletion	RNAi	Preclinical	Melanoma; breast cancer	Possemato et al. (2011), Locasale et al. (2011)
Arginine	Depletion	Arginine deaminase conjugated to PEG	Phase II	HCC (phase II/III); melanoma (phase I/II)	Ni et al. (2008)
ODC	Depletion	DMFO	Phase II	Neuroblastoma	Koomoa et al. (2008)
Nucleic acid synthesis					
DHFR	Depletion	Methotrexate	Approved	Various types of cancer	Goricar et al. (2014), Neradil et al. (2012), Bertino (2009)
Nucleoside analogues	Depletion	5-FU	Approved	Solid cancer	Ghiringhelli and Apetoh (2014)
RNR	Depletion	Gemcitabine	Approved	Pancreatic cancer	Cerqueira et al. (2007)

of the metabolic fluxes (which are the conversion rates of metabolites throughout the metabolic pathways) toward a normal metabolism as defined by healthy cells in the same tissue. In the concept of *depletion*, metabolic drugs inhibit a pathway that is predominately essential for the tumor cells, and thus they drain the metabolic requirements of the tumor.

The concept of *depletion* has a long-standing history in cancer treatment with antifolates, nucleoside analogues, and asparaginase (Walling 2006; Muller and Boos 1998; Galmarini et al. 2002) as examples. The underlying principle for the use of antifolates and nucleoside analogues is the dependency of fast-proliferating cells on de novo DNA synthesis. Since not only cancer cells can display a fast proliferation, these drugs lead to a general collateral damage. Nevertheless, their efficiency and side effects are similar to other non-metabolic chemotherapeutic agents such as paclitaxel, which inhibits mitosis (Kumar et al. 2010; Baldo and Pagani 2014). The expression of asparaginase is a more specific therapy than nucleoside analogues, since acute lymphoblastic leukemia is asparagine auxotrophs and depends on the uptake of sufficient amounts of asparagine. Thus the expression of asparaginase *depletes* the availability of asparagine because it degrades it to aspartate (Muller and Boos 1998).

The same concept of *depletion* applies to more recently found inhibitors of fatty acid synthase or choline kinase (Ross et al. 2008; Yalcin et al. 2010), which are in various (pre)clinical phases. Thereby, the concept of *depletion* goes always along with the risk that either the metabolites that are synthesized can be taken up from the environment or that the pathway is also essential for non-tumorigenic cells.

The concept of *normalization* is built upon the fact that some metabolic pathways such as glycolysis are also needed in healthy cells and that cancer cells display a hyperactivation of such pathways. A preclinical example for the former is 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3, which is a positive regulator of the glycolytic enzyme phosphofructokinase. Inhibition of this regulator decreases but does not inhibit glycolysis. Thus, *normalization* is sufficient to lead to proliferation inhibition, while healthy cells with lower glycolysis are unaffected (De Bock et al. 2013). Accordingly, targeting the 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 in hyper-sprouting blood vessels leads to a normalization of the blood vessels (Schoors et al. 2014).

Not only the *normalization* of hyperactivated metabolic pathways leads to a therapeutic benefit but also the reactivation of pathways that are active in healthy tissue. This is exemplified by the potency of reactivating gluconeogenesis in liver and kidney cancer. In the healthy liver glucocorticoids promote gluconeogenesis. However, in liver cancer cells, the enzymes that convert the glucocorticoids into their active form are aberrantly regulated, resulting in the insensitivity of liver cancer cells to endogenous glucocorticoids (Ma et al. 2013). Yet, treating liver cancers with dexamethasone, which is a synthetic and active glucocorticoid, led to the reactivation of gluconeogenesis and increased therapeutic efficacy (Ma et al. 2013). Recently, it was also shown that the re-expression of the gluconeogenic enzyme fructose-1,6-bisphosphatase 1 in renal cell carcinoma antagonized its glycolytic phenotype (Aleksandrova et al. 2014).

Examples for novel drug targets, which are in preclinical and clinical trials and follow the concept of *normalization*, are inhibitors of the mutant form of isocitrate dehydrogenase. Point mutations in isocitrate dehydrogenase isoform 1 or isoform 2 are highly abundant in glioma, glioblastoma, and acute myeloid leukemia. The presence of mutant isocitrate dehydrogenase in the cell leads to the production of 2-hydroxyglutarate from α-ketoglutarate, which is the product of the non-mutated isocitrate dehydrogenase reaction. Since 2-hydroxyglutarate is an endpoint metabolite that is not further converted, it rapidly accumulates in the cells and outcompetes the structurally similar α-ketoglutarate as a cofactor for α-ketoglutarate-dependent dioxygenases (Yan et al. 2009; Dang et al. 2010). This leads to an inhibition of the α-ketoglutarate-dependent dioxygenases and consequently to hypermethylation (Turcan et al. 2012). The altered methylation patterns thereby promote dedifferentiation of the tumors. Consequently, the specific inhibition of the mutant isocitrate dehydrogenase enzyme *normalizes* the methylation pattern in the tumor and leads to differentiation of tumor cells and inhibition of tumor proliferation (Popovici-Muller et al. 2012; Wang et al. 2013; Lu et al. 2012). Mutant isocitrate dehydrogenase inhibitors thereby constitute an ideal case of a drug target, since the mutation is only present in the tumor but not in any healthy tissue throughout the body.

In conclusion, metabolism offers a wide range of drug targets that can be exploited for cancer therapy. Yet, the current challenge is to overcome the idea that metabolism is a single and consistent entity and to analyze cancer metabolism in the context of the tissue of origin, the (epi)genetics of the individual tumors, signaling aberrations, cancer cell heterogeneity (including cancer associate cells), and the associated microenvironment. Thus, metabolic drugs require that we move from a general standard therapy toward personalized medicine. Given the dramatic variance in tissue and specific metabolism, the possibility of a targeted delivery of drugs opens another horizon for metabolism-based therapeutic strategies.

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References

Adamis S, Varkarakis IM (2014) Defining prostate cancer risk after radical prostatectomy. Eur J Surg Oncol 40(5):496–504

Adeva M et al (2013) Enzymes involved in l-lactate metabolism in humans. Mitochondrion 13 (6):615–629

Albers MJ et al (2008) Hyperpolarized 13C lactate, pyruvate, and alanine: noninvasive biomarkers for prostate cancer detection and grading. Cancer Res 68:8607–8615

Aleksandrova K et al (2014) Inflammatory and metabolic biomarkers and risk of liver and biliary tract cancer. Hepatology 60:858–871

Alvarez JV et al (2014) Oncogene pathway activation in mammary tumors dictates [18F]-FDG-PET uptake. Cancer Res 74:7583–7598

Amann T et al (2009) GLUT1 expression is increased in hepatocellular carcinoma and promotes tumorigenesis. Am J Pathol 174(4):1544–1552
Antalis CJ et al (2010) High ACAT1 expression in estrogen receptor negative basal-like breast cancer cells is associated with LDL-induced proliferation. Breast Cancer Res Treat 122 (3):661–670
Audard V et al (2007) Cholestasis is a marker for hepatocellular carcinomas displaying beta-catenin mutations. J Pathol 212(3):345–352
Badrick E, Renehan AG (2014) Diabetes and cancer: 5 years into the recent controversy. Eur J Cancer 50(12):2119–2125
Baldo BA, Pagani M (2014) Adverse events to nontargeted and targeted chemotherapeutic agents: emphasis on hypersensitivity responses. Immunol Allergy Clin North Am 34(3):565–596, viii
Banerji S et al (2012) Sequence analysis of mutations and translocations across breast cancer subtypes. Nature 486(7403):405–409
Bauer DE et al (2005) ATP citrate lyase is an important component of cell growth and transformation. Oncogene 24(41):6314–6322
Ben Sahra I, Tanti J-F, Bost F (2010) The combination of metformin and 2-deoxyglucose inhibits autophagy and induces AMPK-dependent apoptosis in prostate cancer cells. Autophagy 6:670–671
Berger MF et al (2011) The genomic complexity of primary human prostate cancer. Nature 470 (7333):214–220
Berkers CR et al (2013) Metabolic regulation by p53 family members. Cell Metab 18(5):617–633
Bertino JR (2009) Cancer research: from folate antagonism to molecular targets. Best Pract Res Clin Haematol 22(4):577–582
Bianchini F, Kaaks R, Vainio H (2002) Overweight, obesity, and cancer risk. Lancet Oncol 3 (9):565–574
Birsoy K et al (2013) MCT1-mediated transport of a toxic molecule is an effective strategy for targeting glycolytic tumors. Nat Genet 45(1):104–108
Bruix J, Sherman M, American Association for the Study of Liver Diseases (2011) Management of hepatocellular carcinoma: an update. Hepatology 53(3):1020–1022
Budczies J et al (2014) Glutamate enrichment as new diagnostic opportunity in breast cancer. Int J Cancer 136:1619–1628
Budhu A et al (2013) Integrated metabolite and gene expression profiles identify lipid biomarkers associated with progression of hepatocellular carcinoma and patient outcomes. Gastroenterology 144(5):1066–1075, e1
Cairns RA, Harris IS, Mak TW (2011) Regulation of cancer cell metabolism. Nat Rev Cancer 11 (2):85–95
Calvisi DF et al (2011) Increased lipogenesis, induced by AKT-mTORC1-RPS6 signaling, promotes development of human hepatocellular carcinoma. Gastroenterology 140(3):1071–1083
Canape C et al (2014) Probing treatment response of glutaminolytic prostate cancer cells to natural drugs with hyperpolarized [5- C]glutamine. Magn Reson Med
Cancer Genome Atlas Network (2012) Comprehensive molecular portraits of human breast tumours. Nature 490(7418):61–70
Cao H et al (2008) Identification of a lipokine, a lipid hormone linking adipose tissue to systemic metabolism. Cell 134(6):933–944
Carracedo A, Cantley LC, Pandolfi PP (2013) Cancer metabolism: fatty acid oxidation in the limelight. Nat Rev Cancer 13(4):227–232
Carver BS (2014) Strategies for targeting the androgen receptor axis in prostate cancer. Drug Discov Today 19:1493–1497
Cerqueira NM, Fernandes PA, Ramos MJ (2007) Understanding ribonucleotide reductase inactivation by gemcitabine. Chemistry 13(30):8507–8515
Chandler JD et al (2003) Expression and localization of GLUT1 and GLUT12 in prostate carcinoma. Cancer 97:2035–2042

Chen J et al (2011) Shikonin and its analogs inhibit cancer cell glycolysis by targeting tumor pyruvate kinase-M2. Oncogene 30(42):4297–4306
Chen P et al (2014) Higher dietary folate intake reduces the breast cancer risk: a systematic review and meta-analysis. Br J Cancer 110(9):2327–2338
Chesney J et al (2014) Fructose-2,6-bisphosphate synthesis by 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 4 (PFKFB4) is required for the glycolytic response to hypoxia and tumor growth. Oncotarget 5(16):6670–6686
Choi J et al (2013) Metabolic interaction between cancer cells and stromal cells according to breast cancer molecular subtype. Breast Cancer Res 15(5):R78
Clem B et al (2008) Small-molecule inhibition of 6-phosphofructo-2-kinase activity suppresses glycolytic flux and tumor growth. Mol Cancer Ther 7(1):110–120
Costello LC et al (2004) Role of zinc in the pathogenesis and treatment of prostate cancer: critical issues to resolve. Prostate Cancer Prostatic Dis 7:111–117
Costello LC, Franklin RB, Feng P (2005) Mitochondrial function, zinc, and intermediary metabolism relationships in normal prostate and prostate cancer. Mitochondrion 5:143–153
Dang CV (2012a) Links between metabolism and cancer. Genes Dev 26(9):877–890
Dang CV (2012b) MYC on the path to cancer. Cell 149(1):22–35
Dang L et al (2009) Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. Nature 462 (7274):739–744
Dang L, Jin S, Su SM (2010) IDH mutations in glioma and acute myeloid leukemia. Trends Mol Med 16(9):387–397
De Bock K et al (2013) Role of PFKFB3-driven glycolysis in vessel sprouting. Cell 154 (3):651–663
De Schrijver E et al (2003) RNA interference-mediated silencing of the fatty acid synthase gene attenuates growth and induces morphological changes and apoptosis of LNCaP prostate cancer cells. Cancer Res 63:3799–3804
DeBerardinis RJ, Thompson CB (2012) Cellular metabolism and disease: what do metabolic outliers teach us? Cell 148(6):1132–1144
Dennison JB et al (2013) Lactate dehydrogenase B: a metabolic marker of response to neoadjuvant chemotherapy in breast cancer. Clin Cancer Res 19(13):3703–3713
Di Tommaso L et al (2009) The application of markers (HSP70 GPC3 and GS) in liver biopsies is useful for detection of hepatocellular carcinoma. J Hepatol 50(4):746–754
DiPaola RS et al (2008) Therapeutic starvation and autophagy in prostate cancer: a new paradigm for targeting metabolism in cancer therapy. Prostate 68:1743–1752
Dimitrov L, Hong CS, Yang C, Zhuang Z, Heiss JD (2015) New developments in the pathogenesis and therapeutic targeting of the IDH1 mutation in glioma. Int J Med Sci 12(3):201–213
Dohnal V, Wu Q, Kuca K (2014) Metabolism of aflatoxins: key enzymes and interindividual as well as interspecies differences. Arch Toxicol 88(9):1635–1644
Doyen J et al (2014) Expression of the hypoxia-inducible monocarboxylate transporter MCT4 is increased in triple negative breast cancer and correlates independently with clinical outcome. Biochem Biophys Res Commun 451(1):54–61
Draoui N, Feron O (2011) Lactate shuttles at a glance: from physiological paradigms to anti-cancer treatments. Dis Model Mech 4:727–732
Dunbar EM et al (2014) Phase 1 trial of dichloroacetate (DCA) in adults with recurrent malignant brain tumors. Invest New Drugs 32(3):452–464
El Gammal AT et al (2010) Chromosome 8p deletions and 8q gains are associated with tumor progression and poor prognosis in prostate cancer. Clin Cancer Res 16(1):56–64
El-Serag HB (2011) Hepatocellular carcinoma. N Engl J Med 365(12):1118–1127
Eroles P et al (2012) Molecular biology in breast cancer: intrinsic subtypes and signaling pathways. Cancer Treat Rev 38(6):698–707
Evans MJ et al (2005) Target discovery in small-molecule cell-based screens by in situ proteome reactivity profiling. Nat Biotechnol 23(10):1303–1307

Falvella FS et al (2002) Stearoyl-CoA desaturase 1 (Scd1) gene overexpression is associated with genetic predisposition to hepatocarcinogenesis in mice and rats. Carcinogenesis 23 (11):1933–1936
Farazi PA, DePinho RA (2006) Hepatocellular carcinoma pathogenesis: from genes to environment. Nat Rev Cancer 6(9):674–687
Feldman BJ, Feldman D (2001) The development of androgen-independent prostate cancer. Nat Rev Cancer 1:34–45
Fendt SM et al (2013a) Reductive glutamine metabolism is a function of the alpha-ketoglutarate to citrate ratio in cells. Nat Commun 4:2236
Fendt S-M et al (2013b) Metformin decreases glucose oxidation and increases the dependency of prostate cancer cells on reductive glutamine metabolism. Cancer Res 73:4429–4438
Fiaschi T et al (2012) Reciprocal metabolic reprogramming through lactate shuttle coordinately influences tumor-stroma interplay. Cancer Res 72:5130–5140
Flavin R et al (2010) Fatty acid synthase as a potential therapeutic target in cancer. Future Oncol 6 (4):551–562
Foulkes WD, Smith IE, Reis-Filho JS (2010) Triple-negative breast cancer. N Engl J Med 363 (20):1938–1948
Franklin RB, Costello LC (2007) Zinc as an anti-tumor agent in prostate cancer and in other cancers. Arch Biochem Biophys 463:211–217
Franz MC et al (2013) Zinc transporters in prostate cancer. Mol Aspects Med 34(2–3):735–741
Friday E et al (2012) Role of glutamate dehydrogenase in cancer growth and homeostasis. In: Dehydrogenases. InTech, Rijeka, Croatia, pp 181–190
Fulda S, Galluzzi L, Kroemer G (2010) Targeting mitochondria for cancer therapy. Nat Rev Drug Discov 9(6):447–464
Fung C, Dale W, Mohile SG (2014) Prostate cancer in the elderly patient. J Clin Oncol 32:2523–2530
Galluzzi L et al (2013) Metabolic targets for cancer therapy. Nat Rev Drug Discov 12(11):829–846
Galmarini CM, Mackey JR, Dumontet C (2002) Nucleoside analogues and nucleobases in cancer treatment. Lancet Oncol 3(7):415–424
Gao SP et al (2007) Mutations in the EGFR kinase domain mediate STAT3 activation via IL-6 production in human lung adenocarcinomas. J Clin Invest 117(12):3846–3856
Gao P et al (2009) c-Myc suppression of miR-23a/b enhances mitochondrial glutaminase expression and glutamine metabolism. Nature 458(7239):762–765
Ghiringhelli F, Apetoh L (2014) Enhancing the anticancer effects of 5-fluorouracil: current challenges and future perspectives. Biomed J
Giatromanolaki A et al (2012) The metabolic interactions between tumor cells and tumor-associated stroma (TAS) in prostatic cancer. Cancer Biol Ther 13:1284–1289
Giatromanolaki A et al (2014) Autophagy proteins in prostate cancer: relation with anaerobic metabolism and Gleason score. Urol Oncol 32:39.e11–8
Giovannucci E et al (2010) Diabetes and cancer: a consensus report. CA Cancer J Clin 60 (4):207–221
Glen A et al (2008) iTRAQ-facilitated proteomic analysis of human prostate cancer cells identifies proteins associated with progression. J Proteome Res 7:897–907
Goidts V et al (2012) RNAi screening in glioma stem-like cells identifies PFKFB4 as a key molecule important for cancer cell survival. Oncogene 31(27):3235–3243
Goldhirsch A et al (2011) Strategies for subtypes–dealing with the diversity of breast cancer: highlights of the St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2011. Ann Oncol 22(8):1736–47
Gong Z et al (2014) Associations of dietary folate, vitamins B6 and B12 and methionine intake with risk of breast cancer among African American and European American women. Int J Cancer 134(6):1422–1435
Goricar K et al (2014) Influence of the folate pathway and transporter polymorphisms on methotrexate treatment outcome in osteosarcoma. Pharmacogenet Genomics 24:514–521

Grasso CS et al (2012) The mutational landscape of lethal castration-resistant prostate cancer. Nature 487(7406):239–243
Groheux D et al (2011) Correlation of high 18F-FDG uptake to clinical, pathological and biological prognostic factors in breast cancer. Eur J Nucl Med Mol Imaging 38(3):426–435
Gross MI et al (2014) Antitumor activity of the glutaminase inhibitor CB-839 in triple-negative breast cancer. Mol Cancer Ther 13(4):890–901
Gurel B et al (2010) NKX3.1 as a marker of prostatic origin in metastatic tumors. Am J Surg Pathol 34(8):1097–1105
Hamanaka RB, Chandel NS (2012) Targeting glucose metabolism for cancer therapy. J Exp Med 209(2):211–215
Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144(5):646–674
Harris HR, Bergkvist L, Wolk A (2012) Folate intake and breast cancer mortality in a cohort of Swedish women. Breast Cancer Res Treat 132(1):243–250
Hatzivassiliou G et al (2005) ATP citrate lyase inhibition can suppress tumor cell growth. Cancer Cell 8(4):311–321
Heidenreich A et al (2014a) EAU guidelines on prostate cancer. Part 1: screening, diagnosis, and local treatment with curative intent-update 2013. Eur Urol 65(1):124–137
Heidenreich A et al (2014b) EAU guidelines on prostate cancer. Part II: treatment of advanced, relapsing, and castration-resistant prostate cancer. Eur Urol 65(2):467–479
Hensley CT, Wasti AT, DeBerardinis RJ (2013) Glutamine and cancer: cell biology, physiology, and clinical opportunities. J Clin Invest 123(9):3678–3684
Horiuchi D et al (2012) MYC pathway activation in triple-negative breast cancer is synthetic lethal with CDK inhibition. J Exp Med 209(4):679–696
Howlader N et al (2014) US incidence of breast cancer subtypes defined by joint hormone receptor and HER2 status. J Natl Cancer Inst 106(5):1–8
Hu W et al (2010) Glutaminase 2, a novel p53 target gene regulating energy metabolism and antioxidant function. Proc Natl Acad Sci U S A 107(16):7455–7460
Huang Q et al (2014) CD147 promotes reprogramming of glucose metabolism and cell proliferation in HCC cells by inhibiting the p53-dependent signaling pathway. J Hepatol 61(4):859–866
Hunt MC, Tillander V, Alexson SE (2014) Regulation of peroxisomal lipid metabolism: the role of acyl-CoA and coenzyme A metabolizing enzymes. Biochimie 98:45–55
Jadvar H (2011) Prostate cancer: PET with 18F-FDG, 18F- or 11C-acetate, and 18F- or 11C-choline. J Nucl Med 52:81–89
Jadvar H et al (2005) Glucose metabolism of human prostate cancer mouse xenografts. Mol Imaging 4:91–97
Jadvar H et al (2013) Baseline 18F-FDG PET/CT parameters as imaging biomarkers of overall survival in castrate-resistant metastatic prostate cancer. J Nucl Med 54:1195–1201
Jans J et al (2010) Expression and localization of hypoxia proteins in prostate cancer: prognostic implications after radical prostatectomy. Urology 75:786–792
Jemal A et al (2011) Global cancer statistics. CA Cancer J Clin 61(2):69–90
Jeon HM et al (2013) Expression of cell metabolism-related genes in different molecular subtypes of triple-negative breast cancer. Tumori 99(4):555–564
Jin Q et al (2010) Fatty acid synthase phosphorylation: a novel therapeutic target in HER2-overexpressing breast cancer cells. Breast Cancer Res 12(6):R96
Kapanda CN et al (2012) Synthesis and pharmacological evaluation of 2,4-dinitroaryldithiocarbamate derivatives as novel monoacylglycerol lipase inhibitors. J Med Chem 55(12):5774–5783
Karantanos T, Corn PG, Thompson TC (2013) Prostate cancer progression after androgen deprivation therapy: mechanisms of castrate resistance and novel therapeutic approaches. Oncogene 32(49):5501–5511
Keibler MA, Fendt SM, Stephanopoulos G (2012) Expanding the concepts and tools of metabolic engineering to elucidate cancer metabolism. Biotechnol Prog 28(6):1409–1418
Kennecke H et al (2010) Metastatic behavior of breast cancer subtypes. J Clin Oncol 28 (20):3271–3277

Keshari KR et al (2013) Metabolic reprogramming and validation of hyperpolarized 13C lactate as a prostate cancer biomarker using a human prostate tissue slice culture bioreactor. Prostate 73:1171–1181
Khan MA et al (2000) Positron emission tomography scanning in the evaluation of hepatocellular carcinoma. J Hepatol 32(5):792–797
Kim YR et al (2013) HOXB13 downregulates intracellular zinc and increases NF-kappaB signaling to promote prostate cancer metastasis. Oncogene 33:4558–4567
Kitamura K et al (2011) Proliferative activity in hepatocellular carcinoma is closely correlated with glucose metabolism but not angiogenesis. J Hepatol 55(4):846–857
Klarer AC et al (2014) Inhibition of 6-phosphofructo-2-kinase (PFKFB3) induces autophagy as a survival mechanism. Cancer Metab 2(1):2
Koo HR et al (2014) 18F-FDG uptake in breast cancer correlates with immunohistochemically defined subtypes. Eur Radiol 24(3):610–618
Koomoa DL et al (2008) Ornithine decarboxylase inhibition by alpha-difluoromethylornithine activates opposing signaling pathways via phosphorylation of both Akt/protein kinase B and p27Kip1 in neuroblastoma. Cancer Res 68(23):9825–9831
Koukourakis MI et al (2014) Lactate dehydrogenase 5 isoenzyme overexpression defines resistance of prostate cancer to radiotherapy. Br J Cancer 110:2217–2223
Kridel SJ et al (2004) Orlistat is a novel inhibitor of fatty acid synthase with antitumor activity. Cancer Res 64:2070–2075
Kumar S et al (2010) Clinical trials and progress with paclitaxel in ovarian cancer. Int J Womens Health 2:411–427
Kumar-Sinha C et al (2004) Elevated alpha-methylacyl-CoA racemase enzymatic activity in prostate cancer. Am J Pathol 164(3):787–793
Kung HN, Marks JR, Chi JT (2011) Glutamine synthetase is a genetic determinant of cell type-specific glutamine independence in breast epithelia. PLoS Genet 7(8):e1002229
Labuschagne CF et al (2014) Serine, but not glycine, supports one-carbon metabolism and proliferation of cancer cells. Cell Rep 7(4):1248–1258
Larsson SC, Wolk A (2007) Overweight, obesity and risk of liver cancer: a meta-analysis of cohort studies. Br J Cancer 97(7):1005–1008
Lee GY et al (2014a) Comparative oncogenomics identifies PSMB4 and SHMT2 as potential cancer driver genes. Cancer Res 74(11):3114–3126
Lee JM et al (2014b) beta-Catenin signaling in hepatocellular cancer: implications in inflammation, fibrosis, and proliferation. Cancer Lett 343(1):90–97
Lee YH et al (2014c) Antitumor effects in hepatocarcinoma of isoform-selective inhibition of HDAC2. Cancer Res 74(17):4752–4761
Leiblich A et al (2006) Lactate dehydrogenase-B is silenced by promoter hypermethylation in human prostate cancer. Oncogene 25:2953–2960
Levitsky LL et al (1994) GLUT-1 and GLUT-2 mRNA, protein, and glucose transporter activity in cultured fetal and adult hepatocytes. Am J Physiol 267(1 Pt 1):E88–E94
Li C et al (2011) Green tea polyphenols control dysregulated glutamate dehydrogenase in transgenic mice by hijacking the ADP activation site. J Biol Chem 286(39):34164–34174
Lim KS et al (2014) Inhibition of monocarboxylate transporter-4 depletes stem-like glioblastoma cells and inhibits HIF transcriptional response in a lactate-independent manner. Oncogene 33 (35):4433–4441
Liu Y (2006) Fatty acid oxidation is a dominant bioenergetic pathway in prostate cancer. Prostate Cancer Prostatic Dis 9:230–234
Liu Y, Zuckier LS, Ghesani NV (2010) Dominant uptake of fatty acid over glucose by prostate cells: a potential new diagnostic and therapeutic approach. Anticancer Res 30:369–374
Liu X, Fu YM, Meadows GG (2011) Differential effects of specific amino acid restriction on glucose metabolism, reduction/oxidation status and mitochondrial damage in DU145 and PC3 prostate cancer cells. Oncol Lett 2(2):349–355

Liu W et al (2012a) Reprogramming of proline and glutamine metabolism contributes to the proliferative and metabolic responses regulated by oncogenic transcription factor c-MYC. Proc Natl Acad Sci U S A 109(23):8983–8988
Liu Y et al (2012b) A small-molecule inhibitor of glucose transporter 1 downregulates glycolysis, induces cell-cycle arrest, and inhibits cancer cell growth in vitro and in vivo. Mol Cancer Ther 11(8):1672–1682
Locasale JW (2013) Serine, glycine and one-carbon units: cancer metabolism in full circle. Nat Rev Cancer 13(8):572–583
Locasale JW et al (2011) Phosphoglycerate dehydrogenase diverts glycolytic flux and contributes to oncogenesis. Nat Genet 43(9):869–874
Long J et al (2011) Expression level of glutamine synthetase is increased in hepatocellular carcinoma and liver tissue with cirrhosis and chronic hepatitis B. Hepatol Int 5(2):698–706
Lu C et al (2012) IDH mutation impairs histone demethylation and results in a block to cell differentiation. Nature 483(7390):474–478
Lund K, Merrill DK, Guynn RW (1985) The reactions of the phosphorylated pathway of L-serine biosynthesis: thermodynamic relationships in rabbit liver in vivo. Arch Biochem Biophys 237 (1):186–196
Lu-Yao GL et al (2009) Outcomes of localized prostate cancer following conservative management. JAMA 302:1202–1209
Ma R et al (2013) Switch of glycolysis to gluconeogenesis by dexamethasone for treatment of hepatocarcinoma. Nat Commun 4:2508
Mano Y et al (2014) Correlation between biological marker expression and fluorine-18 fluorodeoxyglucose uptake in hepatocellular carcinoma. Am J Clin Pathol 142(3):391–397
Martinez-Outschoorn UE et al (2010a) Tumor cells induce the cancer associated fibroblast phenotype via caveolin-1 degradation: implications for breast cancer and DCIS therapy with autophagy inhibitors. Cell Cycle 9(12):2423–2433
Martinez-Outschoorn UE et al (2010b) Autophagy in cancer associated fibroblasts promotes tumor cell survival: role of hypoxia, HIF1 induction and NFkappaB activation in the tumor stromal microenvironment. Cell Cycle 9(17):3515–3533
Massie CE et al (2011) The androgen receptor fuels prostate cancer by regulating central metabolism and biosynthesis. EMBO J 30:2719–2733
McBrayer SK et al (2012) Multiple myeloma exhibits novel dependence on GLUT4, GLUT8, and GLUT11: implications for glucose transporter-directed therapy. Blood 119(20):4686–4697
McCleland ML et al (2012) An integrated genomic screen identifies LDHB as an essential gene for triple-negative breast cancer. Cancer Res 72(22):5812–5823
Mena E et al (2012) 11C-Acetate PET/CT in localized prostate cancer: a study with MRI and histopathologic correlation. J Nucl Med 53:538–545
Metallo CM, Vander Heiden MG (2013) Understanding metabolic regulation and its influence on cell physiology. Mol Cell 49(3):388–398
Migita T et al (2014) Inhibition of ATP citrate lyase induces triglyceride accumulation with altered fatty acid composition in cancer cells. Int J Cancer 135(1):37–47
Mihelich BL et al (2011) miR-183-96-182 cluster is overexpressed in prostate tissue and regulates zinc homeostasis in prostate cells. J Biol Chem 286(52):44503–44511
Minamimoto R et al (2011) The potential of FDG-PET/CT for detecting prostate cancer in patients with an elevated serum PSA level. Ann Nucl Med 25:21–27
Moeini A, Cornella H, Villanueva A (2012) Emerging signaling pathways in hepatocellular carcinoma. Liver Cancer 1(2):83–93
Moon J-S et al (2011) Androgen stimulates glycolysis for de novo lipid synthesis by increasing the activities of hexokinase 2 and 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 2 in prostate cancer cells. Biochem J 433:225–233
Muller HJ, Boos J (1998) Use of L-asparaginase in childhood ALL. Crit Rev Oncol Hematol 28 (2):97–113

Neradil J, Pavlasova G, Veselska R (2012) New mechanisms for an old drug; DHFR- and non-DHFR-mediated effects of methotrexate in cancer cells. Klin Onkol 25(Suppl 2):2s87–2s92
Ni Y, Schwaneberg U, Sun ZH (2008) Arginine deiminase, a potential anti-tumor drug. Cancer Lett 261(1):1–11
Nielsen SF, Nordestgaard BG, Bojesen SE (2012) Statin use and reduced cancer-related mortality. N Engl J Med 367(19):1792–1802
Niva CC, Lee JM, Myohara M (2008) Glutamine synthetase gene expression during the regeneration of the annelid *Enchytraeus japonensis*. Dev Genes Evol 218(1):39–46
Nomura DK et al (2010) Monoacylglycerol lipase regulates a fatty acid network that promotes cancer pathogenesis. Cell 140(1):49–61
Oppong BA et al (2014) The effect of metformin on breast cancer outcomes in patients with type 2 diabetes. Cancer Med 3(4):1025–1034
Oyama N et al (1999) The increased accumulation of [18F]fluorodeoxyglucose in untreated prostate cancer. Jpn J Clin Oncol 29:623–629
Pértega-Gomes N et al (2014) A lactate shuttle system between tumour and stromal cells is associated with poor prognosis in prostate cancer. BMC Cancer 14:352
Pierobon M, Frankenfeld CL (2013) Obesity as a risk factor for triple-negative breast cancers: a systematic review and meta-analysis. Breast Cancer Res Treat 137(1):307–314
Petrut B, Trinkaus M, Simmons C, Clemons M (2008) A primer of bone metastases management in breast cancer patients. Curr Oncol 15(Suppl 1):S50–S57
Polanski R et al (2014) Activity of the monocarboxylate transporter 1 inhibitor AZD3965 in small cell lung cancer. Clin Cancer Res 20(4):926–937
Pollari S et al (2011) Enhanced serine production by bone metastatic breast cancer cells stimulates osteoclastogenesis. Breast Cancer Res Treat 125(2):421–430
Popovici-Muller J et al (2012) Discovery of the first potent inhibitors of mutant IDH1 that lower tumor 2-HG in vivo. ACS Med Chem Lett 3(10):850–855
Porporato PE et al (2011) Anticancer targets in the glycolytic metabolism of tumors: a comprehensive review. Front Pharmacol 2:49
Possemato R et al (2011) Functional genomics reveal that the serine synthesis pathway is essential in breast cancer. Nature 476(7360):346–350
Puszyk WM et al (2013) Linking metabolism and epigenetic regulation in development of hepatocellular carcinoma. Lab Invest 93(9):983–990
Reinicke K et al (2012) Cellular distribution of glut-1 and glut-5 in benign and malignant human prostate tissue. J Cell Biochem 113:553–562
Ren JG et al (2010) Induction of erythroid differentiation in human erythroleukemia cells by depletion of malic enzyme 2. PLoS One 5(9):1–12
Ren JG et al (2014) Knockdown of malic enzyme 2 suppresses lung tumor growth, induces differentiation and impacts PI3K/AKT signaling. Sci Rep 4:5414
Rizos CV, Elisaf MS (2013) Metformin and cancer. Eur J Pharmacol 705(1–3):96–108
Rohle D et al (2013) An inhibitor of mutant IDH1 delays growth and promotes differentiation of glioma cells. Science 340(6132):626–630
Ros S, Schulze A (2013) Balancing glycolytic flux: the role of 6-phosphofructo-2-kinase/fructose 2,6-bisphosphatases in cancer metabolism. Cancer Metab 1:8
Ros S et al (2012) Functional metabolic screen identifies 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 4 as an important regulator of prostate cancer cell survival. Cancer Discov 2:328–343
Ross J et al (2008) Fatty acid synthase inhibition results in a magnetic resonance-detectable drop in phosphocholine. Mol Cancer Ther 7(8):2556–2565
Rossi S et al (2003) Fatty acid synthase expression defines distinct molecular signatures in prostate cancer. Mol Cancer Res 1:707–715
Roychowdhury S, Chinnaiyan AM (2013) Advancing precision medicine for prostate cancer through genomics. J Clin Oncol Off J Am Soc Clin Oncol 31:1866–1873
Rubin MA et al (2002) alpha-Methylacyl coenzyme A racemase as a tissue biomarker for prostate cancer. JAMA 287(13):1662–1670

Rubin MA, Maher CA, Chinnaiyan AM (2011) Common gene rearrangements in prostate cancer. J Clin Oncol 29(27):3659–3668
Sanità P et al (2014) Tumor-stroma metabolic relationship based on lactate shuttle can sustain prostate cancer progression. BMC Cancer 14:154
Santidrian AF et al (2013) Mitochondrial complex I activity and NAD+/NADH balance regulate breast cancer progression. J Clin Invest 123(3):1068–1081
Schlaepfer IR et al (2014) Lipid catabolism via CPT1 as a therapeutic target for prostate cancer. Mol Cancer Ther 13:2361–2371, p. molcanther. 0183.2014
Schoors S et al (2014) Partial and transient reduction of glycolysis by PFKFB3 blockade reduces pathological angiogenesis. Cell Metab 19(1):37–48
Shen MM, Abate-Shen C (2010) Molecular genetics of prostate cancer: new prospects for old challenges. Genes Dev 24:1967–2000
Shonk CE, Morris HP, Boxer GE (1965) Patterns of glycolytic enzymes in rat liver and hepatoma. Cancer Res 25:671–676
Shrubsole MJ et al (2011) Dietary B vitamin and methionine intakes and breast cancer risk among Chinese women. Am J Epidemiol 173(10):1171–1182
Sonveaux P et al (2008) Targeting lactate-fueled respiration selectively kills hypoxic tumor cells in mice. J Clin Invest 118(12):3930–3942
Sotgia F et al (2011) Understanding the Warburg effect and the prognostic value of stromal caveolin-1 as a marker of a lethal tumor microenvironment. Breast Cancer Res 13(4):213
Stein M et al (2010) Targeting tumor metabolism with 2-deoxyglucose in patients with castrate-resistant prostate cancer and advanced malignancies. Prostate 70:1388–1394
Stein E et al (2014) Clinical safety and activity in a phase I trial of AG-221, a first in class, potent inhibitor of the IDH2-mutant protein, in patients with IDH2 mutant positive advanced hematologic malignancies. In: Proceedings of the 105th annual meeting of the American Association for Cancer Research
Sung J et al (2003) Fluorodeoxyglucose positron emission tomography studies in the diagnosis and staging of clinically advanced prostate cancer. BJU Int 92:24–27
Sweeney MJ et al (1963) Comparative biochemistry hepatomas. IV. Isotope studies of glucose and fructose metabolism in liver tumors of different growth rates. Cancer Res 23:995–1002
Swinnen JV et al (2002) Overexpression of fatty acid synthase is an early and common event in the development of prostate cancer. Int J Cancer 98:19–22
Taketa K et al (1988) Profiles of carbohydrate-metabolizing enzymes in human hepatocellular carcinomas and preneoplastic livers. Cancer Res 48(2):467–474
Taylor BS et al (2010) Integrative genomic profiling of human prostate cancer. Cancer Cell 18 (1):11–22
Tchou J et al (2010) Degree of tumor FDG uptake correlates with proliferation index in triple negative breast cancer. Mol Imaging Biol 12(6):657–662
Telang S et al (2014) Discovery of a PFKFB3 inhibitor for phase I trial testing that synergizes with the B-Raf inhibitor vemurafenib. Cancer Metab 2(Suppl 1):P14
Tennakoon JB et al (2013) Androgens regulate prostate cancer cell growth via an AMPK-PGC-1α-mediated metabolic switch. Oncogene 33:5251–5261
Tennant DA, Duran RV, Gottlieb E (2010) Targeting metabolic transformation for cancer therapy. Nat Rev Cancer 10(4):267–277
Tessem M-B et al (2008) Evaluation of lactate and alanine as metabolic biomarkers of prostate cancer using 1H HR-MAS spectroscopy of biopsy tissues. Magn Reson Med 60:510–516
Thorgeirsson SS, Grisham JW (2002) Molecular pathogenesis of human hepatocellular carcinoma. Nat Genet 31(4):339–346
Timmerman LA et al (2013) Glutamine sensitivity analysis identifies the xCT antiporter as a common triple-negative breast tumor therapeutic target. Cancer Cell 24(4):450–465
Tomlinson IP et al (2002) Germline mutations in FH predispose to dominantly inherited uterine fibroids, skin leiomyomata and papillary renal cell cancer. Nat Genet 30(4):406–410

Torizuka T et al (1995) In vivo assessment of glucose metabolism in hepatocellular carcinoma with FDG-PET. J Nucl Med 36(10):1811–1817
Tsouko E et al (2014) Regulation of the pentose phosphate pathway by an androgen receptor-mTOR-mediated mechanism and its role in prostate cancer cell growth. Oncogenesis 3:e103
Tsuchiyama K et al (2013) Expression of microRNAs associated with Gleason grading system in prostate cancer: miR-182-5p is a useful marker for high grade prostate cancer. Prostate 73 (8):827–834
Tsui KH et al (2011) p53 downregulates the gene expression of mitochondrial aconitase in human prostate carcinoma cells. Prostate 71(1):62–70
Tsui KH et al (2013) Hypoxia upregulates the gene expression of mitochondrial aconitase in prostate carcinoma cells. J Mol Endocrinol 51(1):131–141
Turcan S et al (2012) IDH1 mutation is sufficient to establish the glioma hypermethylator phenotype. Nature 483(7390):479–483
Van de Sande T et al (2002) Role of the phosphatidylinositol 3′-kinase/PTEN/Akt kinase pathway in the overexpression of fatty acid synthase in LNCaP prostate cancer cells. Cancer Res 62 (3):642–646
Van de Sande T et al (2005) High-level expression of fatty acid synthase in human prostate cancer tissues is linked to activation and nuclear localization of Akt/PKB. J Pathol 206(2):214–219
Vander Heiden MG (2011) Targeting cancer metabolism: a therapeutic window opens. Nat Rev Drug Discov 10(9):671–684
Vander Heiden MG et al (2010) Identification of small molecule inhibitors of pyruvate kinase M2. Biochem Pharmacol 79(8):1118–1124
Vaz CV et al (2012) Androgen-responsive and nonresponsive prostate cancer cells present a distinct glycolytic metabolism profile. Int J Biochem Cell Biol 44:2077–2084
Visser WF et al (2007) Metabolite transport across the peroxisomal membrane. Biochem J 401 (2):365–375
Voduc KD et al (2010) Breast cancer subtypes and the risk of local and regional relapse. J Clin Oncol 28(10):1684–1691
Walling J (2006) From methotrexate to pemetrexed and beyond. A review of the pharmacodynamic and clinical properties of antifolates. Invest New Drugs 24(1):37–77
Walsh MJ et al (2010) ML265: a potent PKM2 activator induces tetramerization and reduces tumor formation and size in a mouse xenograft model. In: Probe reports from the NIH Molecular Libraries Program, Bethesda, MD
Walsh AL et al (2014) Long noncoding RNAs and prostate carcinogenesis: the missing 'linc'? Trends Mol Med 20(8):428–436
Wang JB et al (2010) Targeting mitochondrial glutaminase activity inhibits oncogenic transformation. Cancer Cell 18(3):207–219
Wang B et al (2012) Stat3-mediated activation of microRNA-23a suppresses gluconeogenesis in hepatocellular carcinoma by down-regulating glucose-6-phosphatase and peroxisome proliferator-activated receptor gamma, coactivator 1 alpha. Hepatology 56(1):186–197
Wang F et al (2013) Targeted inhibition of mutant IDH2 in leukemia cells induces cellular differentiation. Science 340(6132):622–626
Weber G, Ashmore J (1958) Absent fructose-1,6-diphosphatase activity in hepatoma. Exp Cell Res 14(1):226–228
Weber G, Cantero A (1955) Glucose-6-phosphatase activity in normal, pre-cancerous, and neoplastic tissues. Cancer Res 15(2):105–108
Weber G, Morris HP (1963) Comparative biochemistry of hepatomas. III. Carbohydrate enzymes in liver tumors of different growth rates. Cancer Res 23:987–994
Weischenfeldt J et al (2013) Integrative genomic analyses reveal an androgen-driven somatic alteration landscape in early-onset prostate cancer. Cancer Cell 23(2):159–170
Wu JM, Skill NJ, Maluccio MA (2010) Evidence of aberrant lipid metabolism in hepatitis C and hepatocellular carcinoma. HPB (Oxford) 12(9):625–636

Xu X, Chen J (2009) One-carbon metabolism and breast cancer: an epidemiological perspective. J Genet Genomics 36(4):203–214
Yalcin A et al (2010) Selective inhibition of choline kinase simultaneously attenuates MAPK and PI3K/AKT signaling. Oncogene 29(1):139–149
Yan H et al (2009) IDH1 and IDH2 mutations in gliomas. N Engl J Med 360(8):765–773
Yang B et al (2003) Aberrant promoter methylation profiles of tumor suppressor genes in hepatocellular carcinoma. Am J Pathol 163(3):1101–1107
Yang Y et al (2007) Metabonomic studies of human hepatocellular carcinoma using high-resolution magic-angle spinning 1H NMR spectroscopy in conjunction with multivariate data analysis. J Proteome Res 6(7):2605–2614
Yang C et al (2009) Glioblastoma cells require glutamate dehydrogenase to survive impairments of glucose metabolism or Akt signaling. Cancer Res 69(20):7986–7993
Yeh SDJ et al (1996) Detection of bony metastases of androgen-independent prostate cancer by PET-FDG. Nucl Med Biol 23:693–697
Yeruva L et al (2010) Perillyl alcohol and methyl jasmonate sensitize cancer cells to cisplatin. Anticancer Drugs 21(1):1–9
Yoshimoto S et al (2013) Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. Nature 499(7456):97–101
Yue S et al (2014) Cholesteryl ester accumulation induced by PTEN loss and PI3K/AKT activation underlies human prostate cancer aggressiveness. Cell Metab 19(3):393–406
Zadra G, Photopoulos C, Loda M (2013) The fat side of prostate cancer. Biochim Biophys Acta Mol Cell Biol Lipids 1831:1518–1532
Zaugg K et al (2011) Carnitine palmitoyltransferase 1C promotes cell survival and tumor growth under conditions of metabolic stress. Genes Dev 25(10):1041–1051
Zecchini V et al (2014) Nuclear ARRB1 induces pseudohypoxia and cellular metabolism reprogramming in prostate cancer. EMBO J 33:1365–1382
Zha S et al (2005) Peroxisomal branched chain fatty acid beta-oxidation pathway is upregulated in prostate cancer. Prostate 63:316–323
Zhang SM et al (2003) Plasma folate, vitamin B6, vitamin B12, homocysteine, and risk of breast cancer. J Natl Cancer Inst 95(5):373–380
Zhang CX et al (2011) Dietary folate, vitamin B6, vitamin B12 and methionine intake and the risk of breast cancer by oestrogen and progesterone receptor status. Br J Nutr 106(6):936–943
Zhang WC et al (2012) Glycine decarboxylase activity drives non-small cell lung cancer tumor-initiating cells and tumorigenesis. Cell 148(1–2):259–272
Zhang C et al (2013) IDH1/2 mutations target a key hallmark of cancer by deregulating cellular metabolism in glioma. Neuro Oncol 15(9):1114–1126
Zheng Q et al (1995) Glucose regulation of glucose transporters in cultured adult and fetal hepatocytes. Metabolism 44(12):1553–1558
Zheng MF, Shen SY, Huang WD (2013) DCA increases the antitumor effects of capecitabine in a mouse B16 melanoma allograft and a human non-small cell lung cancer A549 xenograft. Cancer Chemother Pharmacol 72(5):1031–1041
Zou J et al (2011) hZIP1 zinc transporter down-regulation in prostate cancer involves the overexpression of ras responsive element binding protein-1 (RREB-1). Prostate 71:1518–1524

Metabolic Syndrome, Type 2 Diabetes, and Cancer: Epidemiology and Potential Mechanisms

Sarit Ben-Shmuel, Ran Rostoker, Eyal J. Scheinman, and Derek LeRoith

Contents

Abstract

Obesity is associated with multiple metabolic disorders that drive cardiovascular disease, T2D and cancer. The doubling in the number of obese adults over the past 3 decades led to the recognition of obesity as a "disease". With over 42 million children obese or overweight, this epidemic is rapidly growing worldwide. Obesity and T2D are both associated together and independently with an increased risk for cancer and a worse prognosis. Accumulating evidence from epidemiological studies revealed potential factors that may explain the

S. Ben-Shmuel • R. Rostoker • E.J. Scheinman • D. LeRoith (✉)
Clinical Research Institute at Rambam (CRIR), Diabetes and Metabolism Clinical Research Center of Excellence, Rambam Health Care Campus, Haifa, Israel
e-mail: Derek.leroith@mssm.edu

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association between obesity-linked metabolic disorders and cancer risk. Studies based on the insulin resistance MKR mice, highlighted the roe of the insulin receptor and its downstream signaling proteins in mediating hyperinsulinemia's mitogenic effects. Hypercholesterolemia was also shown to promote the formation of larger tumors and enhancement in metastasis. Furthermore, the conversion of cholesterol into 27-Hydroxycholesterol was found to link high fat diet-induced hypercholesterolemia with cancer pathophysiology. Alteration in circulating adipokines and cytokines are commonly found in obesity and T2D. Adipokines are involved in tumor growth through multiple mechanisms including mTOR, VEGF and cyclins. In addition, adipose tissues are known to recruit and alter macrophage phenotype; these macrophages can promote cancer progression by secreting inflammatory cytokines such as TNF-α and IL-6.

Better characterization on the above factors and their downstream effects is required in order to translate the current knowledge into the clinic, but more importantly is to understand which are the key factors that drive cancer in each patient. Until we reach this point, policies and activities toward healthy diets and physical activities remain the best medicine.

Keywords
Cancer · Hyperinsulinemia · Obesity

1 Introduction

Growing evidence, over the past decade or more, has provided us with the knowledge that obesity and type 2 diabetes (T2D) are associated with an increased risk of many types of cancer and more importantly, perhaps, increased mortality in these individuals. The major emphasis today is focused on the epidemic of obesity and, secondarily, T2D and the metabolic derangements of these metabolic disorders. In addition, there are numerous ongoing studies attempting to understand the etiology of the vascular complications resulting from obesity and T2D, as well as potential preventative medications. On the other hand, given the increase in cases of obesity and T2D worldwide, focus should also be directed towards a major complication, namely, cancer risk and cancer mortality, which undoubtedly increases in parallel with the obesity/T2D epidemic.

In this review we present the epidemiological evidence for the association between these metabolic disorders and cancer risk, we will describe the studies identifying causative factors, and we will try to relate the metabolic aspects of both obesity and T2D and how they impact on cancer.

2 Epidemic of Obesity and T2D and the Increased Cancer Risk

The epidemic of obesity is now recognized as a worldwide problem. While the USA still leads the world in the percent of overweight and obese individuals, many westernized countries are fast catching up, and developing countries are increasingly being affected. According to the World Health Organization, in 2008, over 1.4 billion adults (11%) worldwide are overweight, of which ~300 million were obese (Haslam and James 2005), and the incidence is rising rapidly. The universally accepted definition of overweight and obesity is a body mass index (BMI) of >25 and >30 kg/m^2, respectively. Using these criteria, more than 60% of adults in the USA are overweight and at least half of these are obese. In Southeast Asian countries, due to different body habitus, it is clearly more appropriate to define obesity using waist circumference, >40 inches in men and >35 inches in women. These measurements correlate better with visceral adiposity, and visceral adiposity closely tracks with elements of metabolic syndrome such as dyslipidemia, hypertension, and glucose intolerance.

The concern over the growing epidemic of obesity and the health ramifications has recently motivated the American Medical Association to designate obesity as a "disease," whereas previously it was considered a "disorder," a condition that carries less concern. The clear evidence that obesity predisposes to cardiovascular disease and cancer, for example, warrants this refocus on obesity as a disease entity.

This obesity epidemic is driving a similar trend in T2D, and the International Diabetes Federation (IDF) data suggests that ~8% of the world's population has diabetes with levels as high as ~10% in the Middle East and North Africa. By the year 2030, this number will more than double, since many obese individuals are unaware they have diabetes.

2.1 Obesity and Cancer

The increased association of obesity and metabolic syndrome with cancer risk and cancer mortality has become evident over the past decade, from numerous epidemiological studies. Thus, in countries where obesity prevalence has increased rapidly, such as the USA, a significant proportion (~20%) of all new cancers may be attributable to obesity (Calle et al. 2003; Jemal et al. 2007). Specific examples of common cancers include breast, endometrial, colon, and prostate cancers. In one study of over 33,000 men, the presence of metabolic syndrome was associated with a 56% enhanced risk of cancer mortality over the following 14 years of follow-up (Jaggers et al. 2009; Pothiwala et al. 2009). While the Nurses' Health Study suggested that central adiposity determined by waist circumference and waist to hip ratio was associated with an increased risk of postmenopausal breast cancer (Huang et al. 1999), more recent studies have claimed that premenopausal obesity is also a risk factor for breast cancer risk (Pierobon and Frankenfeld 2013; Robinson et al. 2014). The Million Women Study in the UK and the Cancer Prevention Study

II in the USA similarly reported increased cancer mortality in obese individuals (Petrelli et al. 2002; Reeves et al. 2007).

Strong supporting evidence for the association of obesity and cancer risk and mortality was obtained from a number of bariatric surgery studies, which dramatically reverse obesity. In the Swedish Obesity Subjects (SOS) study, following more than 30% weight loss, a reduction of cancer in women of about 41% was recorded (Sjostrom et al. 2009). A similar effect was seen in the Utah obesity study (Adams et al. 2009). Furthermore, in the Women's Intervention Nutrition Study (WINS), a 24% reduction in breast cancer was seen after only a 4% reduction in weight over 5 years (Prentice et al. 2006). The actual mechanisms involved in this effect are as yet undefined, but most likely reflect a correction of factors that are abnormal in cases of obesity and metabolic syndrome.

2.2 Children/Adolescents

The obesity epidemic has not spared children and adolescents. While definitions of obesity and metabolic syndrome are less well defined in children and adolescents, the increase is clear (Cook et al. 2003; Weiss et al. 2004). Furthermore, nonalcoholic fatty acid liver disease (NAFLD, another complication of obesity) is increased from 2.6% in normal weight children to 77% in overweight children (Franzese et al. 1997; Schwimmer et al. 2006). In line with this increase in NAFLD is the increase in adult hepatocellular carcinoma (HCC) in individuals who were obese during their childhood years, with a hazard ratio (HR) of 1.2–1.3 (Berentzen et al. 2014). Other studies have shown that BMI in the upper quartile in children from 2 to 14 years of age was associated with increased cancer risk in adulthood by 40% (Park et al. 2012), particularly colorectal, ovarian, cervical, and kidney cancer.

2.3 T2D Epidemic

The obesity epidemic is driving an epidemic of T2D worldwide. While there exists a group of healthy obese individuals, the majority demonstrate features of metabolic syndrome or prediabetes and, in genetically predisposed, progress to frank T2D (Cornier et al. 2008). Different manifestations of metabolic syndrome have different diagnostic criteria, but most include an increased waist circumference, dyslipidemia, hypertension, and even elevated fasting plasma glucose. Almost 70–80% of individuals with T2D are obese; moreover, several long-term prospective studies have shown a higher risk of T2D with increasing body weight. A National Health and Nutrition Examination Survey (NHANES) 25 years ago highlighted an association between being overweight and suffering from diabetes (Van Itallie 1985). Recently, it has been discovered that even apparently metabolically healthy overweight or obese men are still at significantly higher risk of developing T2D (Arnlov et al. 2011).

2.4 T2D and Cancer

Evidence is now emerging for a direct association between T2D and a higher risk of cancer mortality independent of the effects of obesity. (Coughlin et al. 2004; Verlato et al. 2003; Yancik et al. 2001a, b). It was found that hyperinsulinemia and insulin resistance, as shown by elevated levels of circulating C-peptide (a commonly used biomarker for insulin secretion in T2D), were significant risk factors for breast cancer (Bruning et al. 1992; Gunter et al. 2009; Verheus et al. 2006). A prospective study in Sweden of 80,000 women (average age 64.2 years) found that individuals with T2D had an increased incidence of breast cancer (Weiderpass et al. 1997). In another prospective study in 2003, an increased risk of estrogen receptor-positive breast cancer, postmenopausally, was found to be associated with T2D (Michels et al. 2003). Although T2D patients are known to be at high risk of developing pancreatic cancer (Coughlin et al. 2004; Pisani 2008; Rousseau et al. 2006), it has also been discovered that pancreatic cancer precedes diabetes (Isaksson et al. 2003; Permert et al. 1994). Colorectal cancer is also positively associated positively with T2D. A case-control study of around 10,000 adults in the UK showed that the risk of both colonic and rectal cancers is increased in both male and female diabetic patients (odds ratio = 1.42) (Yang et al. 2005), as was also shown in the Physicians' Health Study, where the relative risk of colorectal cancer in men with T2D was 1.5 (Sturmer et al. 2006). Endometrial cancer risk has also been shown to be associated with high circulating insulin or C-peptide levels, and mortality risk is relatively high in diabetes compared to some other cancers (relative risk $\geq$2) (Folsom et al. 2004).

In contrast to most epithelial cancers, T2D has been found in several meta-analyses to be inversely associated with prostate cancer risk (Bonovas et al. 2004; Coughlin et al. 2004; Kasper and Giovannucci 2006). On the other hand, obese males with high levels of circulating C-peptide, who develop prostate cancer, are at higher risk of dying from the disease, suggesting a relationship between insulin and aggressive, high-grade prostatic tumors specifically (Ma et al. 2008).

3 Potential Mechanisms

Following the numerous observations that obesity and diabetes are clearly associated with an increased risk of cancer and cancer-related mortality, there has been increased interest in establishing the causal factors involved in this effect. Some of the possible factors are listed in Table 1. In-depth analyses of the observational studies have strongly suggested that insulin, insulin-like growth factor 1 (IGF-1) and insulin-like growth factor 1 receptor (IGF-1R), leptin, inflammatory cytokines, caloric intake, and lipids are examples of potential causal factors that may explain the association between metabolic disorders and cancer risk. To study these various factors, a number of groups have developed appropriate mouse models for these preclinical experiments.

Table 1 Potential causal factors of the association between metabolic disorders and cancer risk

Factor	Associated with	References
Hyperinsulinemia	Insulin resistance	Ferguson et al. (2013), Novosyadlyy and LeRoith (2010), Xu et al. (2014)
IGF-1/IGF-1R	Nutrition	Djiogue et al. (2013), Lee and Yee (1995), Levine et al. (2006)
Leptin	Obesity	Ntikoudi et al. (2014), Uddin et al. (2014), Vansaun (2013)
Adiponectin (low)	Obesity	Grossmann and Cleary (2012), Vansaun (2013)
Glucose	Diabetes	Noto et al. (2013), Sciacca et al. (2013), Tseng and Tseng (2014)
Inflammatory cytokines	Inflammation	Del Prete et al. (2011), Elinav et al. (2013), Landskron et al. (2014)
Estrogen and androgens	Breast, endometrial, and prostate cancers	Crawford (2009), McNamara and Sasano (2015), Rizner (2013), Wang and Di (2014)

4 Animal Models

4.1 Obesity

High-fat diet (HFD)-induced obesity has been used extensively in mice to study the ramifications on cancer growth. Using this model it was demonstrated that the growth of Lewis lung carcinoma and mouse colon 38 adenocarcinoma cell lines was increased. This was associated with insulin resistance, hyperinsulinemia, glucose intolerance, and hyperleptinemia. Male mice demonstrated more severe metabolic derangements compared to female mice, and the effect on tumor growth was more pronounced. Interestingly, when female obese mice underwent ovariectomy, they developed the same degree of metabolic derangement as the male obese mice, and the effect on tumor growth was markedly enhanced. This suggested that estradiol was protective against metabolic derangements in the face of HFD-induced obesity (Yakar et al. 2006). Similarly, in ovariectomized mice fed with a HFD or a calorie-restricted (CR) diet, HFD-induced obesity had the largest tumors whereas CR mice had the smallest (Rondini et al. 2011). In other models of diet-induced obesity and CR diet, it was demonstrated that prostate, pancreatic, and skin, in addition to colon and breast, cancers are affected by these manipulations of the metabolic changes (Blando et al. 2011; Ford et al. 2013; Lashinger et al. 2013; Moore et al. 2012; Olivo-Marston et al. 2014). The factors mediating these effects include hyperinsulinemia, leptin, inflammatory cytokines, and, as recently demonstrated, hypercholesterolemia.

4.2 Hypercholesterolemia

In mice fed with HFD to induce obesity, Nelson et al. showed that mammary tumor growth was increased and dependent on a metabolite of cholesterol, namely, 27-OH cholesterol, as well as the presence of the estrogen receptor. The conversion of cholesterol to its 27-OH metabolite occurred both in tumor-associated macrophages and tumor cells as well (Nelson et al. 2013). In a separate study, ApoE knockout (ApoE-/-) mice, which demonstrate hypercholesterolemia and hypertriglyceridemia, were shown to have enhanced mammary tumor growth and lung metastases (Alikhani et al. 2013). Interestingly, ApoE-/- mice are insulin sensitive and have normal glucose homeostasis, making them an excellent model to study the isolated effects of lipid abnormalities as their circulating insulin levels are also normal if somewhat lower than wild-type controls (Kawashima et al. 2009). These observations, along with studies in prostate cancer cells (Pelton et al. 2012), have identified cholesterol as tumor promoters and support the epidemiological studies that show an association of hyperlipidemia and cancer growth and mortality and the reduction of mortality in statin users (Nielsen et al. 2012).

4.3 Hyperinsulinemia

The MKR mice were developed by engineering muscle insulin resistance through a transgenic overexpression of a defective IGF-1R that interferes with insulin receptor (IR) as well, through hybrid receptors, resulting in severe insulin resistance. Male MKR mice are diabetic with severe hyperglycemia, hyperinsulinemia, and hyperlipidemia, but without being obese, since the diabetes is not brought about by HFD-induced obesity and diabetes. Female MKR mice, on the other hand, do not demonstrate hyperlipidemia and hyperglycemia but still have hyperinsulinemia; thus they are extremely useful for studying the effect of isolated hyperinsulinemia on cancer. Using multiple oncogenic-induced mouse tumor models, both by orthotopic injections of cancer cell lines and crossing with mammary tumor transgenic mice (Table 2), we were able to demonstrate that hyperinsulinemia was causative in the growth and progression of mammary tumors as well as metastases to the lungs. The role of hyperinsulinemia was demonstrated by reducing the circulating levels of insulin. This was achieved by the use of a β_3-adrenergic receptor agonist (CL-316,243) previously shown to improve the insulin resistance and hyperinsulinemia in male MKR mice (Kim et al. 2006). The reduced insulin levels led to a marked reduction in breast tumor growth (Fierz et al. 2010a). Since insulin may affect tumor growth via IR or IGF-1R, we blocked the tyrosine kinase activity of these receptors using a small molecule tyrosine kinase inhibitor (BMS-536924). Receptor activity inhibition resulted in reduced tumor growth. A similar result was obtained with the use of low-dose picropodophyllin (PPP), a compound designed as an IGF-1R-specific inhibitor, but in our hands PPP inhibits both IR and IGF-1R (Rostoker et al. 2013).

Table 2 Mouse mammary tumor models

Chemicals	Cell lines	Transgenic mice	Oncogenes	References
DMBA				Middleton (1965)
MPA				Lanari et al. (2009)
Nitrosourea compounds (MNU, ENU, etc.)				Williams et al. (1981), Imamura et al. (1984)
	MVT-1		c-myc/VEGF	Pei et al. (2004)
		MMTV-c-myc	c-myc	Stewart et al. (1984)
	Met-1		PyVMT	Borowsky et al. (2005)
		MMTV-PyVMT		Guy et al. (1992)
	MCNeuA		NeuA	Campbell et al. (2002)
		rtTA-Neu (tetracycline inducible)		Gunther et al. (2002), McHenry et al. (2010)
		MMTV-c-Neu	NeuC	Muller et al. (1988)
	M-Wnt mesenchymal E-Wnt epithelial		Wnt	Dunlap et al. (2012)
		MMTV-Wnt1		Tsukamoto et al. (1988)
	E0771			Ewens et al. (2005), Sirotnak et al. (1984)
		MMTV-v-Ha-ras	v-Ha-ras	Sinn et al. (1987)
		WAP-TGF-α		Sandgren et al. (1995)
		WAP-IGF-1		Hadsell et al. (1996)
		WAP-Tag	SV40	Tzeng et al. (1993)

DMBA 7,12-dimethylbenz-(a)anthracene, *ENU* *N*-ethylnitrosourea, *MMTV* mouse mammary tumor virus, *MNU* *N*-methyl-*N*-nitrosourea, *MPA* medroxyprogesterone acetate, *PyVMT* polyoma-virus middle T antigen, *rtTA* reverse tetracycline-dependent transactivator, *SV40* Simian virus 40, *Tag* SV40 T antigen, *TGF-α* transforming growth factor-α, *VEGF* vascular endothelial growth factor, *v-Ha-ras* Harvey rat sarcoma viral oncogene, *WAP* whey acidic protein promoter

Since mTOR is activated by insulin and is a central mediator of tumor progression (Fig. 1), we studied the impact of mTOR inhibition (by rapamycin) on mammary tumor progression and the metabolic state of the mice. Mammary tumor progression was studied in the double transgenic, MMTV-PyVmT/MKR, and two orthotopic models using the Met-1 and MCNeuA cells. In both the wild-type and MKR (insulin-resistant, hyperinsulinemic) mice, glucose intolerance and hypertriglyceridemia worsened significantly after rapamycin treatment. Nonetheless, tumor growth was inhibited in all three mammary tumor models, despite the worsening of insulin resistance and higher levels of circulating insulin (Fierz et al. 2010b). Inhibition of phosphatidylinositol 3-kinase (PI3K) alone, using the

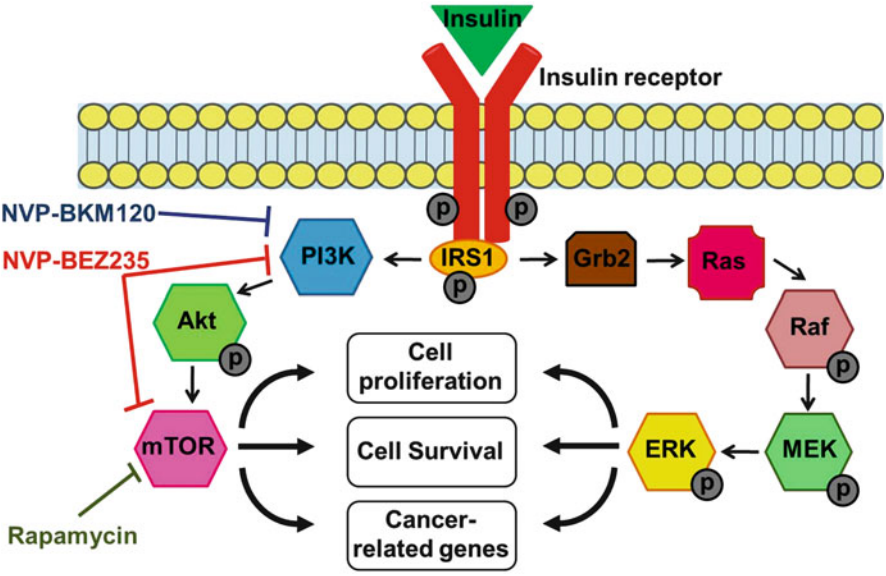

Fig. 1 Insulin receptor/PI3K/mTOR signaling pathway. *ERK* extracellular signal-regulated kinase, *Grb2* growth factor receptor-bound protein 2, *IRS-1* insulin receptor substrate 1, *MEK* mitogen-activated protein kinase kinase, *P* phosphate, *Raf* rapidly accelerated fibrosarcoma kinase, *Ras* rat sarcoma protein

oral pan-class I PI3K inhibitor (NVP-BKM120), or together with mTOR, using NVP-BEZ235, inhibited tumor growth. However, in regard to the metabolic effects, inhibiting PI3K alone led to more severe metabolic derangements with increased insulin resistance, hyperinsulinemia, and hyperglycemia, in comparison to the dual inhibitor of PI3K/mTOR (Gallagher et al. 2012).

4.4 Adipokines

Circulating adipokines are commonly altered in obesity and T2D (Nalabolu et al. 2014). Leptin is classically elevated in these conditions, whereas adiponectin is reduced. Leptin has been shown to stimulate cancer cell growth in cell cultures, suggesting a role in the effect of obesity in enhancing cancer growth and prognosis (Park and Scherer 2011; Somasundar et al. 2003). The role of leptin in cancer progression has been studied in genetically induced obese mouse models. The Lep^{Ob}/Lep^{Ob} mice are obese, insulin resistant, and hyperinsulinemic, with *low* leptin levels. These competing features may explain the varying results when studying cancer in these mice. On the other hand, Lep^{db}/Lep^{db} mice, carrying a leptin receptor mutation that results in obesity with elevated leptin levels, show more marked cancer growth and metastases. Interestingly, serum from Lep^{Ob}/Lep^{Ob} induces a mesenchymal phenotype in the B16 melanoma cells that may explain

the enhanced pulmonary metastases in the absence of increased primary tumor growth (Kushiro and Nunez 2011).

On the other hand, adiponectin has been shown to induce cancer cell apoptosis and may explain the effect of low adiponectin levels in obesity and T2D on cancer prognosis (Kelesidis et al. 2006; Körner et al. 2006). In a model of chemical (azoxymethane, AOM)-induced colon carcinogenesis and adiponectin and adiponectin receptor knockout mice, HFD increased the AOM effect (Fujisawa et al. 2008). Adiponectin administration inhibited tumor growth through multiple mechanisms, including inhibition of cell proliferation and inhibition of mTOR, VEGF, and cyclins (Moon et al. 2013).

Interestingly, there seems to be an interplay between the effect of adiponectin and cholesterol on tumor growth (Liu et al. 2013). In bigenic mice (MMTV-PyVmT and adiponectin deficient) fed with high-fat high-cholesterol diet, the resultant hypercholesterolemia and increased expression of the low density lipoprotein (LDL) receptor (LDLR) increased tumor cholesterol content and enhanced tumor growth. Adiponectin downregulated the LDLR in vitro and in vivo and demonstrated anti-cancer effects. Thus, the potential effects of adiponectin deficiency in obesity and T2D on both metabolism and enhanced cancer growth may be explained by a number of mechanisms. Insulin resistance associated with reduced adiponectin may secondarily lead to enhanced LDLR expression in cancer cells and increased uptake of cholesterol, leading to enhanced cancer growth. This may partially explain the increased LDLR expression seen in triple negative breast cancers and the worse prognosis (Antalis et al. 2011; Rudling et al. 1986).

4.5 Inflammatory Cytokines

Both obesity and T2D have been labeled "inflammatory disorders." Adipose tissue from obese individuals shows a marked increase in macrophage infiltration and dysregulation of these macrophages in the tumor environment, suggesting that local tumor-associated macrophages (TAM) may regulate tumor progression (Fig. 2). TAMs are recruited by monocyte chemotactic protein-1 (MCP-1) found in tumor tissues and are generally of the M1 macrophage phenotype that is pro-inflammatory. The switch from M2 (anti-inflammatory) to M1 macrophages is commonly seen in obese individuals and rodents. These macrophages are also capable of secreting pro-inflammatory factors that affect the adipose tissue and cancer cells leading to cancer progression. Thus, in the case of breast cancer, obese women have increased circulating levels of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6). Elevated levels of these cytokines are associated with increased cancer progression. Moreover, local breast adipose tissue may similarly affect tumor growth via cytokines. Reversal of these effects was seen with low-calorie diets, low-fat diets, and weight loss. Similarly, cyclooxygenase (COX) and prostaglandin E_2 (PGE_2) inhibitors may prove to be useful as they inhibit the elevated levels of COX-2 and PGE_2 found in white adipose tissue inflammation (Howe et al. 2013). These drugs have additional relevant effects,

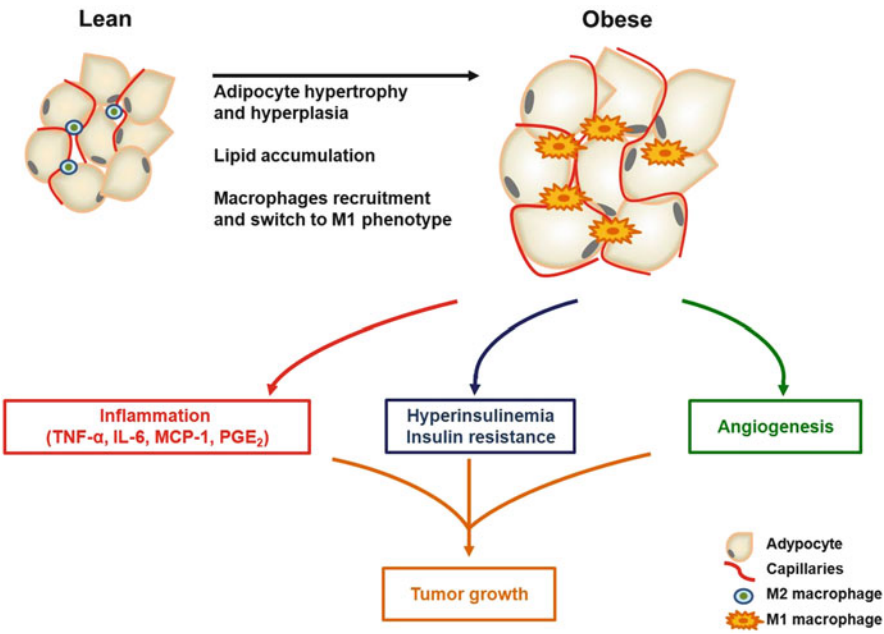

Fig. 2 Adipocyte and macrophage dysregulation favor tumor development

including activation of adenosine monophosphate-activated protein kinase (AMPK, which inhibits mTOR) and nuclear factor-κB (NF-κB) antagonism that may be important in inhibiting the inflammatory processes.

5 Metformin, Insulin, and Cancer

Metformin is a biguanide that has multiple biological properties, many of which may be beneficial in reducing cancer risk or cancer progression. Metformin reduces the activity of complex I in the respiratory chain in hepatocytes, causing energetic stress, which in turn activates liver kinase B1 (LKB1)/AMPK pathway and inhibits gluconeogenesis (Shaw et al. 2005). Reduced hepatic gluconeogenesis lowers blood glucose levels and, secondarily, circulating insulin concentrations. Since hyperinsulinemia has been shown to be associated with cancer and cancer-related mortality in obese and pre-diabetic individuals, this may be one explanation for metformin's reduction of cancer risk and mortality in these situations, as demonstrated by epidemiological studies (Wang et al. 2014; Zhang et al. 2013). Other effects of metformin on adipokines and inflammatory cytokines were recently revealed. These include resistin and adiponectin (Gomez-Diaz et al. 2012; Singh et al. 2012). What effect these may have on insulin resistance and secondarily on cancer remains to be determined.

Effects of metformin on cancer may also be via direct mechanisms. Otto Warburg demonstrated increased glycolysis in cancer cells, also known as the Warburg effect (Warburg 1956); however, a significant amount of ATP is derived from oxidative phosphorylation, the latter being affected by metformin-induced stress and AMPK activation. AMPK, in turn, inhibits cancer cell growth by inhibiting fatty acid synthesis and mTOR-induced protein translation (Algire et al. 2010; Larsson et al. 2012). p53 mutations in certain cancers may make them more sensitive to biguanides, due to increased oxidative phosphorylation. Finally, metformin may play a role in inhibiting tumor-initiating cells that are commonly resistant to therapy (Zhu et al. 2014).

6 Conclusions

While the epidemiological documentation of a relationship between obesity, metabolic syndrome, T2D, and cancer risk and prognosis is becoming clearer, the causal factors remain to be defined. Specific animal models and human studies have identified strong contenders, such as hyperinsulinemia, hyperlipidemia, IGF-1, and leptin levels; the relevance of each of these may vary depending on the model. Identifying, quantifying, and proving which factors are of greater importance are critical if investigators wish to target molecules to be used as adjunct chemotherapy, especially for chemoresistant- or radiation-resistant cancers.

References

Adams TD, Stroup AM, Gress RE, Adams KF, Calle EE, Smith SC, Halverson RC, Simper SC, Hopkins PN, Hunt SC (2009) Cancer incidence and mortality after gastric bypass surgery. Obesity (Silver Spring) 17:796–802

Algire C, Amrein L, Zakikhani M, Panasci L, Pollak M (2010) Metformin blocks the stimulative effect of a high-energy diet on colon carcinoma growth in vivo and is associated with reduced expression of fatty acid synthase. Endocr Relat Cancer 17:351–360

Alikhani N, Ferguson RD, Novosyadlyy R, Gallagher EJ, Scheinman EJ, Yakar S, LeRoith D (2013) Mammary tumor growth and pulmonary metastasis are enhanced in a hyperlipidemic mouse model. Oncogene 32:961–967

Antalis CJ, Uchida A, Buhman KK, Siddiqui RA (2011) Migration of MDA-MB-231 breast cancer cells depends on the availability of exogenous lipids and cholesterol esterification. Clin Exp Metastasis 28:733–741

Arnlov J, Sundstrom J, Ingelsson E, Lind L (2011) Impact of BMI and the metabolic syndrome on the risk of diabetes in middle-aged men. Diabetes Care 34:61–65

Berentzen TL, Gamborg M, Holst C, Sorensen TI, Baker JL (2014) Body mass index in childhood and adult risk of primary liver cancer. J Hepatol 60:325–330

Blando J, Moore T, Hursting S, Jiang G, Saha A, Beltran L, Shen J, Repass J, Strom S, DiGiovanni J (2011) Dietary energy balance modulates prostate cancer progression in Hi-Myc mice. Cancer Prev Res (Phila) 4:2002–2014

Bonovas S, Filioussi K, Tsantes A (2004) Diabetes mellitus and risk of prostate cancer: a meta-analysis. Diabetologia 47:1071–1078

Borowsky AD, Namba R, Young LJ, Hunter KW, Hodgson JG, Tepper CG, McGoldrick ET, Muller WJ, Cardiff RD, Gregg JP (2005) Syngeneic mouse mammary carcinoma cell lines: two closely related cell lines with divergent metastatic behavior. Clin Exp Metastasis 22:47–59

Bruning PF, Bonfrer JM, van Noord PA, Hart AA, de Jong-Bakker M, Nooijen WJ (1992) Insulin resistance and breast-cancer risk. Int J Cancer 52:511–516

Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ (2003) Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. N Engl J Med 348:1625–1638

Campbell MJ, Wollish WS, Lobo M, Esserman LJ (2002) Epithelial and fibroblast cell lines derived from a spontaneous mammary carcinoma in a MMTV/neu transgenic mouse. In Vitro Cell Dev Biol Anim 38:326–333

Cook S, Weitzman M, Auinger P, Nguyen M, Dietz WH (2003) Prevalence of a metabolic syndrome phenotype in adolescents: findings from the third National Health and Nutrition Examination Survey, 1988–1994. Arch Pediatr Adolesc Med 157:821–827

Cornier MA, Dabelea D, Hernandez TL, Lindstrom RC, Steig AJ, Stob NR, Van Pelt RE, Wang H, Eckel RH (2008) The metabolic syndrome. Endocr Rev 29:777–822

Coughlin SS, Calle EE, Teras LR, Petrelli J, Thun MJ (2004) Diabetes mellitus as a predictor of cancer mortality in a large cohort of US adults. Am J Epidemiol 159:1160–1167

Crawford ED (2009) Understanding the epidemiology, natural history, and key pathways involved in prostate cancer. Urology 73:S4–S10

Del Prete A, Allavena P, Santoro G, Fumarulo R, Corsi MM, Mantovani A (2011) Molecular pathways in cancer-related inflammation. Biochem Med (Zagreb) 21:264–275

Djiogue S, Nwabo Kamdje AH, Vecchio L, Kipanyula MJ, Farahna M, Aldebasi Y, Seke Etet PF (2013) Insulin resistance and cancer: the role of insulin and IGFs. Endocr Relat Cancer 20:R1–R17

Dunlap SM, Chiao LJ, Nogueira L, Usary J, Perou CM, Varticovski L, Hursting SD (2012) Dietary energy balance modulates epithelial-to-mesenchymal transition and tumor progression in murine claudin-low and basal-like mammary tumor models. Cancer Prev Res (Phila) 5:930–942

Elinav E, Nowarski R, Thaiss CA, Hu B, Jin C, Flavell RA (2013) Inflammation-induced cancer: crosstalk between tumours, immune cells and microorganisms. Nat Rev Cancer 13:759–771

Ewens A, Mihich E, Ehrke MJ (2005) Distant metastasis from subcutaneously grown E0771 medullary breast adenocarcinoma. Anticancer Res 25:3905–3915

Ferguson RD, Gallagher EJ, Scheinman EJ, Damouni R, LeRoith D (2013) The epidemiology and molecular mechanisms linking obesity, diabetes, and cancer. Vitam Horm 93:51–98

Fierz Y, Novosyadlyy R, Vijayakumar A, Yakar S, LeRoith D (2010a) Insulin-sensitizing therapy attenuates type 2 diabetes-mediated mammary tumor progression. Diabetes 59:686–693

Fierz Y, Novosyadlyy R, Vijayakumar A, Yakar S, LeRoith D (2010b) Mammalian target of rapamycin inhibition abrogates insulin-mediated mammary tumor progression in type 2 diabetes. Endocr Relat Cancer 17:941–951

Folsom AR, Anderson KE, Sweeney C, Jacobs DR Jr (2004) Diabetes as a risk factor for death following endometrial cancer. Gynecol Oncol 94:740–745

Ford NA, Nunez NP, Holcomb VB, Hursting SD (2013) IGF1 dependence of dietary energy balance effects on murine Met1 mammary tumor progression, epithelial-to-mesenchymal transition, and chemokine expression. Endocr Relat Cancer 20:39–51

Franzese A, Vajro P, Argenziano A, Puzziello A, Iannucci MP, Saviano MC, Brunetti F, Rubino A (1997) Liver involvement in obese children. Ultrasonography and liver enzyme levels at diagnosis and during follow-up in an Italian population. Dig Dis Sci 42:1428–1432

Fujisawa T, Endo H, Tomimoto A, Sugiyama M, Takahashi H, Saito S, Inamori M, Nakajima N, Watanabe M, Kubota N, Yamauchi T, Kadowaki T, Wada K, Nakagama H, Nakajima A (2008) Adiponectin suppresses colorectal carcinogenesis under the high-fat diet condition. Gut 57:1531–1538

Gallagher EJ, Fierz Y, Vijayakumar A, Haddad N, Yakar S, LeRoith D (2012) Inhibiting PI3K reduces mammary tumor growth and induces hyperglycemia in a mouse model of insulin resistance and hyperinsulinemia. Oncogene 31:3213–3222

Gomez-Diaz RA, Talavera JO, Pool EC, Ortiz-Navarrete FV, Solorzano-Santos F, Mondragon-Gonzalez R, Valladares-Salgado A, Cruz M, Aguilar-Salinas CA, Wacher NH (2012) Metformin decreases plasma resistin concentrations in pediatric patients with impaired glucose tolerance: a placebo-controlled randomized clinical trial. Metabolism 61:1247–1255

Grossmann ME, Cleary MP (2012) The balance between leptin and adiponectin in the control of carcinogenesis - focus on mammary tumorigenesis. Biochimie 94:2164–2171

Gunter MJ, Hoover DR, Yu H, Wassertheil-Smoller S, Rohan TE, Manson JE, Li J, Ho GY, Xue X, Anderson GL, Kaplan RC, Harris TG, Howard BV, Wylie-Rosett J, Burk RD, Strickler HD (2009) Insulin, insulin-like growth factor-I, and risk of breast cancer in postmenopausal women. J Natl Cancer Inst 101:48–60

Gunther EJ, Belka GK, Wertheim GB, Wang J, Hartman JL, Boxer RB, Chodosh LA (2002) A novel doxycycline-inducible system for the transgenic analysis of mammary gland biology. FASEB J 16:283–292

Guy CT, Cardiff RD, Muller WJ (1992) Induction of mammary tumors by expression of polyomavirus middle T oncogene: a transgenic mouse model for metastatic disease. Mol Cell Biol 12:954–961

Hadsell DL, Greenberg NM, Fligger JM, Baumrucker CR, Rosen JM (1996) Targeted expression of des(1-3) human insulin-like growth factor I in transgenic mice influences mammary gland development and IGF-binding protein expression. Endocrinology 137:321–330

Haslam DW, James WP (2005) Obesity. Lancet 366:1197–1209

Howe LR, Subbaramaiah K, Hudis CA, Dannenberg AJ (2013) Molecular pathways: adipose inflammation as a mediator of obesity-associated cancer. Clin Cancer Res 19:6074–6083

Huang Z, Willett WC, Colditz GA, Hunter DJ, Manson JE, Rosner B, Speizer FE, Hankinson SE (1999) Waist circumference, waist:hip ratio, and risk of breast cancer in the Nurses' Health Study. Am J Epidemiol 150:1316–1324

Imamura N, Yanagihara K, Kusunoki Y (1984) Tumor-associated antigen of spontaneous mammary tumor in rats. Oncology 41:206–209

Isaksson B, Strommer L, Friess H, Buchler MW, Herrington MK, Wang F, Zierath JR, Wallberg-Henriksson H, Larsson J, Permert J (2003) Impaired insulin action on phosphatidylinositol 3-kinase activity and glucose transport in skeletal muscle of pancreatic cancer patients. Pancreas 26:173–177

Jaggers JR, Sui X, Hooker SP, LaMonte MJ, Matthews CE, Hand GA, Blair SN (2009) Metabolic syndrome and risk of cancer mortality in men. Eur J Cancer 45:1831–1838

Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ (2007) Cancer statistics, 2007. CA Cancer J Clin 57:43–66

Kasper JS, Giovannucci E (2006) A meta-analysis of diabetes mellitus and the risk of prostate cancer. Cancer Epidemiol Biomarkers Prev 15:2056–2062

Kawashima Y, Chen J, Sun H, Lann D, Hajjar RJ, Yakar S, Leroith D (2009) Apolipoprotein E deficiency abrogates insulin resistance in a mouse model of type 2 diabetes mellitus. Diabetologia 52:1434–1441

Kelesidis I, Kelesidis T, Mantzoros CS (2006) Adiponectin and cancer: a systematic review. Br J Cancer 94:1221–1225

Kim H, Pennisi PA, Gavrilova O, Pack S, Jou W, Setser-Portas J, East-Palmer J, Tang Y, Manganiello VC, Leroith D (2006) Effect of adipocyte beta3-adrenergic receptor activation on the type 2 diabetic MKR mice. Am J Physiol Endocrinol Metab 290:E1227–E1236

Körner A, Kratzsch J, Kiess W (2006) Effect of adiponectin on proliferation of breast cancer cells. Exp Clin Endocrinol Diabetes 114:P03_043

Kushiro K, Nunez NP (2011) Ob/ob serum promotes a mesenchymal cell phenotype in B16BL6 melanoma cells. Clin Exp Metastasis 28:877–886

Lanari C, Lamb CA, Fabris VT, Helguero LA, Soldati R, Bottino MC, Giulianelli S, Cerliani JP, Wargon V, Molinolo A (2009) The MPA mouse breast cancer model: evidence for a role of progesterone receptors in breast cancer. Endocr Relat Cancer 16:333–350

Landskron G, De la Fuente M, Thuwajit P, Thuwajit C, Hermoso MA (2014) Chronic inflammation and cytokines in the tumor microenvironment. J Immunol Res 2014:149185

Larsson O, Morita M, Topisirovic I, Alain T, Blouin MJ, Pollak M, Sonenberg N (2012) Distinct perturbation of the translatome by the antidiabetic drug metformin. Proc Natl Acad Sci U S A 109:8977–8982

Lashinger LM, Harrison LM, Rasmussen AJ, Logsdon CD, Fischer SM, McArthur MJ, Hursting SD (2013) Dietary energy balance modulation of Kras- and Ink4a/Arf+/–driven pancreatic cancer: the role of insulin-like growth factor-I. Cancer Prev Res (Phila) 6:1046–1055

Lee AV, Yee D (1995) Insulin-like growth factors and breast cancer. Biomed Pharmacother 49:415–421

Levine AJ, Feng Z, Mak TW, You H, Jin S (2006) Coordination and communication between the p53 and IGF-1-AKT-TOR signal transduction pathways. Genes Dev 20:267–275

Liu J, Xu A, Lam KS, Wong NS, Chen J, Shepherd PR, Wang Y (2013) Cholesterol-induced mammary tumorigenesis is enhanced by adiponectin deficiency: role of LDL receptor upregulation. Oncotarget 4:1804–1818

Ma J, Li H, Giovannucci E, Mucci L, Qiu W, Nguyen PL, Gaziano JM, Pollak M, Stampfer MJ (2008) Prediagnostic body-mass index, plasma C-peptide concentration, and prostate cancer-specific mortality in men with prostate cancer: a long-term survival analysis. Lancet Oncol 9:1039–1047

McHenry PR, Sears JC, Herrick MP, Chang P, Heckman-Stoddard BM, Rybarczyk M, Chodosh LA, Gunther EJ, Hilsenbeck SG, Rosen JM, Vargo-Gogola T (2010) P190B RhoGAP has pro-tumorigenic functions during MMTV-Neu mammary tumorigenesis and metastasis. Breast Cancer Res 12:R73

McNamara KM, Sasano H (2015) The intracrinology of breast cancer. J Steroid Biochem Mol Biol. Ahead of print 145:172–178

Michels KB, Solomon CG, Hu FB, Rosner BA, Hankinson SE, Colditz GA, Manson JE, Nurses' Health S (2003) Type 2 diabetes and subsequent incidence of breast cancer in the Nurses' Health Study. Diabetes Care 26:1752–1758

Middleton PJ (1965) The histogenesis of mammary tumours induced in the rat by chemical carcinogens. Br J Cancer 19:830–839

Moon HS, Liu X, Nagel JM, Chamberland JP, Diakopoulos KN, Brinkoetter MT, Hatziapostolou M, Wu Y, Robson SC, Iliopoulos D, Mantzoros CS (2013) Salutary effects of adiponectin on colon cancer: in vivo and in vitro studies in mice. Gut 62:561–570

Moore T, Beltran L, Carbajal S, Hursting SD, DiGiovanni J (2012) Energy balance modulates mouse skin tumor promotion through altered IGF-1R and EGFR crosstalk. Cancer Prev Res (Phila) 5:1236–1246

Muller WJ, Sinn E, Pattengale PK, Wallace R, Leder P (1988) Single-step induction of mammary adenocarcinoma in transgenic mice bearing the activated c-neu oncogene. Cell 54:105–115

Nalabolu MR, Palasamudram K, Jamil K (2014) Adiponectin and leptin molecular actions and clinical significance in breast cancer. Int J Hematol Oncol Stem Cell Res 8:31–40

Nelson ER, Wardell SE, Jasper JS, Park S, Suchindran S, Howe MK, Carver NJ, Pillai RV, Sullivan PM, Sondhi V, Umetani M, Geradts J, McDonnell DP (2013) 27-Hydroxycholesterol links hypercholesterolemia and breast cancer pathophysiology. Science 342:1094–1098

Nielsen SF, Nordestgaard BG, Bojesen SE (2012) Statin use and reduced cancer-related mortality. N Engl J Med 367:1792–1802

Noto H, Goto A, Tsujimoto T, Osame K, Noda M (2013) Latest insights into the risk of cancer in diabetes. J Diabetes Investig 4:225–232

Novosyadlyy R, LeRoith D (2010) Hyperinsulinemia and type 2 diabetes: impact on cancer. Cell Cycle 9:1449–1450

Ntikoudi E, Kiagia M, Boura P, Syrigos KN (2014) Hormones of adipose tissue and their biologic role in lung cancer. Cancer Treat Rev 40:22–30

Olivo-Marston SE, Hursting SD, Perkins SN, Schetter A, Khan M, Croce C, Harris CC, Lavigne J (2014) Effects of calorie restriction and diet-induced obesity on murine colon carcinogenesis, growth and inflammatory factors, and microRNA expression. PLoS One 9:e94765

Park J, Scherer PE (2011) Leptin and cancer: from cancer stem cells to metastasis. Endocr Relat Cancer 18:C25–C29

Park MH, Falconer C, Viner RM, Kinra S (2012) The impact of childhood obesity on morbidity and mortality in adulthood: a systematic review. Obes Rev 13:985–1000

Pei XF, Noble MS, Davoli MA, Rosfjord E, Tilli MT, Furth PA, Russell R, Johnson MD, Dickson RB (2004) Explant-cell culture of primary mammary tumors from MMTV-c-Myc transgenic mice. In Vitro Cell Dev Biol Anim 40:14–21

Pelton K, Freeman MR, Solomon KR (2012) Cholesterol and prostate cancer. Curr Opin Pharmacol 12:751–759

Permert J, Larsson J, Westermark GT, Herrington MK, Christmanson L, Pour PM, Westermark P, Adrian TE (1994) Islet amyloid polypeptide in patients with pancreatic cancer and diabetes. N Engl J Med 330:313–318

Petrelli JM, Calle EE, Rodriguez C, Thun MJ (2002) Body mass index, height, and postmenopausal breast cancer mortality in a prospective cohort of US women. Cancer Causes Control 13:325–332

Pierobon M, Frankenfeld CL (2013) Obesity as a risk factor for triple-negative breast cancers: a systematic review and meta-analysis. Breast Cancer Res Treat 137:307–314

Pisani P (2008) Hyper-insulinaemia and cancer, meta-analyses of epidemiological studies. Arch Physiol Biochem 114:63–70

Pothiwala P, Jain SK, Yaturu S (2009) Metabolic syndrome and cancer. Metab Syndr Relat Disord 7:279–288

Prentice RL, Caan B, Chlebowski RT, Patterson R, Kuller LH, Ockene JK, Margolis KL, Limacher MC, Manson JE, Parker LM, Paskett E, Phillips L, Robbins J, Rossouw JE, Sarto GE, Shikany JM, Stefanick ML, Thomson CA, Van Horn L, Vitolins MZ, Wactawski-Wende J, Wallace RB, Wassertheil-Smoller S, Whitlock E, Yano K, Adams-Campbell L, Anderson GL, Assaf AR, Beresford SA, Black HR, Brunner RL, Brzyski RG, Ford L, Gass M, Hays J, Heber D, Heiss G, Hendrix SL, Hsia J, Hubbell FA, Jackson RD, Johnson KC, Kotchen JM, LaCroix AZ, Lane DS, Langer RD, Lasser NL, Henderson MM (2006) Low-fat dietary pattern and risk of invasive breast cancer: the Women's Health Initiative Randomized Controlled Dietary Modification Trial. JAMA 295:629–642

Reeves GK, Pirie K, Beral V, Green J, Spencer E, Bull D, Million Women Study C (2007) Cancer incidence and mortality in relation to body mass index in the Million Women Study: cohort study. BMJ 335:1134

Rizner TL (2013) Estrogen biosynthesis, phase I and phase II metabolism, and action in endometrial cancer. Mol Cell Endocrinol 381:124–139

Robinson WR, Tse CK, Olshan AF, Troester MA (2014) Body size across the life course and risk of premenopausal and postmenopausal breast cancer in Black women, the Carolina Breast Cancer Study, 1993–2001. Cancer Causes Control. Ahead of print 25(9):1101–1117

Rondini EA, Harvey AE, Steibel JP, Hursting SD, Fenton JI (2011) Energy balance modulates colon tumor growth: Interactive roles of insulin and estrogen. Mol Carcinog 50:370–382

Rostoker R, Bitton-Worms K, Caspi A, Shen-Orr Z, LeRoith D (2013) Investigating new therapeutic strategies targeting hyperinsulinemia's mitogenic effects in a female mouse breast cancer model. Endocrinology 154:1701–1710

Rousseau MC, Parent ME, Pollak MN, Siemiatycki J (2006) Diabetes mellitus and cancer risk in a population-based case-control study among men from Montreal, Canada. Int J Cancer 118:2105–2109

Rudling MJ, Stahle L, Peterson CO, Skoog L (1986) Content of low density lipoprotein receptors in breast cancer tissue related to survival of patients. Br Med J (Clin Res Ed) 292:580–582

Sandgren EP, Schroeder JA, Qui TH, Palmiter RD, Brinster RL, Lee DC (1995) Inhibition of mammary gland involution is associated with transforming growth factor alpha but not c-myc-induced tumorigenesis in transgenic mice. Cancer Res 55:3915–3927

Schwimmer JB, Deutsch R, Kahen T, Lavine JE, Stanley C, Behling C (2006) Prevalence of fatty liver in children and adolescents. Pediatrics 118:1388–1393

Sciacca L, Vigneri R, Tumminia A, Frasca F, Squatrito S, Frittitta L, Vigneri P (2013) Clinical and molecular mechanisms favoring cancer initiation and progression in diabetic patients. Nutr Metab Cardiovasc Dis 23:808–815

Shaw RJ, Lamia KA, Vasquez D, Koo SH, Bardeesy N, Depinho RA, Montminy M, Cantley LC (2005) The kinase LKB1 mediates glucose homeostasis in liver and therapeutic effects of metformin. Science 310:1642–1646

Singh S, Akhtar N, Ahmad J (2012) Plasma adiponectin levels in women with polycystic ovary syndrome: impact of metformin treatment in a case-control study. Diabetes Metab Syndr 6:207–211

Sinn E, Muller W, Pattengale P, Tepler I, Wallace R, Leder P (1987) Coexpression of MMTV/v-Ha-ras and MMTV/c-myc genes in transgenic mice: synergistic action of oncogenes in vivo. Cell 49:465–475

Sirotnak FM, DeGraw JI, Schmid FA, Goutas LJ, Moccio DM (1984) New folate analogs of the 10-deaza-aminopterin series. Further evidence for markedly increased antitumor efficacy compared with methotrexate in ascitic and solid murine tumor models. Cancer Chemother Pharmacol 12:26–30

Sjostrom L, Gummesson A, Sjostrom CD, Narbro K, Peltonen M, Wedel H, Bengtsson C, Bouchard C, Carlsson B, Dahlgren S, Jacobson P, Karason K, Karlsson J, Larsson B, Lindroos AK, Lonroth H, Naslund I, Olbers T, Stenlof K, Torgerson J, Carlsson LM (2009) Effects of bariatric surgery on cancer incidence in obese patients in Sweden (Swedish Obese Subjects Study): a prospective, controlled intervention trial. Lancet Oncol 10:653–662

Somasundar P, Yu AK, Vona-Davis L, McFadden DW (2003) Differential effects of leptin on cancer in vitro. J Surg Res 113:50–55

Stewart TA, Pattengale PK, Leder P (1984) Spontaneous mammary adenocarcinomas in transgenic mice that carry and express MTV/myc fusion genes. Cell 38:627–637

Sturmer T, Buring JE, Lee IM, Gaziano JM, Glynn RJ (2006) Metabolic abnormalities and risk for colorectal cancer in the physicians' health study. Cancer Epidemiol Biomarkers Prev 15:2391–2397

Tseng CH, Tseng FH (2014) Diabetes and gastric cancer: the potential links. World J Gastroenterol 20:1701–1711

Tsukamoto AS, Grosschedl R, Guzman RC, Parslow T, Varmus HE (1988) Expression of the int-1 gene in transgenic mice is associated with mammary gland hyperplasia and adenocarcinomas in male and female mice. Cell 55:619–625

Tzeng YJ, Guhl E, Graessmann M, Graessmann A (1993) Breast cancer formation in transgenic animals induced by the whey acidic protein SV40 T antigen (WAP-SV-T) hybrid gene. Oncogene 8:1965–1971

Uddin S, Hussain AR, Khan OS, Al-Kuraya KS (2014) Role of dysregulated expression of leptin and leptin receptors in colorectal carcinogenesis. Tumour Biol 35:871–879

Van Itallie TB (1985) Health implications of overweight and obesity in the United States. Ann Intern Med 103:983–988

Vansaun MN (2013) Molecular pathways: adiponectin and leptin signaling in cancer. Clin Cancer Res 19:1926–1932

Verheus M, Peeters PH, Rinaldi S, Dossus L, Biessy C, Olsen A, Tjonneland A, Overvad K, Jeppesen M, Clavel-Chapelon F, Tehard B, Nagel G, Linseisen J, Boeing H, Lahmann PH, Arvaniti A, Psaltopoulou T, Trichopoulou A, Palli D, Tumino R, Panico S, Sacerdote C, Sieri S, van Gils CH, Bueno-de-Mesquita BH, Gonzalez CA, Ardanaz E, Larranaga N, Garcia CM, Navarro C, Quiros JR, Key T, Allen N, Bingham S, Khaw KT, Slimani N, Riboli E, Kaaks

R (2006) Serum C-peptide levels and breast cancer risk: results from the European Prospective Investigation into Cancer and Nutrition (EPIC). Int J Cancer 119:659–667
Verlato G, Zoppini G, Bonora E, Muggeo M (2003) Mortality from site-specific malignancies in type 2 diabetic patients from Verona. Diabetes Care 26:1047–1051
Wang L, Di LJ (2014) BRCA1 and estrogen/estrogen receptor in breast cancer: where they interact? Int J Biol Sci 10:563–573
Wang Z, Lai ST, Xie L, Zhao JD, Ma NY, Zhu J, Ren ZG, Jiang GL (2014) Metformin is associated with reduced risk of pancreatic cancer in patients with type 2 diabetes mellitus: a systematic review and meta-analysis. Diabetes Res Clin Pract 106(1):19–26
Warburg O (1956) On the origin of cancer cells. Science 123:309–314
Weiderpass E, Gridley G, Persson I, Nyren O, Ekbom A, Adami HO (1997) Risk of endometrial and breast cancer in patients with diabetes mellitus. Int J Cancer 71:360–363
Weiss R, Dziura J, Burgert TS, Tamborlane WV, Taksali SE, Yeckel CW, Allen K, Lopes M, Savoye M, Morrison J, Sherwin RS, Caprio S (2004) Obesity and the metabolic syndrome in children and adolescents. N Engl J Med 350:2362–2374
Williams JC, Gusterson B, Humphreys J, Monaghan P, Coombes RC, Rudland P, Neville AM (1981) N-methyl-N-nitrosourea-induced rat mammary tumors. Hormone responsiveness but lack of spontaneous metastasis. J Natl Cancer Inst 66:147–155
Xu CX, Zhu HH, Zhu YM (2014) Diabetes and cancer: associations, mechanisms, and implications for medical practice. World J Diabetes 5:372–380
Yakar S, Nunez NP, Pennisi P, Brodt P, Sun H, Fallavollita L, Zhao H, Scavo L, Novosyadlyy R, Kurshan N, Stannard B, East-Palmer J, Smith NC, Perkins SN, Fuchs-Young R, Barrett JC, Hursting SD, LeRoith D (2006) Increased tumor growth in mice with diet-induced obesity: impact of ovarian hormones. Endocrinology 147:5826–5834
Yancik R, Ganz PA, Varricchio CG, Conley B (2001a) Perspectives on comorbidity and cancer in older patients: approaches to expand the knowledge base. J Clin Oncol 19:1147–1151
Yancik R, Wesley MN, Ries LA, Havlik RJ, Edwards BK, Yates JW (2001b) Effect of age and comorbidity in postmenopausal breast cancer patients aged 55 years and older. JAMA 285:885–892
Yang YX, Hennessy S, Lewis JD (2005) Type 2 diabetes mellitus and the risk of colorectal cancer. Clin Gastroenterol Hepatol 3:587–594
Zhang P, Li H, Tan X, Chen L, Wang S (2013) Association of metformin use with cancer incidence and mortality: a meta-analysis. Cancer Epidemiol 37:207–218
Zhu P, Davis M, Blackwelder AJ, Bachman N, Liu B, Edgerton S, Williams LL, Thor AD, Yang X (2014) Metformin selectively targets tumor-initiating cells in ErbB2-overexpressing breast cancer models. Cancer Prev Res (Phila) 7:199–210

Antilipidemic Drug Therapy Today and in the Future

Werner Kramer

Contents

Abstract

The armamentarium for the treatment of dyslipidemia today comprises six different modes of action with overall around 24 different drugs. The treatment of lipid disorders was revolutionized with the introduction of statins which have become the most important therapeutic option available today to reduce and prevent atherosclerosis and its detrimental consequences like cardiovascular

W. Kramer (✉)
Institute of Biochemistry, Biocenter, Goethe-Universität Frankfurt, Max-von-Laue-Str. 9, Frankfurt, Germany
e-mail: w.kramer.mainz@arcor.de

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diseases and stroke. With and optimized reduction of elevated LDL levels with statins, the risk for cardiovascular diseases (CVD) can be reduced by 30%, indicating a residual remaining risk of 70% for the development and progression of CVD notifying still a high medical need for more effective antilipidemic drugs. Consequently, the search for novel lipid-modifying drugs is still one of the most active areas in research and development in the pharmaceutical industry. Major focus lies on approaches to LDL-lowering drugs superior to statins with regard to efficacy, safety, and patient compliance and on approaches modifying plasma levels and functionality of HDL particles based on the clinically validated inverse relationship between high-plasma HDL levels and the risk for CVD. The available drugs today for the treatment of dyslipidemia are small organic molecules or nonabsorbable polymers for binding of bile acids to be applied orally. Besides small molecules for novel targets, biological drugs such as monoclonal antibodies, antisense or gene-silencing oligonucleotides, peptidomimetics, reconstituted synthetic HDL particles and therapeutic proteins are novel approaches in clinical development are which have to be applied by injection or infusion. The promising clinical results of several novel drug candidates, particularly for LDL cholesterol lowering with monoclonal antibodies raised against PCSK9, may indicate more than a decade after the statins, the entrance of new breakthrough therapies to treat lipid disorders.

Keywords
Drug development pipeline · Dyslipidemia · Novel lipid-modifying drugs

Abbreviations

ABCA1	ABC Transporter A1
ACC2	Acetyl-CoA carboxylase 2
ACS	Acute coronary syndrome
AMPK	AMP-activated protein kinase
ANPTL3	Angiopoietin-like 3
ApoA-I	Apolipoprotein A-I
ApoA-II	Apolipoprotein A-II
ApoB	Apolipoprotein B
ApoC-III	Apolipoprotein C-III
ApoE	Apolipoprotein E
ASO	Antisense oligonucleotides
BET-protein	Bromodomain and extracellular domain protein
CABG	Coronary artery bypass graft
CAD	Coronary artery disease
CE	Cholesterol ester

CETP	Cholesterol ester transfer protein
CHD	Coronary heart disease
CRP	C-reactive protein
CVD	Cardiovascular disease
DGAT	Diacylglycerol acyl transferase
EL	Endothelial lipase
FGF 21	Fibroblast growth factor 21
FH	Familial hypercholesterolemia
FXR	Farnesoid X receptor
GI	Gastrointestinal
GLP-1	Glucagon-like peptide-1
GWAS	Genome-wide association study
heFH	Heterozygous familial hypercholesterolemia
HDL	High-density lipoprotein
HDL-C	High-density lipoprotein cholesterol
HL	Hepatic lipase
HMG-CoA	Hydroxymethylglutaryl coenzyme A
hoFH	Homozygous familial hypercholesterolemia
IBAT	Ileal bile acid transporter
IDL	Intermediate-density lipoprotein
LCAT	Lecithin–cholesterol acyl transferase
LDL	Low-density lipoprotein
LDL-C	Low-density lipoprotein cholesterol
Lp(a)	Lipoprotein (a)
LPL	Lipoprotein lipase
Lrh-1	Liver receptor homolog-1
moAb	Monoclonal antibody
MTP	Microsomal lipid transfer protein
NASH	Nonalcoholic steatohepatitis
NPC1L1	Niemann–Pick C1-like protein 1
OATP1B1	Organic anion transporting peptide 1B1
PAI-1	Plasminogen activator inhibitor-1
PBC	Primary biliary cirrhosis
PCSK9	Proprotein convertase subtilisin/kexin type 9
PEG	Polyethylene glycol
PGD2R	Prostaglandin D2 receptor
PK	Pharmacokinetic
PPAR	Peroxisome proliferator-activated receptor
PSC	Primary sclerosing cholangitis
RCT	Reverse cholesterol transport
RNAi	RNA interference
RXR	Retinoid X receptor
S1P	Site-1 protease
S2P	Site-2 protease
SARS	Severe acute respiratory syndrome

SCD-1	Stearoyl-coenzyme A desaturase 1
SHP	Small heterodimer partner
SR-BI	Scavenger receptor BI
Srebp-1	Sterol regulatory element-binding protein 1
TG	Triglyceride
VLDL	Very-low density lipoprotein

1 Introduction

Worldwide cardiovascular diseases (CVD including coronary heart disease (CHD) and stroke are the leading causes of mortality with an increasing prevalence (National Clinical Guideline 2014); atherosclerotic cardiovascular disease accounts for 17.3 million deaths/year – more than 4.3 million in Europe (Stone et al. 2013) and more than 30% of all deaths in the United States (Graham et al. 2007a; Lloyd-Jones et al. 2010) – and is projected to increase to 23.6 death cases in 2030 (Mendis et al. 2011). The overall risk for CHD is determined by multiple parameters such as dyslipidemia, hypertension, smoking, diabetes mellitus, obesity, coagulopathies, diet, and sedentary lifestyle as well as hereditary genetic variations (Roger et al. 2012; Prospective Studies Collaboration 1995). These risk factors account for more than 90% of the population-attributable risk for CVD (Yusuf et al. 2004), and up to 80% of CVD could be prevented by prevention or treatment of these risk modalities (Mendis et al. 2011). Dyslipidemia, mainly hypercholesterolemia, plays a causal role in the pathogenesis of atherosclerosis being responsible for the development and progression of CHD, stroke, and peripheral vascular disease (Roger et al. 2012). Out of nine independent risk factors for CVD, dyslipidemia is associated with the highest population-attributable risk, and 55% of CVD cases can directly be assigned to lipids (Yusuf et al. 2004). Consequently, lowering or modifying plasma lipid levels has become one of the most important options for treatment and prevention to combat the leading "killing disease no. 1" in the world (Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults 2001), taking 17.1 million lives a year (Rozman and Monostory 2010).

2 Dyslipidemia and Cardiovascular Disease

2.1 Low-Density Lipoproteins and CVD Risk

Dyslipidemia is defined as a spectrum of deviations from lipid homeostasis characterized by elevations in plasma concentrations of low-density lipoproteins (LDL), lipoprotein a (Lp(a)), apolipoproteins B and triglycerides (TG), as well as decreased plasma levels of high-density lipoproteins (HDL) or apolipoprotein A1 (ApoA-I). Primary dyslipidemias result from mutations in proteins involved in critical pathways involved in lipid and lipoprotein metabolism such as the LDL

receptor (familial hypercholesterolemia FH), apolipoprotein B (familial defective ApoB-100), or PCSK9 (variant FH). Most cases of dyslipidemia are of secondary cause, resulting from other diseases or health conditions such as diabetes, hypothyroidism, or a sedentary lifestyle with overcaloric nutrition, decreased physical exercise, or excessive alcohol consumption. Aging of the general population also contributes to the growing incidence of hypercholesterolemia because plasma levels of LDL-C exert a natural rise with increasing lifetime (Schaefer et al. 1994). Plasma cholesterol levels are lowest immediately after birth with 50 mg/dL total cholesterol and 30 mg/dL LDL-C (Parker et al. 1983; Dietschy and Turley 2004). With onset of breast feeding, total cholesterol levels rapidly increase to 180 mg/dL due to the high cholesterol content of breast milk.

Epidemiological studies [NHANES III] show that mean total cholesterol levels in adolescents aged 4–19 is 165 mg/dL; during lifetime cholesterol levels gradually increase, and in healthy subjects consuming typical Western diets, mean total cholesterol levels rise to around 200 mg/dL (Cohen et al. 2010; Martin et al. 1986). Compared to "wild-forging," non-primates with LDL-C levels of 30–50 mg/dL and total cholesterol of 70–100 mg/dL humans have much higher plasma cholesterol concentrations. This is probably the result from changes in the eating habits from a low-carbohydrate/high protein rich diet to a greater consumption of grain and animal meat rich in saturated fat (Mann 2000).

From pathological examination, we know that the earliest stages of the atherogenic process can be detected in coronary arteries already in adolescence and early adulthood (Strong and McGill 1969; Newman et al. 1986); 75% of young men (mean age 22 years) killed in the Korean and Vietnam war had already detectable fibrous plaques (McNamara et al. 1971). Pathological examination of 2,876 individuals in the age range of 15–34 years died from noncardiovascular reasons revealed an increase of fatty streaks and lesions with increasing age and a positive correlation of the lesions with plasma cholesterol levels (McGill et al. 2000).

Epidemiological studies have shown a continuous relationship between total plasma cholesterol levels and the risk of coronary heart disease (National Heart Foundation of Australia and New Zealand 2001). 1% reduction in plasma LDL-C/non-HDL-C correlates to a 1% reduction in CVD events, whereas the increase of HDL-C by 1% decreases CVD events by 3% (O'Keefe et al. 2004; Sacks and Expert group on HDL Cholesterol 2002). The major causative role of elevated levels of LDL cholesterol in atherosclerotic vascular disease has been unequivocally proven by clinical landmark studies where reduction of plasma LDL cholesterol levels by statins – inhibitors of cholesterol biosynthesis (Scandinavian Simvastatin Survival Study Group 1994; Downs et al. 1998; Shepherd et al. 1995) – or surgical interruption of the enterohepatic circulation of bile acids (Buchwald et al. 1990) reduced cardiovascular morbidity and death. A meta-

analysis of 27 clinical trials with 170,000 participants has revealed significant risk reduction by statin therapy (Cholesterol Treatment Trialists' (CTT) Collaborators et al. 2012):

1. −21% for CVD events
2. −27% for coronary events (Ridker 2014)
3. −15% for stroke (Ridker 2014)
4. +24% for coronary revascularization (Ridker 2014)

for a reduction of plasma LDL-C levels by 1 mmol/L (38.7 mg/dL).

In the Cochrane review from 18 randomized clinical trials with 56,934 participants, statin therapy resulted in a reduction of 14% in total mortality and 25% of combined fatal and nonfatal CVD events (Taylor et al. 2013), i.e., primary prevention of dyslipidemia is working. In the year 2000, 341,745 fewer death cases from coronary heart disease compared to 1980 were registered in the United States after introduction of statin therapy into clinical practice, whereby a percentage of 24% could directly be aligned to a decrease of total plasma cholesterol levels (Ford et al. 2007). Similarly, a 35% decrease in CHD mortality was reported in Canada between 1994 and 2005, 48% of this reduction being associated with improved control of lipids and blood pressure (Wijeysundera et al. 2010). The mean age of participants in these statin trials was 63 years (Wijeysundera et al. 2010), raising the question whether the efficacy of LDL-C lowering therapy in reduction of CHD events would be higher if initiated earlier in life based on the observational studies with a correlation of atherosclerotic lesions to plasma cholesterol levels with increasing lifetime (Strong and McGill 1969; Newman et al. 1986; McNamara et al. 1971; McGill et al. 2000), and the findings that elevated cholesterol levels in early adulthood are associated with CVD in later life (Pearson et al. 1990). The observation that lowering of LDL-C levels can stop the progression of coronary atherosclerotic lesions is obviously linearly correlated with plasma LDL-C levels achieved, indicating that more intensive lowering of LDL-C leads to less plaque progression which suggests that lowering of elevated LDL-C levels early in life should be more effective in the prevention/progression of CHD/CVD than onset of therapy in later life (Ference and Mahajan 2013). Despite the quantum leap in the treatment of dyslipidemias by the introduction of statins into clinical practice, major gaps in the management of lipid disorders persist. Statins are undoubtedly the 1st-line therapy for the prevention of CVD in almost all population groups except those with severe renal or cardiac failure (Saydah et al. 2004) but do not address the entire spectrum of CVD risk, and the residual risk of major vascular events remains high with more than 20% over 5 years despite optimal management of LDL-C levels with statins (Cholesterol Treatment Trialists' (CTT) Collaboration et al. 2010). However, only 30–70% of high-risk patients will attain standard LDL-C targets recommended in the guidelines; for example, patients with FH and those with mixed dyslipidemias secondary to the metabolic syndrome are at an increased risk due to other risk factors such as low HDL, high TGs, or non-lipid

risk factors (Barnett et al. 2013). Particularly, in patients with complex metabolic diseases like diabetes mellitus, significant improvements in the control of risk factors are necessary; only 7.3% of adults with diabetes in the NHANES 1999–2000 survey attained the recommended targets for treatment of hyperglycemia, dyslipidemia, and hypertension (Saydah et al. 2004).

2.2 High-Density Lipoproteins and CVD

The relationship between total plasma cholesterol and CHD risk is continuous but not linear; the risk for CHD rises more strongly at higher LDL-C levels (Schaefer et al. 1994), indicating the involvement of further risk factors. However, unequivocal evidence from randomized clinical trials that reduction of other risk factors like low HDL or HDL-C, elevated TG, or non-lipid influences like hypertension, inflammation, and diabetes reduces the risk of CHD and therefore the number of patients with CVD is only available for hypertension (Sever et al. 2003). In a recent meta-analysis of 46 lipid GWAS-studies with more than 100,000 individuals of European descent, 95 genetic loci associated with serum lipid traits were identified, many of them harboring genes involved in lipid metabolism like HMG-CoA reductase, NPC1L1, cholesterol-7α hydroxylase, or ANPTL3/4 proteins, all of them being targets of available lipid-lowering drugs, but many other newly identified genes representing possible new risk factors or pharmacological targets to monitor or treat CAD (Teslovich et al. 2010). HDL is a mediator of cholesterol transport from peripheral tissues to the liver and is considered as an important regulator of CHD risk (Parker et al. 1983; Dietschy and Turley 2004; Cohen et al. 2010) with an inverse relationship of HDL levels to CHD risk (Martin et al. 1986). HDL-C is an independent risk factor for CVD and superior than LDL-C as a predictor of CV events (Sever et al. 2003; Heart Protection Study Collaborative Group 2002; Colhoun et al. 2004). An increase of HDL-C by 1 mg/dL (0,026 mmol/L) was associated with a reduction of CVD risk by 2–3% (Curb et al. 2004). Paradoxically, despite the unequivocal role of HDL as an independent risk factor for CVD events, the failure of recent clinical trials with drugs aiming to increase HDL-C levels may suggest that the functionality of the HDL particles rather than the height of HDL-C levels is more relevant for the cardio- and atheroprotective efficacy of HDL particles. Due to the complex metabolism of HDL particles involving many different proteins and interaction with other lipoprotein particles, the impact of mutations in key proteins of the HDL-particle pathways and CV risk is not fully understood today. The role of HDL as a predictor of CV diseases is still valid in the sense that HDL-C is a predictor of incident CV events in the setting of secondary prevention in individuals who have already been diagnosed with CVD (Rader and Hovingh 2014). As a conclusion, low HDL-C plasma level may serve as a strong biomarker for CVD risk but does not predict HDL functionality or composition. Consequently, measuring of HDL-C as a metric for monitoring pharmacological efficacy for reduction of CVD events by modulation of HDL particles is not sufficient to evaluate and predict of the efficacy of a

drug approach elevating HDL levels (Kingwell et al. 2014). Recently, the results of a population study involving 2,924 adults free of CVD with a mean follow-up of 9.4 years investigating the association between HDL levels and functional parameters to primary endpoint of CVD (nonfatal MI, nonfatal stroke, or coronary revascularization or death from CVD) were published (Rohatgi et al. 2014). Whereas baseline levels of HDL were not found to be associated with CV events, the cholesterol efflux capacity (from macrophages) as a key component of the RCT process was identified as a reliable biomarker being strongly and inversely associated with the incidence of CV events in a population cohort. Little correlation was found between cholesterol efflux capacity and traditional CV risk factors, coronary artery calcium, or markers of inflammation. Cholesterol efflux from macrophages is predominantly catalyzed by ABCA1, underlining the importance of this ABC transporter for RCT. From these findings, it can be concluded that measurements of macrophage-specific cholesterol efflux using fluorescently labeled cholesterol could be used in the future as a reliable method to assess the severity of atherosclerosis and its clinical consequences and to predict the efficacy of a drug approach affecting HDL metabolism for reduction of CVD risk.

2.3 Plasma Triglycerides and CVD

Regarding a causative role of TG for CVD events, the picture is as well not really clear. Observational epidemiological studies and clinical trials using drugs targeting elevated TG levels and low HDL-C levels indicate a causative and predictive role of elevated TG plasma levels for the development of primary CHD. A recent systematic review and meta-regression analysis of 40 randomized controlled trials of lipid-modifying drugs with CHD events as outcome revealed that changes in plasma TG levels are predictive of CV events in randomized controlled clinical trials being significantly in primary prevention populations but not in secondary prevention populations (Stauffer et al. 2013). Because plasma TG levels and HDL-C are metabolically and mechanistically interconnected via lipid metabolizing and exchanging proteins like CETP, LPL, or EL, it is methodically difficult to determine the relative contribution of either TG or HDL-C on CVD risk. Loss-of-function mutations in the ABCA1 transporter decrease plasma HDL-C without an effect on TG levels or risk for CHD arguing against a direct relationship between HDL-C and CHD risk. As a potential explanation for the difference in the prediction power of changes in TG levels for CV events in primary and secondary prevention trials elevated plasma TG levels could still be a risk factor in secondary populations but of lower importance for the total risk of coronary events.

3 Current Treatment of Dyslipidemia

Today the armamentarium introduced in medical practice for the treatment of lipid disorders comprises five different modes of action and respective molecular targets with overall 24 different drug entities on the market, some of them available only in selected countries. The majority of approaches focus on the reduction of LDL-C levels by inhibition of cholesterol biosynthesis (HMG-CoA reductase inhibitors – statins), interruption of the enterohepatic circulation of bile acids (bile acid sequestrants), or inhibition of intestinal cholesterol absorption (ezetimibe). Nicotinic acid and derivatives thereof as well as agonists of the peroxisome proliferator activator receptor PPAR-α (fibrates) are efficacious drugs to increase HDL-C levels and to decrease elevated TG levels. Table 1 summarizes the main characteristics of these drug classes with regard to their activity on plasma lipid parameters.

3.1 HMG-Co-Reductase Inhibitors (Statins)

The molecular target of statins is the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A reductase (EC 1.1.1.34), the rate-limiting enzyme in the biosynthesis pathway from mevalonate to cholesterol, sterols, isoprenoids, and other lipids like dolichol and ubiquinone. For a detailed overview about statins, some of the recent review articles are recommended (Sirtori 2014; Shitara and Sugiyama 2006). Statins are competitive enzyme inhibitors competing with 3-hydroxy-3-methylglutaryl-CoA for the catalytic binding site, thereby reducing biosynthesis rate of mevalonate and its downstream

Table 1 Lipid-modifying drugs on the market

Class	Mode of action	Efficacy parameters			
		LDL-C (%)	HDL-C (%)	Triglycerides (%)	LIT
Statins	Inhibition of HMG-CoA reductase	−(18–55)	+(5–15)	−(7–30)	Toth (2010)
Ezetimibe	Inhibition of cholesterol absorption	−(18–20)	+(1–4)	−8	Toth (2010)
Sequestrants	Inhibition of bile acid reabsorption	−(9–28)	+(0–9)	+ (2–16)	Hou and Goldberg (2009); Martinez-Hervas et al. (2011)
Fibrates	PPAR-alpha	−(5–20)	+(10–20)	−(20–50)	Toth (2010)
Nicotinic acid	Multiple targets?	−(5–25)	+(15–35)	−(20–50)	Toth (2010)

Table 2 Efficacy of Hmg-Coa reductase inhibitors (Ewang-Emukowhate and Wierzbicki 2013)

Drug	Total C (%)	LDL-C (%)	HDL-C (%)	TG (%)
Atorvastatin	−(27–39)	−(37–51)	+ (2–6)	−(20–28)
Rosuvastatin	−(33–40)	−(46–55)	+ (8–10)	−(20–26)
Simvastatin	−(20–33)	−(28–46)	+ (5–7)	−(12–18)
Fluvastatin	−(13–19)	−(17–23)	+ (0.9)	−(5–13)
Lovastatin	−(21–36)	−(29–48)	+ (7–8)	−(2–13)
Pravastatin	−(15–22)	−(20–30)	+ (3–6)	−(8–13)
Pitavastatin	−(28–32)	−(39–44)	+ (4–6)	−(14–19)

Adverse side effects
Mild elevation of transaminases in 1–3% of treated patients
Myalgia in 15–20% of treated patients (Toth et al. 2008)
Frequency of rhabdomyopathy 1/7,428 of statin-treated patients (Cholesterol Treatment Trialists' (CTT) Collaborators et al. 2008b)
Around 50% discontinuation after 1 year of treatment (Jackevicius et al. 2002)

products like cholesterol. The decrease in the intracellular cholesterol regulatory pool leads to an upregulation of LDL receptors in the hepatocyte plasma membrane, leading to an increased clearance rate of LDL particles from the blood and therefore a fall of plasma LDL-C levels. Overall, statins reduce LDL-C levels by 17–55%, concomitantly with an increase of HDL-C by 1–10% and a decrease of TG by 2–28% (Vigna and Fellin 2010; Toth 2010; Ewang-Emukowhate and Wierzbicki 2013) (Table 2). Based on dosage, atorvastatin and rosuvastatin are the most potent statins; the more polar compounds pravastatin and rosuvastatin are not or only metabolized by cytochrome P450 enzymes, particularly Cyp3A4, and are therefore less prone to drug–drug interactions with other medications due to cytochrome P450 metabolism. With high-dose therapy of statins, reduction of LDL-C up to 60% can be achieved. The findings however that whenever the statin dose is doubled LDL-C levels are reduced by only additional 6% ("the role of 6%") limits the clinical efficacy of statin use because of a steep increase in the incidence of adverse side effects by high statin doses; elevations of plasma transaminase levels and muscle toxicity by myalgia or severe rhabdomyolysis have been associated with high statin doses, thereby limiting the tolerable dose of a statin and the achievable reduction of plasma LDL-C levels. In addition to the beneficial effects of statins on plasma lipid profiles, further activities of statins are discussed to contribute to their efficacy in reduction of CVD events, but a final proof of the causative role of these pleiotropic statin effects still has to be proven: statins improve endothelial function leading to vasodilation (Förstermann and Li 2011), inhibit inflammatory processes with reduction of circulating CRP levels (Plenge et al. 2002), as well as stabilize plaques maybe by inhibition of myocyte infiltration and inhibition of metalloproteinase secretion (Massaro et al. 2010). Since inhibition of the biosynthesis cascade to cholesterol by statins occurs at a very early step, a potential risk was seen in this approach due to the decrease of other lipids like ubiquinone and dolichol and manifestation of these decreases of non-sterol lipids in adverse side effects in different organs including the liver, muscle, eye, or brain. Today, after more than 25 years of broad clinical use with millions of patients treated,

statins can be considered as very effective and safe drugs; currently more than 25 million American people with dyslipidemia are taking statins, and according to a recent recommendation for use, another 13 million are expected to benefit from statin treatment; for all patients with known CVD, the new guidelines by the American College of Cardiology and the American Heart Association for the management of cholesterol recommend statin treatment regardless of their LDL-C levels (Pencina et al. 2014). If the public health guidelines being currently discussed in the USA and UK would be fully implemented, one third of the middle-aged and adult population in these countries will be recommended for statin therapy (Pencina et al. 2014). Safety and tolerance of statin treatment is good with the exception of myalgia as the major adverse side effect. Indeed, statin-induced myalgia is a very frequent phenomenon in clinical practice observed in 15–20% of patients treated with statins (Toth et al. 2008), whereas fortunately, the frequency of the severe and life-threatening rhabdomyolysis is low (1 out of 7,428 patients treated) (Cholesterol Treatment Trialists' (CTT) Collaborators et al. 2008). Statins are taken up in the liver into hepatocytes by the organic anion transporting polypeptide OATP1B, and it was found that genetic variants of the SLCO1B1 gene are associated with the risk of myopathy upon statin treatment (Carr et al. 2013).

Some of these variants exert a diminished affinity and transport activity for statins, leading to a reduced hepatic uptake resulting in higher blood levels and increased muscle exposure with an increased off-target activity at the neuromuscular endplate: statins are powerful inhibitors of chloride channels at muscle cell membranes (Pierno et al. 2006) potentially leading to paralysis; additional mechanism possibly being involved in statin-induced myopathy are diminished ubiquinone levels in plasma and muscle due to inhibition of their synthesis in the liver, induction of atrogen-1 being involved in skeletal muscle atrophy (Hanai et al. 2007), or direct antagonistic effects of statins on muscle differentiation (Martini et al. 2009). Mild elevation of serum transaminase occurs in only 1–4% of statin-treated patients dose-related, indicating a high liver safety of statins (Vigna and Fellin 2010). Evidences for a slightly increased incidence for type 2 diabetes and acute renal failure were reported from observational data: 1 additional case of type 2 diabetes may occur for every 225 individuals treated with statins for 2 years, indicating a 20–30% increase risk to develop diabetes (Sattar et al. 2010). Acute renal failure maybe a dose-related adverse side effect of statins of low incidence (Dormuth et al. 2013). During development of statins, cataracts were an issue due to findings from dog studies; a recent retrospective analysis gave evidence for a slightly increased incidence for cataract formation in patients treated with statins (Leuschen et al. 2013). Today there are no evidences regarding early concerns that statin use may impair cognitive functions (Shepherd et al. 2002), but in contrast, patients treated with statins were shown to have a reduced risk to develop Alzheimer's disease (Corrao et al. 2013). As well, no evidence for an increased risk for cancer or cancer mortality was found for treatment with statins (Ridker 2014). The introduction of inhibitors of HMG-CoA reductase, the statins, into clinical practice has dramatically changed the landscape and treatment regimens for the treatment of lipid disorders. Coronary events and stroke can be

reduced by 25–30% and 10–15%, respectively (Law et al. 2003), and several meta-analyses of statin trial revealed reduction of CVD risk for each 1 mmol/L LDL-lowering by 21% (Cholesterol Treatment Trialists' (CTT) Collaborators et al. 2012) and of overall mortality by 14%, respectively (Taylor et al. 2013). Nevertheless, the overall mortality by CHD will increase in the future due to the increasing incidence of metabolic disorders like obesity, diabetes, and the metabolic syndrome.

Despite optimized drug therapy with statins, a significant proportion of patients fail to achieve the recommended levels of LDL cholesterol (Schectman and Hiatt 1996; Hsu et al. 1995) as defined by the European and US guidelines (Catapano 2009). The reasons for these treatment failures are manifold including the following factors:

- A poor compliance due to discomfort and side effects; still around 50% of statin-treated patients discontinue their therapy after only 1 year (Jackevicius et al. 2002).
- The recommended target for LDL-C in high-risk patients and FH patients (1.8–2 mmol/L) cannot be achieved with tolerable statin doses due to the "rule of 6%" phenomenon, leading to insufficient dosing to avoid a steep increase of adverse side effects upon dose escalation.
- Inefficiency of a drug class or statin intolerance (Hsu et al. 1995; LaRosa et al. 2005).

3.2 Bile Acid Sequestrants

Bile acid sequestrants are polymeric anion-binding resins that strongly bind the negatively charged bile acids in the lumen of the small intestine, thereby interrupting the enterohepatic circulation of bile acids (Out et al. 2012). By blocking intestinal bile acid reabsorption, the amount and/or composition of bile acids recirculating to the liver is changed, leading to an increase in hepatic bile acid synthesis from cholesterol via involvement of the nuclear receptors FXR, SHP, and Lrh-1; the depletion of the regulatory hepatic cholesterol pool induces a stimulation of cholesterol biosynthesis via upregulation of HMG-CoA reductase and LDL-receptor expression at the hepatocyte surface, the latter leading to an increase in hepatocellular uptake of LDL particles with a consequent decrease of plasma LDL-cholesterol levels.

Currently there are three bile acid sequestrants on the market – cholestyramine, colestipol, and colesevelam. Cholestyramine and colestipol are used in daily dosages up to 30 g, whereas recommended daily dose of colesevelam is 3.75 g; the high necessary doses of cholestyramine and colestipol are caused by occupation of the majority of the anion-binding sites by chloride anions in competition to anionic bile acids. Whereas cholestyramine and colestipol show a preference for dihydroxy bile acids, colesevelam binds bile acids by both ionic and hydrophobic interactions, thereby increasing binding affinity and specificity resulting in

significantly lower necessary dose. The treatment with sequestrants is safe, and due to their nonsystemic mode of action within the lumen of the intestine, sequestrants do not show systemic toxic effects; because of their non-absorbability, they can also be used in pregnancy. The necessary high doses of the 1st-generation sequestrants cholestyramine and colestipol are responsible for the major adverse side effects in the gastrointestinal tract with constipation, abdominal pain, flatulence, and nausea being the cause for the compliance issues and high rate of treatment discontinuation up to 40–60% with first-generation sequestrants (Andrade et al. 1995); colesevelam due to its improved binding characteristics has only few adverse side effects and a high compliance of 88–93% (Davidson et al. 1999). Because of the binding characteristics for anionic and hydrophobic compounds, chronic use of sequestrants may decrease the absorption of fat-soluble vitamins and nutrients and interfere with the intestinal absorption of drugs and influencing their pharmacodynamics, particularly important for drugs with a narrow therapeutic window such as anticoagulants, digitalis, β-blockers, thiazides, or thyroxine. In these cases, a time lack between intake of the respective drug and the sequestrant is recommended. By monotherapy, sequestrants can decrease LDL-C levels by 9–28% and increase HDL-C levels up to 9% (Hou and Goldberg 2009). First-generation sequestrants may lead to an undesired increase of plasma triglyceride levels which is not or significantly less the case with colesevelam. By the change in the composition of the bile acid pool with an enrichment of trihydoxy bile acids due to a higher binding affinity of dihydroxy bile acids to cholestyramine and colestipol, the less potent trihydroxy bile acids as FXR agonists lead to a decreased FXR activity such as a decrease in the expression of ApoC-II as an activator of lipoprotein lipase or reduction of the FXR-mediated inhibition of hepatic TG synthesis via Shp and Srebp1c (Kast et al. 2001). As a result, the increased TG synthesis and the decreased TG clearance in the vasculature induces an overall increase of circulating TG. Bile acid sequestrants have proven their efficacy in reducing CHD (Hou and Goldberg 2009). CVD events were reduced by cholestyramine by 18% with an additional 8% effect upon coadministration to a statin (The Lipid Research Clinics Investigators 1992). The additive activity of bile acid sequestrants makes them ideal partners for co-medication to statins in patients where statin therapy is not sufficient enough: coadministration of colesevelam to statins significantly lowered LDL-C levels by additional 16% so that more than 39% of the patients reached their therapeutic target of $<$100 mg/dL LDL-C compared to only 10% treated with a statin alone (Bays et al. 2006). Colesevelam is able to reduce as well serum levels of CRP (Bays et al. 2006) and to increase LDL particle size, indicating an additional inhibitory effect on inflammatory processes involved in atherogenesis (Deveraj et al. 2006).

Recent studies have strengthened early observations that bile acid sequestrants in addition to their cholesterol-lowering activities can improve glycemic control in patients with type 2 diabetes (Handelsman et al. 2010), and colesevelam was approved as the first bile acid sequestrant for the treatment of type 2 diabetes: in a 16-week clinical trial with prediabetic individuals, colesevelam in addition to its improvement of lipid profiles reduced fasting blood glucose levels by 4% and HbA1c values by 2%, respectively. Fasting blood glucose levels and HbA1c values

in patients treated with antidiabetic drugs could be further reduced by 10% upon coadministration of colesevelam (Goldberg et al. 2008). The mechanisms whereby bile acid sequestrants improve glycemic control are not yet fully understood but may involve the following contributions:

- Increased secretion of incretins like GLP-1 due to spillover of bile acids into the colon (Suzuki et al. 2007)
- Changes in the composition of the bile acid pool with activation of FXR downstream activities like repression of hepatic gluconeogenesis and increasing of insulin sensitivity (Zhang et al. 2006)
- Activation of TGR5 receptor in the colon by bile acid spillover with stimulation of energy expenditure (Thomas et al. 2009)

The proven efficacy of bile acid sequestrants on improvement of atherogenic lipid profiles and reduction of CHD events, their safety profile and nonsystemic mode of action, and their beneficial effects on glucose homeostasis strengthens the role and value of bile acid sequestrants particularly for the treatment of patients with statin intolerance, type 2 diabetes, or the metabolic syndrome.

3.3 Ezetimibe

Ezetimibe exerts its hypolipidemic action by inhibiting intestinal cholesterol absorption. Ezetimibe targets the NPC1L1 pathway which is a key regulator of cholesterol uptake from enterocyte brush border membranes and reuptake of cholesterol from bile into hepatocytes.

Cholesterol solubilized in mixed micelles is transferred to the enterocyte brush border membrane via a protein-mediated process (Cai et al. 2002; Kramer et al. 2005) and moved to detergent-resistant microdomains from which cholesterol absorption occurs then by clathrin-dependent receptor-mediated endocytosis involving a complex of clathrin, adaptor protein 2 complex, and NPC1L1 (Wang and Song 2012) with storage in the so-called endocytic recycling compartment. NPC1L1 is a cholesterol-sensing protein cycling between this compartment and the brush border membrane dependent on the cholesterol levels sensed in the brush border membrane; when intracellular cholesterol levels are low, NPC1L1 is with the aid of microfilaments recycled to the brush border membrane, thereby initiating and stimulating the microdomain endocytotic process. Ezetimibe specifically inhibits this NPC1L1 pathway. There is a controversy with regard to the primary molecular target of ezetimibe. It is postulated that the molecular target of ezetimibe is the NPC1L1 protein itself (Betters and Yu 2010). However, a direct binding of ezetimibe to NPC1L1 by labeling techniques was never really demonstrated. By use of a fluorescent ezetimibe glucuronide, a specific binding to the surface of HEK 293 cells expressing NPC1L1 was shown (Garcia-Calvo et al. 2005), but this can as well be explained by a role of NPC1L1 in forming of the protein complex necessary for clathrin-mediated endocytosis (Wang and Song 2012), thereby allowing access

of ezetimibe to its primary molecular target putatively being different from NPC1L1. In contrast, a direct and specific binding of ezetimibe to aminopeptidase N (CD13) was demonstrated by extensive photoaffinity labeling studies with various photoreactive ezetimibe analogues as well as by isolation of the ezetimibe-binding protein by affinity chromatography with an ezetimibe affinity matrix. Sequence analysis of the purified radiolabeled ezetimibe-binding protein or the affinity-purified ezetimibe-binding protein unequivocally demonstrated its identity with aminopeptidase N (CD13), whereas no amino acid sequences of NPC1L1 were found in the isolated ezetimibe-binding proteins (Kramer et al. 2004, 2005; Frick et al. 2003), indicating that aminopeptidase N is the primary target for ezetimibe in the process of NPC1L1-mediated endocytosis of cholesterol from cholesterol-rich microdomains of the enterocyte brush border membrane (Skov et al. 2010):

a. Membrane impermeable ezetimibe analogues are able to inhibit cholesterol uptake into $CaCo_2$ cells as well in vivo, indicating that the binding to the outside of the enterocyte brush border membrane is sufficient for inhibition of cholesterol internalization (Kramer et al. 2004, 2005).
b. The cellular localization of APN between the brush border membrane and an intracellular storage compartment – the so-called deep apical tubules – is cholesterol-dependent as is the case for NPC1L1.
c. Besides its enzymatic activity, APN can act as a receptor involved in endocytotic processes (Hansen et al. 2003) and is involved in the endocytosis of various virus classes like corona, SARS, or cytomegaly (Yeager et al. 1992).
d. The uptake of cholesterol from mixed micelles by $CaCo_2$ cells can specifically be inhibited by masking APN with APN-specific antibodies (Kramer et al. 2004, 2005) independently whether the cells were cholesterol-depleted with cyclodextrin or not, whereby the concentration of NPC1L1 in the brush border membrane is largely changed by cyclodextrin treatment.

These findings indicate that specific binding of ezetimibe to APN blocks the internalization of the cholesterol-rich microdomains, thereby preventing cholesterol absorption by interruption of the NPC1L1-cholesterol sensing pathway (Skov et al. 2010).

Ezetimibe is the only drug available inhibiting intestinal cholesterol absorption. In monotherapy, ezetimibe decreases serum LDL-C up to 20%, decreases TG up to 8%, and has a small increasing effect on HDL-C by 1–4% (Bruckert et al. 2003). Ezetimibe does not influence intestinal absorption of bile acids, fat-soluble vitamins, or carotenes. Ezetimibe does not show any significant adverse side effects and has no influence on the activities of cytochrome P-450 enzymes, therefore being devoid of significant pharmacokinetic interactions with other drugs. The effect of ezetimibe on LDL-C is additive to statins; therefore, combination of ezetimibe with a statin can overcome the "rule of 6%" by doubling of a statin dose. Ezetimibe/simvastatin combinations with 10 mg ezetimibe and 10, 20, 40, or 80 mg simvastatin reduced LDL-C by 51%, 57%, and 59%, respectively

(Ballantyne et al. 2005). The additive effect to statins and the lack of necessary titration of the statin dose makes combinations of ezetimibe with statins an important options of patients resistant or intolerant to statins. Large clinical trials, however, like ENHANCE in patients with heterozygous FH did not reveal a statistically significant effect on carotid intima media thickness compared to simvastatin alone despite a higher LDL-C lowering efficacy of the combination (Kastelein et al. 2008). In two further trials SEAS (Simvastatin and Ezetimibe in Aortic Stenosis) and SHARP (Study of Heart and Renal Protection), however, a significant risk reduction for CVD events was observed proportional to the reduction of LDL-C (Rossebo et al. 2008; Baigent et al. 2011). A conclusive answer regarding a clinically relevant beneficial effect of ezetimibe on cardiovascular morbidity and mortality is awaited by the ongoing IMPROVE-IT study covering 18,000 patients with acute coronary syndrome, comparing treatment regimens of simvastatin vs a combination with ezetimibe.

3.4 Fibrates

Peroxisome proliferator activator receptor α (PPARα, NR1C1 (nuclear receptor subfamily 1, group C)) is a nuclear transcription factor being a major regulator of lipid metabolism in the liver, exerting its activity by modifying the expression of a large number of target genes predominantly involved in fatty acid transport and metabolism and gluconeogenesis. Endogenous ligands comprise fatty acids and eicosanoids including leukotriene B4. Binding of an agonist to PPARα to the ligand-binding domain induces a conformational change, leading to the sequestration of a number of co-activator and corepressor proteins activating the receptor and heterodimerization with retinoid X receptor (RXR) followed by binding to PPAR responsive elements (PPREs) in the promoters of the target genes initiating modification of their expression. Fibrates are synthetic ligands selectively mimicking natural fatty acid ligands activating PPARα and thereby orchestrating via the PPARα target genes the adaption of the body to energy deprivation with stimulation of mitochondrial and peroxisomal β-oxidation, inhibition of hepatic VLDL production by inhibition of DGAT-2 activity, and activation of LPL to deliver free fatty acids for metabolism and energy generation, the latter mediated by reduction in the expression of the LPL inhibitor ApoC-III and induction of the LPL-activator ApoC-II. As a result, TG hydrolysis in VLDL and chylomicron particles is stimulated leading to a strong decrease in circulating TG levels; the resulting TG-poor LDL particles and remnants show a higher affinity to the LDL receptor resulting in an increased clearance with an accompanying decrease in LDL-C levels; fibrates modify LDL-particle size from small dense LDL-particles – characteristic for diabetic dyslipidemia – to large less atherogenic ones (Gazi et al. 2007). Additionally, HDL-C levels are increased by fibrates due to a stimulation in hepatic expression and production of apolipoproteins ApoA-I and ApoA-II ----as well as by a diminished loading of HDL particle with TG whereby the resulting HDL particles become less sensitive to catabolism by hepatic lipase.

Overall, reverse cholesterol transport is stimulated by these processes. Currently three fibrates are in clinical use – fenofibrate, gemfibrozil, and bezafibrate: fenofibrate is the preferred drug owing to its lack of PK interaction with statins. In clinical use, fibrates efficiently reduce serum TG concentration by 20–50%, increase HDL-C levels by 10–20%, and have a modest LDL-C lowering effect of 5–20%. Due to their lipid-modifying profile, fibrates can be particularly useful in monotherapy or in combination with statins for the treatment of patients with diabetic dyslipidemia or the metabolic syndrome (Fievet and Staels 2009). In large outcome studies in diabetic patients – FIELD (fenofibrate intervention and event lowering in diabetes) involving 9,795 type 2 diabetic patients (The FIELD study Investigators 2005) and ACCORD (action to control cardiovascular risk in diabetes) with 5,518 diabetic patients receiving either simvastatin (40 mg/day) or a combination of 40 mg/day simvastatin with 160 mg/day fenofibrate (The ACCORD Study Group 2010) – no reduction in macrovascular events like CHD, coronary death, or nonfatal MI could be demonstrated. However, microvascular events including retinopathy, nephropathy, and neuropathy were significantly reduced. Meta-analysis revealed that patients with low HDL-C (<0.9 mmol/L) and high TG plasma levels (>2.3 mmol/L) benefit from treatment with fenofibrate (Sacks et al. 2010). The tolerability and safety of fibrates are acceptable: Adverse side effects include myopathy, upper GI-symptoms, mild elevation of serum transaminase and homocysteine levels, and an increased incidence for cholesterol gallstones (Prueksaritanont et al. 2002a). The most severe adverse side effect is muscle toxicity with rhabdomyolysis (Davidson et al. 2007). Particularly gemfibrozil treatment in combination with statins is associated with an increased risk for myopathy and hepatotoxicity caused by its competition with statins for the glucuronidation elimination pathway, thereby decreasing the elimination of statins (Prueksaritanont et al. 2002b).

3.5 Nicotinic Acid

The lipid profile-modifying effects of vitamin B3 – nicotinic acid or niacin – are since nearly 60 years introduced into clinical practice (Carlson 2005). At pharmacological doses up to 2 g/day, niacin exerts significant changes in most of the different lipoproteins: increases in HDL-C of 25–30% and Apo-AI production (Lamon-Fava et al. 2008) combined with decreases of LDL-C by 15–20%, TG by 20–40%, Lp(a) by about 30%, as well as reduction of fibrinogen and plasminogen activator inhibitor 1 (PAI 1). Niacin is currently the most potent drug on the market to increase HDL-C and the only approved drug with significant reduction of the independent CV risk factor Lp(a). From that perspective, the profile of niacin with reduction of atherogenic lipoproteins, increase of antiatherogenic lipoproteins, and reduction of prothrombotic factors principally is close to an ideal drug target profile to treat dyslipidemias, particularly in patients with diabetic dyslipidemia.

However, these clinical benefits on lipid profiles of niacin are overshadowed by a number of shortcomings hampering its therapeutic value in clinical practice. These include:

(a) A poor compliance due to the unpleasant side effect of flushing mediated by activation of the prostaglandin receptor PGD2R.
(b) Glucose control is negatively affected with increases of fasting blood glucose (4–17%), HbA1c levels, insulin levels, and a decrease in insulin sensitivity for several weeks at high niacin doses necessary to achieve the above beneficial effects on lipid patterns; normalization of glucose controls was observed after 48 weeks (Goyal et al. 2014) indicating that short- and long-term treatment with niacin worsens glycemic control limiting its use particularly in patients with diabetic dyslipidemia; low doses of nicotinic acid have only minimal effects on glucose tolerance but as well only a moderate effect on lipid profiles.
(c) Increases in plasma levels of uric acid promoting the risk for gout attacks.
(d) Liver toxicity and gastrointestinal side effects. To overcome the clinically most prominent and uncomfortable side effect, flush slow release forms of niacin (niaspan) were developed, improving handling of nicotinic acid and the benefit/adverse side effect ratio.

Another approach to increase the compliance of niacin use was the inhibition of flushing by co-medication with the PGD2 inhibitor laropiprant; the incidence of flush was significantly reduced but did not disappear completely due to the fact that flush can also be induced by prostaglandin E2 (Vosper 2011).

Despite its clinical use since decades, the molecular mechanism(s) by which niacin mediates its pleiotropic effects on lipid mechanism is not fully understood. A whole plethora of molecular targets and mechanisms has been reported for niacin; whereas its effects on TG metabolism can quite well be explained in molecular terms, the mechanisms whereby niacin increases HDL-C are more speculative. Scientific arguments for the following mechanisms for niacin have been reported:

A. Inhibition of TG Levels

Niacin potently inhibits lipolysis in adipose tissue by activation of the nicotinic acid receptor GPR109A, triggering an inhibition of adenylate cyclase, and consequently the decrease of cAMP levels prevents activation of PKA with inhibition of hormone-sensitive lipase resulting in a decrease of lipolysis (Julius and Fischer 2013) and plasma free fatty acids. In the liver, the diminished flux of free fatty acids from adipose tissue leads to a suppression in the expression of PPAR-γ coactivator-1b (PGC-1b) as well as of apolipoprotein C3, resulting in a decreased secretion of VLDL particles. A direct inhibition of the enzyme DGAT-2 by nicotinic acids limits the delivery of TG for secretion as VLDL particles and was proposed as the most relevant molecular target for niacin action of TG synthesis (Kamanna et al. 2013). The decrease in the content of TG in the VLDL particles increases resulting LDL particle size, and additionally, by

stimulation of ApoB catabolism, plasma LDL and VLDL levels were decreased further.

B. Increase of HDL-C Levels

The HDL effects of niacin probably are not related to activation of the nicotinic acid receptor GPR109A; investigations with GPR109A k.o. mice or specific high-affinity ligands for GPR109A demonstrated that this receptor – being clearly involved in niacin's pharmacological activity in TG metabolism – does not play a significant role for the HDL effects of niacin (Lauring et al. 2012). This explains the clinical failure with highly specific GR109A agonists; MK-1903 and SCH900271 strongly inhibited lipolysis and decreased plasma FFA levels but had no significant effects on serum lipid profiles, particularly HDL levels (Lauring et al. 2012). Recent studies explain the niacin effects on HDL by an inhibition of CETP-mediated lipid exchange resulting in cholesterol-enriched HDL particles, reduction of hepatic clearance of HDL particles, as well as a stimulation of reverse cholesterol transport through an increased flux of cholesterol to HDL. Mechanisms suggested to be involved is an increase in ABCA1 expression and production of nascent HDL particles and a reduction in the expression of the putative hepatic HDL-receptor β-chain ATP synthase resulting in an inhibition of HDL-particle clearance (Kamanna et al. 2013).

C. Effect on Flush

The discovery of GPR109A as a specific nicotinic acid receptor strongly triggered the search for non-niacin-related high-affinity ligands for this receptor led by the assumption that the pleiotropic beneficial effects of niacin are mediated via this receptor. Unfortunately, these new drugs failed in increasing HDL levels. GPR109A is not only expressed in adipocytes but as well in immune cells, spleen, and the Langerhans cells of the epidermis (Kamanna et al. 2009). Activation of the latter by nicotinic acid increases the production of PGD2 as the causative agent of the undesired side effect flush; therefore, flush is a non-dissociable adverse side effect from inhibition of lipolysis with GPR109A agonists like niacin, making the search for a "better" niacin with less side effects unlikely.

In large clinical trials, however, niacin failed to exert significant beneficial effects on CVD events. The AIM HIGH study performed in high-risk patients with CVD and low HDL-C, their LDL-C being optimally treated with statins, did not reveal a significant reduction in CVD events despite strong increases in HDL-C and reductions in TG (Investigators AIM-HIGH et al. 2011). On the other hand, a combination of niacin with bile acid sequestrants and lovastatin resulted in a regression of CAD (Kane et al. 1990). In the largest hitherto performed drug trial with niacin – the HPS2-THRIVE trial – in more than 25,000 adults with vascular disease, their LDL-C levels were adjusted with 40 mg simvastatin or 40 mg simvastatin plus 10 mg ezetimibe; afterwards, the patients were randomly assigned either to a treatment group receiving 2 g niacin and 40 mg laropiprant or a placebo arm. Over an observation period of 3.9 years at average, LDL-C was lowered by additional 0.25 mmol/L and HDL-C increased by 0.16 mmol/L. However, no

significant effect on the incidence of major CVD events could be observed (13.2% vs 13.7%). In contrast, discontinuation rate was significantly higher in the niacin/laropiprant group (25.4% vs 16.6%), and adverse side effect reactions were as well increased in the treatment group (55.6% vs 52.7%); patients with diabetes had a 55% proportional increase in disturbances of glucocontrol, and a 32% higher incidence of newly diagnosed diabetes was found in the niacin/laropiprant group (The HPS2-THRIVE Collaborative Group 2014). As a conclusion, the addition of a slow-release niacin-laropiprant combination to patients treated with statins had no additional beneficial effect on vascular events. This unfavorable outcome finally led to the withdrawal of niacin/statin/laropiprant combinations from the market, leaving behind significant doubts regarding benefits of niacin treatment for dyslipidemic patients.

4 Novel Approaches for the Treatment of Dyslipidemias

Various meta-analyses of clinical trials performed with statins indicate that even with an optimized LDL-C reduction by use of statins, the risk for CVD can be reduced by about 30%, indicating persistence of a 70% residual risk for the development and progression of CHD. Despite the fundamental change in the treatment of lipid disorders by the introduction of statins being the standard treatment today, a number of open questions remain, and for prevention of atherosclerosis, new treatments are needed:

- Will an reduction of LDL-C by other mode of actions yield similar outcome benefits on CVD morbidity and mortality?
- Will lowering of TG-rich lipoproteins and remnant cholesterol result in a reduction of major CVD events?
- Will pharmacological stimulation of reverse cholesterol transport lead to an improvement of CVD risk?

Particularly, an improvement regarding the following shortcomings of statin therapy should be addressed by new lipid-modifying drugs:

- Achievement of target LDL-C in high-risk patients, i.e., improved efficacy and additional beneficial effects such as lowering of Lp(a)
- More efficient treatment of patients with FH
- Overcoming the “rule-of 6%” hurdle of statins
- Effective treatment and reaching LDL-C targets in patients with statin intolerance/resistance

Therefore, an ideal drug for the treatment of dyslipidemias would concomitantly more efficaciously lower LDL-C and TG as well as stimulate reverse cholesterol transport (RCT) via the HDL pathway with less side effects than the existing therapies defining the medical need for future lipid-modifying drugs:

- A higher efficiency in lowering of LDL cholesterol
- Addressing additional risk factors for cardiovascular diseases
- Less adverse side effects
- Inhibition of atherosclerosis progression or even reversion
- Higher responder rate
- Higher compliance
- Low costs

For approval of each new agent to treat lipid disorders, a positive benefit-to-risk ratio for CHD development and progression probably will have to be clinically demonstrated. With statins as the current standard therapy, most novel hypolipidemic investigational drugs will have to be tested probably as an add-on therapy to statins or in patient cohorts not being efficiently treatable with statins.

As of April 2015 (analyzed by publically available sources and the actual official development pipelines published on the homepages of more than 70 pharmaceutical companies), 53 novel drug candidates ((NME) new medical entities) acting on around 27 different molecular targets or pathways are reported to be in clinical development (Tables 3, 4, 5, and 6). The tissue localization of the mode of action of the drug candidates in clinical development is illustrated in Fig. 1.

Table 3 Lipid-modifying drugs in clinical development: LDL-lowering (as primary target)

Target	Approach	Drug candidate	Company	Development stage
PCSK 9	MoAb	Alirocumab	Sanofi/Regeneron	Phase III, under approval
		Evolocumab	Amgen	Phase III, under approval
		Bococizumab	Pfizer/Rinat	Phase III
		RG7652	Roche/Genentech	Phase II
		LY-3015014	Eli Lilly	Phase II
	Adnectin	BMS-962476	BMS	Phase I
	siRNA	ALN-PCS	Alnylam	Phase I
	Small molecule	CAT-2003	Catabasis Pharmaceuticals	Phase II
		K-312 (PCSK9/CETP-Inh)	Kowa	Phase I
MTP	Small molecule	Lomitapide	Aegerion	Approved
		SLx-4090/KD-206	Surface Logix/Kadmon	Phase II
AMPK/ATP-citrate lyase	Small molecule	ETC-1002	Esperion Therapeutics	Phase II
TR-β1 receptor	Small molecule	MGL-3196/VIA-3196	Madrigal Pharmaceuticals	Phase I
ApoB	Antisense	Mipomersen	Sanofi	Approved
Apo(a)	Antisense	ISIS-APOARx	ISIS Pharmaceuticals	Phase II

Table 4 Lipid-modifying drugs in clinical development: HDL-increasing (as primary target)

Target	Approach	Drug candidate	Company	Development stage
CETP	Small molecule	Anacetrapib	Merck	Phase III
		Evacetrapib	Eli Lilly	Phase III
		DRL-17822	Dr Reddy	Phase II
		DEZ-001/TA-8995	Dezima/Mitsubishi Tanabe	Phase II
	Vaccine	ATH-03	AFFIRIS	Phase I
CETP/ PCSK9	Small molecule	K-312	Kowa	Phase I
BET	Small molecule	RVX-208	Resverlogix	Phase II
Niacin analogue	Small molecule	ARI-3037MO	Arisaph Pharmaceuticals	Phase II
HDL mimetics	Peptide-lipid complexes	MDCO-216	The Medicines Company	Phase II
		CSL 112	CSL Ltd.	Phase II
		CER 001	Cerenis Therapeutics	Phase II
ApoE mimetic	Peptide	AEM-28	Capstone Therapeutics	Phase I
rec-LCAT	Protein replacement	MEDI6012 (ACP-501)	MedImmune/ AlphaCore	Phase I
FGF-21 analogues	Protein	LY2405319	Eli Lilly	Phase I
	Protein-PEG conjugate	?	BMS	Phase II

Whereas the today used drugs for treatment of lipid disorders are all orally taken and are with the exception of the bile acid sequestrants throughout small organic molecules, the current clinical development pipeline shows a much greater heterogeneity with regard to the characteristics of the drug candidates: Out of the 52 clinical candidates, 20 of them are biologics (Table 7):

- Six (monoclonal) antibodies: five addressing the PCSK9 pathway, one being a vaccine against CETP
- One monobody (adnectin): PCSK9
- Five oligonucleotide drugs: PCSK9 (1), ApoB (1), Apo(a) (1), ApoC-III (1), ANGPTL3 (1)
- Three HDL mimetics: complexes of ApoA-I with phospholipids
- One peptidomimetic: ApoE
- Three therapeutic recombinant proteins: LCAT, FGF21, FGF21-PEG conjugate
- One gene therapy: lipoprotein lipase

All of these drug candidates have to be applied by an invasive delivery, i.e., by s.c. injection or infusions. The balance between efficacy and safety of these new

Table 5 Lipid-modifying drugs in clinical development: triglyceride lowering (as primary target)

Target	Approach	Drug candidate	Company	Development stage
Lipoprotein lipase	Gene therapy	Glybera	UniQure	Approved
DGAT-1	Small molecule	Pradigastat	Novartis	Phase II
		P7435	Piramal Enterprises	Phase I
LpPLA2	Small molecule	Darapladib	GSK	Phase III
Apo CIII	Antisense	ISIS-APOC-IIIRx	ISIS Pharmaceuticals	Phase III
ANGPTL3	Antisense	ISIS-ANGPTL3Rx	ISIS Pharmaceuticals	Phase I
PPAR-alpha/delta	Small molecule	GFT-505	Genfit	Phase II
PPAR-alpha	Small molecule	ZYH7	Cydus Cadia	Phase I
		K-877	Kowa	Phase II/III
		NP-136	Mitsubishi Tanabe	Phase I
PPAR-delta	Small molecule	MBX-8025	CymaBay Therapeutics	Phase II
		CER-002	Cerenis Therapeutics	Phase I

biological approaches to reduce CVD events and the discomfort for the patients to receive repeated injections or infusions (at least once a month) either by medical doctors or self-medication with appropriate injection devices will finally determine the acceptability of these new treatment regimens for lipid disorders.

According to the mode of action, the drug candidates can be classified with regard to their primary focus in their interference with lipoprotein metabolism:

LDL-increase:

- Inhibition of cholesterol and triglyceride synthesis
- Inhibition of lipoprotein assembly
- Enhancement of lipoprotein clearance
- Antisense approaches

HDL-increase:

- Inhibition of lipoprotein assembly
- Enhancers of ApoA-I action

Concerning the mechanisms and molecular targets, there are two top activities in the lipid field: (a.) the development of approaches inhibiting the action of PCSK9 for LDL-C lowering and (b.) the development of inhibitors of CETP to increase HDL-C levels.

Table 6 Lipid-modifying drugs in clinical development: miscellaneous targets and indications

Drug candidate	Company	Target	Approach	Indication	Development stage
SHP626/LUM002	Shire	IBAT-inhibitor	Small molecule	NASH	Phase I
SHP625/LUM001	Shire	IBAT-inhibitor	Small molecule	PBC	Phase II
Elobixbat	Albireo/Ferring/Ajinomoto	IBAT-inhibitor	Small molecule	Chronic obstipation	Phase III
A4250	Albireo	IBAT-inhibitor	Small molecule	Cholestatic liver disease	Phase II
2330672	GSK	IBAT-inhibitor	Small molecule	T2 diabetes, cholestatic pruritus	Phase II
Px-104	Phenex Pharmaceuticals	FXR-agonist	Small molecule	NASH	Phase II
Obeticholic acid	Intercept	FXR-agonist	Small molecule	NASH, PBC, PSC	Phase II/III
S-556971	Shionogi-Kotobuki	NPC1L1-pathway	Small molecule	Hypercholesterolemia	Phase II
BMS-852927	BMS/Exelixis	LXR-Agonist	Small molecule	Metabolic Syndrome	Phase I
BMS-823778	BMS	? (MGAT2-Inh.?)	Small molecule	Dyslipidemia	Phase I
PF-06427878	Pfizer	?	Small molecule	Hyperlipidemia	Phase I

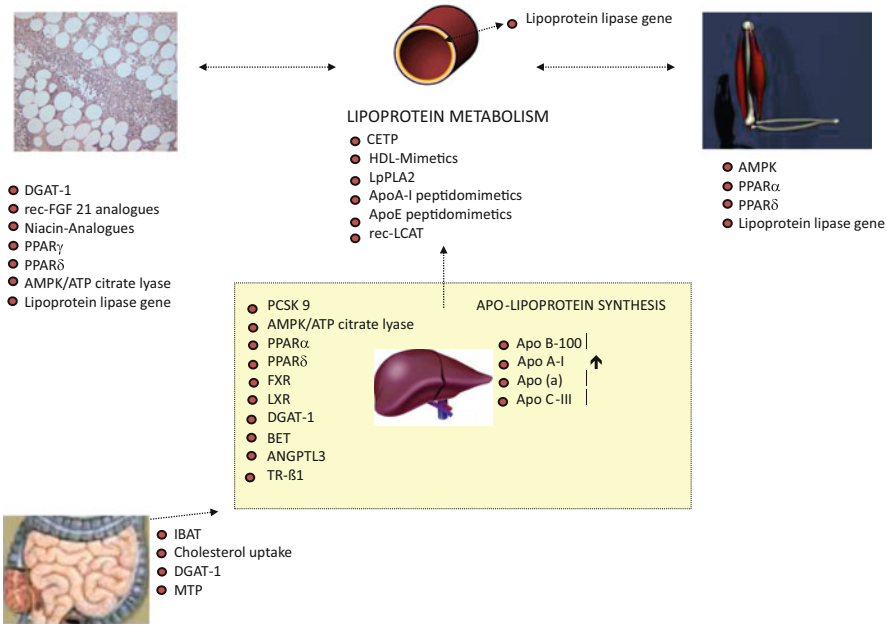

Fig. 1 Organ localization of the targets and mode of actions of lipid-modifying drugs currently in clinical development

4.1 LDL- and Apolipoprotein B-Lowering Approaches

Familial hypercholesterolemia (FH) is an inherited lipid disorder with a prominent elevation of plasma LDL-C levels and CV risk caused by loss-of-function mutations in the genes for the LDL receptor, apolipoprotein B-100, or gain-of-function mutations for proprotein convertase subtilisin/kexin type 9 (PCSK9). With a prevalence of 1:500 in western populations, heterozygous FH is one of the most abundant inherited diseases, whereas homozygous FH is very rare (1 in a million) (Yuan et al. 2006). Current therapy options for the FH patients rely on statins and their combinations with ezetimibe and/or bile acid sequestrants, but still 5–10% of these patients do not reach LDL-C target levels. LDL apheresis is today the only available therapy for hoFH patients which is effective, but very costly with a limited access for patients to clinical centers performing apheresis. Therefore, inherited forms of hypercholesterolemia caused by mutations in the genes for apolipoprotein-B and PCSK9 are principally ideally suited for pharmacological approaches blocking these proteins, either by inhibition of the respective protein or by a gene-silencing therapy, whereas homozygous forms with loss of the LDL-receptor function can only be approached by gene therapy.

Table 7 Classes of lipid-modifying drugs in clinical development

	Number of targets	Small molecules	Biologics						Sum of NCE's
			MoAb	Peptides	Proteins	Oligonuc	Vaccine	Gene therapy	
LDL-lowering	6	6	6			3			15
HDL-increasing	7	6		4	3		1		14
TG-lowering	8	9				2		1	12
Miscellaneous	6	11							11
	27	32	6	4	3	5	1	1	52

Overall
52 NCE's with 27 targets/modes of action
32 Small molecules
20 Biologics

4.1.1 Inhibition of Cholesterol and Triglyceride Synthesis

Inhibitors of Diacylglycerol Acyltransferases (DGAT)

A rapidly growing field in drug discovery for the treatment of dyslipidemia, obesity, diabetes, and the metabolic syndrome is the search for DGAT inhibitors. DGATs catalyze the final step in the biosynthesis of TG. In mammals, two nonhomologous enzymes encoded by different genes – DGAT-1 and DGAT-2 – are involved in TG synthesis; DGAT-1 is localized predominantly in the endoplasmic reticulum, whereas DGAT-2 also associates with lipid droplets (Liu et al. 2012). The interest in DGAT inhibitors was stimulated by the phenotype observed in DGAT k.o. mice. DGAT-1 k.o. mice have reduced TG levels, are resistant to diet-induced obesity, and show an increased insulin sensitivity (Smith et al. 2000; Chen and Farese 2000; Chen et al. 2002). DGAT-2 k.o. mice show a similar phenotype but exert severe skin abnormalities questioning the usefulness as a potential therapeutic target. However, antisense approaches to DGAT-2 have demonstrated decreases in body weight, adipose tissue, hepatic TG, and insulin resistance without skin abnormalities which may indicate that DGAT-2 inhibitors may be beneficial for the treatment of metabolic diseases, particularly of hepatic steatosis and nonalcoholic steatohepatitis (NASH). Currently at least four small molecule DGAT-1 inhibitors are in clinical and preclinical development, LCQ-908 in phase III being mostly advanced for the treatment of the rare familial chylomicronemia syndrome (hyperlipoproteinemia type I) (www.novartis.com/downloads/newsroom/corporate-fact-sheet/2a_Pharmaceuticals_EN.pdf-2013-01-22). In randomized, placebo controlled clinical studies, pradigastat given to healthy human volunteers for 14 days suppressed postprandial TG dose-dependently with a maximal suppression of 90%; concomitantly, the number of postprandial chylomicron particles measured by ApoB48 levels was also reduced associated with modest reductions in fasting TG and cholesterol levels (Myers et al. 2012a). In a small clinical study with six patients suffering from familial chylomicronemia syndrome FCS, a 3-week treatment with 20 mg pradigastat was able to reduce plasma TG levels from 1,212 mg/dL by 38.4% nearly exclusively by a reduction in chylomicron TG; the compound was well tolerated at all doses tested (10–40 mg) with mild and transient gastrointestinal side effects in all patients. No serious adverse side effects or discontinuation from the study occurred (Myers et al. 2012b). Dosing for 14–28 days caused weight loss, improved insulin sensitivity, decreased lipid disposal after a meal, and reduced hepatic steatosis (Aicher et al. 2010). For inhibition of DGAT-2, an antisense drug is in preclinical development as an attempt for the treatment of NASH awaiting filing of an IND application early 2013 (www.isispharm.com/Pipeline/index.htm; Drugs in development).

Activation of AMPK and Inhibition of ATP-Citrate Lyase

ETC-1002 (8-hydroxy-2,2,14,14-tetramethylpentadodecan-1,15-dioic acid) is a novel regulator of lipid and carbohydrate metabolism exerting a unique dual mode of action, namely, activation of AMPK and inhibition of ATP-citrate lyase, the latter mechanism exerted by formation of a coenzyme A thioester of ETC-1002

within the liver which is a direct inhibitor of ATP-citrate lyase (Pinkovsky et al. 2013). ETC-1002 was investigated in various clinical trials including four phase IIa double-blind placebo-controlled studies in cohorts of hypercholesterolemic patients of different characteristics (Newton et al. 2014a).

a. One hundred and seventy-seven hypercholesterolemic patients with normal or elevated TG levels receiving 40, 80, or 120 mg ETC-1002 for 12 weeks showed reductions of 17.9–26.6% in LDL-C and 20% in hsCRP vs baseline.
b. In 60 type 2 diabetes patients receiving up to 120 mg for 4 weeks, LDL-C and hsCRP levels were reduced by −42.9% and 40.5%, respectively.
c. Fifty-six patients with statin intolerance receiving up to 240 mg ETC-1002 for 2 weeks showed reduction of 32 and 42% in LDL-C and hsCRP vs baseline.
d. In 58 hypercholesterolemic patients treated with 10 mg/day atorvastatin, additional treatment with up to 240 mg/day ETC-1002 could reduce plasma levels of LDLD-C and hsCRP by further 21.9% and 23.5% vs baseline.

The drug was well tolerated, and hypercholesterolemic patients with a history of statin intolerance treated with ETC-1002 had compared to placebo lower rates of muscle-related adverse events (27% vs 32%) and fewer number of discontinuation because of muscle-related adverse effects (0% vs 16%) and did not show signs of liver toxicity (increases in transaminases $<3 \times$ ULN, CPK $> 5 \times$ ULN) (Newton et al. 2014b). In a phase 2b study including 349 hypercholesterolemic patients for 12 weeks, the efficacy of ETC-1002 was investigated as monotherapy vs ezetimibe and in combination with ezetimibe. In monotherapy, ETC-1002 at 120 or 180 mg/day lowered LDL-C by 27% and 30% and hdCRP by 30% and 40% vs baseline compared to 21% and 11% for 10 mg ezetimibe. Patients treated with 10 mg ezetimibe receiving additional 120 or 180 mg/day ETC-1002 showed reduction in LDL-C and hsCRP of 43–48% and 28–38%, respectively (Press Release Esperion 2014). No clinically relevant changes in HDL-C or TG levels were observed, and the compound was well tolerated and not associated with any dose-limiting side effects. These data indicate that ETC-1002 is a potential safe alternative treatment of hypercholesterolemic patients.

4.1.2 Inhibition of Lipoprotein Assembly

Inhibition of the Microsomal Triglyceride Transfer Protein (MTP)

The assembly of VLDL particles from apolipoprotein B and lipids in the endoplasmic reticulum of hepatocytes and enterocytes is catalyzed by MTP, and consequently, inhibition of this protein would reduce the secretion of VLDL particles by the liver and of chylomicrons by the small intestine. Due to the catabolism of these lipoproteins to LDL, an inhibition of MTP results in a strong decrease of plasma LDL particles. MTP inhibitors are very efficacious in lowering both atherogenic ApoB-containing lipoproteins and plasma triglycerides, but their development is hampered and limited by its mechanism-based adverse side effects of lipid accumulation in the liver and the intestine with the drawbacks of liver toxicity by

hepatic steatosis and its sequelae. However, patients with homozygous FH are unresponsive to current lipid therapy and have to rely on LDL apheresis only. For these patients, the reduction in the synthesis rate of LDL particles by inhibition of VLDL/chylomicron assembly would be a new therapeutic option. In December 2012, the FDA approved lomitapide for the treatment of patients with homozygous FH as an adjunct to diet and other lipid-lowering therapies including LDL apheresis. In a phase III clinical trial, an average reduction of 40% in LDL-C was achieved after 26 weeks with LDL reduction up to 80% in patients receiving 40–60 mg of lomitapide daily. In addition, triglycerides were reduced by 54% compared to placebo. Most patients in the study showed mild to moderate gastrointestinal adverse effects with abdominal discomfort, diarrhea, and nausea (Cuchel et al. 2013). In order to limit the mechanism-based appearance of hepatic steatosis, SLx-4090 inhibits MTP in the enterocytes only because the compound is inactivated upon entry into systemic circulation by metabolization to an inactive metabolite. Results of a phase II clinical trial of SLx-4090 in combination with metformin for 12 weeks in type 2 diabetes patients indicate a significant reduction of plasma triglycerides by 35%, reduction of postprandial free fatty acids and HbA1c, and a weight loss of 1.3% (Tong et al. 2010).

4.1.3 Enhancement of Lipoprotein Clearance

Phospholipase A2 Inhibitors

Phospholipases are involved in the reshaping of lipoproteins and the pathogenesis of atherosclerosis. Phospholipase A2 (PLA2) occurs in two forms – secretory PLA2 (sPLA2) and associated to lipoproteins (LpPLA2). Overall, PLA2 occurs in 12 isoforms and 6 of them have been found associated with atherosclerotic lesions (Rosenson and Hurt-Camejo 2012). Elevated LpPLA2 levels are linked to an increased CVD risk (Packard 2009). PLA2 hydrolyzes glycophospholipids at the surface of VLDL and LDL particles, yielding lysophosphatides, oxidized unesterified free fatty acids, and eicosanoids, the latter being further processed to proinflammatory mediators like prostanoids, leukotrienes, and lipoxins. Additionally, the resulting VLDL and LDL particles are less efficiently cleared by the ApoB and ApoE receptors in the liver leading to an increased LDL-C level. These PLA2-modified LDL particles are avidly bound to macrophages with subsequent receptor-mediated internalization of cholesterol fostering foam cell formation. Due to these, functions of PLA2 in atherogenesis inhibitors to both forms of PLA2 have been in clinical development for a long time. Darapladib was tested overall in more than 23,000 patients whether it could significantly reduce the rate of strokes and heart attacks. In the IBIS-2 study, no effect on inflammatory markers, arterial stiffness, or atheroma volume could be discovered (Serruys et al. 2008). In patients with CHD, darapladib showed a dose-dependent inhibition of LpPLA2 activity up to 66% accompanied with a mean decrease of the inflammatory markers IL-6 and CRP by 12.3% and 13%, respectively. After 12 months of treatment with 160 mg/day darapladib, however, no differences between treatment and placebo groups were found with regard to plaque stability and plasma high-sensitive CRP. Whereas the

necrotic core volume of the plaques increased in the placebo group, darapladib produced a decrease in the core volume by 5.2 mm^3 without significant differences in total atheroma volume (Serruys et al. 2008).

Recently published data from clinical trials with PLA2 inhibitors were disappointing. Neither with varespladib nor with darapladib (SOLID-TIMI 52 trial with 13,026 patients with acute coronary syndrome; STABILITY trial with 15,000 patients with chronic CHD) significant reductions on major coronary events could be found (The STABILITY Investigators 2014). A meta-analysis of 32 studies with PLA2 inhibitors involving overall 79,000 patients showed a correlation of both, PLA2 mass and PLA2 activity, with CVD events; however, after adjustment of ApoB levels, there was no longer a correlation.

Since statins reduce the number of ApoB-containing lipoproteins, a potential additional effect of PLA2 inhibition probably is marginal. Furthermore, inflammatory pathways are highly redundant, and pro- and anti-inflammatory effects may overlap, imposing significant doubts on the rationale and probability of success of this approach.

Thyromimetics

Thyroid hormones, particularly triiodothyronine T3, have profound effects on lipid metabolism via various mechanisms such as increase in hepatic LDL-receptor expression, stimulation of cholesterol conversion to bile acids by activation of the cholesterol-7 alpha hydroxylase pathway, increase in hepatic cholesterol secretion by upregulation of ABC transporters ABCG5/ABCG8, and stimulation of RCT. Consequently, a liver-selective thyromimetic compound shows principally all desired actions for the treatment of dyslipidemias: decreases in LDL-C and TG and stimulation of RCT. Thyroid hormones exert these pleiotropic activities by means of thyroid receptors. The cholesterol-lowering activity is mainly mediated by the TRβ-1 receptor isoform predominantly expressed in hepatocytes, whereas the TRα-1 receptor is responsible for the heart rate increasing effects; therefore, a high selectivity for the TRβ-1 receptor is mandatory to avoid any CV adverse side effects (Gullberg et al. 2002). After initial trials with naturally occurring thyroid hormones, the observed spectrum of beneficial effects for dyslipidemias stimulated the search for safe and liver-selective thyromimetics. Eprotirome (KB-2115) was investigated in phase III trials for the treatment of hypercholesterolemia and dyslipidemia. A 12-week study in 189 patients on statin therapy with 25–100 μg eprotirome/day showed promising results with reductions of LDL-C, TG, and Lp (a) of 22–32%, 27–43%, and 16–33%, respectively, with a small decrease in HDL-C by 6%. No significant adverse side effects on heart and cardiovascular function were observed (Ladenson et al. 2010). The development of the compound was terminated due to safety concerns by reason of cartilage damage after a 12-month toxicology study in dogs (Press Release Karo 2012). Another thyroid hormone receptor agonist, soberitome, was investigated in a phase I clinical trial, showing LDL-C reductions up to 41% in healthy volunteers (Scanlan 2010), but the compound is also no longer in development. The continuing interest in thyroid hormone analogues to overcome the mechanism-based issues is documented by the

recent entry of two new thyromimetic compounds – MGL-3196/VIA-3196 and ZYT1 – into phase I clinical trials. MGL-3196 is a liver-directed β-selective TR agonist lacking activity on the α-TR receptor without any observed cardiovascular activity. In a phase I clinical trial in 48 healthy volunteers receiving daily doses of 50–200 mg MGL-3196 for 2 weeks, the compound was found to be safe without significant adverse side effects: Plasma lipid profiles were beneficially influenced with decreases of 30% in LDL-C, 28% in non-HDL-C, 24% in ApoB, and 60% in TG, indicating a promising profile for the treatment of mixed dyslipidemias (Taub et al. 2013). The major challenge for thyromimetics for the treatment of dyslipidemias remains the unequivocal demonstration of their long-term safety.

Inhibitors of Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9)

The clinical efficacy of statins is limited by the so-called rule of 6%: each time the dose of a statin is doubled, the resulting decrease in LDL-C lowering is only additional 6%. Elevations of plasma transaminase levels and muscle toxicity have been associated with high statin doses, thereby limiting the tolerated dose of a statin and the achievable reduction in plasma LDL-C levels. As a consequence, a significant percentage of statin-treated patients does not reach the recommended LDL-C target levels. The findings that around 3% of Afro-Americans are heterozygous for loss-of-function mutations of PCSK9 associated with very low plasma LDL cholesterol levels and a reduction of CV events by 80–90% (Konrad et al. 2011) has made PCSK9 one of the most intensively investigated novel targets to treat hypercholesterolemia. PCSK9 is a serine protease synthesized by the liver and the intestine as a 692 amino acid precursor protein. After autocatalytic cleavage in the endoplasmic reticulum, the cleaved prodomain acts as a chaperone tightly bound to the mature 62 kDa PCSK9 protein permitting the entry of this complex into the secretory pathway (Lambert et al. 2012). The action of PCSK9 is twofold: (a) in the post-endoplasmic reticulum compartment, PCSK9 guides the LDL receptor for degradation into lysosomes and (b) mature 62 kDa PCSK9-protein is secreted and binds to the LDL receptor at the surface of the hepatocyte. The complex composed of PCSK9, LDL receptor, and the adaptor protein ARH is then internalized into endosomes. Binding of PCSK9 prevents the LDL receptor from being recycled back to the cell surface, shifting the PCSK9/LDL-receptor complex to the acidic compartment of lysosomes for degradation. By this dual action, PCSK9 reduces the surface expression of LDL receptors and thus decreases the clearance rate of LDL particles resulting in an increase of LDL-C plasma levels. The height of circulating PCSK9 levels is predictive for cardiovascular events in patients with CAD based on its positive correlation to plasma TG levels (Werner et al. 2014), and thus plasma PCSK9 levels may be useful as a biomarker for CVD risk assessment. It is obvious from these mechanisms that by inhibition of the action of PCSK9, the endogenous "brake" in LDL-receptor-mediated LDL clearance can be released. The activity of PCSK9 is not restricted to the LDL receptor but also to a variety of other receptors like VLDLR, ApoER2 (LRP8), LRP1, BACE1, and hepatic receptor CD81 (Norata et al. 2014); the degradation of these receptors probably involves specific cellular pathways not directly linked with cholesterol

metabolism, such as modulation of genes involved in proliferative, apoptotic, and inflammatory pathways (Norata et al. 2014).

Various observations contribute in a convergent manner to the high attractivity of PCSK9 as a (the ?) major regulator to treat hypercholesterolemia:

- Natural loss-of-function mutations in human lead to maximal reduction in plasma LDL-cholesterol levels without apparent adverse side effects. However, until now homozygous mutations have been described only in a few cases not allowing a generalization of safety prospectives to large populations being treated with a PCSK9 inhibitor. Additionally to its function in regulating cholesterol homeostasis PCSK9 may be involved in further cellular processes: Controversial results have been published with regard to a potential increase in BACE 1 and β-amyloid levels in the brain of PCSK9 k.o. mice raising the possibility of an increased risk to develop Alzheimer's disease upon PCSK9 inhibition treatment (Jonas et al. 2008; Liu et al. 2010).
- PCSK9 may be necessary for a normal β-cell function and therefore, loss of PCSK9 may predispose to diabetes treatment (Mbikay et al. 2010).
- PCSK9 reduces the expression of CD 81, the receptor mediating the cellular entry of the hepatitis C virus (Labonte et al. 2009) indicating a potential risk for HCV infection upon treatment with PCSK9 inhibitors.
- In one study it was shown that PCSK9 is necessary for liver regeneration (Zaid et al. 2008). This is particularly important in the light of co-medication with drugs having a potential for liver toxicity.
- PCSK9 inhibits also the VLDL receptor and PCSK9 k.o. mice show adipocyte hypertrophy and fat accumulation (Roubtsova et al. 2011).

So far no reports regarding these potential adverse effects have been reported in humans. Nevertheless, these potential physiological roles of PCSK9 have to be further investigated to ensure a safe long-term treatment with PCSK9 inhibitors. The mode of action of PCSK9 by extracellular binding to the LDL receptor avoids the necessity of a hepatocyte or organelle-specific drug targeting. Cholesterol-lowering drugs can increase plasma levels of PCSK9, thereby limiting their pharmacological efficacy and explaining the underlying biochemistry of the "rule of 6%" found with doubling of a statin dose (Mayne et al. 2008; Dubuc et al. 2010). PCSK9 expression is mainly modulated by sensing intracellular cholesterol levels and subsequent activation of SREBP-2 leading to a concomitant increase in the expression of LDL receptor, HMG-CoA reductase, and PCSK9 to ensure cellular cholesterol homeostasis (Dong et al. 2010). Statins, particularly at higher dosages, can strongly induce PCSK9 up to 47% with 80 mg/day atorvastatin (Welder et al. 2010). For fibrates and ezetimibe, controversial results regarding their effect to increase PCSK9 levels were reported (Konrad et al. 2011). An optimal therapy to lower LDL-C levels would therefore be possible by combination of a statin or other LDL-C-lowering drugs with a PCSK9 inhibitor to counteract attenuation of statin potency.

Due to the mechanisms of PCSK9 action, the following approaches are principal options for drugs interfering with the PCSK9 pathway:

A. Silencing of PCSK9 expression
B. Inhibition of autocatalytic processing of the PCSK9 precursor protein
C. Prevention of PCSK9 secretion
D. Prevention of the interaction of secreted PCSK9 with the LDL receptor
E. Prevention of endosome targeting to lysosomes

Monoclonal Antibodies Against PCSK9

Most advanced is approach D aiming to inhibit the interaction of secreted PCSK9 with the LDL receptor, either by neutralizing antibodies against PCSK9 or peptidomimetics mimicking the contact points between PCSK9 and the LDL receptor. Currently there are at least 5 different monoclonal anti-PCSK9 antibodies in clinical development. For the three most advance antibodies – evolocumab (AMG-145), alirocumab (SAR236553/REGN727), and aococizumab (RIN 316) – extensive data from clinical studies in phase I and II (all 3) as well as III for evolocumab and alirocumab have been published (an excellent overview about all performed and running clinical data can be found in the paper of Dadu et al. (2014). In phase I clinical trials in monotherapy, these antibodies reduced LDL-C by 61–81% concomitantly with a reduction of ApoB by 48–59%. A further cardiovascular risk factor, Lp(a), was reduced by all three drugs by 27–50%. Alirocumab additionally decreased TG levels by 16% and increased HDL-C by 18%, whereas no effect on these parameters were found for evolocumab. No significant differences regarding adverse side effects were found for all three antibodies: the most common adverse side effects were injection site reaction with pain and localized rash (2–9%), upper respiratory tract infection (6–10%), nasopharyngitis (4–15%), and gastrointestinal disturbance like diarrhea (4%) and nausea (4–6%). When used in combination with other lipid-lowering drugs like statins or ezetimibe, the PCSK9 antibodies could further decrease LDL-C by 50–60%. In phase II clinical trials in combination with statins or ezetimibe, the outstanding efficacy of blocking PCSK9 with antibodies could be further underpinned: In addition to statins, alirocumab reduced LDL-C by 35–68% and Lp(a) by 13–32% compared to 42–66% and 11–29% for evolocumab, respectively. The effect of both drugs on HDL-C with increases of 13–32% for alirocumab and 11–29% for evolocumab and reductions of TG levels by 6–18% and 4–33% were as well comparable. In a placebo-controlled trial in statin-treated patients after 24 weeks, bococizumab at doses of 150 or 300 mg twice monthly produced mean changes of LDL-C of 67% and 55% from baseline, respectively (Ballantyne et al. 2014). Whereas in the phase II trials primarily patients with a primary hypercholesterolemia on stable statin doses were included, the ongoing and planned phase III trials include a much wider spectrum of patients with dyslipidemias such as statin-intolerant and naive on lipid therapy with and without combinations with statins, ezetimibe, or fibrates.

The recently published data from the first two Phase III clinical trials allow a first anticipation regarding the potential impact and quantum leap in the treatment of lipid disorders and potential prevention of atherosclerosis. In the ODYSSEY long-term trial, 2,431 patients of a high CV risk (18% of them with heterogeneous FH) being treated with a statin or another lipid-modifying therapy were treated for 78 weeks in a placebo-controlled study with 150 mg of alirocumab s.c. every fortnight. After 24 weeks, mean reduction in LDL-C was 61% vs. 0.8 increase in the placebo group. Seventy-nine percent of treated patients achieved the target of at least 50% reduction in LDL-C from baseline, and discontinuation rate was similar in the verum and placebo arms with 6.2% vs 5.5%, respectively. The alirocumab group shows a 54% risk reduction in the absolute rate of CV events (1.4% vs 3%) (Robinson et al. 2015). Treatment of hypercholesterolemic patients with 150 or 300 mg alirocumab every 4 weeks resulted in similar reductions of LDL-C as fortnightly injections (Press Release Sanofi 2015).

Within the DESCARTES study 901, hypercholesterolemic patients were stratified into four groups treated with diet alone, 10 mg atorvastatin, 80 mg atorvastatin, or 80 mg atorvastatin plus 10 mg ezetimibe, and received additional 420 mg of evolocumab s.c. every 4 weeks. Mean add-on reductions of 55.7%, 61.2%, 56.8%, and 48.5% could be achieved with supplementation of evolocumab with additional reduction in ApoB, non-HDL-C, Lp(a), and TG plasma levels (Blom et al. 2014). In patients with hoFH, PCSK9 antibodies are also efficacious as shown recently by the TESLA study: In 50 patients with a mean LDL-C of 348 mg/dL after 12 weeks of treatment with 420 mg of evolocumab, LDL-C were reduced by 30.9% compared with an increase by 8% in the nontreated group, in patients with mutations in the LDL receptor, a reduction by 41% could be demonstrated, whereas the drug had no effect in patients devoid of the LDL receptor (Raal et al. 2015). Huge phase III clinical outcome trials are running for alirocumab and evolocumab involving 23,500 (ODYSSEY) and 22,000 (FOURIER) patients, respectively, and for bococizumab as well a phase III trial involving 22,000 patients is planned (for a detailed overview regarding the clinical trials with PCSK9 antibodies, see the excellent article by Dadu et al. (2014). Conclusive results from these trials are expected in 2018, allowing a definite judgment regarding the clinical benefit of PCSK9 inhibition for cardiovascular diseases. Primary outcome parameters of these megatrials will be the time of occurrence of one of the following clinical events: death from CHD, nonfatal myocardial infarction, stroke, or hospitalization due to unstable angina. In summary, the clinical findings obtained so far with PCSK9 neutralizing antibodies are encouraging. It will have to be demonstrated that these impressive reductions in LDL-C and Lp(a) levels are translated into measurable clinical benefits for the patients in terms of reduction of mortality and prevention from CHD and that this antibody therapy possesses a high long-term safety.

Alternative Approaches to Inhibit PCSK9 Activity

An alternative approach to monoclonal antibodies for blocking the protein–protein interaction between PCSK9 and the LDL receptor is the design of peptides or peptidomimetics interfering with the different contact surfaces between the two proteins. Drug candidates mimicking the C-terminus of PCSK9 (SX-PCK9), the EGF-A domain of the LDL receptor, or an adnectin based on a scaffold for human fibronectin with an engineered domain to prevent the interaction between PCSK9 and the LDL receptor (BMS-962476) are evaluated in early preclinical and clinical trials, respectively (Mullard 2012). Both monoclonal antibodies and PCSK9 mimetics tackle the function of the secreted PCSK9 protein only. Since PSCK9 is also intracellularly involved in the regulation of LDL-receptor surface expression approaches, silencing the PCSK9 gene should theoretically be even more effective. Both an antisense (BMS-PCSK9Rx-2) and gene-silencing approaches are evaluated. Most advanced in this regard is the investigational drug CAT-2003 in phase 2 clinical trials: CAT-2003 is an orally active small molecule – a covalent conjugate between niacin and eicosapentaenoic acid. The molecule is systemically inert, after uptake into cells the prodrug is intracellularly hydrolyzed into its pharmacologically active drugs. The molecular mechanism whereby CAT-2003 inhibits PCSK9 is the inhibition of the processing of the SREBP 120 kDa precursor protein into active SREBPs, thereby inhibiting the activation of SREBP-regulated target genes such as PCSK9 (Zimmer et al. 2014). In HepG2 cells, expression of SREBP2 is reduced by 50% as well as PCSK9-mRNA. Due to the pleiotropic action of SREBPs, the actions of other genes like HMG-CoA-reductase, SCD-1, ACC-2, S1P, or S2P are as well inhibited. In ApoA3 Leiden mice being treated for 16 weeks with the compound plasma levels of PCSK9, total cholesterol and TG were reduced by 61%, 41%, and 33%, respectively; histological evaluation of the livers showed an increase in the expression of LDL receptor by 104%. In a phase I clinical trial (PATHWAYS I), normal healthy volunteers ($N = 99$) receiving 300–2,000 mg CAT-2003 for up to 14 days showed a decrease of TG levels up to 90% at each dose tested with a concomitant reduction of thr plasma levels of PCSK9, non-HDL-C and LDL-C (Donovan et al. 2014). Plasma levels of NEFAs were not increased, indicating that the compound did not activate the nicotinic acid receptor GPR109a. Furthermore, no flushing was observed, demonstrating no systemic activity of niacin via the PDGR2 receptor. Further clinical trials are ongoing, among them a 12-week study in patients with chylomicronemia (hyperlipoproteinemia type I) to asses further efficacy and safety.

A small molecule originally designed as a CETP inhibitor K-312 – currently in phase 1 clinical trials – was found to suppress also the production of PCSK9; in the hepatocyte cell line, HepG2 K-312 decreased PCSK9 mRNA by 90% by inhibiting SREBP binding to the sterol response element SRE in the promoter region of the PCSK9 gene and suppressing SREBP expression. As a potential mechanism of K-312 was suggested that it interferes with the binding of the transcription factors SREBP ½, HNFα/β, and HINFP to their closely neighboring binding sites in the PCSK9 promoter region (Shibata et al. 2012; Miyosawa et al. 2012).

Apolipoprotein E Mimetics

AEM-28 is a synthetic 28mer peptide combing the a 18mer amphipathic lipid-binding domain of ApoE and the binding domain for the human ApoE receptor. AEM-28 has the ability to insert into the phospholipid surface of ApoB containing lipoproteins, targeting them to the liver where it is docked via the heparansulfate proteoglycan receptor (syndecan 1). In normocholesterolemic and hypercholesterolemic cynomolgus monkeys, AEM-28 was able to reduce total cholesterol levels up to 70% after a 2-h infusion (Goldberg et al. 2014). In a first-in-man study, the compound showed an acceptable safety profile with reductions of VLDL and TG up to 76% vs baseline within the first 12 h after infusion (Press Release Capstone 2014). Treatment with AEM-28 maybe an effective treatment option for patients that lack a functional or dysfunctional LDL-receptor pathway such as homozygous and heterozygous FH or severe refractory hypercholesterolemia.

4.1.4 Inhibition of Lipoprotein Lp(a)

Epidemiological and genetic studies as well as meta-analyses have manifested a causal and independent role of apolipoprotein Lp(a) for CVD, particularly myocardial infarction and stroke (Kolski and Tsimikas 2012) with Lp(a) levels linearly linked to the risk of CVD. Lp(a) levels show a strong heterogeneity primarily due to genetic variations between individuals and cannot be modulated by diet or lifestyle changes. Lp(a) mediates its atherogenicity through both its LDL and its apolipoprotein (a) moiety, the latter being atherogenic because of the proinflammatory properties of the Kringle domains. The most effective therapy to reduce elevated Lp(a) levels is plasma apheresis resulting in Lp(a) reductions up to 75%. Currently no drugs are approved for specific lowering of Lp(a), but some drugs like nicotinic acid show a certain efficacy in lowering of Lp(a) levels. Several lipid-modifying drugs in clinical development are as well investigated for their effects on Lp(a). Mipomersen recently approved for the treatment of FH showed a Lp(a) reduction by 42% and 50% in patients with heterozygous and homozygous familial FH, respectively (Visser et al. 2012). A specifically designed antisense nucleotide against apolipoprotein A was able to reduce its expression in animals more than 80% (Merki et al. 2011). The MTP inhibitor lomitapide decreased Lp (a) modestly by 17% (Samaha et al. 2008). Antibodies against PCSK9 like AMG-145 and SAR236553 reduced Lp(a) levels in hypercholesterolemic patients by 15–44% (Dias et al. 2012; Stein et al. 2012a). The CETP inhibitor anacetrapib or the thyromimetic eprotirome also reduced Lp(a) levels by up to 40%. A novel and efficacious approach to reduce Lp(a) seems possible with agonists of the farnesoid X receptor (FXR) (Chennamsety et al. 2011); obeticholic acid (Neuschwander-Tetri et al. 2014) and Px-104 (Press Release Phenex 2012) are FXR agonists currently in clinical development for the treatment of NASH.

4.1.5 Gene Modulation Approaches

Antisense and Gene Silencing

Patients with statin intolerance and heterozygous FH do not reach the ATP III goals for LDL-C (Rana and Boeckholdt 2010), indicating a significant medical need for efficacious lipid management for these patients. Principally, antisense oligonucleotide (ASO) or RNA silencing technologies are ideally suited to achieve a high specificity in inhibiting the specific function of a particular protein by downregulating its synthesis on the translation level, thereby minimizing the risk of drug–drug interactions due to lack of involvement of cytochrome P450 enzymes in drug metabolism. This is of particular importance for multimorbid patients often receiving a multidrug therapy. Major disadvantages of antisense-directed therapies are their limited stability against serum and cellular nucleases, their limited permeability across biological membranes, as well as their off-target effects by interacting with Toll-like receptors (Jones 2011). Therefore, RNA-based and antisense drugs have to be applied parenterally, and thus the delivery to their target tissues and cells is still a major challenge and obstacle for RNA and ASO drugs. After an i.v. injection, the majority of ASOs are accumulated in the liver and the kidney, thus making therapeutically interesting drug targets localized in these organs a priority for ASO therapy. On the other hand, this organotropism remains a major challenge for RNA and DNA-based drugs acting in other tissues than the liver and the kidney. Fortunately, the liver is the major organ regulating lipid homeostasis and metabolism, and therefore ASO therapies are a reliable approach to treat dyslipidemias by blocking the expression of target genes localized in the hepatocyte such as apolipoprotein B-100, apolipoprotein(a), PCSK9, or MTP. In particular, locked nucleic acid-derived ASOs are promising candidates for a wider scope of application due to their improved compound characteristics such as cellular uptake via gymnosis (Stein et al. 2010) and the avoidance of liposome technologies with their associated toxic lipid reagents.

Apolipoprotein B

ApoB Antisense Approaches

The most advanced nucleic acid-based drug to treat dyslipidemias is mipomersen (Toth 2011), which obtained FDA approval for treatment of patients with homozygous FH in early 2013 (Press Release Sanofi 2013). Mipomersen is an Apo B100 ASO made of 20 2′-O-methyl-ethyl-nucleotides. Several clinical trials have demonstrated the efficacy of mipomersen to reduce Apo B-100, LDL-C, total cholesterol levels, as well as Lp(a) levels. In healthy volunteers, Apo B-100 and LDL-C levels were reduced by 21% and 34% (Akdim et al. 2010). Addition of 200 mg mipomersen s.c. once weekly to heterozygous FH patients being treated with the maximal possible statin doses reduced their LDL-C levels further by $-28 \pm 5.2\%$, and 49% of treated patients achieved their LDL-C target of 100 mg/dL (2.6 mM) compared to only 4.9% in the group treated with statins only. Efficacy of mipomersen was better in females than in men (mean LDL-C reduction −40.6%

vs −20%). The dropout rate of 10.8% was mainly caused by injection-site reactions, and 4.9% of treated patients showed an increase in liver fat (Stein et al. 2012b). In patients with homozygous FH treated with the maximally tolerated dose of statins, 200 mg of mipomersen reduced LDL-C by further 24.7% (Raal et al. 2010).

Adverse side effects were elevations of transaminases, flu-like symptoms, and injection site reactions as well as signs of hepatic steatosis frequently found in FH patients with genetic deficiencies of Apo B-100, limiting the maximal tolerable dose. The FDA voted for approval of mipomersen as an add-on therapy for patients with homozygous FH being already treated with another lipid-lowering therapy on a low-fat diet (Press Release Sanofi 2013), whereas the European Agency EMA did not approve mipomersen due to safety concerns. Mipomersen is a new and attractive alternative to LDL apheresis for these patients.

ApoB RNAi-Approaches

For apolipoprotein B-100, several RNA-silencing drugs are investigated in clinical trials. Using the SNALP technology (Rozema et al. 2007), the liposomal siRNA drug PRO-040201 (Press Release Tekmira 2009) tested at doses of 0.03–1 mg/kg in 23 probands was well tolerated without severe adverse side effects or injection site reactions; one proband expressed "flu-like symptoms" probably caused by an innate immune response to the ApoB SNALP drug product with dose-limiting cytokine release leading to termination of its development (Press Release 2009). The development of a follow-up compound with adjustments to the ApoB siRNA (TKM-ApoB) to minimize any immune stimulatory properties is reported to continue.[1]

PCSK9

PCSK9 RNAi Approaches

For PCSK9, several RNA-based drug approaches are in preclinical and clinical development. ALN-PCS is an RNAi drug delivered as a stable nucleic acid lipid particle (SNALP delivery technology) (Rozema et al. 2007) aiming to silence the PCSK9 gene. A phase I trial was performed with healthy volunteers having elevated LDL-C levels >110 mg/dL with a single injection of doses of 0.015–0.4 mg/kg. After 4 weeks, plasma levels for PCSK9 and LDL-C were reduced up to 84% and 50%, respectively, with no effect on HDL-C levels. The compound was reported to be safe without severe adverse side effects (Fitzgerald et al. 2014). Importantly, no elevations in liver enzymes were observed tempting to speculate that PCSK9 may be a better target for antisense approaches to lower LDL cholesterol levels with regard to liver toxicity compared to apolipoprotein B. In a multidose study with a dose of 2 mg/kg, circulating PCSK9 levels were reduced by 95% leading to a 67%

[1] Homepage Tekmira Pharmaceuticals Corporation. http://www.tekmira.com/programs/Products.asp#apob

decrease in LDL-C (Fitzgerald et al. 2014). With a new drug delivery platform based on GalNAC-siRNA conjugates, subcutaneous application with a long duration of action become possible; in nonhuman primates, a single dose of 3–10 mg/kg of ALN-PCS s.c. knocked down PCSK9 levels up to 96% and LDL-C up to a 77% with a reduction of LDL-C of >50% even 90 days after injection. These data support attempts for an effective treatment of hypercholesterolemia with injection of a PCSK9 RNAi drug only once a month or even once per quarter (Press Release Alnylam 2015). On a preclinical level, a 19mer gene-silencing oligonucleotide for PCSK9 mRNA shows after s.c. administration a significant specific reduction in the concentration of PCSK9 mRNA without affecting expression of ABCA1, ABCG1, and LXR (Press Release Idera 2011).

PCSK9 Antisense Approaches

Two antisense drug candidates for PCSK9 – Santaris SPC 5001 BMS/ISIS-394814, both being phosphothioates to work via an RNAse H mechanism – have been investigated in phase I clinical trials, but their development has been terminated for undisclosed reasons. Recently, a case of acute kidney injury (with recovery) was reported in one patient having received weekly injections of SPC-5001 for 3 weeks with multifocal tubular necrosis and signs of oligonucleotide accumulation in the kidney (van Poelgeest et al. 2013). It is known that phosphothiate ASOs bind particularly to proteins in plasma and various tissues interacting with polyanions such as PDGF or VEGF resulting in high concentrations in these tissues and therefore a high efficacy. On the other hand, the resulting high concentrations in the liver, kidney, and spleen can lead to cellular toxicity with transient inhibition of the clotting cascade (Kurreck 2003), thereby limiting the therapeutic window of phosphorothioate-based ASOs.

Other RNA-Based Drug Candidates

Antisense Approaches

Further molecular targets for antisense approaches for the treatment of dyslipidemias in development are apolipoprotein CIII (ISIS-ApoC-IIIRx), lipoprotein Lp(a) (ISIS-Apo(a)Rx, and angiopoietin-like III (ISIS-ANGPTL3Rx). Chylomicronemia is a rare inherited disease with 3–5,000 patients worldwide; due to increased levels of apolipoprotein CIII as a noncompetitive inhibitor of lipoprotein lipase, TG-rich lipoproteins cannot be catabolized leading to extremely high TG plasma levels up to 2,000 mg/dL strongly increasing the risk for acute pancreatitis and diabetes. Polymorphisms in ApoC-III have been associated with hypertriglyceridemia; ApoC-III loss-of-function results in higher TG hydrolysis rates and consequent increased TG clearance leading to lower TG and VLDL levels in heterozygous individuals of ApoC-III loss-of-function, and recently it was shown that rare loss-of-function variants in ApoC-III seem to be cardioprotective (Tachmazidou et al. 2013). In a phase II clinical program, ISIS-APOC-IIIRx was investigated in patients with extremely high TG levels, type 2 diabetics, and FCS (familial chylomicronemia syndrome) receiving 300 mg weekly. In single therapy after 13 weeks, plasma levels of ApoC-III and TG

were reduced by 88% and 69%, respectively, whereas HDL-C increased by 42%. In addition, glucose control was significantly improved with reduction of 1.2% in HbA1c (placebo-adjusted) as well as reductions in serum fructosamine and glycated albumin (Alexander et al. 2014), suggesting a potential of this drug to improve peripheral insulin sensitivity in patients with diabetic dyslipidemia. ISIS-APO(a)Rx investigated in a phase I trial in healthy volunteers with Lp(a) levels ranging from 10 to 98 mg/dL decreased Lp(a) levels dose-dependently by 95% and reduced oxidized pro-inflammatory phospholipids by up to 59%.[2]

RNAi Approaches

Using the GalNAC siRNA conjugate platform RNAi drugs silencing PCSK9 (see above), ApoC-III (ALN-AC3), and ANGPTL3 are investigated in preclinical investigations. In mouse models matching human genetics, a single s.c.-administration of ALN-AC3 knocked down ApoC-III levels by >95% and reduced TG levels up to 68% with a persistence of the pharmacodynamic effect for more than 20 days. ANGPTL3 is an independent risk factor for CV diseases, regulating lipid, glucose, and energy metabolism; individuals with increased levels of ANGPTL3 exert hyperlipidemia with an increased risk of heart attacks, increased arterial wall thickness, and metabolic disease including insulin resistance. ANGPTL3 is an inhibitor of cellular lipases produced by the liver, and genetic investigations have demonstrated that loss-of-function in ANGPTL3 is associated with decreased levels of both TG and LDL-C (Musunuru et al. 2010). S.c. applications of ALN-ANG to ob/ob mice resulted in 95% knockdown of ANGPTL3 and decreases in TG, LDL-C, and total C levels of 95%, 85%, and 60%, respectively. In addition to the RNAi drug ALN-ANG, also an antisense drug ISIS-ANGPTL3Rx is in phase 1 clinical trials. If these findings could be confirmed in humans, silencing/k.o. of ANGPTL3 could become an important therapeutic option for the treatment of severe hypertriglyceridemia and mixed dyslipidemias.

Gene Therapy

For the treatment of patients with the rare disease FCS ("familial chylomicronemia syndrome," hyperlipoproteinemia type 1a) being deficient in the enzyme lipoprotein lipase which is essential to breakdown circulating chylomicron particles, the drug adipogene tiparvovec (Glybera) was approved in 2012 in Europe as the first gene therapy drug (Melchiorri et al. 2013) to restore LPL activity. Glybera contains the gene for the human LPL-variant S447X with a tissue-specific promoter in a non-replicating AAV1 vector with a high affinity for muscle tissue. For gene therapy, Glybera was applied by multiple injections at doses of 10^{11} to 10^{12} gene copies/kg body weight into muscles of the lower extremities under spinal anesthesia or strong sedation of the patients. Three days before and 12 weeks after Glybera injection, an immunosuppressive therapy with cyclosporin (3 mg/kg × day) and mycophenolat mofetil (2 × 1 g/day) is recommended to avoid antibody formation.

[2] Homepage ISIS Pharmaceuticals. www.isispharma.com/Pipeline/index.htm

Overall, 27 patients received the gene therapy; 2–12 weeks after injection in some patients, reduction of fasting TG levels >40% could be achieved, and after 4 months, the starting TG levels reappeared. Muscle biopsies after 6 months demonstrated the expression of the LPL gene and the presence of secreted active LPL enzyme. A follow-up observation of 12 patients with earlier episodes of pancreatitis up to 3 years indicated a tendency to less frequent and less severe acute attacks of pancreatitis (www.ema.europa.eu/docs/de_DE/document_library/EPAR-Product_Information/human/002145/WC500135472.pdf.).

4.2 Novel Approaches to Increase HDL

The inverse relationship of HDL-C levels and the incidence of CHD (Parhofer 2005; Gordon et al. 2000) indicate that an elevation of HDL levels should exert antiatherogenic properties. A major mechanism for the antiatherosclerotic properties of HDL is their role in RCT as the key pathway to transport cholesterol from peripheral tissues such as cholesterol-laden macrophages to the liver for excretion into bile. RCT is a very complex pathway not fully understood involving a sequence of events:

1. Synthesis of ApoA-1 and formation of nascent pre-β-HDL particles.
2. Uptake of cholesterol from peripheral cells (macrophages) by mediation through the ABC transporter ABCA1 and ABCG1.
3. Esterification of cholesterol taken up to cholesterol esters (CE) by lecithin–cholesterol acyl transferase (LCAT) to avoid cholesterol efflux from the HDL particles and leading to formation of CE-rich α2 HDL and α3 HDL.
4. CE transfer from HDL particles to ApoB-rich lipoproteins in exchange for triglycerides catalyzed by CETP resulting in the formation of pre-α and pre-α1 HDL particles. The resulting TG-rich HDL is susceptible to lipolytic modification by hepatic lipase and endothelial lipase leading to the formation of smaller HDL particles more sensitive to faster catabolism.
5. Hydrolytic release of phospholipids from these HDL particles by the enzymes hepatic lipase (HL), endothelial lipase (EL), and soluble phospholipase A2 (sPLA2).
6. Hepatic uptake of remaining CE from HDL particles by the scavenger receptor SR-BI releasing ApoA-1 for cholesterol reloading in peripheral tissues or being filtered in the kidney into urine.

There are three monogenic causes known of elevated HDL levels with loss of function of CETP, EL, and ApoC-III (Larach et al. 2013):

– Loss of function of CETP blocks the transfer of CE to ApoB-containing lipoproteins and prevents therefore the formation of more atherogenic VLDL and LDL particles.

- Loss of function of ApoC-III as a noncompetitive inhibitor of lipoprotein lipase stimulates hydrolysis of TG-laden lipoprotein particles, and due to the reciprocal interrelationship of high TG and low HDL, plasma levels of HDL are increased.
- Endothelial lipase catalyses the hydrolysis of phospholipids in HDL particles, thereby destabilizing HDL particles which show an increased catabolism; loss of function of EL prevents this EL-mediated catabolism of HDL particles.

However, the role of HDL particles is more complex than just being a carrier for water-insoluble lipids in plasma in the course of RCT. In addition to this key function, HDL particles exert antioxidative, anti-inflammatory, and antithrombotic activities (Grunfeld and Feingold 2008) as well as improvement of endothelial function (Terasaka et al. 2008). The functionality of HDL particles is as important as the magnitude of plasma HDL levels for protection from atherosclerosis. HDL is also able to inhibit the formation of oxidized LDL, and lipid peroxides from oxidized LDL particles (oxLDL) can be transferred via CETP to HDL particles leading to a rapid clearance of the resulting lipid peroxide-laden HDL particles from circulation, indicating a role of HDL in the elimination of oxidized LDL as well (Garner et al. 1998). Additionally, HDL and ApoA-1 can protect endothelial cells from injury by inhibiting signaling pathways involved in apoptosis of endothelial cells triggered by oxLDL or proinflammatory cytokines (Suc et al. 1997). The findings that people with certain ApoA-1 mutations are not at a higher risk for CVD despite very low overall HDL levels underline the essential role of HDL particle functionality. The complex physiology of the HDL metabolism offers in a variety of drug approaches such as:

- Prevention of HDL particle reshaping by inhibition of lipid-transfer proteins like CETP or inhibition of phospholipid-hydrolyzing enzymes like HL, EL, or sPLA2.
- Increasing production of ApoA-I.
- Substitution by exogenous reconstituted HDL particles or ApoA-I peptidomimetics.
- Stimulation of cholesterol efflux from macrophages by increasing the expression of ABCA1 and ABCG1.
- Stimulation of hepatic CE uptake mediated by the scavenger receptor SR-BI.
- In addition, modulation of the antioxidative, anti-inflammatory, or antithrombotic properties of HDL particles by interference with targets like the apolipoproteins E, J, A2, or A3 and enzymes like paraoxonase (PON1) or LCAT can be considered.

The negative outcome with the CETP inhibitor torcetrapib may indicate that not every way of raising HDL cholesterol levels automatically translates into a clinical benefit. The nearly complete and irreversible inhibition of CETP may have eliminated the portion of RCT of CE by VLDL and LDL to such an extent that any beneficial effect was eliminated (Tall 2007). It may be that the dynamics of lipoprotein modulation have to be maintained.

This complexity of HDL pathways shows that simply measuring HDL levels is not a viable parameter for drug efficacy and more sophisticated methods to monitor the different aspects of HDL function are needed as was recently demonstrated by a detailed analysis of HDL parameters and their correlation to endpoints of CVD in a population cohort (Rohatgi et al. 2014).

4.2.1 Inhibitors of Cholesterol Ester Transfer Protein (CETP)

CETP catalyzes the transfer of CE from HDL particles to VLDL or LDL, yielding cholesterol-rich apoB-containing lipoproteins which contribute to an increased deposition of cholesterol into the peripheral arterial wall. The reverse translocation of TG from VLDL and LDL to HDL yields TG-rich HDL particles which are more prone to hydrolysis of their lipids by hepatic lipase. By this mechanism, the HDL particle is destabilized and more rapidly cleared by the kidney, resulting finally in decreased HDL plasma levels. Consequently, a high CETP activity leads to decreased plasma HDL levels. Therefore, a shift in partitioning of cholesterol between LDL/VLDL and HDL particles by blocking of CETP is one option to increase plasma HDL levels. The finding that certain individuals with loss-of-function mutations in the CETP gene showed elevated HDL levels and a decreased incidence of CHD (Koizumi et al. 1985; Inazu et al. 1990) triggered the search for CETP inhibitors. Other findings, however, showed that certain mutations in the CETP gene are associated with an increased risk of CHD despite high HDL-C levels (Zhong et al. 1996). Additionally, animal experiments also show conflicting results with regard to beneficial and detrimental effects of CETP inhibition. Torcetrapib was the first CETP inhibitor being evaluated in human clinical trials. Monotherapy with a dose of 60 mg/day was able to increase HDL, HDL2, and HDL3 levels by 33%, 74%, and 26%, respectively, with a reduction of LDL-C by 8% (McKenney et al. 2006). A large clinical trial with 60 mg/day torcetrapib on top of 10–80 mg/day atorvastatin showed an increase of HDL-C levels by 51.9% with an additional lowering of LDL-C of 20.6% compared to atorvastatin alone (Kastelein et al. 2007). In these and further studies, however, no effect of torcetrapib on progression or regression of atherosclerosis could be demonstrated despite a 60% increase in HDL-C levels. Torcetrapib increased systolic and diastolic blood pressure by 1.3–2.2 and 0.9–1.1 mmHg and was associated with an increase in the number of deaths from both cardiovascular and noncardiovascular causes in the torcetrapib–atorvastatin group compared to the atorvastatin group alone (McKenney et al. 2006; Davidson et al. 2006), finally leading to the termination of its clinical development. Another CETP inhibitor, dalcetrapib, weaker in its pharmacological efficacy compared to torcetrapib, showed HDL-C increases by 25–30% without any effects on blood pressure. Later on it could be demonstrated that the hypertensive activity of torcetrapib is independent on its mechanism on CETP and is an off-target effect possibly by induction of elevated aldosterone and cortisol levels (Forrest et al. 2008). Dalcetrapib was investigated in a phase III clinical trial with 15,000 patients for its ability to reduce cardiovascular morbidity and mortality in patients with a recent acute coronary syndrome event (Schwartz et al. 2009). After an interim analysis, the development of the drug was

discontinued not because of adverse side effects but due to disappointing results in the efficacy outcome not disclosed further yet. Despite these failures, the search for efficacious and safe CETP inhibitors is still one of the most active drug discovery fields for novel anti-atherosclerotic drugs. At least four small molecule CETP inhibitors are currently in clinical investigation. Anacetrapib now in phase III showed in a double-blind, placebo-controlled 18-month trial at a dose of 100 mg/day on top of a statin an increase of HDL-C by 138% and a decrease in LDL-C of 40% without any effect on blood pressure, serum electrolytes, or aldosterone levels without any cardiac adverse effects. Additionally, Lp(a) levels were decreased by 36.4% (Cannon et al. 2010). In a direct comparative trial, 300 mg/day anacetrapib could increase HDL-C by 100% compared to 30% with 2 g/day nicotinic acid (Yvan-Charret et al. 2010), the most potent HDL-C increasing drug available today. A huge trial involving 30,000 patients (REVEAL, NCT01252953) is under way to elucidate whether anacetrapib can reduce the rate of cardiovascular events in patients with optimized statin therapy. Evacetrapib in monotherapy increased HDL-C by 53.6–128.8% concomitantly with a decrease of LDL-C by 13.6–35.9% at doses of 30–500 mg/day. In combination with a statin a dose of 100 mg/day, evacetrapib increased HDL-C by 78.5–88.5% with an additional decrease of LDL-C of 11.2–13.9% (Nicholls et al. 2011a). These clinical trials with anacetrapib and evacetrapib demonstrate a clear beneficial change of the lipid profiles. However, both drugs also reduce LDL-C and Lp(a) which makes it difficult to differentiate and elaborate the contribution of the beneficial effect of HDL increase and potential reduction of CVD risk due to CETP inhibition.

Masking of CETP action with an antibody raised against a fusion protein of a certain CETP epitope linked to a T cell epitope of tetanus toxin (CETi-1) is an alternative approach. In a phase II study, 90% of treated patients showed 1 year after vaccination with CETi-1 an immune response with an increase of HDL-C by 8% (Omori 2004). CETi-1 is no longer in development, but a new CETP vaccination approach with ATH-03 using a small peptide fragment of the CETP protein acting as a B cell epitope has entered phase I trials to assess its safety and immunogenicity (NCT01284582) (Homepage AFFiRiS 2009). An interesting approach is the combination of the CETP-inhibitory action a with PCSK9 downregulatory activity in one molecule. K-312, currently in phase 1 clinical trials, has a unique mode of action to suppress PCSK9 transcription in part by inhibiting SREBP binding to SRE on the PCSK9 promoter and suppression of SREBP expression (Shibata et al. 2012; Miyosawa et al. 2012). In summary, it still remains to be demonstrated whether a pharmacological intervention raising plasma HDL levels by inhibition of the enzymatic activity of CETP exerts a robust reduction of atherosclerosis and cardiovascular morbidity and mortality.

4.2.2 Enhancer of Apolipoprotein A-1 Activity

Owing to the key role of ApoA-1, several approaches aim to increase its contribution to RCT, either by stimulation of its biosynthesis or by mimicking HDL function with synthetic ApoA-1 mimetics or reconstituted HDL formulations.

HDL Replacement Therapy

A mutant form of ApoA-1 – ApoA-1 Milano – was found to be associated with very low HDL-C levels without an increased risk for CVD (Sirtori et al. 2001; Franceschini et al. 1980). A small clinical trial with an i.v. application of ApoA-1 Milano reconstituted in phospholipid vesicles was reported to induce a significant reduction of coronary atherosclerosis in patients with ACS after only 6 weeks of treatment with a decrease of atheroma volume by 14.1% (Nissen et al. 2003). This surprising result triggered the research for efficient HDL replacement therapies. The ApoA-1 Milano phospholipid formulation MDCO-216 was investigated in a phase 1 clinical trial for the treatment of atherothrombotic diseases and ACS as well as for prevention of restenosis after CABG and balloon angioplasty.[3] Twenty-four healthy individuals and 24 patients with confirmed CAD received a 2-h infusion of MDCO-216 in doses of 5–40 mg/kg and followed for 30 days. Half-lifetime ranged from 45 to 59 h. In both groups ApoA-1, phospholipids and prebeta HDL levels were increased with an increase in HDL particle size accompanied by decreases in ApoE; in dosages above 20 mg/kg, however, increases in TG and decreases in HDL-C were observed (Bellibas et al. 2014). Overall the drug was well tolerated with no serious adverse events or safety issues. CSL-111 is a synthetic HDL particle reconstituted from ApoA-1 isolated from human plasma with soybean phosphatidylcholine. The compound failed in phase II trials to reduce plaque volume in coronary arteries in patients suffering from a recent episode of ACS (Tardif et al. 2007). In patients with ACS receiving a single infusion of 80 mg/kg CS-111 over 4 h, no significant difference could be detected in the surrogate markers of forearm venous occlusion plethysmography despite a 64% increase in HDL-C and a 23% decrease in LDL-C (Chenevard et al. 2012). A reformulation of CSL-111 – named CSL-112 – is currently in phase II clinical trials in stable CAD patients (Gille et al. 2012; Diditchenko et al. 2012). In 57 healthy volunteers, having received a single dose of 5–135 mg/kg CSL-112 increased prebeta 1-HDL by 3,600% and stimulated macrophage cholesterol efflux mediated by ABCA1 by 630% and increased efflux capacity by 192% (Gille et al. 2014). Mechanistic investigations suggest that the infused CSL-112 particles fuse with HDL in plasma with subsequent release of lipid-poor ApoA-1 (Diditchenko et al. 2014). The effects lasted at least for 72 h indicating that a once or twice weekly infusion could be an appropriate treatment regimen (Gille et al. 2012). Additionally, CSL-112 exerted anti-inflammatory effects with a strong inhibition in the expression of the adhesion molecule ICAM-1 (CD-54) on monocytes and neutrophils and a decrease in proinflammatory cytokines (TNF-α, Il-1β, Il-6) and chemokines (Il-8, RANTES, Mip-1β) (Diditchenko et al. 2012). CER-001 is an HDL-mimetic, comprise of recombinant ApoA-I complexed with phospholipids and designed to mimic the beneficial properties of natural nascent pre-β HDL particles. In healthy volunteers, CER-001 increased plasma ApoA-1 levels after a single infusion in a dose-dependent manner with similar increases in HDL-C (Keyserling et al. 2011). In

[3] Homepage The Medicines Company. http://www.themedicinescompany.com/page/pipeline

rabbits CER-001 was at least 10–20 times more potent than ETC-216 to mobilize cholesterol from macrophages (Kastelein 2015). In a placebo-controlled phase 2b study (CHI-SQUARE) with 507 patients with ACS given CER-001 for 6 weeks with weekly infusions, the drug failed to show that this HDL mimetic can reduce total atheroma volume; intravenous ultrasound analysis (IVUS) scans conducted 3 weeks after the last infusion showed that CER-01 reduced total atheroma volume from baseline, but the change was not significantly different from the change from baseline seen in the placebo group (Press Release Cerenis 2014a; Tardif et al. 2014). In a further clinical trial in patients with familial primary hypoalphalipoproteinemia, however, CER-001 was able to reduce carotid artery mean vessel wall area as measured by magnetic resonance imaging after treatment of 23 patients with 9 doses of CER-001 over 4 weeks. Furthermore, in 23 patients with hoFH having received biweekly infusions of CER-001 over 6 months on top of optimized LDL-C, lowering therapy including apheresis carotid artery mean vessel wall area was as well statistically reduced (Press Release Cerenis 2014b; Kootte et al. 2015) leading to orphan drug approval by the European Medicines Agency EMA in September 2014.

As of today, the clinical trials with HDL replacement therapy have delivered mixed results. The efficacy, safety, and cost-effectiveness of this approach has to be shown in future studies. As well, the invasive route of administration will limit its widespread use both due to reasons regarding patient compliance and the need for a physician for intervention.

Apolipoprotein A-I Peptidomimetics

Several ApoA-1 mimetics mimicking at least one of the amphipathic helices of ApoA-1 and therefore being able to associate lipids showed promising results in animal studies after oral application such as stimulation of RCT, LCAT activity, and off-loading of cholesterol in the liver via the SR-BI receptor (Navab et al. 2004; Carballo-Jane et al. 2010). The 18mer peptide D-4 F containing four D-amino acids orally applied to mice was able to inhibit lipid peroxidation, to increase PON1 activity, and to stimulate cholesterol efflux (Navab et al. 2006; Xie et al. 2010; Vakili et al. 2010). A phase I clinical trial in 50 patients with CAD receiving 30–500 mg of APP-018 (D-4 F) orally showed only a very low bioavailability (Bloedon et al. 2008; Watson et al. 2011). Efficacy data in this study and a related one using the peptide L-4 F are inconsistent with regard to changes in total cholesterol, LDL-C, HDL-C, or TG despite some decrease in the HDL inflammatory index for D-4 F. FX-5A is a bihelical peptide derived from ApoA-I where five nonpolar amino acids on the hydrophobic side of the helix were substituted with alanine, thereby reducing the lipid affinity of the second helix. This peptide modification was found to reduce the peptide's cytotoxicity and increase its specificity to remove cholesterol from cells. I.v. application of FX-5A peptide complexed with phospholipids to ApoE k.o. mice was able to raise HDL, promote RCT, and reduce atherosclerosis (Reuters 2012).

ApoA-1 Upregulators

A completely new and innovative approach to increase HDL-C is pursued by Resverlogix's drug RVX-208 which exerts its activity via an epigenetic mechanism (Bailey et al. 2010). RVX-208 is an inhibitor of BET proteins (bromodomain and extracellular domain) including BRD4, a member of the BET protein family (Belkina and Denis 2012). One of the key epigenetic mechanisms involves the modification of chromosomal proteins by acetylation, methylation, or phosphorylation catalyzed by specific enzymes. The modified amino acids of histones serve as baits for other proteins including BET proteins for binding and reading this epigenetic code. BET proteins contain two specific sites – the bromodomain and extracellular domain – which can recognize acylated lysine residues of histones bound to DNA. By binding of BET proteins to these modified histones, additional cofactors regulating gene activity are recruited leading to an increase in gene transcription as the ApoA-1 apolipoprotein. RVX-208 mimics the binding of acylated lysine residues to BET, thereby triggering the cascade of increased ApoA-1 gene transcription and ApoA-1 formation. A phase IIb clinical trial (SUSTAIN) with 176 patients with established atherosclerosis RVX-208 significantly raised HDL-C with increases in ApoA-1 levels and large HDL particles, thereby stimulating RCT (Nicholls et al. 2011b). Statin-treated patients with CHD being treated with 50–150 mg/day RVX-208 for 12 weeks showed an increase of 0.1–5.6% for ApoA-I, 3.2–8.3% in HDL-C, and 11.1–21.1% in the number of large HDL particles (Nicholls et al. 2011b). In 10% of patients, transient and significant increases in liver transaminases were observed. In a phase 2b placebo-controlled clinical trial, 323 patients with low HDL levels and established CHD were randomized to a placebo group ($n = 80$) or a treatment group ($n = 243$). All patients received either 10–40 mg/day atorvastatin or 5–20 mg/day rosuvastatin, whereas the treatment group received 100 mg bid RVX-208 for 6 months. RVX-208 failed in achieving its primary and secondary endpoints to increase HDL-C and ApoA-I or to reduce arterial plaques (European Society of Cardiology (ESC) 2013). HDL-C and ApoA-I were increased by 10.9 and 12.8% vs 7.7 and 10.6% in the placebo group. No differences were found for LDL-C (−16 vs −17.6), large HDL particles (38.1 vs 38), or hsCRP (−32.7% vs −33.8%), whereas liver transaminase increases were more often reported in the RVX-208 group (7% vs 0%). In the primary endpoint, the medium change percent of atheroma volume, the RVX-208 group showed a change of −0.4% vs −0.3% in the placebo group. The reasons for failure are not yet clear: lack of efficacy, a too short duration of treatment, or the inability to improve to beneficial effects of statin treatment. Due to their crucial role in cell gene control, BET inhibitors may depress transcription of endogenous human retroviruses because the BET protein-containing transcription regulator complexes can also exert corepressor function (Belkina and Denis 2012). Since the efficacy data of RVX-208 on HDL-C are so far disappointing, further studies will be necessary to determine the validity of this epigenetic approach to increase HDL levels. Overall, the mystery regarding HDL continues (Virani and Ballantyne 2011), and the efficacy of HDL increases by pharmacological intervention as an

approach to reduce cardiovascular morbidity and mortality has still to be demonstrated in further clinical endpoint trials.

4.3 Therapeutic Proteins

4.3.1 LCAT Replacement

Lecithin–cholesterol acyltransferase (LCAT) increases plasma levels of HDL by converting free cholesterol into cholesterol esters, the latter becoming substrates for CETP-catalyzed transfer to TG-rich ApoB-containing lipoproteins in exchange for TG, thereby generating pre-α and pre-α1 particles. Therefore, increasing of LCAT activity by stimulation, increase of expression, or exogenous delivery could be a way to increase functional plasma HDL levels and stimulate RCT. A proof of concept was recently demonstrated in one patient with the rare genetic disease familial LCAT deficiency (125 patients so far reported). Patients with LCAT deficiency have dramatically low levels of HDL-C with a pathophysiological phenotype including corneal opacities ("fish-eye disease"), anemia, splenomegaly, and severe kidney disease, the latter being the major cause of morbidity and mortality. A 53-year-old patient with baseline HDL levels lower than 5 mg/dL (<0.13 mmol/L) received increasing doses of recombinant LCAT (ACP-501) (0.9, 3, and 9 mg/kg) as single infusions for 2 weeks followed by an infusion of 9 mg/kg every 1–2 weeks. After each dose, LCAT levels increased, remaining stable for 4 days. Plasma HDL-C levels rose up to 24 mg/ml (0.62 mmol/L); markers for renal function improved significantly (30% decrease in blood urea, 17% decrease of cystatin C, 25% improvement of anemia) without any significant adverse side effects (Shamburek et al. 2013). From a phase 1 single-dose escalation study with 16 volunteers with stable atherosclerosis receiving a single dose of ACP-501, an elevation of HDL-C with no adverse side effects was reported after 28 days of follow-up observation (Business Wire Press 2012). These findings may stimulate the search for compounds upregulating or stimulating LCAT activity as a novel approach to increase HDL levels and RCT.

4.3.2 FGF 21 Analogues

Fibroblast growth factor 21 (FGF 21) is a 181 amino acid protein secreted by the liver, white and brown adipose tissue, and exocrine pancreas, regulating insulin sensitivity and lipid and energy metabolism (Kharitonenkov et al. 2005). Administration of exogenous FGF 21 to obese diabetic rhesus monkeys exerted profound beneficial effects on glucose and lipid levels as well as on body weight (Kharitonenkov et al. 2007). LY2405319 is a stable analogue of FGF 21 and was investigated in a placebo-controlled clinical trial in 47 diabetic patients who received daily s.c. injection of 3–20 mg for 28 days. LDL-C levels at doses of 10 and 20 mg were reduced by 29.5% and 20.2% and of total cholesterol by 19.2% and 15.4%, respectively. Apolipoproteins B and CIII were reduced by 25.1% and 34% (ApoB) and 21.6% and 35.4% (ApoC-III). Furthermore, HDL-C levels were increased by 15–20%, whereas TG levels were strongly decreased by 46.2% and

44.6%. Additionally, the 4-week treatment was accompanied by a weight loss of 1.3–1.5 kg and a strong increase in circulating adiponectin levels, whereas no significant effects were observed for fasting plasma glucose levels (Gaich et al. 2013). These beneficial efficacy of LY2405319 on multiple metabolic parameters deviated in metabolic disorders may indicate that FGF 21 analogues could evolve if proven to be a safe as novel effective drugs for the treatment of dyslipidemia associated with metabolic disorders like diabetes, obesity, or the metabolic syndrome.

5 Conclusions

The introduction of statins into clinical practice with lovastatin as the first drug of this class in 1987 (Henwood and Heel 1988) has revolutionized the treatment of lipid disorders and made reduction of LDL-C as the primary goal in the treatment of dyslipidemia and prevention of CVD (National Cholesterol Education Program 2002; Graham et al. 2007b). The tremendous clinical efficacy of antibodies against PCSK9 to reduce LDL-C far beyond what was possible with statins marks a potential quantum leap in the treatment of elevated LDL cholesterol levels by modification of the PCSK9 pathway. Despite the clinically established inverse relationship between HDL and CVD risk, the enormous efforts have not yet translated these findings into effective HDL-modifying drug approaches with unequivocal clinical benefit on CVD morbidity and mortality. Recent findings highlighting the functionality of HDL as probably the most relevant factors underlying the protective effects of HDL will help to develop HDL-modifying drugs with beneficial clinical efficacy on cardiovascular endpoints to prevent and combat atherosclerosis and its clinical consequences. A major need for the clinical development of novel lipid-modifying drugs is the development of new noninvasive measurements and biomarkers for monitoring their efficacy in halting the progression or inducing regression of atherosclerosis to allow smaller, focused clinical trials with stratified patient populations for clinical proof-of-concept of novel drug approaches.

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References

Aicher TD, Boyd SA, McVean M, Celeste A (2010) Novel therapeutics and targets for the treatment of diabetes. Exp Rev Clin Pharmacol 3:209–229

Akdim F, Visser ME, Tribble DL, Baker BF, Stroes ES, Yu R et al (2010) Effect of mipomersen, an apolipoprotein B synthesis inhibitor, on low-density lipoprotein cholesterol in patients with familial hypercholesterolemia. Am J Cardiol 105:1413–1419

Alexander V, Gaudet D, Cheng W, Flaim J, Hughes S, Singleton W et al (2014) An antisense inhibitor of apolipoprotein C-III significantly decreases apolipoprotein C-III, triglycerides, very-low-density lipoprotein cholesterol and particle number, and increases high-density

lipoprotein cholesterol and particle number in hypertriglyceridemic patients on fibrate. J Am Coll Cardiol 63(112):A1453, Abstract A1453

Andrade SE, Walker AM, Gottlieb LK, Hollenberg NK, Testa MA, Saperia GM, Platt R (1995) Discontinuation of antihyperlipidemic drugs—do rates reported in clinical trials reflect rates in primary care settings? N Engl J Med 332:1125–1131

Baigent C, Landray MJ, Reith C, Emberson J, Wheeler DC, Tomson C, SHARP Investigators et al (2011) The effects of lowering LDL cholesterol with simvastatin plus ezetimibe in patients with chronic kidney disease (Study of Heart and Renal Protection): a randomized placebo-controlled trial. Lancet 377:2181–2192

Bailey D, Jahagirdar R, Gordon A, Hafiane A, Campbell S, Chatur S et al (2010) RVX-208: a small molecule that increases apolipoprotein A-I and high-density lipoprotein cholesterol in vitro and in vivo. J Am Coll Cardiol 55:2580–2589

Ballantyne CM, Abate N, Yuan Z, King TR, Palmisano J (2005) Dose-comparison study of the combination of ezetimibe and simvastatin (Vytorin) versus atorvastatin in patients with hypercholesterolemia: the Vytorin Versus Atorvastatin (VYVA) study. Am Heart J 149:464–473

Ballantyne CM, Neutel J, Cropp A, Duggan W, Wang E, Plowchalk D et al. (2014) Efficacy and safety of bococizumab (RIN316/PF-04950615), a monoclonal antibody against proprotein convertase subtilisin/kexin type 9 in statin-treated hypercholesterolemic subjects: results from a randomized, placebo-controlled, dose-ranging study. J Am Coll Cardiol 63(12), Abstract A1374

Barnett J, Viljoen A, Wierzbicki AS (2013) The need for combination drug therapies in patients with complex dyslipidemia. Curr Cardiol Rep 15:391. doi:10.1007/s11886-013-0391-1. Review

Bays HE, Davidon M, Jones MR, Abby SL (2006) Effects of colesevelam hydrochloride on low-density lipoprotein cholesterol and high-density C-reactive protein when added to statins in patients with hypercholesterolemia. Am J Cardiol 97:1198–1205

Belkina AC, Denis GV (2012) BET domain co-regulators in obesity, inflammation and cancer. Nat Rev Cancer 12:465–477

Bellibas SE, Kallend D, Bobillier A, Kempen H, Wijngaard PL (2014) MDCO-216 (ApoA-I Milano/POPC) induces reverse cholesterol transport in stable coronary artery disease patients with a dose proportional pharmacokinetics after single ascending doses. Circulation 130, A13357 (Abstract 13357)

Betters JL, Yu L (2010) NPC1L1 and cholesterol transport. FEBS Lett 584:2740–2747

Bloedon LT, Dunbar R, Duffy D, Pinell-Salles P, Norris R, DeGroot BJ et al (2008) Safety, pharmacokinetics, and pharmacodynamics of oral apoA-I mimetic peptide D-4 F in high-risk cardiovascular patients. J Lipid Res 49:1344–1352

Blom DJ, Hala T, Bolognese M, Lillestol MJ, Toth PD, Burgess L et al (2014) A 52-week placebo-controlled trial of evolocumab in hyperlipidemia. N Engl J Med 370:1809–1819

Bruckert E, Giral P, Tellier P (2003) Perspectives in cholesterol-lowering therapy: the role of ezetimibe, a new selective inhibitor of intestinal cholesterol absorption. Circulation 107:3124–3128

Buchwald H, Varco RL, Matts JP, Long JM, Fitch LL, Campbell JS et al (1990) Effect of partial ileal bypass surgery on mortality and morbidity from coronary heart disease in patients with hypercholesterolemia. Report of the program on the surgical control of the hyperlipidemias (POSCH). N Engl J Med 323:946–955

Business Wire Press Release (2012) AlphaCore reports positive Phase 1 results for ACP-501 (rhLCAT) in patients with stable atherosclerosis. http://www.businesswire.com/news/home/20121009005461/en/AlphaCore-Reports-Positive-Phase-1-Results-ACP-501#.VQiGW

Cai TQ, Guo Q, Wong B, Milot D, Zhang L, Wright SD (2002) Protein-disulfide isomerase is a component of an NBD-cholesterol monomerizing protein complex from hamster small intestine. Biochim Biophys Acta 1581:100–108

Cannon CP, Shah S, Dansky HM, Davidson M, Brinton EA, Gotto AM et al (2010) Safety of anacetrapib in patients with or a high risk for coronary heart disease. N Engl J Med 363:2406–2415

Carballo-Jane E, Chen Z, O'Neill E, Wang J, Burton C, Chang CH et al (2010) Apo A-I mimetic peptides promote pre-beta HDL formation in vivo causing remodeling of HDL and triglyceride accumulation at higher dose. Biorg Med Chem 18:8669–8678

Carlson LA (2005) Nicotinic acid: the broad-spectrum lipid drug. A 50th anniversary review. J Intern Med 258:94–114

Carr DF, O'Meara H, Jorgensen AL, Campbell J, Hobbs M, McCann G et al (2013) Slco1b1 genetic variant associated with statin-induced myopathy: a proof-of-concept study using the clinical practice research datalink. Clin Pharmacol Ther 94:695–701

Catapano AL (2009) Perspectives on low-density lipoprotein goal achievement. Curr Med Res Opin 25:431–447

Chen HC, Farese RV Jr (2000) DGAT and triglyceride synthesis: a new target for obesity treatment? Trends Cardiovasc Med 10:188–192

Chen HC, Ladha Z, Farese RV Jr (2002) Deficiency of acyl coenzyme a: diacylglycerol acyltransferase 1 increases leptin sensitivity in murine obesity models. Endocrinology 143:2893–2898

Chenevard R, Hurlimann D, Spieker L, Bechir M, Enseleit F, Hermann M et al (2012) Reconstituted HDL in acute coronary syndromes. Cardiovasc Ther 30:e51–e57

Chennamsety I, Claudel T, Kostner KM, Baghdasarayan A, Kratky D, Levak-Frank S et al (2011) Farnesoid X receptor represses hepatic human APOA gene expression. J Clin Invest 121:3724–3734

Cholesterol Treatment Triallists' (CTT) Collaboration, Baigent C et al (2010) Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170.000 participants in 26 randomized trials. Lancet 376:1670–1681

Cholesterol Treatment Trialists' (CTT) Collaborators, Kearney PM, Blackwell L, Collins R, Keech A, Simes J, Petro R, Armitage J, Baigent C (2008) Efficacy of cholesterol-lowering in 18,686 people with diabetes in 14 randomised trials of statins: a meta-analysis. Lancet 371:117–125

Cholesterol Treatment Trialists' (CTT) Collaborators, Mihaylova B, Emberson J, Blackwell L et al (2012) The effects of lowering LDL cholesterol with statin therapy in people at low risk of vascular disease: meta-analysis of individual data from 27 randomized trials. Lancet 380:581–590

Cohen JD, Cziraky MJ, Cai Q, Wallace A, Wasser T, Crouse JR, Jacobson TA (2010) 30-Year trends in serum lipids among United States adults: results from the National Health and Nutrition Examination Surveys II, III, and 1999–2006. Am J Cardiol 106:969–975

Colhoun HM, Betteridge DJ, Durrington PN, Hitman GA, Neil HA, Livingstone SJ et al (2004) Primary prevention of cardiovascular disease with atorvastatin in type 2 diabetes in the Collaborative Atorvastatin Diabetes Study (CARDS): multicentre randomized placebo-controlled trial. Lancet 364:685–696

Corrao G, Ibrahim B, Nicotra F, Zambon A, Mertino L, Pasini TS et al (2013) Long-term use of statins reduces the risk of hospitalization for dementia. Atherosclerosis 230:171–176

Cuchel M, Meagher EA, du Toit Theron H, Blom DJ, Marais AD, Hegele RA et al (2013) Efficacy and safety of a microsomal triglyceride transfer protein inhibitor in patients with homozygous familial hypercholesterolaemia: a single-arm, open- label, phase 3 study. Lancet 381:40–46

Curb JD, Abbott RD, Rodriguez BL, Masaki KH, Chen R, Popper JS et al (2004) High density lipoprotein cholesterol and the risk of stroke in elderly men. Am J Epidemiol 160:150–157

Dadu RT, Ballantyne CM (2014) Lipid lowering with PCSK9 inhibitors. Nat Rev Cardiol 11:563–575

Davidson MH, Dillon MA, Gordon B, Jones P, Weiss SJ, Isaacsohn S et al (1999) Colesevelam hydrochloride (cholestagel): a new, potent bile acid sequestrant associated with a low incidence of gastrointestinal side effects. Arch Intern Med 159:1893–1900

Davidson MH, McKenney JM, Shear CL, Revkin JH (2006) Efficacy and safety of torcetrapib, a novel cholesteryl ester transfer protein inhibitor, in individuals with below-average high-density lipoprotein cholesterol levels. J Am Coll Cardiol 48:1774–1781

Davidson MH, Armani A, McKenney JM, Jacobson TA (2007) Safety considerations with fibrate therapy. Am J Cardiol 99:S3–S18

Deveraj S, Autret B, Jialal I (2006) Effects of colesevelam hydrochloride (WelChol) on biomarkers of inflammation in patients with mild hypercholesterolemia. Am J Cardiol 98:641–643

Dias CS, Shaywitz AJ, Wasserman SM, Smith BP, Gao B, Stolman DS et al (2012) Effect of AMG-145 on low-density lipoprotein cholesterol levels: results from 2 randomized. Double-blind, placebo-controlled, ascending-dose phase 1 studies in healthy volunteers and hypercholesterolemic subjects on statins. J Am Coll Cardiol 60:1888–1898

Diditchenko S, Navdaev AV, Spycher MO, Gille A, Wright SD (2014) Mechanism of high density lipoprotein remodeling induced by CSL112. Circulation 130, A15370 (Abstract 15370)

Diditchenko S, Schenk S, Pragst I, Wright S (2012) CSL112, a novel formulation of apolipoprotein A-I exhibits potent anti-inflammatory activity in whole blood. Circulation 126, A11838 (Abstract 11838)

Dietschy JM, Turley SD (2004) Thematic review series: brain lipids. Cholesterol metabolism in the central nervous system during early development and in mature animal. J Lipid Res 45:1375–1397

Dong B, Wu M, Li H, Kraemer FB, Adeli K, Seidah NG et al (2010) Strong induction of PCSK9 gene expression through HNF1-alpha and SREBP2: mechanism for the resistance to LDL-cholesterol lowering effect of statins in dyslipidemic hamsters. J Lipid Res 51:1486–1495

Donovan J, Dunbar R, Biernat L, Logan D, Curtis M, Mancini M et al (2014) Phase 1 single and multiple ascending dose study of CAT-2003, a novel activator of lipoprotein lipase, demonstrates reductions in postprandial triglycerides. J Am Coll Cardiol 63(12):A1450, Abstract A 1450

Dormuth CR, Hemmelgarn BR, Paterson JM, James MT, Teare GF, Raymond CB et al (2013) Use of high potency statins and rates of admission for acute kidney injury: multicenter, retrospective observational analysis of administrative databases. BMJ 346:f880. doi:10.1136/bmj.f880

Downs J, Clearfield M, Weis S, Whitney E, Shapiro DR, Beere PA et al (1998) Primary prevention of acute coronary events with lovastatin in men and women with average cholesterol levels: results of AFCAPS/TexCAPS. Air Force/Texas Coronary Atherosclerosis Prevention Study. JAMA 279:1615–1622

Dubuc G, Tremblay M, Paré G, Jacques H, Hamelin J, Benjannet S et al (2010) A new method for measurement of total plasma PCSK9: clinical applications. J Lipid Res 51:140–149

European Society of Cardiology (ESC) (2013) Press Release September 2, 2013. ASSURE. Effect of an oral agent inducing Apo A-I synthesis on progression of coronary atherosclerosis. http://www.escardio.org/about/press/press-releases/esc13-amsterdam/Pages/hotline-three-assure.aspx

Ewang-Emukowhate M, Wierzbicki AS (2013) Lipid-lowering agents. J Cardiovasc Pharmacol Ther 18:401–411

Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (2001) Executive summary of the third report of the national Cholesterol Education Program (NCEP) (Adult treatment panel III). JAMA 285:2486–2497

Ference BA, Mahajan N (2013) The role of early LDL lowering to prevent the onset of atherosclerotic disease. Curr Atheroscler Rep 15:312. doi:10.1007/s11883-013-0312-1

Fievet C, Staels B (2009) Combination therapy of statins and fibrates in the management of cardiovascular risk. Curr Opin Lipidol 20:505–511

Fitzgerald K, Frank-Kamenetsky M, Shulga-Morskaya S, Liebow A, Bettencourt BR, Sutherland JE et al (2014) Effect of an RNA interference drug on the synthesis of proprotein convertase

subtilisin/kexin type 9 PCSK9) and the concentration of serum LDL cholesterol in healthy volunteers: a randomized, single-blind, placebo- controlled, phase 1 trial. Lancet 383:60–68
Ford ES, Ajani UA, Croft JB et al (2007) Explaining the decrease in U.S. deaths from coronary disease, 1980–2000. N Engl J Med 356:2388–2398
Forrest MJ, Bloomfield D, Briscoe RJ, Brown PN, Cumiskey AM, Ehrhart J et al (2008) Torcetrapib-induced blood pressure elevation is independent of CETP inhibition and is accompanied by increased circulating levels of aldosterone. Br J Pharmacol 154:1465–1473
Förstermann U, Li H (2011) Therapeutic effect of enhancing endothelial nitric oxide synthase (eNOS) expression and preventing enos uncoupling. Br J Pharmacol 164:213–223
Franceschini G, Sirtori CR, Capurso A 2nd, Weisgrabewr KH, Mahley RW (1980) A-IMilano apoprotein. Decreased high density lipoprotein cholesterol levels with significant lipoprotein modifications and without clinical atherosclerosis in an Italian family. J Clin Invest 66:892–900
Frick W, Bauer-Schäfer A, Bauer J, Girbig F, Corsiero D, Heuer W, Kramer W (2003) Synthesis of a biotin-tagged photoaffinity probe of 2-azetidinone cholesterol absorption inhibitors. Biorg Med Chem 11:1639–1642
Gaich G, Chien JY, Fu H, Glass LC, Deeg MA, Holland WL et al (2013) The effects of LY2405319, an FGF21 analog, in obese human subjects with type 2 diabetes. Cell Metab 18:333–340
Garcia-Calvo M, Lisnock J, Bull HG, Hawes BE, Burnett DA, Braun MP et al (2005) The target of ezetimibe is Niemann-Pick C1-Like 1 (NPC1L1). Proc Natl Acad Sci U S A 102:8132–8137
Garner B, Waldeck AR, Witting PK, Rye A, Stocker R (1998) Oxidation of high density lipoproteins. II. Evidence for direct reduction of lipid peroxides by methionine residues of apolipoproteins AI and AII. J Biol Chem 273:6088–6095
Gazi IF, Tsimihodimos V, Tselepis AD, Elisaf M, Mikhailidis DP (2007) Clinical importance and therapeutic modulation of small dense low-density lipoprotein particles. Expert Opin Biol Ther 7:53–72
Gille A, Easton R, Wright S, Shear C (2012) CSL112, a novel formulation of human apolipoprotein A-I, dramatically increases cholesterol efflux capacity in healthy subjects: a placebo-controlled, double-blinded, randomized single ascending dose study. Circulation 126, A11855 (Abstract 11855)
Gille A, Easton R, D'Andrea D, Wright SD, Shear CL (2014) CSL112 enhances biomarkers of reverse cholesterol transport after single and multiple infusions in healthy subjects. Arterioscler Thromb Vasc Biol 34:2106–2114. doi:10.1161/ATVBAHA.114.303720
Goldberg RB, Fonseca VA, Truitt KE, Jones MR (2008) Efficacy and safety of colesevelam in patients with type 2 diabetes mellitus and inadequate glycemic control receiving insulin-based therapy. Arch Intern Med 168:1531–1540
Goldberg D, Appt S, Morrel E, Friden P, Kaplan J, Anantharamaiah GM, Register T (2014) Effect of apolipoprotein E mimetic (AEM-28) on lipoprotein metabolism in cynomolgus monkeys. Circulation 130, A11348 (Abstract 11348)
Gordon T, Castelli W, Hjortland MC, Kannel WBm Dawber TR (2000) High density lipoprotein as a protective factor against coronary heart disease. The Framingham Study. Am J Med 62:707–714
Goyal P, Igel LI, LaScalea K, Borden WB (2014) Cardiometabolic impact of non- statin lipid lowering therapies. Curr Atheroscler Rep 16:390. doi:10.1007/s11883- 013-0390-0
Graham I, Atar D, Borch-Johnsen K, Boysen G, Burell G, Cifkova R et al (2007a) European guidelines on cardiovascular disease prevention in clinical practice: executive summary. Fourth joint task force of the european society of cardiology and other societies on cardiovascular prevention in clinical practice (constituted by representatives of nine societies and by invited experts). Eur Heart J 28:2375–2414
Graham I, Atar D, Borch-Johnsen K, Boysen G, Burell G, Cifkova R et al (2007b) European guidelines on cardiovascular disease prevention in clinical practice: full text. Fourth Joint Task Force of the European Society of Cardiology and other societies on cardiovascular disease

prevention in clinical practice (constituted by representatives of nine societies and by invited experts). Eur J Cardiovasc Prev Rehabil 14(Suppl 2):S1–S113
Grunfeld C, Feingold KR (2008) HDL and innate immunity: a tale of two apolipoproteins. J Lipid Res 49:1605–1606
Gullberg H, Rudling M, Salto C, Forest D, Angelin B, Vennström B (2002) Requirement for thyroid hormone receptor beta in T3 regulation of cholesterol metabolism in mice. Mol Endocrinol 16:1767–1777
Hanai J, Cao P, Tanksale P, Imamura S, Koshimizu E, Zhao J et al (2007) The muscle-specific ubiquitin ligase atrogin-1/MAFbx mediates statin-induced muscle toxicity. J Clin Invest 117:3940–3951
Handelsman Y, Goldberg RB, Garvey WT, Fonseca VA, Rosenstock J, Jones MR et al (2010) Colesevelam hydrochloride to treat hypercholesterolemia and improve glycemia in prediabetes: a randomized, prospective study. Endocr Pract 16:617–628
Hansen GH, Pedersen J, Niels-Christiansen LL, Immerdal L, Danielsen EM (2003) Deep-apical tubules: dynamic lipid-raft microdomains in the brush-border region of enterocytes. Biochem J 373:125–132
Heart Protection Study Collaborative Group (2002) MRC/BHF Heart protection study of cholesterol lowering with simvastatin in 20 536 high risk individuals: a randomized placebo-controlled trial. Lancet 360:7–22
Henwood JM, Heel RC (1988) Lovastatin. A preliminary review of its pharmacodynamic properties and therapeutic use in hyperlipidaemia. Drugs 36:429–454
Homepage AFFiRiS AG (2009) Press Release May 27, 2009. Entwicklung einer Atherosklerose-Impfung von EU unterstützt. www.affiris.com/pdf/presse_medien/pressemeldungen/09_Ew
Hou R, Goldberg AC (2009) Lowering low-density lipoprotein cholesterol: statins, ezetimibe, bile acid sequestrants, and combinations: comparative efficacy and safety. Endocrinol Metab Clin North Am 38:79–97
Hsu I, Spinler SA, Johnson NE (1995) Comparative evaluation of the safety and efficacy of HMG-CoA reductase inhibitor monotherapy in the treatment of primary hypercholesterolemia. Ann Pharmacother 29:743–759
Inazu A, Brown ML, Hesler CB, Agellon LB, Koizumi J, Takata K et al (1990) Increased high-density lipoprotein levels caused by a common cholesteryl-ester transfer protein gene mutation. N Engl J Med 323:1234–1238
Investigators AIM-HIGH, Boden WE, Probstfield JL, Anderson T, Chaitman BR, Desvignes-Nickens P, Koprowicz K et al (2011) Niacin in patients with low HDL cholesterol levels receiving intensive statin therapy. N Engl J Med 365:2255–2267
Jackevicius CA, Mamdani M, Tu JV (2002) Adherence with statin therapy in elderly patient with and without acute coronary syndromes. JAMA 288:462–467
Jonas MC, Costantini C, Puglielli L (2008) PCSK9 is required for the disposal of non- acetylated intermediates of the nascent membrane protein BACE1. EMBO Rep 9:916–922
Jones D (2011) The long march of antisense. Nat Rev Drug Discov 10:401–402
Julius U, Fischer S (2013) Nicotinic acid as a lipid-modifying drug – a review. Atheroscler Suppl 14:7–13
Kamanna VS, Ganji SH, Kashyap ML (2009) The mechanism and mitigation of niacin-induced flushing. Int J Clin Pract 63:1369–1377
Kamanna VS, Ganji SH, Kashyap ML (2013) Recent advances in niacin and lipid metabolism. Curr Opin Lipidol 24:239–245
Kane JP, Malloy MJ, Ports TA, Phillips NR, Diehl JC, Havel RJ (1990) Regression of coronary atherosclerosis during treatment of familial hypercholesterolemia with combined drug regimens. JAMA 264:3007–3012
Kast HR, Nguyen CM, Sinal CJ, Jones SA, Lafitte BA, Reue K et al (2001) Farnesoid X-activated receptor induces apolipoprotein C-II transcription: a molecular Mechanism linking plasma triglyceride levels to bile acids. Mol Endocrinol 15:1720–1728

Kastelein JJP (2015) High density lipoprotein: treatment target. www.pace-cme.org/legacy/files/Kastelein.pptx

Kastelein JJ, van Leuven SI, Burgess L, Evans GW, Kuizenhoven JA, Barter PJ et al (2007) Effect of torcetrapib on carotid atherosclerosis in familial hypercholesterolemia. N Engl J Med 356:1620–1630

Kastelein JJ, Akdim F, Stroes ES, Zwinderman AH, Bots ML, Stalenhoef AF et al (2008) Simvastatin with or without ezetimibe in familial hypercholesterolemia. N Engl J Med 358:1431–1443

Keyserling CH, Hunt TL, Klepp HM, Scott RA, Barbaras R, Schwendeman A et al (2011) CER-001, a synthetic HDL-mimetic, safely mobilizes cholesterol in healthy dyslipidemic volunteers. Circulation 124(A):15525

Kharitonenkov A, Shiyanova TL, Koester A, Ford AM, Nicanovic R, Galbreath EJ et al (2005) FGF-21 as a novel metabolic regulator. J Clin Invest 115:1627–1635

Kharitonenkov A, Wroblewski VJ, Koester A, Chen YF, Clutinger CK, Tigno XT et al (2007) The metabolic state of diabetic monkeys is regulated by fibroblast growth factor-21. Endocrinology 148:774–781

Kingwell BA, Chapman MJ, Kontush A, Miller NE (2014) HDL-targeted therapies: progress, failures and future. Nat Rev Drug Discov 13:445–464

Koizumi J, Mabuchi H, Yoshimura A, Michishita I, Takeda I, Takeda M, Itoh H et al (1985) Deficiency of cholesteryl-ester transfer activity in patients with familial hyperalphalipoproteinaemia. Atherosclerosis 58:175–186

Kolski B, Tsimikas S (2012) Emerging therapeutic agents to lower lipoprotein (a) levels. Curr Opin Lipidol 23:560–568

Konrad RJ, Troutt JS, Cao G (2011) Effects of currently prescribed LDL-C lowering drugs on PCSK9 and implications for the next generation of LDL-C-lowering agents. Lipids Health Dis 10:38–47

Kootte RS, Smits LP, van der Valk FM, Dasseux JL, Keyserling CH, Barbaras R et al (2015) Effect of open-label infusion of an apoA-I-containing particle (CER-001) on RCT and artery wall thickness in patients with FHA. J Lipid Res 56:703–712

Kramer W, Girbig F, Corsiero D, Pfenninger A, Frick W, Jähne G (2004) Molecular elucidation of intestinal cholesterol absorption. In: Paumgartner G, Leuschner U, Keppler D, Stiehl A (eds) XVIII International Bile Acid Meeting. Stockholm, Sweden, June 17–19, 2004. Springer, Amsterdam, pp 153–167

Kramer W, Girbig F, Corsiero D, Pfenninger A, Frick W, Jähne G et al (2005) Aminopeptidase N (CD13) is a molecular target of the cholesterol absorption Inhibitor ezetimibe in the enterocyte brush border membrane. J Biol Chem 280:1306–1320

Kurreck J (2003) Antisense technologies. Improvement through novel chemical modifications. Eur J Biochem 270:1628–1644

Labonte P, Begley S, Guevin C, Asselin MC, Nassoury N, Mayer G et al (2009) PCSK9 impedes hepatitis C virus infection in vitro and modulates liver CD81 expression. Hepatology 50:17–24

Ladenson PH, Kristensen JD, Ridgeway EC, Olsson AG, Carlsson B, Klein I et al (2010) Use of the thyroid hormone analogue eprotirome in statin-treated dyslipidemia. N Engl J Med 362:906–916

Lambert G, Sjouke B, Choque B, Kastelein JJ, Hovingh GK (2012) The PCSK9 decade. J Lipid Res 53:2515–2524

Lamon-Fava S, Diffenderfer MR, Barrett PH, Buchsbaum A, Nyaku M, Horvath KV et al (2008) Extended-release niacin alters the metabolism of plasma apolipoprotein (Apo) A-I and ApoB-containing lipoproteins. Arteriscler Thromb Vasc Biol 28:1672–1678

Larach DB, Cuchel M, Rader DJ (2013) Monogenic causes of elevated HDL cholesterol and implications for development of new therapeutics. Clin Lipidol 8:635–648

LaRosa JC, Grundy SM, Waters DD, Shear C, Barter P et al (2005) Intensive lipid lowering with atorvastatin in patients with stable coronary disease. N Engl J Med 352:1425–1435

Lauring B, Taggart AK, Tata JR, Dunbar R, Caro L, Cheng K et al (2012) Niacin lipid efficacy in independent of both the niacin receptor GPR109A and free fatty acid suppression. Sci Transl Med 4:1–10

Law MR, Wald NJ, Rudnicka AR (2003) Quantifying effect of statins on low density lipoprotein cholesterol, ischaemic heart disease, and stroke: a systematic review and meta-analysis. BMJ 2003:1423–1429

Leuschen J, Mortensen EM, Frei CR, Mansi EA, Panday V, Mansi I (2013) Association of statin use with cataracts: a propensity score-matched analysis. JAMA Opthalmol 131:1427–1434

Liu M, Wu G, Baysarowich J, Kavana M, Addona GH, Bierilo GH, Bierilo KK et al (2010) (2010) PCSK9 is not involved in the degradation of LDL receptors and BACE1 in the adult mouse brain. J Lipid Res 51:2611–2618

Liu Q, Siloto RM, Lehner R, Stone SJ, Weselake RJ (2012) Acyl-CoA:diacylglycerol acyltransferase: molecular biology, biochemistry and biotechnology. Prog Lipid Res 51 (4):350–377

Lloyd-Jones D, Adams RJ, Todd M, Carnethon M, Dai S, De Simone G et al (2010) Heart disease and stroke statistics: 2010 update – from the American Heart Association. Circulation 121:948–954

Mann N (2000) Dietary lean red meat and human evolution. Eur J Nutr 39:71–79

Martin MJ, Hulley SB, Browner WS, Kuller LH, Wentworth D (1986) Serum cholesterol, blood pressure and mortality: implications from a cohort of 361,662 men. Lancet 2:933–936

Martinez-Hervas S, Carmena R, Ascaso JF (2011) Significance of LDL-C lowering therapy in diabetic patients. Clin Lipidol 6:389–399

Martini C, Trapani I, Narciso I, Marino M, Prentalance A, Pallotini V (2009) 3-Hydroxy-3-methylglutaryl coenzyme a reductase increase is essential for rat muscle differentiation. J Cell Physiol 220:524–530

Massaro M, Zampolli A, Scoditti E, Carluccio MA, Storelli C, Distante A et al (2010) Statins inhibit cyclooxygenase-2 and matrix metalloproteinase-9 in human endothelial cells: anti-angiogenic actions possibly contributing to plaque stability. Cardiovasc Res 86:311–320

Mayne J, Dewpura T, Raymond A, Cousins M, Chaplin A, Lahey KA et al (2008) Plasma PCSK9 levels are significantly modified by statins and fibrates in humans. Lipids Health Dis 7:22. doi:10.1186/1476-511X-7-22

Mbikay M, Sirois F, Mayne J, Wang GS, Chen A, Dewpura TJ et al (2010) PCSK9-deficient mice exhibit impaired glucose tolerance and pancreatic islet abnormalities. FEBS Lett 584:701–706

McGill HC Jr, McMahan CA, Zieske AW et al (2000) Association of coronary heart disease risk factors with microscopic qualities of coronary atherosclerosis in youth. Circulation 102:374–379

McKenney JM, Davidson MH, Shear CL, Revkin JH (2006) Efficacy and safety of torcetrapib, a novel cholesteryl ester transfer protein inhibitor, in individuals with below- average high-density lipoprotein cholesterol levels on a background of atorvastatin. J Am Coll Cardiol 48:1782–1790

McNamara JJ, Molot MA, Stremple JF, Cutting RT (1971) Coronary artery disease in combat casualties in Vietnam. JAMA 216:1185–1187

Melchiorri D, Pani L, Gasparini P, Cossu G, Ancans J, Borg JJ et al (2013) Regulatory evaluation of Glybera in Europe – two committees, one mission. Nat Rev Drug Discov 12:719. doi:10.1038/nrd3835-c1

Mendis S, Puska P, Norrving B, World Health Association (2011) Global Atlas on cardiovascular disease prevention and control: policies, strategies, and intervention. World Health Organization

Merki E, Graham M, Taleb A, Leibundgut G, Yang X, Miller ER et al (2011) Antisense oligonucleotide lowers plasma levels of apolipoprotein (a) and lipoprotein (a) in transgenic mice. J Am Coll Cardiol 57:1611–1621

Miyosawa K, Watanabe Y, Murakami T, Desui D, Matsumoto I, Tanabe S, Aikawa M (2012) A novel CETP inhibitor. K-312, suppresses PCSK9 expression through the modulation of its promoter activity. Circulation 126:A13436, Abstract A 13436

Mullard A (2012) Cholesterol-lowering blockbuster candidates speed into phase III trials. Nat Rev Drug Discov 11:817–819

Musunuru K, Pirruccello JP, Do R, Peloso GM, Guiducci C, Sougnez C et al (2010) Exome sequencing, ANGPTL3 mutations, and familial combined hypolipidemia. N Engl J Med 363:2220–2227

Myers CD, Serrano-Wu M, Amer A, Chen J, Rocheford E, Commerford R et al (2012a) The DGAT1 inhibitor Pradigastat decreases chylomicron secretion and prevents postprandial triglyceride elevation in humans. J Clin Lipidol 6(3):285, Abstract 180

Myers CD, Gaudet D, Tremblay K, Amer A, Chen J, Aimin F (2012b) The DGAT1 inhibitor LCQ908 decreases triglyceride levels in patients with the Familial Chylomicronemia Syndrome. J Clin Lipidol 6(3):266, Abstract 128

National Cholesterol Education Program (2002) Third report of the National Cholesterol Education Program (NCEP) Expert Panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adults Treatment Panel III): final report. Circulation 106:3143–3421

National Clinical Guideline Centre (2014) NICE guideline CG67 (update) lipid modification. http://nice.org.uk/guidance/index.jsp?action=download&o=66547

National Heart Foundation of Australia, The Cardiac Society of Australia and New Zealand (2001) Lipid management guidelines – 2001. Med J Aust 175:S57–S88

Navab M, Anantharamaiah G, Reddy ST, Hama S, Hough G, Grijalva VR et al (2004) Oral D-4 F causes formation of pre-beta high-density lipoprotein and improves high- density lipoprotein-mediated cholesterol efflux and reverse cholesterol transport from macrophages in apolipoprotein E-null mice. Circulation 109:3215–3220

Navab M, Anantharamaiah GM, Reddy S, van Lenten BJ, Datta G, Garber D, Fogelman AM (2006) Potential clinical utility of high-density lipoprotein–mimetic peptides. Curr Opin Lipidol 17:440–444

Neuschwander-Tetri BA, Loomba R, Sanyal AJ, Lavine JE, Van Natta ML, Abdelmalek MF et al (2014) Farnesoid X nuclear receptor ligand obeticholic acid for non- Cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicenter, randomized, placebo-controlled trial. Lancet 385:956–965

Newman WP III, Freedman DS, Voors AW et al (1986) Relation of serum lipoprotein levels and systolic blood pressure to early atherosclerosis: the Bogalusa Heart Study. N Engl J Med 314:138–144

Newton RS, Ballantyne CM, Thompson PD, MacDougall DE, Hanselman JC, Margulies JR et al (2014a) ETC-1002 lowers LDL-cholesterol and is well tolerated in hypercholesterolemic patients across four Phase 2a studies. J Clin Lipidol 8:339, Abstract 155

Newton RS, Thompson PD, MacDougall DE, Margulies JR, Hanselman JC, McBride JS et al (2014b) ETC-1002 lowers LDL-cholesterol and is well tolerated in hypercholesterolemic patients with statin intolerance. J Clin Lipidol 8:340, Abstract 156

Nicholls SJ, Brewer HB, Kastelein JJ, Krueger KA, Wang MD, Shao M et al (2011a) Effects of the CETP inhibitor evacetrapib administered as monotherapy or in combination with statins on HDL and LDL cholesterol: a randomized controlled trial. JAMA 16:2099–2109

Nicholls SJ, Gordon A, Johansson J, Wolski K, Ballantyne CM, Kastelein JJ et al (2011b) Efficacy and safety of a novel inducer of apolipoprotein a-I synthesis in statin-treated patients with stable coronary artery disease a randomized controlled trial. J Am Coll Cardiol 57:1111–1119

Nissen SE, Tsunoda T, Tuzcu EM, Schoenhagen P, Cooper CJ, Yasin M et al (2003) Effect of recombinant ApoA-IMilano on coronary atherosclerosis in patients with acutecoronary syndromes: a randomized controlled trial. JAMA 290:2292–2300

Norata GD, Tibolla G, Catapano AL (2014) PCSK9 inhibition for the treatment of hypercholesterolemia: promises and emerging challenges. Vascul Pharmacol 62:103–111

O'Keefe JH Jr, Cordain L, Harris WH, Moe RM, Vogel R (2004) Optimal low-density lipoprotein is 50–70 mg/dL. J Am Coll Cardiol 43:2142–2146
Omori T (2004) CETi. AVANT. Curr Opin Investig Drugs 5:334–338
Out C, Groen AK, Brufau G (2012) Bile acid sequestrants: more than simple resins. Curr Opin Lipidol 23:43–55
Packard CJ (2009) Lipoprotein-associated phospholipase A2 as a biomarker of coronary heart disease and a therapeutic target. Curr Opin Cardiol 24:358–363
Parhofer KG (2005) Beyond LDL-cholesterol: HDL-cholesterol as a target for atherosclerosis prevention. Exp Clin Endocrinol Diabetes 113:414–417
Parker CR Jr, Carr BR, Simpson ER, MacDonald PC (1983) Decline in the concentration of low-density lipoprotein-cholesterol in human fetal plasma near term. Metabolism 32:919–923
Pearson TA, LaCroix AZ, Mead LA et al (1990) The prediction of midlife Coronary heart disease and hypertension in young adults: the John Hopkins multiple risk equations. Am J Prev Med 6 (2 suppl):23–28
Pencina MJ, Navar-Boggan AM, D'Agostino RB Sr, Williams K, Neely B, Sniderman AD, Peterson ED (2014) Application of new cholesterol guidelines to a population-based sample. N Engl J Med 370:1422–1431
Pierno S, Didonna MP, Cippone V, De Luca A, Pisoni M, Frigeri A et al (2006) Effects of chronic treatment with statins and fenofibrate on rat skeletal muscle: a biochemical, histological and electrophysiological study. Br J Pharmacol 149:909–919
Pinkovsky SL, Filippov S, Srivastava RA, Hanselman JC, Bradshaw CD, Hurley TR et al (2013) AMP-activated protein kinase and ATP-citrate lyase are two distinct molecular targets for ETC-1002, a novel small molecule regulator of lipid and carbohydrate metabolism. J Lipid Res 54:134–151
Plenge JK, Hernandez TL, Weil KM, Poirier P, Grunwald GK, Marcovina SM et al (2002) Simvastatin lowers c-reactive protein within 14 days: an effect independent of low-density lipoprotein cholesterol reduction. Circulation 106:1447–1452
Press Release Alnylam (2015) Alnylam launches "Alnylam 2020" guidance for advancement and commercialization of RNAi therapeutics
Press Release Capstone Therapeutics (2014) Capstone therapeutics announces Phase 1b/2a results from AEM-28 showing safety and biomarker efficacy signals. http://olgc.client.shareholder.com/releasedetail.cfm?ReleaseID=887878
Press Release Cerenis Therapeutics (2014a) Cerenis reports top-line Phase II results for its HDL mimetic CER-001. www.cerenis.com/news.asp
Press Release Cerenis Therapeutics (2014b) Cerenis reports two positive Phase II studies for HDL mimetic CER-001 at EAS. www.cerenis.com/news.asp
Press Release Esperion Therapeutics (2014) Esperion therapeutics announces positive top-line Phase 2b results for ETC-1002, an investigational therapy for patients with hypercholesterolemia
Press Release Idera Pharmaceuticals (2011) Idera presents preclinical data of its gene-silencing oligonucleotides (GSO) at Cell Symposium on regulatory RNAs
Press Release Karo Bio (2012) Karo Bio terminates the eprotirome program. http://karobio.com/investormedia/pressreleaser/pressrelease?pid=639535
Press Release Phenex Pharmaceuticals (2012) Phenex reports successful completion of Phase I studies with FXR-agonist Px-102 and publishes research progress in two independent papers
Press Release Sanofi (2013) Genzyme and ISIS announce the approval of KYNAMRO (mipomersen sodium) injection for the treatment of homozygous familial hypercholesterolemia. http://en.sanofi.com/Images/31810_20130129_KYNAMRO-FDA-APPROVAL_en.pdf
Press Release Sanofi (2015) Sanofi and Regeneron announce positive topline results from first phase 3 trials evaluating monthly dosing of Alirocumab in patients with hypercholesterolemia. Primary efficacy endpoints met in two trials of alirocumab administered every four weeks
Press Release Tekmira (2009) Tekmira pharmaceuticals presents ApoB SNALP and PLK1 SNALP data at leading scientific conference. http://investor.tekmirapharma.com/releases.cfm?Year=&ReleasesType=&Pages

Prospective Studies Collaboration (1995) Cholesterol, diastolic pressure and stroke: 13 000 strokes in 450 000 people in 45 prospective cohorts. Lancet 346:1647–1653

Prueksaritanont T, Zhao JJ, Ma B, Roadcap BA, Tang C, Qiu Y et al (2002a) Mechanistic studies on metabolic interactions between gemfibrozil and statins. J Pharmacol Exp Ther 301:1042–1051

Prueksaritanont T, Subramanian R, Fang X, Ma B, Qiu Y, Lin JH et al (2002b) Glucuronidation of statins in animals and humans: a novel mechanism of statin Lactonization. Drug Metab Dispos 30:505–512

Raal FJ, Santos RD, Blom DJ, Marais AD, Charng MJ, Cromwell WC et al (2010) Mipomersen, an apolipoprotein B synthesis inhibitor, for lowering of LDL cholesterol concentrations in patients with homozygous familial hypercholesterolemia: a randomized, double-blind, placebo-controlled trial. Lancet 375:998–1006

Raal FJ, Honarpor N, Blom DJ, Hovingh GK, Xu F, Scott R et al (2015) Inhibition of PCSK9 with evolocumab in homozygous familial Hypercholesterolemia ((TESLA Part B): a randomized, double-blind, placebo-controlled trial. Lancet 385:341–350

Rader DJ, Hovingh GK (2014) HDL and cardiovascular disease. Lancet 384:618–625

Rana JS, Boeckholdt SM (2010) Should we change our lipid management strategies to focus on non-high-density lipoprotein cholesterol? Curr Opin Cardiol 25:622–626

Reuters (2012) Novel atherosclerosis-reversing, heart disease therapy wins NIH clinical development grant. http://www.reuters.com/article/2012/04/10/idUS118932 + 10-Apr-2012 + BW20120410

Ridker PM (2014) LDL cholesterol: controversies and future directions. Lancet 384:607–614

Robinson JG, Farnier M, Krempf M, Bergeron J, Luc G, Averna M et al (2015) Efficacy and safety of Alirocumab in reducing lipids and cardiovascular events. N Engl J Med 372(16):1489–1499

Roger VL, Go AS, Lloyd-Jones DM, Benjamin EJ, Berr JD, Borden WB et al (2012) Heart disease and stroke statistics—2012 update: a report from the American Heart Association. Circulation 125:e2–e220

Rohatgi A, Khera A, Berry JD, Givens EG, Ayers CR, Wedin KE et al (2014) HDL cholesterol efflux capacity and incident cardiovascular events. N Engl J Med 371:2383–2393

Rosenson RS, Hurt-Camejo E (2012) Phospholipase A2 enzymes and the risk of atherosclerosis. Eur Heart J 33:2899–2909

Rossebo AB, Pedersen TR, Boman K, Brudi P, Chambers JB, Egstrup K, SEAS Investigators et al (2008) Intensive lipid lowering with simvastatin and ezetimibe in aortic stenosis. N Engl J Med 359:1343–1356

Roubtsova A, Munkonda MN, Awan Z, Marcinkiewicz J, Chamberland A, Lazure C et al (2011) Circulating proprotein convertase subtilisin/kexin 9 (PCSK9) regulates VLDLR protein and triglyceride accumulation in visceral adipose tissue. Arterioscler Thromb Vasc Biol 31:785–791

Rozema DB, Lewis DL, Wakefield DH, Wong SC, Klein JJ, Roesch PL et al (2007) Dynamic PolyConjugates for targeted in vivo delivery of siRNA to hepatocytes. Proc Natl Acad Sci U S A 104:12982–12987

Rozman D, Monostory K (2010) Perspectives of the non-statin hypolipidemic agents. Pharmacol Ther 127:19–40

Sacks FM, Expert group on HDL Cholesterol (2002) The role of high-density lipoprotein (HDL) cholesterol in the prevention and treatment of coronary heart disease: expert group recommendations. Am J Cardiol 90:139–143

Sacks FM, Carey VJ, Fruchart J (2010) Combination lipid therapy in type 2 diabetes. N Engl J Med 363:692–694

Samaha FF, McKenney J, Bloedon LT, Sasiela WJ, Rader DJ (2008) Inhibition of microsomal triglyceride transfer protein alone or with ezetimibe in patients with moderate hypercholesterolemia. Nat Clin Cardiovasc Med 5:497–505

Sattar N, Preiss D, Murray HM, Welsh P, Buckley BM, Craen A et al (2010) Statins and the risk of incident diabetes: a collaborative meta-analysis of randomized statin trials. Lancet 375:735–742

Saydah SH, Fradkin J, Cowie CC (2004) Poor control of risk factors for vascular disease among adults with previously diagnosed diabetes. JAMA 291:335–342

Scandinavian Simvastatin Survival Study Group (1994) Randomized trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). Lancet 344:1383–1389

Scanlan TS (2010) A case history of bench-to-clinic drug discovery and development. Heart Fail Rev 15:177–182

Schaefer EJ, Lamon-Fava S, Cohn SD, Schaefer MM, Ordovas JM, Castelli WP et al (1994) Effects of age, gender, and menopausal status on plasma low density lipoprotein cholesterol and apolipoprotein B levels in the Framingham Offspring Study. J Lipid Res 35:779–792

Schectman G, Hiatt J (1996) Drug therapy for hypercholesterolemia in patients with cardiovascular disease: factors limiting achievement of lipid goals. Am J Med 100:197–204

Schwartz GG, Olsson AG, Ballantyne CM, Barter PJ, Holme IM, Kallend D et al (2009) Rationale and design of the dal-OUTCOMES trial: efficacy and safety of dalcetrapib in patients with recent acute coronary syndrome. Am Heart J 158:896–901.e3

Serruys PW, Garcia-Garcia HM, Buszman P, Erne P, Verheye S, Aschermann M et al (2008) Effects of the direct lipoprotein-associated phospholipase A2 inhibitor darapladib on human coronary atherosclerotic plaque. Circulation 118:1172–1182

Sever PS, Dahlöf B, Poulter NR, Wedel H, Beevers G, Caulfield M et al (2003) Prevention of coronary and stroke events with atorvastatin in hypertensive patients who have average or lower-than-average cholesterol concentrations, in the Anglo-Scandinavian Cardiac Outcomes Trial—Lipid Lowering Arm (ASCOT-LLA): a multicentre randomized controlled trial. Lancet 361:1149–1158

Shamburek R, Bakker-Arkema R, Krause B, Auerbach B, Freeman L, Homan R, Asztalos B, Schaefer E (2013) First report of enzyme replacement therapy in a patient with familial LCAT deficiency. Rapid improvement of lipid and clinical manifestations. 81st Congress of The European Atherosclerosis Society, Lyon (France), June 2–5 2013. Abstract 1635

Shepherd J, Cobbe SM, Ford I, Isles CG, Ross Lorimer A, MacFarlane PW, West of Scotland Coronary Prevention Study Group et al (1995) Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. N Engl J Med 333:1301–1307

Shepherd J, Blau GJ, Murphy MB, Bollen EL, Buckley BM, Cobbe SM et al (2002) Pravastatin in elderly individuals at risk of vascular disease (prosper): a randomized controlled trial. Lancet 360:1623–1630

Shibata H, Murakami K, Murakami T, Miyosawa K, Iwashita M, Yamazaki H et al (2012) A novel and potent CETP inhibitor K-312 with PCSK9 inhibitory property, exerts strong reduction of LDL-C and anti-atherosclerotic effects. Circulation 126:A13436, Abstract A 13436

Shitara Y, Sugiyama Y (2006) Pharmacokinetic and pharmacodynamic alterations of 3-hydroxy-methylglutaryl coenzyme A (HMG.CoA) reductase inhibitors: drug-drug interactions and interindividual differences in transporter and metabolic enzyme functions. Pharmacol Rev 112:71–105

Sirtori CR (2014) The pharmacology of statins. Pharmacol Res 88:3–11

Sirtori CR, Calabresi L, Franceschini G, Baldasarre D, Amato M, Johansson J et al (2001) Cardiovascular status of carriers of the apolipoprotein A-I (Milano) mutant: the Limone sul Garda study. Circulation 103:1949–1954

Skov M, Tonnesen CK, Hansen GH, Danielsen EM (2010) Dietary cholesterol induces trafficking of intestinal Niemann-Pick Type C1 Like 1 from the brush border to endosomes. Am J Physiol Gastrointest Liver Physiol 300:G33–G40

Smith SJ, Cases R, Jensen DR, Chen HC, Sande E, Tow B et al (2000) Obesity resistance and multiple mechanisms of triglyceride synthesis in mice lacking dgat. Nat Genet 25:87–90

Stauffer ME, Weisenfluh L, Morrison A (2013) Association between triglycerides and cardiovascular events in primary populations: a meta-regression analysis and synthesis of evidence. Vasc Health Risk Manag 9:671–680

Stein CA, Bo Hansen J, Lai J, Wu S, Voskresenskiy A, Hog A et al (2010) Efficient gene silencing by delivery of locked nucleic acid antisense oligonucleotides, unassisted by transfection reagents. Nucleic Acids Res 38:e3. doi:10.1093/nar/gkp841

Stein EA, Gipe D, Bergeron J, Gaudet G, Weiss R, Dufour R et al (2012a) Effect of a monoclonal antibody to PCSK9, REGN727/SAR236553, to reduce low-density lipoprotein cholesterol in patients with heterozygous familial hypercholesterolemia on stable statin dose with or without ezetimibe therapy: a phase 2 randomized controlled trial. Lancet 380:29–36

Stein EA, Dufour R, Gagne C, Gaudet D, East C, Donovan JM et al (2012b) Apolipoprotein B synthesis inhibition with mipomersen in heterozygous familial hypercholesterolemia: results of a randomized, double-blind, placebo-controlled trial to assess efficacy and safety as add-on therapy in patients with coronary artery disease. Circulation 126:2283–2292

Stone NJ et al (2013) ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the American College of Cardiology/American Heart Association Task Force on practice guidelines. J Am Coll Cardiol 63:2889–2934. doi:10.1016/j.jacc.2013.11.002

Strong JP, McGill HC Jr (1969) The natural history of atherosclerosis. Am J Pathol 40:37–49

Suc I, Escargueil-Blanc I, Troly M, Salvayre R, Negre-Salvayre A (1997) HDL and ApoA prevent cell death of endothelial cells induced by oxidized LDL. Arterioscler Thromb Vasc Biol 17:2158–2166

Suzuki T, Oba K, Igari Y, Matsumura N, Watanabe K, Futami-Suda S, Yasuoka H et al (2007) Colestimide lowers plasma glucose levels and increase plasma glucagon-like PEPTIDE-1 (7–36) levels in patients with type 2 diabetes mellitus complicated by hypercholesterolemia. J Nippon Med Sch 74:338–343

Tachmazidou I, Dedoussis G, Southam L, Farmaki AL, Ritchie GR, Xifara DK et al (2013) A rare functional cardioprotective APOC3 variant has risen in frequency in distinct population isolates. Nat Commun 4:2872. doi:10.1038/ncomms3872

Tall AR (2007) CETP inhibitors increase HDL cholesterol levels. N Engl J Med 356:1364–1366

Tardif JC, Grégoire J, LÁllier PL, Ibrahim R, Lespereance J, Heinonen J, Heinonen TM et al (2007) Effects of reconstituted high-density lipoprotein infusions on coronary atherosclerosis: a randomized controlled trial. JAMA 297:1675–1682

Tardif JC, Ballantyne CM, Barter P, Dasseux JL, Fayad ZA, Guertin MC et al (2014) Effects of the high-density lipoprotein mimetics agent CER-001 on coronary atherosclerosis in patients with acute coronary syndromes: a randomized trial. Eur Heart J 35:3277–3286

Taub R, Chiang E, Chabot-Blanchet M, Kelly MJ, Reeves RA, Guerin MC, Tardif JC (2013) Lipid lowering in healthy volunteers treated with multiple doses of MGL-3196, a liver-targeted thyroid hormone receptor-β-agonist. Atherosclerosis 230:373–380

Taylor F, Huffman MD, Macedo AF et al (2013) Statins for the primary prevention of cardiovascular disease. Cochrane Database Syst Rev 1, CD004816

Terasaka N, Yu S, Yvan-Charvet L, Wang N, Mzhavia N, Langlois R et al (2008) ABCG1 and HDL protect against endothelial dysfunction in mice fed a high- cholesterol diet. J Clin Invest 118:3701–3713

Teslovich TM, Musunuru K, Smith AV, Edmondson AC, Stylianou IM, Koseki M et al (2010) Biological, clinical and population relevance of 95 loci for blood lipids. Nature 466:707–713

The ACCORD Study Group (2010) Effects of combination lipid therapy in type 2 diabetes mellitus. N Engl J Med 362:1563–1574

The FIELD study Investigators (2005) Effects of long term fenofibrate therapy on cardiovascular events in 9795 people with type 2 diabetes mellitus (the FIELD study): randomized controlled trial. Lancet 366:1849–1861

The HPS2-THRIVE Collaborative Group (2014) Effects of extended-release niacin with Laropiprant in high-risk patients. N Engl J Med 371:203–212

The Lipid Research Clinics Investigators (1992) The lipid research clinics coronary primary prevention trial. Results of 6 years of post-trial follow-up. Arch Intern Med 152:1399–1410
The STABILITY Investigators (2014) Darapladib for preventing ischemic events in stable coronary heart disease. N Engl J Med 370:1702–1711
Thomas C, Gioiello A, Noriega L, Strehle A, Oury J, Rizzo G et al (2009) TGR5-mediated bile acid sensing controls glucose homeostasis. Cell Metab 10:167–177
Tong W, Paradise E, Kim E, Didio K, Schueller O, Ferkany J, Sweetnam P (2010) Clinical investigations of SLx-4090 in combination with metformin in type 2 diabetics. American Diabetes Association 70th Scientific Session 2010, Abstract 651-P
Toth PP (2010) Drug treatment of hyperlipidemia: a guide to the rational use of lipid-lowering drugs. Drugs 70:1363–1379
Toth PP (2011) Antisense therapy and emerging applications for the management of dyslipidemia. J Clin Lipidol 5:441–449
Toth PP, Harper CR, Jacobson TA (2008) Clinical characterization and molecular mechanisms of statin myopathy. Expert Rev Cardiovasc Ther 6:955–969
Vakili L, Hama S, Kim JB, Tien D, Safarpoor S, Ly N et al (2010) The effect of HDL mimetic peptide 4 F on PON1. Adv Exp Med Biol 660:167–172
van Poelgeest EP, Swart RM, Betjes MG, Moerland M, Weening M, Weening JL, Tessier Y et al (2013) Acute kidney injury during therapy with an antisense oligonucleotide directed against PCSK9. Am J Kidney Dis 62(4):796–800
Vigna GB, Fellin R (2010) Pharmacotherapy of dyslipidemia in the adult population. Expert Opin Pharmacother 11:3041–3052
Virani SS, Ballantyne CM (2011) Update on therapies targeting HDL: the mystery continues. Curr Opin Lipidol 22:514–516
Visser ME, Mitzum JL, Stroes ES, Kastelein JJ (2012) Antisense oligonucleotides for the treatment of dyslipidemia. Eur Heart J 33:1451–1458
Vosper H (2011) Extended release niacin-laropiprant in patients with hypercholesterolemia or mixed dyslipidemias improves clinical parameters. Clin Med Insights Cardiol 5:85–101
Wang LJ, Song BL (2012) Niemann-Pick C1-Like 1 and cholesterol uptake. Biochim Biophys Acta 1821:964–972
Watson CE, Weissbach N, Kjems L, Ayalasomayajula S, Zhang Y, Chang I, Navab M et al (2011) Treatment of patients with cardiovascular disease with L-4 F, an apo A-I mimetic, did not improve select biomarkers of HDL function. J Lipid Res 52:361–373
Welder G, Zineh I, Pacanowski MA, Troutt JS, Cao G, Konrad RJ et al (2010) High-dose atorvastatin causes a rapid increase in human serum PCSK9 and disrupts its correlation with LDL cholesterol. J Lipid Res 51:2714–2721
Werner C, Hoffmann MM, Winkler K, Bohm M, Laufs U (2014) Risk prediction with proprotein convertase subtilisin/kexin type 9 (PCSK9) in patients with stable coronary disease on statin treatment. Vascul Pharmacol 62:94–102
Wijeysundera HC, Machado M, Farahati F et al (2010) Association of temporal trends in risk factors and treatment uptake with coronary heart disease mortality, 1994–2005. JAMA 303:1841–1847
Xie Q, Zhao SP, Li F (2010) D-4 F, an apolipoprotein A-I mimetic peptide, promotes cholesterol efflux from macrophages via ATP-binding cassette transporter A1. Tohoku J Exp Med 220:223–228
Yeager CL, Ashmun RA, Williams RK, Caredllichio CB, Shapiro LH, Look AT, Holmes KV (1992) Human aminopeptidase N is a receptor for human coronavirus 229E. Nature 357:420–422
Yuan G, Wang J, Hegele RA (2006) Heterozygous familial hypercholesterolemia: an underrecognized cause of early cardiovascular disease. CMAJ 174:1124–1129
Yusuf S, Hawken S, Ounpuu S et al (2004) Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case–control study. Lancet 364:937–952

Yvan-Charret L, Kling J, Pagler T, Li H, Hubard B, Fisher T et al (2010) Cholesterol efflux potential and antiinflammatory properties of high-density lipoprotein after treatment with niacin or anacetrapib. Arterioscler Thromb Vasc Biol 30:1430–1438

Zaid A, Roubtsova A, Esalmani R, Marcinkiewicz J, Chamberland J, Chamberland A, Hamelin J et al (2008) Proprotein convertase subtilisin/kexin type 9 (PCSK9): hepatocyte-specific low-density lipoprotein receptor degradation and critical role in mouse liver regeneration. Hepatology 48:646–654

Zhang Y, Lee FY, Barrera G, Lee H, Vales C, Gonzalez FJ et al (2006) Activation of the nuclear receptor FXR improves hyperglycemia and hyperlipidemia in diabetic mice. Proc Natl Acad Sci U S A 103:1006–1011

Zhong S, Sharp DS, Grove JS, Bruce C, Yano Km Curb JD et al (1996) Increased coronary heart disease in Japanese-American men with mutation in the cholesteryl ester transfer protein gene despite increased HDL levels. J Clin Invest 97:2917–2923

Zimmer M, Picarella D, Liu F, Bista P, Lee D, Vu C, Jirousek M (2014) CAT-2003 is a novel small molecule that inhibits proprotein convertase subtilisin/kexin type 9 production and lowers non-high-density lipoprotein cholesterol. Arterioscler Thromb Vasc Biol 34:A6, Abstract A6

Current and Emerging Treatment Options in Diabetes Care

Christoffer Clemmensen, Timo D. Müller, Brian Finan, Matthias H. Tschöp, and Richard DiMarchi

Contents

C. Clemmensen • T.D. Müller • B. Finan • M.H. Tschöp (✉)
Institute for Diabetes and Obesity and Helmholtz Diabetes Center, Helmholtz Zentrum München, German Research Center for Environmental Health (GmbH), Neuherberg, Germany

Division of Metabolic Diseases, Department of Medicine, Technische Universität München, Munich, Germany
e-mail: tschoep@helmholtz-muenchen.de

R. DiMarchi
Department of Chemistry, Indiana University, Bloomington, IN, USA

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Abstract

Diabetes constitutes an increasing threat to human health, particularly in newly industrialized and densely populated countries. Type 1 and type 2 diabetes arise from different etiologies but lead to similar metabolic derangements constituted by an absolute or relative lack of insulin that results in elevated plasma glucose. In the last three decades, a set of new medicines built upon a deeper understanding of physiology and diabetic pathology have emerged to enhance the clinical management of the disease and related disorders. Recent insights into insulin-dependent and insulin-independent molecular events have accelerated the generation of a series of novel medicinal agents, which hold the promise for further advances in the management of diabetes. In this chapter, we provide a historical context for what has been accomplished to provide perspective for future research and novel emerging treatment options.

Keywords

Co-agonist · Combination therapies · Diabetes · Glucose · Insulin · Metabolism · Obesity · Pharmacology · Therapeutics

1 Introduction

Globally, diabetes affects more than 387 million people and is an escalating threat to personal health and national economies (Guariguata et al. 2014; IDF 2014). In 2014 alone, ca. 5 million patients died as a consequence of diabetes (IDF 2014). As a result, the development of safe and effective treatment options has become an international enterprise. Type 1 diabetes (T1D, representing ca. 10% of diabetes cases) and type 2 diabetes (T2D, representing ca. 90% of diabetes cases) constitute the majority of the disease and are generally viewed as two different, yet biologically related disorders. T1D is an autoimmune disease with a prominent genetic component, and T2D is an age- and lifestyle-related disease associated with obesity and inactivity (Kahn et al. 2006; van Belle et al. 2011). Despite having different etiologies, T1D and T2D lead to similar metabolic dysfunctions and long-term complications. One hallmark of diabetes is an absolute or relative lack of insulin, which leads to an increase in plasma glucose levels. If left uncontrolled, diabetes induces multiple acute and chronic complications such as ketoacidosis, kidney failure, heart disease, retinopathy, and various vascular complications (Kahn et al. 2006; van Belle et al. 2011).

T2D currently accounts for ~90% of diabetic cases (Scully 2012) and most T2D patients will eventually require insulin replacement therapy at a later stage of the disease. A deeper molecular understanding of T2D pathophysiology has facilitated a number of medicinal strategies that hold promise to prevent, intervene in, or halt the progression of the disease. Substantial evidence implicates insulin-independent mechanisms with an array of circulating factors, as well as the brain's powerful

glucoregulatory control in glucose disposal as part of the disease (Schwartz et al. 2013). These insights, combined with a deeper understanding of insulin-dependent and insulin-independent molecular events, have accelerated the generation of novel pharmacotherapies for the treatment of T2D. The aim of this chapter is to present a mechanism-based analysis of the therapeutic benefits and pitfalls associated with different classes of medicines for both types of diabetes and an orientation to novel emerging treatment options.

2 Regulation of Glucose Metabolism

2.1 Peripheral Control of Glucose Metabolism

For almost a century, research on glucose homeostatic processes has predominantly focused on the role of peripheral control mechanisms, most notably the role of pancreatic islets as the key organ for regulating glycemic control (Weir and Bonner-Weir 2004). The prevailing dogma is that a meal-induced rise in blood glucose stimulates beta cells in the endocrine pancreas to secrete insulin. Insulin lowers this postprandial glucose surge by acting on the energy-storing organs, such as skeletal muscle and adipose tissue, to facilitate uptake of glucose and to suppress glucose output via inhibition of hepatic gluconeogenesis (Fig. 1a). Conversely, in fasted and hypoglycemic states, the pancreatic alpha cells secrete glucagon, which stimulates hepatic glucose production and opposes the actions of insulin. Under non-diseased physiological conditions, these processes efficiently maintain blood glucose levels within a relatively narrow and stable range (Unger and Cherrington 2012).

Half a century ago, it was discovered that oral ingestion of glucose elicits an enhanced insulin response relative to that of an intravenous glucose infusion (Elrick et al. 1964; McIntyre et al. 1964) This observation, subsequently termed “the incretin effect,” introduced the gut as a metabolically relevant endocrine organ and led to the identification and glucoregulatory impact of many gut-derived peptides (Baggio and Drucker 2007). Thus, in the 1970s and 1980s, the most prominent incretin hormones glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) were identified and their ability to augment glucose metabolism delineated (Dupre et al. 1973; Schmidt et al. 1985). Both GIP and GLP-1 are secreted from the gut in response to ingested nutrients and exhibit insulinotropic actions at pancreatic beta cells, contributing to postprandial glucose homeostasis (Fehmann et al. 1995).

In addition to insulin, glucagon, and the incretin hormones, other humoral factors including epinephrine (adrenaline), glucocorticoids, and growth hormone can impact glucose homeostasis (Gerich 1993). More recently, the field has enlarged with the realization of the glucoregulatory role of an array of more recently discovered factors including fibroblast growth factors (FGFs) (Kharitonenkov et al. 2005), cytokines (Fernandez-Real et al. 1998), and peptides

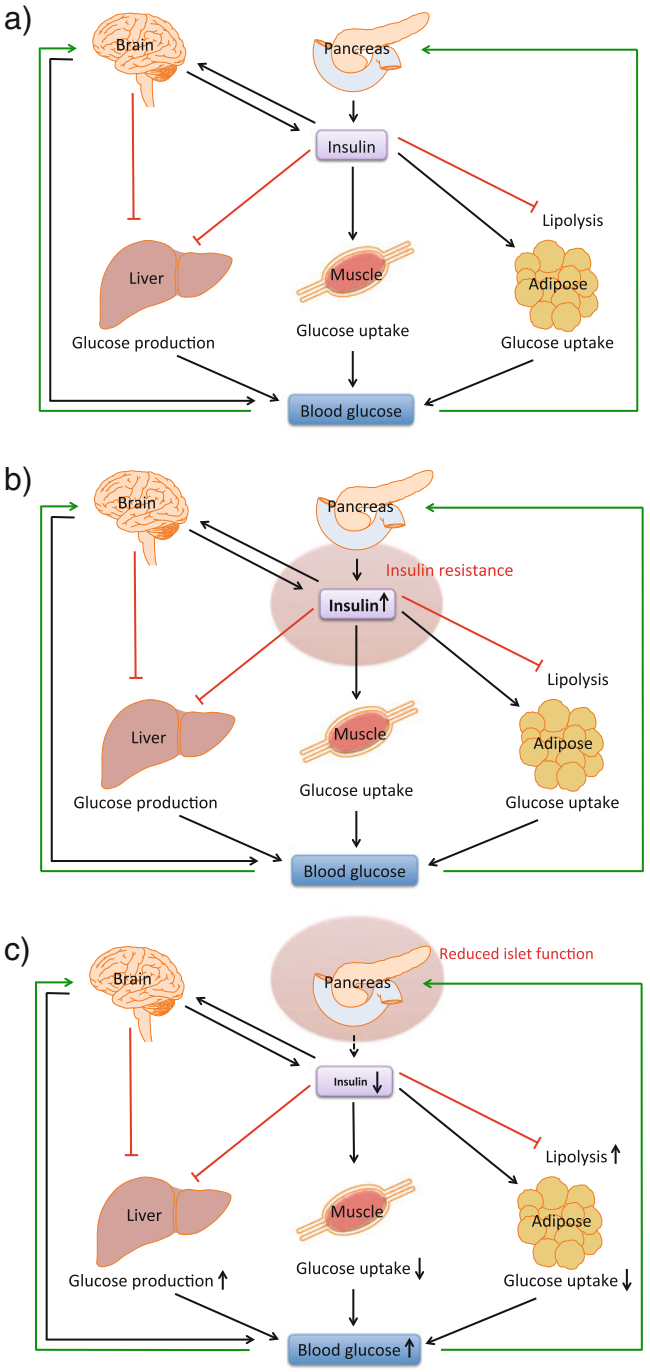

Fig. 1 Schematic overview of normal and pathological glucose homeostasis. Plasma glucose levels are regulated by coordinated interactions between brain- and islet-related mechanisms, involving both insulin-dependent and insulin-independent pathways. (**a**) Under normal conditions,

secreted from muscle (Steensberg et al. 2000), fat (Hotta et al. 2001), and bone (Booth et al. 2013).

2.2 Central Control of Glucose Metabolism

A growing body of evidence has established that the brain directly affects glucose homeostasis through both insulin-dependent and insulin-independent mechanisms (Fig. 1a) (Kleinridders et al. 2014; Schwartz et al. 2013). The mechanisms underlying the ability of centrally acting hormones to lower blood glucose in diabetic animal models are still under investigation but hypothesized to implicate lowering of hepatic glucose production while increasing glucose uptake in skeletal muscle and brown adipose tissue (Rojas and Schwartz 2014; Schwartz et al. 2013). Thus, glucose homeostasis is likely controlled by complex and coordinated interactions between brain-, gut-, and islet-related biological systems. Importantly, as indicated above, our understanding of how factors secreted from other peripheral tissues feed into the major glucoregulatory systems is now starting to be revealed.

3 Pathogenesis and Pathophysiology of Diabetes

3.1 Type 1 Diabetes

T1D is an autoimmune disorder in which the insulin-producing beta cells of the pancreas are selectively destroyed by autoreactive T cells (van Belle et al. 2011). The autoreactive T cells have been shown to recognize islet autoantigens including insulin, glutamic acid decarboxylase (GAD), and zinc transporter 8 (ZnT8) (Bluestone et al. 2010). Eventually, the depleted pancreatic beta-cell function cannot sustain sufficient insulin to maintain euglycemia, and the patients ultimately require insulin replacement therapy. The etiology and pathophysiology of the autoimmunity preceding the diagnosis of T1D are influenced by a combination of genetic and environmental factors (van Belle et al. 2011). Despite a growing understanding of T1D pathogenesis, the driving immune triggers orchestrating the attack of the beta cells remain enigmatic. Autoantibodies can be detected before the clinical onset of T1D. However, the gap between early biochemical alterations and the clinical manifestation complicates the elucidation of causative environmental triggers (van Belle et al. 2011). Until now, environmental triggers proposed to be involved

Fig. 1 (continued) rising plasma glucose levels elicit pancreatic insulin secretion. Insulin then stimulates glucose uptake in adipose tissue and skeletal muscle and suppresses hepatic glucose production. (**b**) Under insulin-resistant conditions, the islets increase insulin secretion in a compensatory manner to maintain glucose homeostasis. (**c**) Loss of beta-cell mass and functionality prevents the necessary insulin secretion needed to overcome the insulin resistance resulting in hyperglycemia and type 2 diabetes

in the disease pathogenesis include viruses, bacteria, and nutrients (Knip et al. 2005). Unraveling how these stimuli might interact with specific molecular targets to initiate the autoimmune cascade is crucial for intervening as early as possible in order to preserve functional beta-cell mass.

3.2 Type 2 Diabetes

Historically, T2D was considered an age-related disease linked to a sedentary lifestyle and hypercaloric diet. It is now acknowledged that genetic factors also play a prominent role for the onset and progression of the disease (Kahn et al. 2012). T2D is a progressive disorder with a pathogenesis that involves a reciprocal interplay of persistent increases in insulin demand and its subsequent production. Insulin resistance is the most well-defined pathological gateway to T2D (Martin et al. 1992) and frequently coincides with excess adipose tissue mass and ectopic lipid deposition in tissues involved in glucose disposal (Kahn et al. 2006). Insulin resistance results from a reduced response of cells in adipose tissue and skeletal muscle to stimulate insulin-mediated glucose uptake as well as a blunted response of cells in the liver to shut down hepatic glucose production. Under normal circumstances, pancreatic beta cells balance the loss of insulin sensitivity by increasing insulin production and release (Fig. 1b). This compensation by pancreatic beta cells often prevents hyperglycemia despite the prevailing insulin-resistant state. However, it is only upon failure of beta cells to fully compensate for the increased insulin demand that hyperglycemia and T2D ensue (Fig. 1c) (Kahn 2003). This loss of beta-cell plasticity is not solely a consequence of cellular loss but also reflects reduced functionality and an impaired response to insulin secretagogues (Kahn 2003). In parallel, without insulin to act as a brake on glucagon secretion from pancreatic alpha cells, elevated glucagon levels and hepatic insulin resistance lead to uncontrolled hepatic glucose production (Fig. 1c). These reciprocal events intensify the metabolic rearrangements and an ever-escalating glucotoxicity that eventually exhausts beta-cell function to amplify the disease cascade (D'Alessio 2011). Additionally, the altered islet biology may impact the glucoregulatory capacity of the brain, which may be further deranged in obese subjects in which central leptin resistance coincides with hampered insulin control (Morton and Schwartz 2011). Ultimately, late-stage, insulin-deficient T2D patients require insulin supplementation to maintain euglycemia.

4 Current Treatments for Diabetes

The primary goal of antidiabetic treatment is to restore or improve glucose control. Hemoglobin A1c (HbA1c) is a biochemical marker that reflects chronic improvements in plasma glucose levels and is frequently employed for the clinical evaluation of therapeutic efficacy (Bonora and Tuomilehto 2011). As outlined above, T2D manifests in numerous states of impaired insulin function, and it is

the failure of the beta cells to secrete sufficient insulin to compensate for the defect that results in hyperglycemia. Accordingly, drugs that can enhance insulin sensitivity as well as compounds that can amplify insulin secretion may serve to improve glycemic control (Cefalu 2007). Current antidiabetic pharmacotherapy primarily consists of insulin, biguanides, sulfonylureas, thiazolidinediones, alpha-glucosidase inhibitors, incretin enhancers, GLP-1 analogs, amylin analogs, sodium-glucose co-transporter 2 inhibitors (SGLT2 inhibitors), and bile acid sequestrants. This multitude of antidiabetic therapeutics allows for a degree of personalized treatment that can be tailored to the glycemic status of the each patient. However, each class of drugs is associated with specific efficacy shortcomings and safety concerns that need to be accounted for when selecting a pharmacotherapy. Furthermore, diabetics (in particular T2D) frequently suffer from comorbidities such as cardiovascular disease and obesity, which may complicate treatment and limit therapeutic options.

Insulin replacement therapy is indispensable for T1D patients. Also, patients suffering from T2D may eventually require exogenous insulin to maintain glycemic control (Fonseca and Haggar 2014). Much progress has been made since the initial discovery of insulin. Insulin analogs with diverse pharmacokinetic properties are now available and employed to tailor individualized regiments in personalizing glycemic control (Fonseca and Haggar 2014). Insulin-induced hypoglycemia is typically not a risk factor for diabetics suffering from insulin resistance, and for T1D patients, the development of insulin analogs with more "peakless" profiles has helped to lower the risk of treatment-induced hypoglycemia (Fonseca and Haggar 2014). Insulin is frequently employed to support the therapeutic efficacy of other antidiabetic compounds including metformin, TZDs, and incretin-based therapies (Barnett 2013; Wulffele et al. 2002). The pharmacological efficacy of these compounds may be significantly hampered if sufficient insulin is not available to support their independent molecular action.

Having the highest benefit-risk profile compared to other available medications, metformin is the most frequently used biguanide and the first-in-line oral therapy for treating T2D (Bennett et al. 2011). Metformin reduces fasting glucose levels by inhibiting hepatic glucose output and stimulating uptake and utilization of glucose in skeletal muscle (Bailey and Turner 1996; Viollet et al. 2012). The underlying cellular mechanisms of action are being investigated but remain somewhat elusive to date (Viollet et al. 2012). Metformin is often used in combination with drugs that can complement its pharmacological profile, such as insulin secretagogues or insulin sensitizers (Bennett et al. 2011). Interestingly, diabetics treated with metformin have a relatively lower risk of developing cancers as compared to patients treated with insulin or sulfonylureas (Bowker et al. 2006). This protective effect is sustained in combination therapies involving metformin (Currie et al. 2009). The most common adverse effects associated with metformin treatment are dose-related gastrointestinal disturbances.

Thiazolidinediones (TZDs) bind to and activate the peroxisome proliferator-activated receptor gamma (PPARγ) to enhance insulin sensitivity and reduce hyperglycemia (Hauner 2002; Saltiel and Olefsky 1996). TZDs exert a number of pleiotropic effects, such as reducing circulating levels of pro-inflammatory cytokines

and increasing adiponectin levels, which may add to the insulin-sensitizing effects associated with their usage (Defronzo et al. 2013; Hauner 2002; Tonelli et al. 2004). However, PPARγ is abundantly expressed in fat cells (also in the muscle and liver), and activation by TZDs initiates lipogenic transcriptional signaling and the most common adverse effect associated with TZDs – weight gain (Fonseca 2003; Smith et al. 2005). Further, an increased risk of congestive heart failure has been associated with the use of TZDs (Hernandez et al. 2011). The FDA has approved adjunctive therapy with TZDs in combination with metformin, insulin, sulfonylureas, and glinides (Derosa and Sibilla 2007; Fuchtenbusch et al. 2000).

Sulfonylureas and glinides improve glycemia by enhancing insulin secretion (Blickle 2006; Proks et al. 2002). Both compounds bind to an ATP-dependent K+ channel, albeit at different sites, expressed on the pancreatic beta-cell membrane. This leads to a membrane depolarization and calcium-mediated insulin secretion (Melander 2004; Proks et al. 2002). The major adverse risk associated with their usage is hypoglycemia (Melander 2004). Moreover, as with TDZs, sulfonylureas and glinides stimulate adiposity and lead to weight gain (Liu et al. 2012).

Inhibitors of dipeptidyl peptidase-IV (DPP-IV), the enzyme responsible for degrading GLP-1, are referred to as incretin enhancers, whereas incretin mimetics refers to the group of synthetic analogs of GLP-1. GLP-1 signals through its receptor on pancreatic beta cells to promote glucose-stimulated insulin secretion. Unlike sulfonylureas, which cause nonspecific insulin secretion, there is little hypoglycemic risk with treatment of incretin-based therapies. They only promote glucose-stimulated insulin secretion, thus offering an internal buffering capacity due to their mechanism of action. While GLP-1 analogs promote clinically relevant, albeit modest, weight loss, DPP-4 inhibitors present a weight-neutral profile (Foley and Jordan 2010; Nathan et al. 2009). GLP-1R agonists may improve cardiovascular risk factors; however, dose-dependent adverse gastrointestinal events and nausea are linked to their usage (Aroda and Ratner 2011; Kanoski et al. 2012).

Alpha-glucosidase is an enzyme involved in the intestinal degradation of complex carbohydrates. Specific enzyme inhibitors protect against postprandial hyperglycemia by delaying carbohydrate absorption in the proximal gut (Lebovitz 1997). However, the interference with nutrient absorption induces gastrointestinal side effects, which have limited their usage. Further, the impact on HbA1c levels is modest, and the alpha-glucosidase inhibitors are less effective in lowering glycemia than metformin and sulfonylureas (Bolen et al. 2007; van de Laar et al. 2005).

The peptide amylin is synthesized in the pancreatic beta cells and co-secreted with insulin in response to a meal (Butler et al. 1990; Moore and Cooper 1991). The administration of amylin analogs is purported to inhibit glucagon secretion from the islet alpha cells leading to a decrease in postprandial glucose excursions (Kruger and Gloster 2004). The reduction in glucagon secretion assists in attenuating hepatic glucose production. Further, amylin analogs slow gastric emptying, elicit hypophagia, and are associated with weight loss (Roth 2013). The effect of amylin-based therapy as measured by HbA1c lowering is modest (Ratner et al. 2004). Consequently, amylin has been approved as adjunctive therapy with insulin for patients who have not achieved glycemic control with insulin monotherapy (Ryan et al. 2005;

Weyer et al. 2001). Amylin decreases body weight in both diabetics and nondiabetics and is currently being investigated for its antiobesity potential (Inzucchi and McGuire 2008; Sadry and Drucker 2013).

Recently, pharmacological inhibitors of sodium-glucose co-transporter 2 (SGLT2) were approved for the treatment of T2D (Elkinson and Scott 2013). Blocking SGLT2 lowers the reabsorption of renal glucose excretion and thus reduces circulating glucose levels (Ferrannini and Solini 2012). Chronic administration lowers HbA1c levels by 0.5–1.5% without the risk of causing hypoglycemia (Nauck 2014). The somewhat distinctive mechanism of action of SGLT2 inhibitors implies a therapeutic opportunity for adjunctive administration with an insulin secretagogue or sensitizing agent. Common adverse events include genital and urinary tract infections; however, more serious safety concerns pertaining to increased cancer risk have recently been raised (Nauck 2014).

Bile acid sequestrants (BASs) were originally developed for treating dyslipidemia (Handelsman 2011). Importantly, BASs were shown to reduce hyperglycemia in patients with coexisting diabetes and dyslipidemia (Garg and Grundy 1994). The glucose-lowering mechanism of BASs remains elusive but seems to involve increasing the circulating bile acid pool, subsequent activation of bile acid receptors such as the farnesoid X receptor (FXR) or Takeda G protein-coupled receptor 5 (TGR5), and the resulting endogenous release of GLP-1 and/or FGF19 (Hylemon et al. 2009). The efficacy of BASs to concurrently improve HbA1c and LDL cholesterol makes them an attractive add-on to the existing glucose-lowering agents. Thus far, reported adverse events associated with their usage primarily relate to mild gastrointestinal discomfort (Handelsman 2011).

As a function of time, the majority of T2D patients receive more than one type of medication (Bailey 2013; Bennett et al. 2011), and designing an individual medicinal strategy entails a multitude of factors for consideration. These include beta-cell functionality and insulin sensitivity but also the ease of use, financial costs, tolerability, disease comorbidities, and the history of diabetes (Bennett et al. 2011; Nathan et al. 2009). Whereas parallel administration of two or more drugs may exhibit additive or synergistic glucose-lowering effects, it may also amplify adverse events, complicating overall medical care. A frequently employed antidiabetic combination therapy is insulin and metformin, which efficaciously lowers hyperglycemia without introducing a concomitant weight gain (Makimattila et al. 1999; Nathan et al. 2009). Conversely, it has been shown that combining insulin therapy with sulfonylureas instead of metformin is associated with increased mortality (Mogensen et al. 2015), underscoring the complexity of prescribing safe and efficacious antidiabetic pharmacotherapies.

5 Novel Avenues for Treating Diabetes

Research programs aiming to illuminate the molecular underpinnings of diabetic pathologies have increased exponentially in recent years. This effort is being directed increasingly toward the development of novel drugs for the treatment of

diabetes and the comorbidities. In addition to the broadened scope of basic discovery research and exploratory pharmacology, investment continues to refine, supplement, and optimize the therapeutic utility of current treatment options. Although there is a broad set of quality options for patients and the prescribing physician, glycemic control in both T1D and T2D remains suboptimal. Additionally, many current medicines possess dose-limiting adverse effects and are of narrow therapeutic index. In the following sections, some of the more prominent and promising preclinical strategies for treating diabetes are reviewed.

5.1 Next-Generation Insulin Analogs

Insulin is a miraculous substance but a dangerous drug. It is the first-in-line treatment for T1D and advanced stages of T2D. Throughout the last decade, we have witnessed a steady progression in the production and quality of insulin to a point where biosynthesis can produce virtually unlimited amounts of insulin in the highest chromatographic purity. Biosynthesis has also been employed to refine the pharmacokinetics of the hormone where site-specific mutations have been introduced to either accelerate or to postpone insulin action (Hirsch 2005). Consequently, the primary objective of cutting-edge research has advanced from pharmacokinetics to pharmacodynamics. The discovery of an insulin that is glucose sensitive is a primary target, much in the manner that an incretin only operates in hyperglycemia. Such an insulin analog or novel formulation would provide for more aggressive treatment of hyperglycemia with less risk of life-threatening hypoglycemia. Simultaneously, the perfection of pump-infused insulin is being attempted through the development of novel glucagon formulations and structural analogs, coupled with continual glucose monitoring (Chabenne et al. 2014; Wu et al. 2011). It is not inconceivable that in the not-so-distant future, a much improved approach to insulin-dependent control of glycemia could emerge. Separately, attempts to minimize body weight in concert with insulin therapy have reached an advanced development state. Obesity is a common feature of advanced, insulin-dependent T2D, and it serves to accelerate pancreatic failure while promoting weight gain. Combination basal insulin therapy with GLP-1 agonism has proven clinically that improved glycemic control, with less hypoglycemia and weight gain, can be achieved (Balena et al. 2013; Garg 2010; Vora 2013). It represents a paradigm shift where it is likely that increased effort will be devoted to further minimize the use of insulin through the identification of additional mechanisms to restore insulin sensitivity and endogenous beta-cell function.

5.2 Pancreatic Transplantation

Although pancreatic transplantation is not a new procedure (Kelly et al. 1967), recent progress in the development and success rate of both pancreatic and islet transplantation procedures have made these invasive therapies increasingly appealing.

The surgeries can be curative and are often employed in T1D patients who are undergoing a renal transplantation or in patients with poorly controlled glycemia or with recurrent hypoglycemia (Gruessner and Sutherland 2005; Gruessner and Gruessner 2013). Improvements in transplantation surgery and immunosuppressive therapy are reflected in a >95% 1-year survival rate and graft survival of close to 85% (Gruessner and Gruessner 2012). Importantly, a successful transplant is more efficient in lowering HbA1c levels and maintaining glycemic control than insulin therapy (Dieterle et al. 2007). An alternative to pancreatic transplantation is the less invasive islet transplants. Despite the obvious appeal of a less invasive procedure, a pancreatic transplant typically has better long-term glycemic outcomes than islet transplants (Gruessner and Gruessner 2013). Sourcing sufficient human islets remains a constant challenge and stem cell technology possesses huge potential to address this need (Bouwens et al. 2013). There still remain sizable issues to scaling the technology for commercial application while addressing a host of safety concerns pertaining to the potential for uncontrolled proliferation and insulin release that might evolve to be non-glucose regulated.

5.3 Leptin

Leptin is an adipocyte-derived hormone that serves to inform the brain of peripheral fuel availability (Zhang et al. 1994). Circulating leptin induces catabolic actions and weight loss by activating specific leptin receptors in the hypothalamus and the hindbrain (Myers et al. 2008). In addition, hypothalamic leptin receptor activation prominently regulates glucose metabolism and can correct diabetes in animal models of both T1D and T2D (Morton and Schwartz 2011). Infusion of leptin into the lateral cerebral ventricle in rats with uncontrolled insulin-deficient diabetes reduces hyperglycemia and improves glucose tolerance, purportedly by inhibiting hepatic glucose production and stimulating glucose uptake (German et al. 2011). Furthermore, leptin therapy corrects hyperglycemia in humans with coexisting lipodystrophy and T1D (Park et al. 2008). Leptin is currently being studied in clinical trials for its ability to improve glycemic control and reduce the requirements for insulin replacement therapy in T1D (NCT01268644).

Despite the capacity of leptin to enhance insulin sensitivity and reduce hyperglycemia in animal models of T2D, clinical trials investigating the efficacy of leptin to correct clinical parameters in obese T2D subjects have been discouraging (Mittendorfer et al. 2011; Moon et al. 2011). Whether the failure of leptin to ameliorate glycemic control in T2D coincides with leptin resistance and excess body weight needs further investigation. Notably, an increasing number of preclinical studies have demonstrated that several agents (FGF21, amylin, exendin-4, and a GLP-1/glucagon co-agonist) can restore leptin sensitivity in diet-induced leptin-resistant models to harvest additional weight-lowering and glycemic benefits of leptin therapy (Clemmensen et al. 2014; Muller et al. 2012; Roth et al. 2008). These studies have spurred new enthusiasm for leptin as an agent in novel combinatorial pharmacotherapies for the treatment of metabolic disorders. However, exogenous

leptin administration has been associated with adverse effects including increased blood pressure and immunogenicity (Kim et al. 2014). These limitations must be resolved before leptin can progress further in the clinic as a drug candidate.

5.4 FGF21

FGF21 is a hormone with profound effects on glucose and lipid metabolism and is currently being investigated as a potential therapy for the treatment of T2D (Kharitonenkov and Adams 2014). It is expressed in multiple tissues including liver, pancreas, adipose, and muscle tissue. Glucagon appears to regulate hepatic FGF21 production (Habegger et al. 2013) as well as PPARalpha agonists (Galman et al. 2008). Fasting (Galman et al. 2008) and dietary macronutrient composition (Laeger et al. 2014) influence circulating levels in a circadian manner (Andersen et al. 2011). Experimental studies have demonstrated that the administration of recombinant FGF21 improves insulin sensitivity in multiple species ranging from rodents to monkeys to man (Kharitonenkov and Adams 2014). The insulin-sensitizing efficacy of FGF21 is associated with an inhibition of hepatic glucose output, increased circulating adiponectin, and a reduction in body fat (Kharitonenkov and Adams 2014). The molecular mechanisms responsible for the metabolic effects of FGF21 are still being investigated, and studies using FGF receptor-mutated mice imply that the majority of the effects are linked to FGF receptor 1 activation in adipose tissue (Adams et al. 2012a). Recently, a novel FGF21 analog was tested in obese subjects with T2D (Gaich et al. 2013), and it was observed to improve an array of metabolic parameters. Discouragingly, no significant improvements in hyperglycemia were observed through the course of 28 days of daily treatment. This may reflect differences in pharmacological properties between native FGF21 and the analog clinically tested or be consequential to the short treatment duration and the small sample size tested in the study. Future clinical trials are needed to confirm these observations and, if validated, to determine the molecular basis.

Despite the wealth of preclinical literature supporting a novel role for FGF21 in treatment of metabolic disease, rodent studies have reported that FGF21 negatively regulates bone metabolism and that such therapy may impose skeletal fragility (Wei et al. 2012). Conversely, a positive relationship between circulating FGF21 levels and bone mineral density has been reported for healthy human subjects (Lee et al. 2013). It is a conundrum that requires additional study, and it is warranted that a balanced analysis of the benefits to metabolism is carefully assessed in the context of bone mineral metabolism.

5.5 Bariatric Surgery

Bariatric surgery provides unquestionably superior body weight and glycemic outcomes when compared to drug therapy in obese patients with poorly controlled

T2D (Schauer et al. 2014). Reports indicate that 60–80% of the patients receiving a Roux-en-Y gastric bypass show a profound reversal of their diabetes (Adams et al. 2012b; Buchwald et al. 2009). The molecular basis of the glycemic improvement constitutes a subject of intense interest as an appreciable degree of it occurs before there is a meaningful difference in body weight. Clinical studies have highlighted changes in multiple gut-secreted peptides such as GLP-1 and ghrelin as a mechanistic explanation for the glycemic benefit of such surgeries (Cummings et al. 2005; Falken et al. 2011; Karamanakos et al. 2008). Studies using genetic animal models have indicated that neither factor alone is crucial for the metabolic benefits (Chambers et al. 2013; Wilson-Perez et al. 2013). Recent, preclinical reports imply that coordinated alteration in multiple systems including bile homeostasis, microbiota, and gut-brain communication functions in concert with humoral alterations to mediate the metabolic effects of surgery (Berthoud et al. 2011; Furet et al. 2010; Lutz and Bueter 2014; Ryan et al. 2014). Identification of these mechanisms could lead to the development of a pharmacological strategy that may reproduce the glycemic control of surgery and render such invasive surgical procedures obsolete.

5.6 Multi-hormone Combination Therapies

It has become increasingly evident that adjusted enteroendocrine responses contribute to the massive and rapid metabolic improvements achieved by bariatric surgeries. Additionally, recent clinical and preclinical advances highlight that parallel targeting of more than one biological mechanism yields superior metabolic efficacy and fewer adverse events compared to traditional monotherapies (Sadry and Drucker 2013). Simultaneous targeting of multiple metabolic pathways can be achieved by coadministration of two distinct hormones (Cegla et al. 2014; Fonseca et al. 2010; Morrow et al. 2011; Muller et al. 2012; Neschen et al. 2015) or through the application of unimolecular polyagonists. These multifunctional hormones combine to embellish certain hormone action profiles but, more importantly, serve to recruit distinct pharmacology that leads to enhanced efficacy and safety (Day et al. 2009; Finan et al. 2012, 2013, 2015; Pocai et al. 2009; Schwenk et al. 2014).

In 2009, the discovery of co-agonist peptides possessing action at the glucagon and the GLP-1 receptors was reported to spectacularly lower body weight and improve glucose metabolism in animal models of obesity and glucose intolerance (Day et al. 2009; Pocai et al. 2009). A follow-up study revealed that GLP-1/glucagon co-agonism reverses leptin resistance in DIO animals (Clemmensen et al. 2014). This observation is provocative and sets the stage for future clinical studies with a central question being at what percent body weight reduction does leptin action return in human subjects. Of note, a recent human study exploring the efficacy of parallel glucagon and GLP-1 receptor agonism showed promising metabolic improvements (Cegla et al. 2014).

While the development of GIP agonists for diabetes has been clouded by the prospect of promoting weight gain, a novel dual incretin co-agonist (GLP-1/GIP) was recently reported to improve glycemic control and enhance insulin secretion in rodents and nonhuman primates (Finan et al. 2013). Furthermore, the enhanced insulinotropic effect of the co-agonist was found in clinical study to substantially reduce HbA1c levels in a dose-dependent improvement (1.1% from baseline) at the highest dose within just 6 weeks. Importantly, the treatment with the co-agonist was not associated with altered gut motility or vomiting, implying that the co-agonist can be dosed to improve efficacy while maintaining a robust safety profile. Follow-up clinical studies are ongoing to probe the efficacy and safety of these unimolecular co-agonists.

The concept of employing multi-agonists or the coadministration of several compounds with complementary mechanisms of action can be expanded to include a multitude of novel treatment protocols. The approach may thus significantly advance the possibility for individualized treatments to finally close the performance gap between drug therapy and surgical procedures.

5.7 Antiobesity Pharmacotherapies

It is well established that excess body fat mediates multiple metabolic disturbances that contribute to insulin resistance and pancreatic secretory defects (Kahn and Flier 2000; Kahn et al. 2006), rendering obesity a prominent role in escalating the diabetes epidemic. Accordingly, several antiobesity pharmacotherapies may have potential in the prevention and management of T2D. Equally, antidiabetic medications display modest antiobesity activity as well (e.g., GLP-1R agonists, amylin analogs, and SGLT2 inhibitors) (Scheen and Van Gaal 2014). Of note, the FDA recently approved the antidiabetic incretin mimetic liraglutide for the treatment of obesity. In contrast to the doses used for treating T2D (1.2 mg or 1.8 mg), the dose for treating obesity is 3.0 mg.

The antiobesity agent orlistat inhibits gastrointestinal lipases and serves to lower the availability of fatty acids for absorption (Hadvary et al. 1988). Orlistat has been shown to improve glycemic control in obese T2D subjects (Hollander et al. 1998) and to exhibit additive glycemic properties when coadministered with metformin (Miles et al. 2002). Similarly, combination therapy of the sympathomimetic amine phentermine and the anticonvulsant agent topiramate results in ~10% weight loss in obese subjects (when provided in conjunction with lifestyle modification) (Rueda-Clausen et al. 2013). Notably, the combination of phentermine and topiramate (± parallel metformin treatment) administered to T2D patients enhances weight loss and improves glycemic control relative to placebo (SEQUEL trial) (Garvey et al. 2012). Lorcaserin is a selective serotonin 2C agonist that lowers body weight in overweight and obese adults (Smith et al. 2010). Coadministration of lorcaserin with metformin and/or a sulfonylurea can improve HbA1c and fasting glucose levels in obese subjects with T2D (O'Neil et al. 2012). Recently, co-treatment with the antidepressant bupropion and the opioid receptor antagonist naltrexone

was approved by the FDA for the treatment of obesity, and this combination therapy may also exhibit meaningful glycemic improvements in obese subjects with T2D (Hollander et al. 2013). Thus, marketed antiobesity therapies may serve as valuable adjuncts in polypharmaceutical treatment options for overweight diabetics.

Evidence supporting the prospect that melanocortin 4 receptor (MC4R) agonism may constitute an effective therapy or co-therapy for diabetes and obesity is accumulating. MC4R is acknowledged to play a seminal role in energy metabolism and MC4R agonism decreases feeding and increases energy expenditure (Tao 2010). Notably, MC4R stimulation also enhances insulin sensitivity and improves glucose tolerance in rodents and nonhuman primates (Kievit et al. 2013; Obici et al. 2001). Currently, MC4R agonists are being evaluated in clinical trials for the treatment of obesity (NCT01749137). Future studies investigating the antidiabetic virtues of MC4R agonism, either as monotherapy or in combination with other agents, seem warranted.

6 Perspectives and Future Directions

Diabetes is a disease that was identified thousands of years ago. How ironic it is that we are currently experiencing a global epidemic of disease. The increased prevalence is associated with enhanced urbanization and increased body weight. Fortunately, through the second half of the last century, a number of effective antidiabetes drugs emerged, and recombinant DNA technology emerged to provide human insulin in virtually unlimited quantity. In concert with advances in glucose monitoring and the full appreciation of hyperglycemic danger, these drugs have been used to provide much improved glycemic control and patient outcomes. Nonetheless, there is much that still needs to be addressed. Insulin remains a drug of exceedingly narrow therapeutic index and the prospect of life-threatening hypoglycemia remains the largest impediment to normalizing plasma glucose. The epidemic of obesity represents a huge challenge, as currently registered antiobesity drugs are only fractionally effective in normalizing body weight. Bariatric surgeries have emerged to address the most advanced forms of obesity, and they are very effective in providing sizable decreases in weight and eliminating diabetes in a sizable percent of patients. However, what is needed is a less invasive approach to manage obesity and preferably one that can be used in adolescents and young adults where T2D has now made its appearance.

There is reason for optimization. Our knowledge of the molecular basis of T2D and obesity has never been greater. The emergence of multiple new antidiabetic medicines demonstrates what can be accomplished when translational research is focused on a specific disease. The first-generation antiobesity drugs have established a foundation from which more effective therapies, and combinations with these first-generation drugs, can be developed to provide more meaningful reductions in body weight with the ultimate goal eliminating the current performance difference relative to gut surgery. Separately, insulin therapy is destined to improve with the renewed emphasis to discover a more glucose-sensitive approach

to therapy. The simultaneous advances in biotechnology, material sciences, synthetic chemistry, and information technology are integrating to provide novel approaches to insulin-dependent diabetes that were impossible as recent as a decade ago. While it is impossible to predict the future with certainty, especially against such lofty goals as outlined in this chapter, the discovery of next-generation medicines with greater transformative impact are certainly plausible. While it is not uncommon for technology to fail in delivering near-term solutions to large medical challenges, when it is viewed over a longer period, it is likely to exceed expectations. If we can maintain the level of interest in addressing diabetes and obesity across academic, biotechnology, and large pharmaceutical companies, then we remain optimistic for the future.

References

Adams AC, Yang C, Coskun T, Cheng CC, Gimeno RE, Luo Y, Kharitonenkov A (2012a) The breadth of FGF21's metabolic actions are governed by FGFR1 in adipose tissue. Mol Metab 2:31–37

Adams TD, Davidson LE, Litwin SE, Kolotkin RL, LaMonte MJ, Pendleton RC, Strong MB, Vinik R, Wanner NA, Hopkins PN, Gress RE, Walker JM, Cloward TV, Nuttall RT, Hammoud A, Greenwood JL, Crosby RD, McKinlay R, Simper SC, Smith SC, Hunt SC (2012b) Health benefits of gastric bypass surgery after 6 years. JAMA 308:1122–1131

Andersen B, Beck-Nielsen H, Hojlund K (2011) Plasma FGF21 displays a circadian rhythm during a 72-h fast in healthy female volunteers. Clin Endocrinol 75:514–519

Aroda VR, Ratner R (2011) The safety and tolerability of GLP-1 receptor agonists in the treatment of type 2 diabetes: a review. Diabetes Metab Res Rev 27:528–542

Baggio LL, Drucker DJ (2007) Biology of incretins: GLP-1 and GIP. Gastroenterology 132:2131–2157

Bailey T (2013) Options for combination therapy in type 2 diabetes: comparison of the ADA/EASD position statement and AACE/ACE algorithm. Am J Med 126:S10–S20

Bailey CJ, Turner RC (1996) Metformin. N Engl J Med 334:574–579

Balena R, Hensley IE, Miller S, Barnett AH (2013) Combination therapy with GLP-1 receptor agonists and basal insulin: a systematic review of the literature. Diabetes Obes Metab 15:485–502

Barnett AH (2013) Complementing insulin therapy to achieve glycemic control. Adv Ther 30:557–576

Bennett WL, Maruthur NM, Singh S, Segal JB, Wilson LM, Chatterjee R, Marinopoulos SS, Puhan MA, Ranasinghe P, Block L, Nicholson WK, Hutfless S, Bass EB, Bolen S (2011) Comparative effectiveness and safety of medications for type 2 diabetes: an update including new drugs and 2-drug combinations. Ann Intern Med 154:602–613

Berthoud HR, Shin AC, Zheng H (2011) Obesity surgery and gut-brain communication. Physiol Behav 105:106–119

Blickle JF (2006) Meglitinide analogues: a review of clinical data focused on recent trials. Diabetes Metab 32:113–120

Bluestone JA, Herold K, Eisenbarth G (2010) Genetics, pathogenesis and clinical interventions in type 1 diabetes. Nature 464:1293–1300

Bolen S, Feldman L, Vassy J, Wilson L, Yeh HC, Marinopoulos S, Wiley C, Selvin E, Wilson R, Bass EB, Brancati FL (2007) Systematic review: comparative effectiveness and safety of oral medications for type 2 diabetes mellitus. Ann Intern Med 147:386–399

Bonora E, Tuomilehto J (2011) The pros and cons of diagnosing diabetes with A1C. Diabetes Care 34(Suppl 2):S184–S190

Booth SL, Centi A, Smith SR, Gundberg C (2013) The role of osteocalcin in human glucose metabolism: marker or mediator? Nat Rev Endocrinol 9:43–55

Bouwens L, Houbracken I, Mfopou JK (2013) The use of stem cells for pancreatic regeneration in diabetes mellitus. Nat Rev Endocrinol 9:598–606

Bowker SL, Majumdar SR, Veugelers P, Johnson JA (2006) Increased cancer-related mortality for patients with type 2 diabetes who use sulfonylureas or insulin. Diabetes Care 29:254–258

Buchwald H, Estok R, Fahrbach K, Banel D, Jensen MD, Pories WJ, Bantle JP, Sledge I (2009) Weight and type 2 diabetes after bariatric surgery: systematic review and meta-analysis. Am J Med 122(248–256):e5

Butler PC, Chou J, Carter WB, Wang YN, Bu BH, Chang D, Chang JK, Rizza RA (1990) Effects of meal ingestion on plasma amylin concentration in NIDDM and nondiabetic humans. Diabetes 39:752–756

Cefalu WT (2007) Pharmacotherapy for the treatment of patients with type 2 diabetes mellitus: rationale and specific agents. Clin Pharmacol Ther 81:636–649

Cegla J, Troke RC, Jones B, Tharakan G, Kenkre J, McCullough KA, Lim CT, Parvizi N, Hussein M, Chambers ES, Minnion J, Cuenco J, Ghatei MA, Meeran K, Tan TM, Bloom SR (2014) Coinfusion of low-dose GLP-1 and glucagon in man results in a reduction in food intake. Diabetes 63:3711–3720

Chabenne J, Chabenne MD, Zhao Y, Levy J, Smiley D, Gelfanov V, Dimarchi R (2014) A glucagon analog chemically stabilized for immediate treatment of life-threatening hypoglycemia. Mol Metab 3:293–300

Chambers AP, Kirchner H, Wilson-Perez HE, Willency JA, Hale JE, Gaylinn BD, Thorner MO, Pfluger PT, Gutierrez JA, Tschop MH, Sandoval DA, Seeley RJ (2013) The effects of vertical sleeve gastrectomy in rodents are ghrelin independent. Gastroenterology 144(50–52):e5

Clemmensen C, Chabenne J, Finan B, Sullivan L, Fischer K, Kuchler D, Sehrer L, Ograjsek T, Hofmann SM, Schriever SC, Pfluger PT, Pinkstaff J, Tschop MH, Dimarchi R, Muller TD (2014) GLP-1/glucagon coagonism restores leptin responsiveness in obese mice chronically maintained on an obesogenic diet. Diabetes 63:1422–1427

Cummings DE, Overduin J, Shannon MH, Foster-Schubert KE, Conference ABSC (2005) Hormonal mechanisms of weight loss and diabetes resolution after bariatric surgery. Surg Obes Relat Dis 1:358–368

Currie CJ, Poole CD, Gale EA (2009) The influence of glucose-lowering therapies on cancer risk in type 2 diabetes. Diabetologia 52:1766–1777

D'Alessio D (2011) The role of dysregulated glucagon secretion in type 2 diabetes. Diabetes Obes Metab 13(Suppl. 1):126–132

Day JW, Ottaway N, Patterson JT, Gelfanov V, Smiley D, Gidda J, Findeisen H, Bruemmer D, Drucker DJ, Chaudhary N, Holland J, Hembree J, Abplanalp W, Grant E, Ruehl J, Wilson H, Kirchner H, Lockie SH, Hofmann S, Woods SC, Nogueiras R, Pfluger PT, Perez-Tilve D, DiMarchi R, Tschop MH (2009) A new glucagon and GLP-1 co-agonist eliminates obesity in rodents. Nat Chem Biol 5:749–757

Defronzo RA, Mehta RJ, Schnure JJ (2013) Pleiotropic effects of thiazolidinediones: implications for the treatment of patients with type 2 diabetes mellitus. Hosp Pract 41:132–147

Derosa G, Sibilla S (2007) Optimizing combination treatment in the management of type 2 diabetes. Vasc Health Risk Manag 3:665–671

Dieterle CD, Arbogast H, Illner WD, Schmauss S, Landgraf R (2007) Metabolic follow-up after long-term pancreas graft survival. Eur J Endocrinol 156:603–610

Dupre J, Ross SA, Watson D, Brown JC (1973) Stimulation of insulin secretion by gastric inhibitory polypeptide in man. J Clin Endocrinol Metab 37:826–828

Elkinson S, Scott LJ (2013) Canagliflozin: first global approval. Drugs 73:979–988

Elrick H, Stimmler L, Hlad CJ Jr, Arai Y (1964) Plasma insulin response to oral and intravenous glucose administration. J Clin Endocrinol Metabol 24:1076–1082

Falken Y, Hellstrom PM, Holst JJ, Naslund E (2011) Changes in glucose homeostasis after Roux-en-Y gastric bypass surgery for obesity at day three, two months, and one year after surgery: role of gut peptides. J Clin Endocrinol Metab 96:2227–2235

Fehmann HC, Goke R, Goke B (1995) Cell and molecular biology of the incretin hormones glucagon-like peptide-I and glucose-dependent insulin releasing polypeptide. Endocr Rev 16:390–410

Fernandez-Real JM, Broch M, Ricart W, Casamitjana R, Gutierrez C, Vendrell J, Richart C (1998) Plasma levels of the soluble fraction of tumor necrosis factor receptor 2 and insulin resistance. Diabetes 47:1757–1762

Ferrannini E, Solini A (2012) SGLT2 inhibition in diabetes mellitus: rationale and clinical prospects. Nat Rev Endocrinol 8:495–502

Finan B, Yang B, Ottaway N, Stemmer K, Muller TD, Yi CX, Habegger K, Schriever SC, Garcia-Caceres C, Kabra DG, Hembree J, Holland J, Raver C, Seeley RJ, Hans W, Irmler M, Beckers J, de Angelis MH, Tiano JP, Mauvais-Jarvis F, Perez-Tilve D, Pfluger P, Zhang L, Gelfanov V, DiMarchi RD, Tschop MH (2012) Targeted estrogen delivery reverses the metabolic syndrome. Nat Med 18:1847–1856

Finan B, Ma T, Ottaway N, Muller TD, Habegger KM, Heppner KM, Kirchner H, Holland J, Hembree J, Raver C, Lockie SH, Smiley DL, Gelfanov V, Yang B, Hofmann S, Bruemmer D, Drucker DJ, Pfluger PT, Perez-Tilve D, Gidda J, Vignati L, Zhang L, Hauptman JB, Lau M, Brecheisen M, Uhles S, Riboulet W, Hainaut E, Sebokova E, Conde-Knape K, Konkar A, DiMarchi RD, Tschop MH (2013) Unimolecular dual incretins maximize metabolic benefits in rodents, monkeys, and humans. Sci Transl Med 5:209ra151

Finan B, Yang B, Ottaway N, Smiley DL, Ma T, Clemmensen C, Chabenne J, Zhang L, Habegger KM, Fischer K, Campbell JE, Sandoval D, Seeley RJ, Bleicher K, Uhles S, Riboulet W, Funk J, Hertel C, Belli S, Sebokova E, Conde-Knape K, Konkar A, Drucker DJ, Gelfanov V, Pfluger PT, Muller TD, Perez-Tilve D, DiMarchi RD, Tschop MH (2015) A rationally designed monomeric peptide triagonist corrects obesity and diabetes in rodents. Nat Med 21:27–36

Foley JE, Jordan J (2010) Weight neutrality with the DPP-4 inhibitor, vildagliptin: mechanistic basis and clinical experience. Vasc Health Risk Manag 6:541–548

Fonseca V (2003) Effect of thiazolidinediones on body weight in patients with diabetes mellitus. Am J Med 115(Suppl 8A):42S–48S

Fonseca VA, Haggar MA (2014) Achieving glycaemic targets with basal insulin in T2DM by individualizing treatment. Nat Rev Endocrinol 10:276–281

Fonseca VA, Handelsman Y, Staels B (2010) Colesevelam lowers glucose and lipid levels in type 2 diabetes: the clinical evidence. Diabetes Obes Metab 12:384–392

Fuchtenbusch M, Standl E, Schatz H (2000) Clinical efficacy of new thiazolidinediones and glinides in the treatment of type 2 diabetes mellitus. Experimental and clinical endocrinology & diabetes. J German Soc Endocrinol German Diabetes Assoc 108:151–163

Furet JP, Kong LC, Tap J, Poitou C, Basdevant A, Bouillot JL, Mariat D, Corthier G, Dore J, Henegar C, Rizkalla S, Clement K (2010) Differential adaptation of human gut microbiota to bariatric surgery-induced weight loss: links with metabolic and low-grade inflammation markers. Diabetes 59:3049–3057

Gaich G, Chien JY, Fu H, Glass LC, Deeg MA, Holland WL, Kharitonenkov A, Bumol T, Schilske HK, Moller DE (2013) The effects of LY2405319, an FGF21 analog, in obese human subjects with type 2 diabetes. Cell Metab 18:333–340

Galman C, Lundasen T, Kharitonenkov A, Bina HA, Eriksson M, Hafstrom I, Dahlin M, Amark P, Angelin B, Rudling M (2008) The circulating metabolic regulator FGF21 is induced by prolonged fasting and PPARalpha activation in man. Cell Metab 8:169–174

Garg SK (2010) The role of basal insulin and glucagon-like peptide-1 agonists in the therapeutic management of type 2 diabetes–a comprehensive review. Diabetes Technol Ther 12:11–24

Garg A, Grundy SM (1994) Cholestyramine therapy for dyslipidemia in non-insulin-dependent diabetes mellitus. A short-term, double-blind, crossover trial. Ann Intern Med 121:416–422

Garvey WT, Ryan DH, Look M, Gadde KM, Allison DB, Peterson CA, Schwiers M, Day WW, Bowden CH (2012) Two-year sustained weight loss and metabolic benefits with controlled-release phentermine/topiramate in obese and overweight adults (SEQUEL): a randomized, placebo-controlled, phase 3 extension study. Am J Clin Nutr 95:297–308

Gerich JE (1993) Control of glycaemia. Bailliere Clin Endocrinol Metab 7:551–586

German JP, Thaler JP, Wisse BE, Oh IS, Sarruf DA, Matsen ME, Fischer JD, Taborsky GJ Jr, Schwartz MW, Morton GJ (2011) Leptin activates a novel CNS mechanism for insulin-independent normalization of severe diabetic hyperglycemia. Endocrinology 152:394–404

Gruessner AC, Gruessner RW (2012) Pancreas transplant outcomes for United States and non United States cases as reported to the United Network for Organ Sharing and the International Pancreas Transplant Registry as of December 2011. Clin Transpl 23–40

Gruessner RW, Gruessner AC (2013) The current state of pancreas transplantation. Nat Rev Endocrinol 9:555–562

Gruessner AC, Sutherland DE (2005) Pancreas transplant outcomes for United States (US) and non-US cases as reported to the United Network for Organ Sharing (UNOS) and the International Pancreas Transplant Registry (IPTR) as of June 2004. Clin Transpl 19:433–455

Guariguata L, Whiting DR, Hambleton I, Beagley J, Linnenkamp U, Shaw JE (2014) Global estimates of diabetes prevalence for 2013 and projections for 2035. Diabetes Res Clin Pract 103:137–149

Habegger KM, Stemmer K, Cheng C, Muller TD, Heppner KM, Ottaway N, Holland J, Hembree JL, Smiley D, Gelfanov V, Krishna R, Arafat AM, Konkar A, Belli S, Kapps M, Woods SC, Hofmann SM, D'Alessio D, Pfluger PT, Perez-Tilve D, Seeley RJ, Konishi M, Itoh N, Kharitonenkov A, Spranger J, DiMarchi RD, Tschop MH (2013) Fibroblast growth factor 21 mediates specific glucagon actions. Diabetes 62:1453–1463

Hadvary P, Lengsfeld H, Wolfer H (1988) Inhibition of pancreatic lipase in vitro by the covalent inhibitor tetrahydrolipstatin. Biochem J 256:357–361

Handelsman Y (2011) Role of bile acid sequestrants in the treatment of type 2 diabetes. Diabetes Care 34(Suppl 2):S244–S250

Hauner H (2002) The mode of action of thiazolidinediones. Diabetes Metab Res Rev 18(Suppl 2): S10–S15

Hernandez AV, Usmani A, Rajamanickam A, Moheet A (2011) Thiazolidinediones and risk of heart failure in patients with or at high risk of type 2 diabetes mellitus: a meta-analysis and meta-regression analysis of placebo-controlled randomized clinical trials. Am J Cardiovasc Drugs 11:115–128

Hirsch IB (2005) Insulin analogues. N Engl J Med 352:174–183

Hollander PA, Elbein SC, Hirsch IB, Kelley D, McGill J, Taylor T, Weiss SR, Crockett SE, Kaplan RA, Comstock J, Lucas CP, Lodewick PA, Canovatchel W, Chung J, Hauptman J (1998) Role of orlistat in the treatment of obese patients with type 2 diabetes. A 1-year randomized double-blind study. Diabetes Care 21:1288–1294

Hollander P, Gupta AK, Plodkowski R, Greenway F, Bays H, Burns C, Klassen P, Fujioka K, Group CO-DS (2013) Effects of naltrexone sustained-release/bupropion sustained-release combination therapy on body weight and glycemic parameters in overweight and obese patients with type 2 diabetes. Diabetes care 36:4022–4029

Hotta K, Funahashi T, Bodkin NL, Ortmeyer HK, Arita Y, Hansen BC, Matsuzawa Y (2001) Circulating concentrations of the adipocyte protein adiponectin are decreased in parallel with reduced insulin sensitivity during the progression to type 2 diabetes in rhesus monkeys. Diabetes 50:1126–1133

Hylemon PB, Zhou H, Pandak WM, Ren S, Gil G, Dent P (2009) Bile acids as regulatory molecules. J Lipid Res 50:1509–1520

IDF (2014) IDF Diabetes Atlas update poster, 6th edn. International Diabetes Federation, Brussels

Inzucchi SE, McGuire DK (2008) New drugs for the treatment of diabetes: part II: incretin-based therapy and beyond. Circulation 117:574–584

Kahn SE (2003) The relative contributions of insulin resistance and beta-cell dysfunction to the pathophysiology of Type 2 diabetes. Diabetologia 46:3–19
Kahn BB, Flier JS (2000) Obesity and insulin resistance. J Clin Invest 106:473–481
Kahn SE, Hull RL, Utzschneider KM (2006) Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature 444:840–846
Kahn SE, Suvag S, Wright LA, Utzschneider KM (2012) Interactions between genetic background, insulin resistance and beta-cell function. Diabetes Obes Metab 14(Suppl. 3):46–56
Kanoski SE, Rupprecht LE, Fortin SM, De Jonghe BC, Hayes MR (2012) The role of nausea in food intake and body weight suppression by peripheral GLP-1 receptor agonists, exendin-4 and liraglutide. Neuropharmacology 62:1916–1927
Karamanakos SN, Vagenas K, Kalfarentzos F, Alexandrides TK (2008) Weight loss, appetite suppression, and changes in fasting and postprandial ghrelin and peptide-YY levels after Roux-en-Y gastric bypass and sleeve gastrectomy: a prospective, double blind study. Ann Surg 247:401–407
Kelly WD, Lillehei RC, Merkel FK, Idezuki Y, Goetz FC (1967) Allotransplantation of the pancreas and duodenum along with the kidney in diabetic nephropathy. Surgery 61:827–837
Kharitonenkov A, Adams AC (2014) Inventing new medicines: the FGF21 story. Mol Metab 3:221–229
Kharitonenkov A, Shiyanova TL, Koester A, Ford AM, Micanovic R, Galbreath EJ, Sandusky GE, Hammond LJ, Moyers JS, Owens RA, Gromada J, Brozinick JT, Hawkins ED, Wroblewski VJ, Li DS, Mehrbod F, Jaskunas SR, Shanafelt AB (2005) FGF-21 as a novel metabolic regulator. J Clin Invest 115:1627–1635
Kievit P, Halem H, Marks DL, Dong JZ, Glavas MM, Sinnayah P, Pranger L, Cowley MA, Grove KL, Culler MD (2013) Chronic treatment with a melanocortin-4 receptor agonist causes weight loss, reduces insulin resistance, and improves cardiovascular function in diet-induced obese rhesus macaques. Diabetes 62:490–497
Kim GW, Lin JE, Blomain ES, Waldman SA (2014) Antiobesity pharmacotherapy: new drugs and emerging targets. Clin Pharmacol Ther 95:53–66
Kleinridders A, Ferris HA, Cai W, Kahn CR (2014) Insulin action in brain regulates systemic metabolism and brain function. Diabetes 63:2232–2243
Knip M, Veijola R, Virtanen SM, Hyoty H, Vaarala O, Akerblom HK (2005) Environmental triggers and determinants of type 1 diabetes. Diabetes 54(Suppl 2):S125–S136
Kruger DF, Gloster MA (2004) Pramlintide for the treatment of insulin-requiring diabetes mellitus: rationale and review of clinical data. Drugs 64:1419–1432
Laeger T, Henagan TM, Albarado DC, Redman LM, Bray GA, Noland RC, Munzberg H, Hutson SM, Gettys TW, Schwartz MW, Morrison CD (2014) FGF21 is an endocrine signal of protein restriction. J Clin Invest 124:3913–3922
Lebovitz HE (1997) alpha-Glucosidase inhibitors. Endocrinol Metab Clin N Am 26:539–551
Lee P, Linderman J, Smith S, Brychta RJ, Perron R, Idelson C, Werner CD, Chen KY, Celi FS (2013) Fibroblast growth factor 21 (FGF21) and bone: is there a relationship in humans? Osteoporos Int 24:3053–3057
Liu SC, Tu YK, Chien MN, Chien KL (2012) Effect of antidiabetic agents added to metformin on glycaemic control, hypoglycaemia and weight change in patients with type 2 diabetes: a network meta-analysis. Diabetes Obes Metab 14:810–820
Lutz TA, Bueter M (2014) The physiology underlying Roux-en-Y gastric bypass: a status report. Am J Physiol Regul Integr Comp Physiol 307:R1275–R1291
Makimattila S, Nikkila K, Yki-Jarvinen H (1999) Causes of weight gain during insulin therapy with and without metformin in patients with Type II diabetes mellitus. Diabetologia 42:406–412
Martin BC, Warram JH, Krolewski AS, Bergman RN, Soeldner JS, Kahn CR (1992) Role of glucose and insulin resistance in development of type 2 diabetes mellitus: results of a 25-year follow-up study. Lancet 340:925–929

McIntyre N, Holdsworth CD, Turner DS (1964) New Interpretation of Oral Glucose Tolerance. Lancet 2:20–21

Melander A (2004) Kinetics-effect relations of insulin-releasing drugs in patients with type 2 diabetes: brief overview. Diabetes 53(Suppl 3):S151–S155

Miles JM, Leiter L, Hollander P, Wadden T, Anderson JW, Doyle M, Foreyt J, Aronne L, Klein S (2002) Effect of orlistat in overweight and obese patients with type 2 diabetes treated with metformin. Diabetes Care 25:1123–1128

Mittendorfer B, Horowitz JF, DePaoli AM, McCamish MA, Patterson BW, Klein S (2011) Recombinant human leptin treatment does not improve insulin action in obese subjects with type 2 diabetes. Diabetes 60:1474–1477

Mogensen UM, Andersson C, Fosbol EL, Schramm TK, Vaag A, Scheller NM, Torp-Pedersen C, Gislason G, Kober L (2015) Sulfonylurea in combination with insulin is associated with increased mortality compared with a combination of insulin and metformin in a retrospective Danish nationwide study. Diabetologia 58:50–58

Moon HS, Matarese G, Brennan AM, Chamberland JP, Liu X, Fiorenza CG, Mylvaganam GH, Abanni L, Carbone F, Williams CJ, De Paoli AM, Schneider BE, Mantzoros CS (2011) Efficacy of metreleptin in obese patients with type 2 diabetes: cellular and molecular pathways underlying leptin tolerance. Diabetes 60:1647–1656

Moore CX, Cooper GJ (1991) Co-secretion of amylin and insulin from cultured islet beta-cells: modulation by nutrient secretagogues, islet hormones and hypoglycemic agents. Biochem Biophys Res Commun 179:1–9

Morrow L, Hompesch M, Guthrie H, Chang D, Chatterjee DJ (2011) Co-administration of liraglutide with insulin detemir demonstrates additive pharmacodynamic effects with no pharmacokinetic interaction. Diabetes Obes Metab 13:75–80

Morton GJ, Schwartz MW (2011) Leptin and the central nervous system control of glucose metabolism. Physiol Rev 91:389–411

Muller TD, Sullivan LM, Habegger K, Yi CX, Kabra D, Grant E, Ottaway N, Krishna R, Holland J, Hembree J, Perez-Tilve D, Pfluger PT, DeGuzman MJ, Siladi ME, Kraynov VS, Axelrod DW, DiMarchi R, Pinkstaff JK, Tschop MH (2012) Restoration of leptin responsiveness in diet-induced obese mice using an optimized leptin analog in combination with exendin-4 or FGF21. J Pept Sci 18:383–393

Myers MG, Cowley MA, Munzberg H (2008) Mechanisms of leptin action and leptin resistance. Annu Rev Physiol 70:537–556

Nathan DM, Buse JB, Davidson MB, Ferrannini E, Holman RR, Sherwin R, Zinman B, American Diabetes A, European Association for Study of D (2009) Medical management of hyperglycemia in type 2 diabetes: a consensus algorithm for the initiation and adjustment of therapy: a consensus statement of the American Diabetes Association and the European Association for the Study of Diabetes. Diabet Care 32:193–203

Nauck MA (2014) Update on developments with SGLT2 inhibitors in the management of type 2 diabetes. Drug Des Devel Ther 8:1335–1380

Neschen S, Scheerer M, Seelig A, Huypens P, Schultheiss J, Wu M, Wurst W, Rathkolb B, Suhre K, Wolf E, Beckers J, Hrabe de Angelis M (2015) Metformin supports the antidiabetic effect of a sodium glucose cotransporter 2 inhibitor by suppressing endogenous glucose production in diabetic mice. Diabetes 64:284–290

Obici S, Feng Z, Tan J, Liu L, Karkanias G, Rossetti L (2001) Central melanocortin receptors regulate insulin action. J Clin Invest 108:1079–1085

O'Neil PM, Smith SR, Weissman NJ, Fidler MC, Sanchez M, Zhang J, Raether B, Anderson CM, Shanahan WR (2012) Randomized placebo-controlled clinical trial of lorcaserin for weight loss in type 2 diabetes mellitus: the BLOOM-DM study. Obesity 20:1426–1436

Park JY, Chong AY, Cochran EK, Kleiner DE, Haller MJ, Schatz DA, Gorden P (2008) Type 1 diabetes associated with acquired generalized lipodystrophy and insulin resistance: the effect of long-term leptin therapy. J Clin Endocrinol Metab 93:26–31

Pocai A, Carrington PE, Adams JR, Wright M, Eiermann G, Zhu L, Du X, Petrov A, Lassman ME, Jiang G, Liu F, Miller C, Tota LM, Zhou G, Zhang X, Sountis MM, Santoprete A, Capito E, Chicchi GG, Thornberry N, Bianchi E, Pessi A, Marsh DJ, SinhaRoy R (2009) Glucagon-like peptide 1/glucagon receptor dual agonism reverses obesity in mice. Diabetes 58:2258–2266

Proks P, Reimann F, Green N, Gribble F, Ashcroft F (2002) Sulfonylurea stimulation of insulin secretion. Diabetes 51(Suppl 3):S368–S376

Ratner RE, Dickey R, Fineman M, Maggs DG, Shen L, Strobel SA, Weyer C, Kolterman OG (2004) Amylin replacement with pramlintide as an adjunct to insulin therapy improves long-term glycaemic and weight control in Type 1 diabetes mellitus: a 1-year, randomized controlled trial. Diabetic Med 21:1204–1212

Rojas JM, Schwartz MW (2014) Control of hepatic glucose metabolism by islet and brain. Diabetes Obes Metab 16(Suppl. 1):33-40

Roth JD (2013) Amylin and the regulation of appetite and adiposity: recent advances in receptor signaling, neurobiology and pharmacology. Curr Opin Endocrinol Diabetes Obes 20:8–13

Roth JD, Roland BL, Cole RL, Trevaskis JL, Weyer C, Koda JE, Anderson CM, Parkes DG, Baron AD (2008) Leptin responsiveness restored by amylin agonism in diet-induced obesity: evidence from nonclinical and clinical studies. Proc Natl Acad Sci U S A 105:7257–7262

Rueda-Clausen CF, Padwal RS, Sharma AM (2013) New pharmacological approaches for obesity management. Nat Rev Endocrinol 9:467–478

Ryan GJ, Jobe LJ, Martin R (2005) Pramlintide in the treatment of type 1 and type 2 diabetes mellitus. Clin Ther 27:1500–1512

Ryan KK, Tremaroli V, Clemmensen C, Kovatcheva-Datchary P, Myronovych A, Karns R, Wilson-Perez HE, Sandoval DA, Kohli R, Backhed F, Seeley RJ (2014) FXR is a molecular target for the effects of vertical sleeve gastrectomy. Nature 509:183–188

Sadry SA, Drucker DJ (2013) Emerging combinatorial hormone therapies for the treatment of obesity and T2DM. Nat Rev Endocrinol 9:425–433

Saltiel AR, Olefsky JM (1996) Thiazolidinediones in the treatment of insulin resistance and type II diabetes. Diabetes 45:1661–1669

Schauer PR, Bhatt DL, Kirwan JP, Wolski K, Brethauer SA, Navaneethan SD, Aminian A, Pothier CE, Kim ES, Nissen SE, Kashyap SR, Investigators S (2014) Bariatric surgery versus intensive medical therapy for diabetes–3-year outcomes. N Engl J Med 370:2002–2013

Scheen AJ, Van Gaal LF (2014) Combating the dual burden: therapeutic targeting of common pathways in obesity and type 2 diabetes. Lancet Diabetes Endocrinol 2:911–922

Schmidt WE, Siegel EG, Creutzfeldt W (1985) Glucagon-like peptide-1 but not glucagon-like peptide-2 stimulates insulin release from isolated rat pancreatic islets. Diabetologia 28:704–707

Schwartz MW, Seeley RJ, Tschop MH, Woods SC, Morton GJ, Myers MG, D'Alessio D (2013) Cooperation between brain and islet in glucose homeostasis and diabetes. Nature 503:59–66

Schwenk RW, Baumeier C, Finan B, Kluth O, Brauer C, Joost HG, DiMarchi RD, Tschop MH, Schurmann A (2014) GLP-1-oestrogen attenuates hyperphagia and protects from beta cell failure in diabetes-prone New Zealand obese (NZO) mice. Diabetologia 58:604–614

Scully T (2012) Diabetes in numbers. Nature 485:S2–S3

Smith SR, De Jonge L, Volaufova J, Li Y, Xie H, Bray GA (2005) Effect of pioglitazone on body composition and energy expenditure: a randomized controlled trial. Metab Clin Exp 54:24–32

Smith SR, Weissman NJ, Anderson CM, Sanchez M, Chuang E, Stubbe S, Bays H, Shanahan WR, Behavioral M, Lorcaserin for O, Obesity Management Study G (2010) Multicenter, placebo-controlled trial of lorcaserin for weight management. New Engl J Med 363:245–256

Steensberg A, van Hall G, Osada T, Sacchetti M, Saltin B, Klarlund Pedersen B (2000) Production of interleukin-6 in contracting human skeletal muscles can account for the exercise-induced increase in plasma interleukin-6. J Physiol 529(Pt 1):237–242

Tao YX (2010) The melanocortin-4 receptor: physiology, pharmacology, and pathophysiology. Endocr Rev 31:506–543

Tonelli J, Li W, Kishore P, Pajvani UB, Kwon E, Weaver C, Scherer PE, Hawkins M (2004) Mechanisms of early insulin-sensitizing effects of thiazolidinediones in type 2 diabetes. Diabetes 53:1621–1629

Unger RH, Cherrington AD (2012) Glucagonocentric restructuring of diabetes: a pathophysiologic and therapeutic makeover. J Clin Invest 122:4–12

van Belle TL, Coppieters KT, von Herrath MG (2011) Type 1 diabetes: etiology, immunology, and therapeutic strategies. Physiol Rev 91:79–118

van de Laar FA, Lucassen PL, Akkermans RP, van de Lisdonk EH, Rutten GE, van Weel C (2005) Alpha-glucosidase inhibitors for patients with type 2 diabetes: results from a Cochrane systematic review and meta-analysis. Diabetes Care 28:154–163

Viollet B, Guigas B, Sanz Garcia N, Leclerc J, Foretz M, Andreelli F (2012) Cellular and molecular mechanisms of metformin: an overview. Clin Sci 122:253–270

Vora J (2013) Combining incretin-based therapies with insulin: realizing the potential in type 2 diabetes. Diabetes Care 36(Suppl 2):S226–S232

Wei W, Dutchak PA, Wang X, Ding X, Wang X, Bookout AL, Goetz R, Mohammadi M, Gerard RD, Dechow PC, Mangelsdorf DJ, Kliewer SA, Wan Y (2012) Fibroblast growth factor 21 promotes bone loss by potentiating the effects of peroxisome proliferator-activated receptor gamma. Proc Natl Acad Sci U S A 109:3143–3148

Weir GC, Bonner-Weir S (2004) Five stages of evolving beta-cell dysfunction during progression to diabetes. Diabetes 53(Suppl 3):S16–S21

Weyer C, Maggs DG, Young AA, Kolterman OG (2001) Amylin replacement with pramlintide as an adjunct to insulin therapy in type 1 and type 2 diabetes mellitus: a physiological approach toward improved metabolic control. Curr Pharm Des 7:1353–1373

Wilson-Perez HE, Chambers AP, Ryan KK, Li B, Sandoval DA, Stoffers D, Drucker DJ, Perez-Tilve D, Seeley RJ (2013) Vertical sleeve gastrectomy is effective in two genetic mouse models of glucagon-like Peptide 1 receptor deficiency. Diabetes 62:2380–2385

Wu Q, Wang L, Yu H, Wang J, Chen Z (2011) Organization of glucose-responsive systems and their properties. Chem Rev 111:7855–7875

Wulffele MG, Kooy A, Lehert P, Bets D, Ogterop JC, Borger van der Burg B, Donker AJ, Stehouwer CD (2002) Combination of insulin and metformin in the treatment of type 2 diabetes. Diabetes Care 25:2133–2140

Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM (1994) Positional cloning of the mouse obese gene and its human homologue. Nature 372:425–432

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