Metabolic Pathways and Cell Signaling

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Metabolism is one of the key features of life, as it allows for the continuous rebuilding of the living structures driven by the extraction of energy from nutrients [1]. As such, metabolism can be thought of as the set of all the chemical reactions that take place in a living organism and are related to the maintenance of life. In principle, many more reactions could happen between the chemical substances that are found in living matter than the number we observe when organisms are actually alive. Those other reactions, albeit possible, would not sustain the maintenance of the living structures. So, the smaller set of reactions that do sustain life—that is, metabolism—is somehow favored among all the chemical possibilities presented by the substances found in organisms. In order words, there must be information on which reactions to promote in living matter. This information is kept in the genes that ultimately encode the proteins that favor the reactions of life—enzymes. They do so by speeding up (i.e. catalyzing) these reactions, so that the substances participating in these reactions are much less available to react otherwise.

1 Overview of Metabolic Pathways

Metabolism is classically divided into catabolic and anabolic reactions. Anabolism comprises the reactions that lead to the formation of the complex and organized structures of living beings from smaller and simpler chemical building blocks. For anabolism to happen, there must be a continuous and substantial supply of chemical energy. This energy supply is derived from the reactions that make up the other branch of metabolism, that is catabolism. Catabolic reactions extract energy from chemical

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substances by selectively and orderly breaking them up into simpler ones. In this process, the energy that kept their atoms together is partially but efficiently funneled to the synthesis of specific molecules, most important among these is adenosine triphosphate (ATP). In anabolism, ATP is used in tandem reactions that involve the transfer, and later removal, of phosphate from it to other molecules, yielding in the end ADP and inorganic phosphate. In this process, enzymes couple this use of ATP and the energy it releases with the synthesis of the complex biological molecules, and thus of the structures of life.

ATP synthesis, be it via substrate-level phosphorylation in the cytosol and mitochondrial matrix or oxidative phosphorylation in the mitochondrial inner membrane, is coupled to the oxidation of substrates, whose electrons are transferred to nicotinamide coenzymes, NADH or NADPH. NADH is an important source of electrons for the electron transport chain in mitochondria and thus of free energy for the chemiosmotic synthesis of ATP [2]. Meanwhile, NADPH is a source of reducing power for reductive processes in the cell, such as fatty acid synthesis and antioxidant defenses. Both NADH and NADPH are constantly recycled in redox reactions:

$$\mathrm{NAD(P)}^{+} + \mathrm{H}^{+} + 1\mathrm{e}^{-} \rightleftharpoons \mathrm{NAD(P)H}.$$

The molecules that are involved in metabolism can be broadly divided into four main classes: proteins, lipids, carbohydrates and nucleic acids. Each of these classes have chemical properties and biological functions that are characteristic of themselves, and each are formed in anabolism from specific small precursor molecules. Nucleic acids are made from nucleotides, which are composed of small nitrogenated bases, a five-carbon sugar and phosphate. The main function of nucleic acids in metabolism is indirect as bearers of genetic information, although they perform a crucial function for protein anabolism in ribosomes. Additionally, one of their nucleotide components is ATP, which also has the fundamental role in metabolism we have just discussed as energetic currency between catabolism and anabolism.

Despite sharing the feature of being also structural scaffolds in the living matter, proteins, carbohydrates and lipids have different functions in energy metabolism. Carbohydrates (sugars and their digestible polymers—starches and glycogen) mostly act as hydrophilic substrates for fast (anaerobic—cytosolic, oxygen-independent) or highly efficient (aerobic—mitochondrial, oxygen-dependent) ATP production. Carbohydrate metabolism can be considered the 'metabolic highway' of eukaryotic cells, as it shares many 'crossroads' with very important metabolic pathways involved with the other main metabolic substrates (Fig. 1). For instance, carbohydrates may be converted into lipids, which are an efficient form of chemical energy storage as, being hydrophobic, lipids (particularly triacylglycerols) do not carry along themselves solvating water molecules, thus reducing the space and weight associated with energy storage, were the organism to store energy only as carbohydrates. Lipids can be mobilized from their stores (mostly from white adipose tissue) in the form of free fatty acids, which also act as highly efficient substrates for ATP synthesis, though their catabolism is restricted to aerobic metabolism.

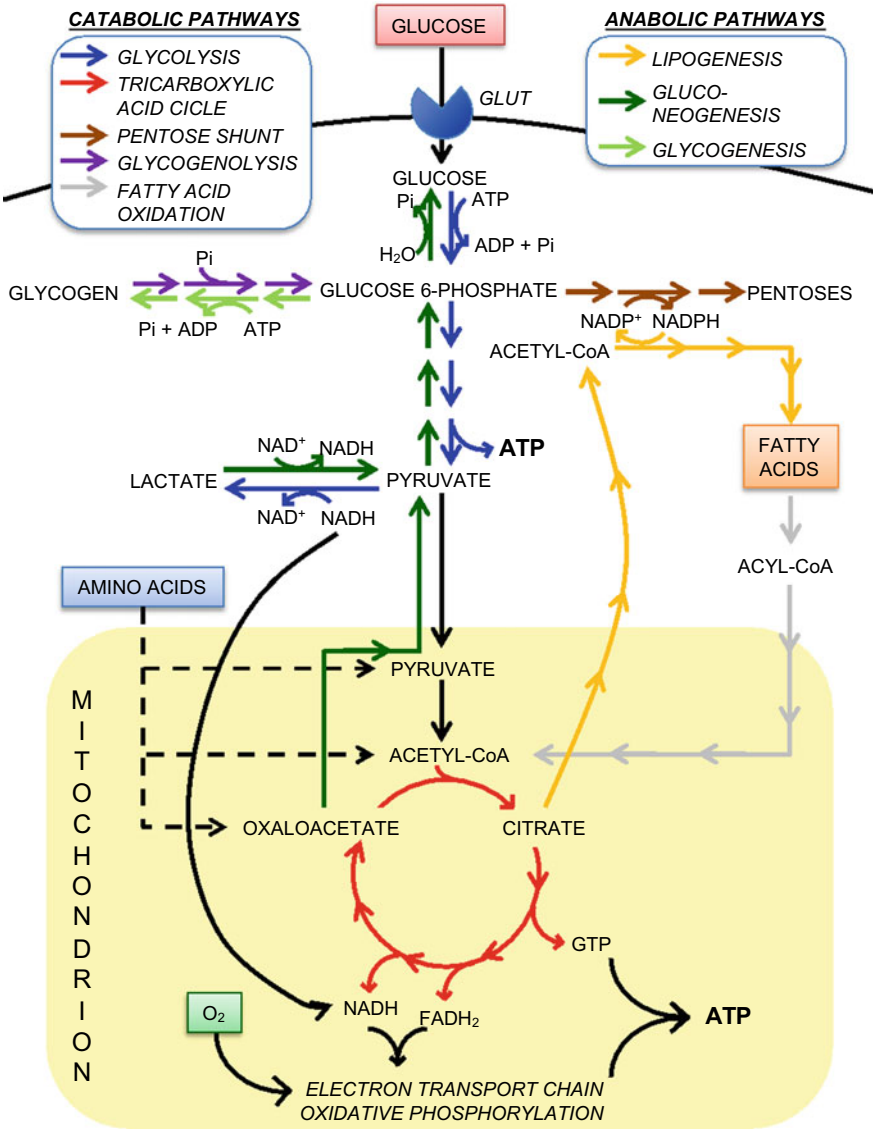

Fig. 1 An overview of the main enzymatic pathways involved in fuel metabolism. Both catabolic and anabolic pathways are sketched. The main substrates are highlighted in pink (glucose, the main carbohydrate), orange (fatty acids) and blue (amino acids)

As Fig. 1 illustrates, metabolic substrates are not freely interconvertible, and this is an issue particularly in the case of proteins. Proteins are not dedicated metabolic substrates, as they develop crucial functions in living organisms, as chemical (enzymes), mechanical (structural proteins), transport (channels and transporters) or communication (receptors) machines. However, their building blocks (amino acids) can yield carbohydrates and, therefore, lipids, via reactions that include the removal of their nitrogen atoms. So, between the three main metabolic substrates, only lipids cannot be converted into any of the others.

A brief analysis of Fig. 1 also demonstrates that metabolic pathways are extensively imbricated, and the final fate of substrates—or even which the substrates are—cannot be easily foreseen. However, despite the apparent complexity, metabolism is a highly dynamic but regulated process. An overview of the main instances of chemical signaling within metabolic regulation, as well as of nutrient and energy sensing by cells, is what lies ahead in this chapter.

2 The Mechanistic Logic of Metabolic Signaling

There are various levels and timeframes in which metabolic control can happen. The most basic type is the one that is intrinsic to a single metabolic pathway, in which the rate of catalysis of one or more enzymes is modulated by the reversible binding of intracellular molecules—usually end-products of the very same pathway—to specific non-catalytic sites of the enzyme. Upon binding, the conformation of the protein or enzyme is changed, either increasing or decreasing its affinity to the substrate(s) and/or its activity. This type of control of protein function, which is not restricted to enzymes, is known as allostery, and the molecules that mediate this phenomenon on a given protein are classified either as their positive or negative allosteric modulators depending on whether they increase or decrease its function [3, 4].

Within a metabolic pathway, allosteric modulation is the main mechanism in which feedback control happens [1]. For instance, in glycolysis, whose metabolic yield results in the net synthesis of 2 ATP molecules per glucose molecule metabolized, ATP is a negative allosteric modulator of the key enzyme phosphofructokinase 1 (PFK1). Conversely, AMP, which can be produced by the reaction of 2 ADP molecules to yield also one ATP by adenylate kinase in situations of ATP depletion, is a positive allosteric modulator of the same enzyme. However, allostery is in many cases also the framework for the integration of interconnected metabolic pathways. Here, again, PFK1 appears as an appropriate example. In situations in which metabolic flow through the tricarboxylic acid (TCA, or Krebs') cycle is much enhanced, such as in increased fatty acid oxidation, citrate can accumulate and allosterically inhibit PFK1, thus shifting ATP production from a carbohydrate to a lipid source [5]. This also stresses another common feature of metabolic control: usually the rate of catalysis of a pathway is determined by and controlled in a single or a few allosteric enzymes, which are effectively the master switches of that pathway.

Other proteins can also be the target of allosteric modulators that translate the metabolic state of the cell, with important signaling consequences within the same cell. For instance, there is a group of potassium ion channels that are allosterically modulated by the intracellular ATP/ADP concentration ratio. Aptly known as K_{ATP} channels, their open state probability is markedly decreased by raises in the [ATP]/[ADP] ratio, thus reducing potassium outflow and consequently causing membrane depolarization. This is the basis of the control of insulin secretion in pancreatic beta-cells by increased glucose availability and metabolism.

Metabolism-derived allosteric modulation of protein function is in this sense a universal feature of life as we know it. However, it is frequently taken for granted nowadays in many studies on metabolism, particularly in those focusing on multicellular organisms. This may be so because allostery could be seen as a phenomenon arising in single cells and affecting exclusively the same cells. However, the above example of K_{ATP} channel control of insulin secretion clearly demonstrates this is not always the case. In any case, allostery as it is commonly approached is a powerful and fast, but short-lived, mechanism of protein function modulation and thus of metabolic control, as it depends on the moment-by-moment levels of the allosteric modulators inside the cell.

Metabolic intercellular signaling is the domain of extracellular chemical mediators and nutrients. These molecules give rise to more perennial changes in the metabolic status of the cells upon which they act. The chemical basis of the sensing of either mediators or nutrients is, in itself, allosteric, as the proteins that first sense the signal transduce its level by changes in their own tertiary structure. However, the cascade of events downstream of this first sensing event tend to last much longer, as they imply in either covalent modifications of proteins (e.g. phosphorylation, dephosphorylation, prenylation etc.), changes in the concentration of intracellular second messengers (e.g. cyclic AMP, diacylglycerol etc.) [6], or modulation of the expression levels of specific proteins [1, 7]. This way, in metabolic intercellular signaling we are considering timeframes of several seconds to hours in principle, when considering covalent modifications and second messengers, to several minutes to days in expression modulation. However, some important intracellular metabolic sensing pathways utilize these same strategies, thus allowing individual cells to adapt to their individual energetic conditions and challenges.

Regarding covalent modifications of proteins in cell signaling, it is important to remember that the same protein may participate in distinct signaling pathways elicited by different extracellular mediators. As an example of that, consider phosphorylation. Phosphate groups from ATP can be incorporated into the structure of proteins as phosphate esters formed with sidechains of amino acids that bear a hydroxyl (OH) moiety, i.e. serine, threonine or tyrosine, in reactions catalyzed by protein kinase enzymes. There are two types of protein kinase enzymes regarding their amino acid substrate specificity: serine/threonine-kinases and tyrosine-kinases. So, a single signaling protein may be differentially modulated by phosphorylation depending on the kind of amino acid that is recognized by the protein kinase that phosphorylates it. Not only that, but within the same type of protein kinases considered, especially in the case of serine/threonine-kinases, a single protein may be phosphorylated in

different residues of the same amino acid, depending on the particular tertiary pattern each kinase recognizes.

Another often observed feature in post-translational modifications of proteins elicited by metabolic intercellular signaling is the fact that they may induce translocation of the targeted protein to different intracellular compartments. For instance, proteins of the signal transducer and activator of transcription (STAT) family dimerize and translocate from the cytoplasm to the nucleus upon specific tyrosine phosphorylation by Janus kinases (JAK). Once in the nucleus, they act as transcription factors and modulate protein expression. Hydrophobic modifications of proteins, such as prenylation or palmitoylation, are also important to localize/restrain signaling cascades to specific organelles or subcellular compartments as they tend to associate the modified proteins to specific membrane domains.

An important point that is often overlooked when discussing cell signaling in general is that, though the activated cascades last longer than the moment-by-moment allosteric modulation of enzyme activity, they do peter out. This has a clear adaptive value, as otherwise cells would be always inadvertently responding to signals and situations long gone. Various strategies of terminating signaling cascades in the absence of their stimulus exist, such as protein phosphatases, ubiquitin–proteasome degradation of activated proteins, degradation of second messengers (as is the case of phosphodiesterase-mediated hydrolysis of cyclic phosphates in cAMP and cGMP), slow intrinsic self-deactivating activity of signaling proteins (for instance the GTPase activity of the Gα subunits of G proteins) or the pumping out of calcium ions from the cytoplasm into either the endoplasmic reticulum (ER) or the extracellular space. Many of these strategies can be activated by the signaling cascade itself, generating negative feedback loops. Appropriately, there are even signaling pathways whose main effector proteins actually promote the deactivation of other signaling cascades, as happens in the inhibition of adenylate cyclase by $G\alpha_i$ proteins. This also sheds light into another outstanding feature of signaling cascades: they crosstalk, again because many signaling molecules and proteins are shared by them, and so the final observed effect is in every moment the result of the balance of their activity in these signaling hubs. As we will see, crosstalk is specially true for metabolic signaling [8–10].

Within this general logic of signaling strategies, in the next sections of this chapter we will briefly overview some examples of important pathways that mediate nutrient sensing in metazoans, as well as the signaling of some crucial hormones and mediators that affect whole-body metabolism. The following discussions do not intend by any means to be exhaustive, but merely to introduce the readers to some examples of the general mechanisms that were presented in this section in the hopes they will illuminate their comprehension of the many other pathways dealt with at depth in the other chapters of this book.

3 Nutrient-Sensing Signaling Pathways

As cell metabolism is basically dependent on ATP availability, which is coupled to NAD^+/NADH redox cycling, it is not surprising that signaling pathways that directly sense both variables have evolved.

In the case of ATP, this is brought about by a signaling system that is centered at a multiunit enzyme called 5'-AMP-dependent protein kinase, or AMPK [9, 11–16]. In situations of ATP depletion, the reaction catalyzed by adenylate kinase:

$$2\text{ADP} \rightleftharpoons \text{ATP} + 5'\text{AMP}$$

is shifted to the right, thereby increasing intracellular AMP content (note that this is not the cyclic form of AMP, cAMP, which is the second messenger produced upon activation of adenylate cyclase by $G\alpha_s$ proteins). AMP can bind to the regulatory γ subunit of AMPK and the consequent conformational change of the complex renders the catalytic α subunit more susceptible to phosphorylation, and thus activation, by liver kinase B1 (LKB1). Various proteins are substrates for active AMPK. This culminates in protein post-translational modifications and gene expression modulation that favor ATP production rather than ATP consuming processes. This way, among other actions, AMPK promotes lipolysis, fatty acid oxidation, glycolysis, glucose transport and autophagy, while inhibiting protein synthesis, lipogenesis, steroidogenesis and gluconeogenesis.

Nicotinamide coenzyme sensing, however, is mediated by another family of enzymes, sirtuins. These are lysine deacetylases that consume NAD^+ in their catalytic cycle, rather than reducing it to NADH as in its function of electron carrier in fuel metabolism. So, in situations in which substrate oxidation is reduced, the NAD^+/NADH ratio increases and consequently the availability of NAD^+ to sirtuins. There are seven sirtuins known in mammals (SIRT1-7), with different affinities for NAD^+, subcellular localization and substrate specificities [8, 16–18]. However, the ones that appear to have NAD^+-sensing abilities in the biological range of NAD^+ concentrations are the nuclear SIRT1 and the mitochondrial SIRT3 and SIRT 5. SIRT1 actions are the best described to date, and mainly involve the modulation of specific genes by deacetylation of specific transcription factors and cofactors, as well as histones. These culminate in the enhancement of mitochondrial function and overall glucose tolerance. Indeed, SIRT1 has been shown to increase the lifespan of animals subjected to high fat diets.

In eukaryotes, most of the ATP production is derived from aerobic mitochondrial metabolism. Consistently, oxygen availability also is sensed by cell signaling systems that impact on metabolism. This is mediated by a family of transcription factors known as hypoxia inducible factors (HIFs) [18–22]. Their function is controlled by an interesting mechanism in which the hydroxylation of HIF-1α, HIF-2α or HIF-3 α by prolyl hydroxylases that use O_2 as substrate signal for HIF proteasomal degradation at normal O_2 tensions. This way, in low O_2 levels, hydroxylation of α HIFs is reduced and the proteins accumulate, whereby they can migrate to the nucleus

and heterodimerize with constitutive HIF-1β and exert transcriptional control of hypoxia responsive element (HRE) genes. Some of these codify glycolytic enzymes and glucose transporters, thus increasing the cell capacity for anaerobic metabolism. On the other hand, it has been shown that HIF-1α in parallel reduces mitochondrial metabolism, both through effects on the conversion of pyruvate into acetyl-CoA and on the activity of TCA cycle enzymes, as well as on the biogenesis of mitochondria, through the indirect downregulation of a mitochondriogenesis-promoting transcriptional coactivator, PGC-1β. However, HIF signaling is not restricted to that, having pleiotropic effects on a multitude of processes important for tissue homeostasis, such as angiogenesis.

Mitochondrial metabolism also creates multiple signals that can be sensed by the cell. For instance, in addition to the ER, mitochondria are important intracellular calcium stores. Calcium mitochondrial content itself is an important regulator of oxidative phosphorylation [23]. However, at high levels and in the presence of oxidants, calcium can induce the formation in the inner mitochondrial membrane of a structure called permeability transition pore which leads to massive efflux of calcium to the cytosol and mitochondrial swelling to a point in which there is also release of cytochrome C. High cytosolic calcium and the presence of cytochrome C in the cytosol are known to trigger the intrinsic apoptotic pathway, for instance. But why would mitochondria be susceptible to oxidant damage? Incomplete reduction of oxygen in sites other than the final complex (IV) of the electron transport chain (ETC) seems to be the main source of superoxide anion, a reactive oxygen species (ROS) [9]. This gets more probable if there is a mismatch between the supply of electrons to the ETC from NADH and the rate of the ETC reactions, causing electrons to accumulate in the complexes of the ETC and increasing the chances they nonenzymatically react with molecular oxygen. This may happen either if the supply of oxygen to complex IV is low, or if the supply of NADH is higher than the capacity of the ETC to draw its electrons and pump protons, which can occur for instance if fatty acid oxidation (β-oxidation) is proceeding at very high rates. Reactive oxygen and nitrogen species are in themselves a feature of metabolism not only as otherwise unwanted byproducts, but also are controllers as well, as they trigger multiple signaling pathways which impact metabolism.

Apart from these mechanisms that we can consider to sense overall cellular energy status, we can also find in mammal cells an array of signaling pathways that specifically sense the three main groups fuel nutrients, the principles of which we will discuss in the next three subsections.

3.1 Lipid Sensing

Cells are endowed with pathways that sense the levels of fatty acids and that sense the availability of cholesterol, both kinds of signaling having major impacts on fuel metabolism.

Fatty acid sensing is mediated both by relatively fast G protein-coupled receptors (GPCRs) [6, 24, 25] and by slower, but longer-lasting, activation of intracellular receptors that are transcription factors, the peroxisome proliferator-activated receptors (PPARs) [26–29]. Fatty acid chemical properties are critical to their ability to activate the different subtypes of each family of fatty acid receptors. Among the GPCRs, GPR40, also known as free fatty acid receptor 1 (FFA1), is activated by medium to long chain fatty acids, particularly n-3 polyunsaturated fatty acids, while saturated fatty acids are more potent to activate it the longer their hydrocarbon chains. GPR40 is coupled to $G\alpha_q$ proteins and thus mediate its intracellular cascade via the activation of phospholipase C and thus the generation of the second messengers IP3, calcium (released from the endoplasmic reticulum) and diacylglycerol, which culminate in the activation of various enzymes, most notably C-type protein kinases (PKCs). More recently, another GPCR that is activated by long chain fatty acids has been described: GPR120 or free fatty acid receptor 4 (FFA4), whose intracellular signaling is similar to that for GPR40. However, in certain tissues GPR40 has been also shown to either stimulate or inhibit cAMP production by associating also to $G\alpha_s$ or $G\alpha_i$ proteins, respectively.

Meanwhile, short chain fatty acid levels, particularly acetate, propionate and butyrate, which are common byproducts of gut bacterial metabolism, are sensed by other GPCRs: GPR43, known also as free fatty acid receptor 2 (FFA2), and GPR41, alias free fatty acid receptor 3 (FFA3) [24]. While GPR43 signals via $G\alpha_i$ and $G\alpha_q$ proteins and GPR41 exclusively via $G\alpha_i$, it has been shown that GPR43 and GPR41 can heterodimerize. Interestingly, the heterodimer loses the ability to inhibit adenylate cyclase, while the raise in intracellular calcium levels is enhanced.

Long-term modulation of cell metabolism by fatty acids is mostly achieved by PPAR activation. Despite their name, PPARs do not restrict their actions only to peroxisomes, which are important organelles for the antioxidant defenses as well as for the metabolism of very long chain fatty acids in mammals and the production of fatty acid-derived signaling molecules. There are three isotypes of PPAR: PPARα, PPARγ and PPARδ (sometimes referred to as PPARβ/δ). Their distribution is not equal between tissues, the liver being the major site of expression of PPARα, while PPARγ is importantly expressed in adipose tissue and PPARδ has a more widespread distribution.

PPARα activation modulates gene expression in ways that enhance mitochondrial fatty acid oxidation not only in the liver, but also in muscle and adipose tissues. Moreover, fatty acid uptake is enhanced in the liver, but fatty acid export to the plasma in the form of lipoproteins is decreased, as well as cholesterol synthesis. This is the basis of the blood cholesterol-lowering effects of the PPARα agonists, fibrates. On the other hand, PPARγ activation (which is markedly promoted by n-3 fatty acids) is an important mediator of adipose tissue expansion and lipogenesis, while reducing leptin expression, and has an overall effect of increasing the whole-body sensitivity to insulin. This has been taken in advantage for the treatment of type 2 diabetes mellitus with the use of specific PPARγ agonists, the glitazones, as antidiabetic drugs. Also, while PPARγ increases liver output of LDL-cholesterol, it also increases apo-A secretion by the gut, thus increasing in parallel the capacity of

cholesterol scavenging by HDL. Both PPAR isotypes also have positive effects on pancreatic β cell function and, thus, on insulin secretion. Meanwhile, PPARδ is the least understood of the three kinds of PPAR so far, but PPARδ agonism has been shown to increase skeletal muscle utilization of glucose and fatty acid oxidation also in muscle, liver and in adipose tissue. Regardless of the PPAR isotype, the signaling involves translocation to the nucleus, where PPAR heterodimerizes with retinoic acid receptor (RXR) to modulate gene expression.

A distinct "flavor" to PPAR signaling is the family of proteins known as PPARγ coactivators (PGC) of transcription [8, 22, 30–32]. Now known not to be restricted as transcriptional coactivators only for PPARγ, PGCs are composed of PGC-1α (6 isoforms), PGC-1β and the PGC-1-related coactivator (PRC). Among them, PGC-1α is the most researched one and has been deemed the master regulator of mitochondriogenesis. In fact, PGC-1α can be considered a booster of whole-body oxygen-dependent oxidative metabolism, as it also promotes angiogenesis, muscle hypertrophy and liver gluconeogenesis.

As mentioned above, there are also sensing mechanisms for cholesterol found in mammalian cells. This is accomplished by a family of receptor transcriptional factors known as sterol regulatory element binding proteins, or SREBPs [1, 33]. Among them, we find two isoforms of SREBP-1, produced by alternative splicing of the SREBP-1 mRNA, and one of SREBP-2. The SREBP-1c isoform is mainly under the control of insulin and thus mediate the majority of the effects this hormone has on the expression of fatty acid metabolism genes in the liver and adipose tissue, mostly promoting lipogenesis. On the other hand, SREPB-2 signals for decreased cell cholesterol availability through an interesting mechanism, in which reduced cholesterol in the ER membrane renders it more available to migration to the Golgi and to proteolytic cleavage there. The N-terminal domain thus released can then migrate to the nucleus where it activates the gene expression of the LDL receptor and of enzymes of the cholesterol biosynthetic pathway.

3.2 Amino Acid Sensing

Though amino acids are known to stimulate both glucagon and insulin secretion, albeit to a much lesser extent than glucose influences the secretion of these metabolism-regulating hormones, a widespread system of cell signaling has evolved in eukaryotes that integrates many intracellular and extracellular signals, including amino acid availability (particularly the essential amino acid leucine in humans). This is centered on the serine/threonine kinase known as mechanistic target of rapamycin (formerly mammalian target of rapamycin), or mTOR [15, 34–36].

Depending on the proteins mTOR associates with, the signals received and the proteins it phosphorylates can be different. Thus, two mTOR complexes are recognized in mammalian cells: mTORC1 and mTORC2. mTORC1 is the complex for which most of the signaling has been described, and integrates various signals to promote anabolic effects, such as enhanced glucose catabolism and protein, lipid and

nucleotide synthesis, while inhibiting autophagy [37]. Overall, mTORC1 actions are an important drive for cell growth. Factors that stimulate mTORC1 activation are amino acids (but also other nutrients in general that increase overall energetic availability), hormones, oxygen and growth factors, while stress signals are inhibitory. On the other hand, mTORC2 seems to be mostly responsive to growth factors and promote cell survival and proliferation, mainly by enhancing glucose metabolism and mediating cytoskeletal rearrangements.

As noted above, the fate of mTOR between these two kinds of complexes is determined by the proteins to which it is associated. In mTORC1, mTOR is bound to RAPTOR, a protein that integrates various upstream signals, as well as to FKBP12, which is the protein when bound to rapamycin inhibits mTORC1 function. In mTORC2, RICTOR plays the role that RAPTOR has of signal integration in mTORC1. Interestingly, rapamycin only decreases mTORC2 activity in chronic treatments.

3.3 Carbohydrate Sensing

Regulation of carbohydrate metabolism is mainly brought about by whole-body coordination of functions through the pancreatic islet hormones, insulin and glucagon. However, it has been shown that in cell preparations lacking either these hormones, glucose independently influences the expression of genes that promote fat storage through a pathway that is mediated by a transcription factor known as carbohydrate responsive element binding protein, or ChREBP [1]. With increased glucose availability, metabolic flux through the pentose pathway also increases, and one of its products, xylulose 5-phosphate, can now activate a protein phosphatase, PP2A, that dephosphorylates ChREBP. This way, ChREBP can then migrate to the nucleus and activate lipogenesis genes, thus favoring the conversion of the excess carbohydrate into fatty acid stores.

4 Extracellular Signals that Regulate Fuel Metabolism

In pluricellular organisms, the concerted control of fuel metabolism in different tissues and organs certainly gave them an adaptive advantage to cope with changes in the environment that would imply either into substrate bounty or scarcity. In vertebrates, and particularly mammals, a sophisticated control system with multiple feedback loops and redundancy evolved that allows them to regulate the extracellular availability of metabolic substrates, particularly glucose, within narrow ranges of concentration. This control system integrates both the nervous and the endocrine systems, and acts via changes in feeding behavior, but most of all by changing the flux of metabolic substrates (sugars, lipids and amino acids) to and from organs

(mainly the liver, skeletal muscle and white adipose tissue) that can produce or store these substrates for their own use or for the disposal of the whole body.

But why glucose, of all the metabolic substrates, was selected by evolution to be the main surrogate for overall nutrient availability in this complex control system? For one side, amino acids are not properly storable, as their polymers are proteins, with their own individual and important functions. In addition, and crucially, there are tissues that are exclusively glycolytic, such as red blood cells, that in this way cannot depend on the mitochondrial oxidation of other substrates for ATP production. (Incidentally, in the case of erythrocytes that would be clearly an adaptive nonsense as they would consume for their own good the main substance their job is to transport across the whole body, oxygen). Moreover, the nervous tissue is in normal situations strictly dependent on glucose metabolism to supply its energetic demand. And this demand can be huge: a simple back of the envelope calculation demonstrates that the amount of glucose available in blood at any time (100 mg/dL in roughly 5 L of blood, so 5 g of glucose) would only suffice to support brain function for just one hour in the unreal situation in which the brain would be the only tissue using that glucose (the mass of glucose daily used by the central nervous system amounts to 120 g, something like a handful of sugar). This testifies to the fact that the flux of glucose to and from the blood is intense and tightly matched. Of course, other nutrients are also sensed by the nervous and endocrine system, such as bodily fat content via the proportional secretion of leptin by the adipose tissue, which signals to the hypothalamus to inhibit feeding behavior and to increase the sympathetic drive to adipose territories where it promotes lipolysis and fatty acid oxidation. Also, amino acids do stimulate insulin and glucagon secretions, albeit to a much smaller extent than the effects brought about on them by changes in blood glucose concentration.

This way, the extracellular signaling landscape can change dramatically and many times during a single day, depending on how many times we eat, what we eat, and how much. At the obvious risk of oversimplification, we can for didactic purposes consider a regular day in the life of a human from the standpoint of glucose metabolism as the alternation of periods when there is an external input of glucose through feeding, and periods when we are fasting, in which this external glucose input is no more.

In the fed state, blood glucose concentration tends to rise, and this increased glucose availability results in increased glucose uptake, metabolism and ATP concentration in pancreatic β cells. ATP closes K_{ATP} channels in these cells, which depolarizes them, leading to the opening of voltage-gated calcium channels and thus to the increased intracellular calcium concentration that triggers the exocytosis of preformed granules of insulin. This glucose sensing mechanism is clearly very fast, producing raises in blood insulin concentration quickly and in phase with the increases in blood glucose. Insulin signaling via its receptor is very fast too and, though it can be found in many tissues, making insulin a general growth factor, the main whole-body effects of this hormone on metabolism are due to its effects on the liver, adipose tissue and skeletal muscle, as illustrated in Fig. 2. Basically, insulin can be regarded as an anabolic hormone that promotes glycogen, protein and fat (triacylglycerol, TAG) synthesis by increasing glucose uptake and/or use in these organs as well as inhibiting glucose production in the liver and free fatty acid

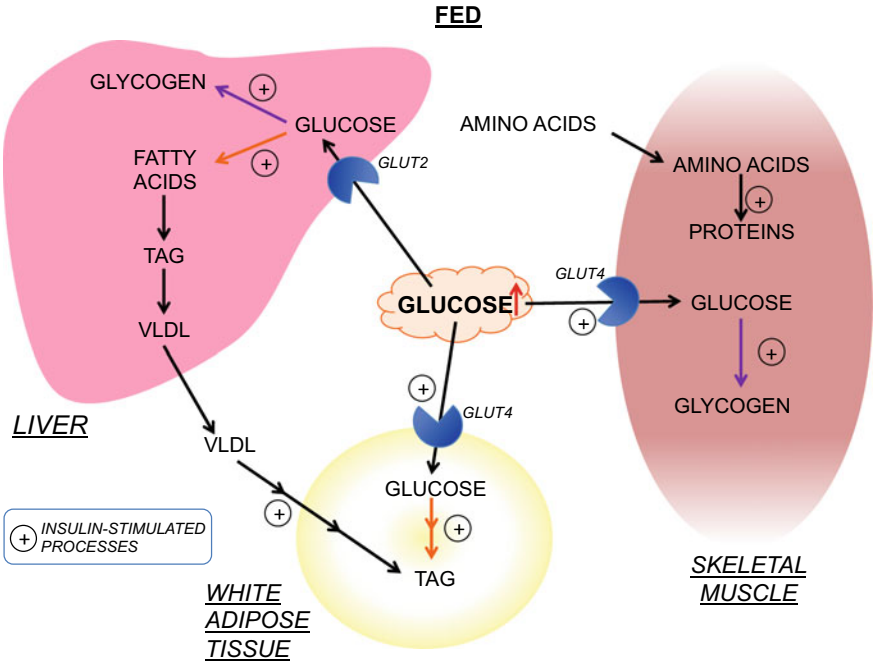

Fig. 2 Interplay between the liver, skeletal muscle and white adipose tissue in the fed state. The colors of the pathways depicted correspond to the same pathways in Fig. 1

output. Using the biochemical jargon, insulin stimulates glycolysis and glycogenesis in liver and muscle, glucose transport in the muscle and adipose tissue, protein synthesis mainly in muscle (in part by the increased intracellular availability and metabolism of glucose), lipogenesis from glucose in liver and adipose tissue, and inhibits glycogenolysis and gluconeogenesis in the liver, and lipolysis in the adipose tissue.

How does insulin mediate all these effects? The insulin receptor is a tyrosine protein kinase that is activated upon insulin binding. Not only does it phosphorylate tyrosine residues in specific substrates (insulin receptor substrate 1, IRS-1, and insulin receptor substrate 2, IRS-2), but it also phosphorylates itself. The phosphotyrosine motifs thus generated are recognized and bound to by proteins that bear the Src homology domain 2 (SH2). The IRS also have this domain and get recruited to the site of the insulin receptor by its autophosphorylation, as well as other proteins containing SH2, such as the p85 regulatory subunit of phosphoinositol-3-kinase (PI3K). This brings the catalytic p110 subunit of PI3K into close vicinity to the plasma membrane, where it can access its substrate, phosphatidylinositol-4,5-bisphosphate, and phosphorylate carbon 3 in the inositol moiety, producing phosphatidylinositol-3,4,5-triphosphate. This is recognized by proteins that have a domain called pleckstrin homology domain, such as the phosphoinositide-dependent kinase 1 (PDK-1). This protein thus activated phosphorylates a most important downstream protein in this

cascade, protein kinase B (PKB), more commonly known as Akt, on its threonine 308. This renders Akt partially active, what is fully achieved by concurrent phosphorylation at serine 473 by mTORC2. Akt itself is a serine/threonine protein kinase and has various possible substrates, including mTORC1 and glycogen synthase kinase 3 (GSK-3). While phosphorylation by Akt activates mTORC1, it inhibits GSK-3 activity. Despite its name, GSK-3 action is not restricted to glycogen synthase, whose phosphorylation by GSK-3 inhibits its function. Incidentally, this release from inhibition of glycogen synthase from GSK-3-mediated phosphorylation by Akt is the mechanism by which insulin induces glycogen synthesis. In addition to these examples of fast modulation of cell metabolism by insulin through Akt, insulin and Akt also have effects on gene expression, such as the aforementioned effect on SREBP-1c.

As insulin actions ensue, blood glucose levels accordingly reduce, ceasing to be a stimulus for further insulin secretion. The reduction of insulin levels itself already brings the insulin-responsive tissues to a more catabolic state. However, falling glucose levels during fasting are also detected by both the central nervous system and the pancreatic α cells that culminate in the release of catabolic mediators that increase glucose levels, namely noradrenaline, adrenaline and glucagon. Other hormones exist that also raise glycemia, and their secretion can also be enhanced by reductions in blood glucose as a stress factor, such as glucocorticoids, growth hormone and the thyroid hormones. This shows that insulin faces rather unfair competition as the single glucose-lowering hormone in humans.

Both glucagon and (nor)adrenaline signal to the metabolism-controlling organs via G protein-coupled receptors associated with $G\alpha_s$ subunits that activate adenylate cyclase to produce cAMP. Increased cAMP activates the serine/threonine protein kinase A (PKA) and, in higher concentrations, also another signaling pathway whose importance has been more recently acknowledged, mediated by cAMP-regulated guanine nucleotide exchange factors (cAMP-GEFs), also known as exchange factors directly activated by cAMP, or Epac. Though the glucagon receptor is found in other tissues, in humans its action is basically restricted to the liver, as it is secreted into the portal bed that drains into the liver, where most of the glucagon secreted is metabolized, making its systemic concentration marginally effective in adipose tissue and muscle.

Cyclic AMP signaling promotes effects that counteract those of insulin in the liver (Fig. 3). For instance, PKA activation stimulates glycogenolysis (via phosphorylation of phosphorylase kinase, which activates glycogen phosphorylase) and inhibits glycogen synthesis (through glycogen synthase phosphorylation, akin to that promoted by GSK-3). Also, gluconeogenesis is enhanced, through the increased availability of amino acids and lactate from muscle protein and glycogen catabolism, respectively. However, direct adrenaline action and cAMP signaling in muscle promotes protein synthesis rather than degradation. Another important substrate for gluconeogenesis is glycerol derived from adipose tissue lipolysis (which is also stimulated by cAMP through (nor)adrenaline). The concurrent increased free fatty acid output from lipolysis in white adipose tissue would lead to increased fatty acid oxidation in the liver, as it happens in other tissues which are thus offered an alternative to glucose as metabolic substrate, thus helping to reduce glucose consumption and thus

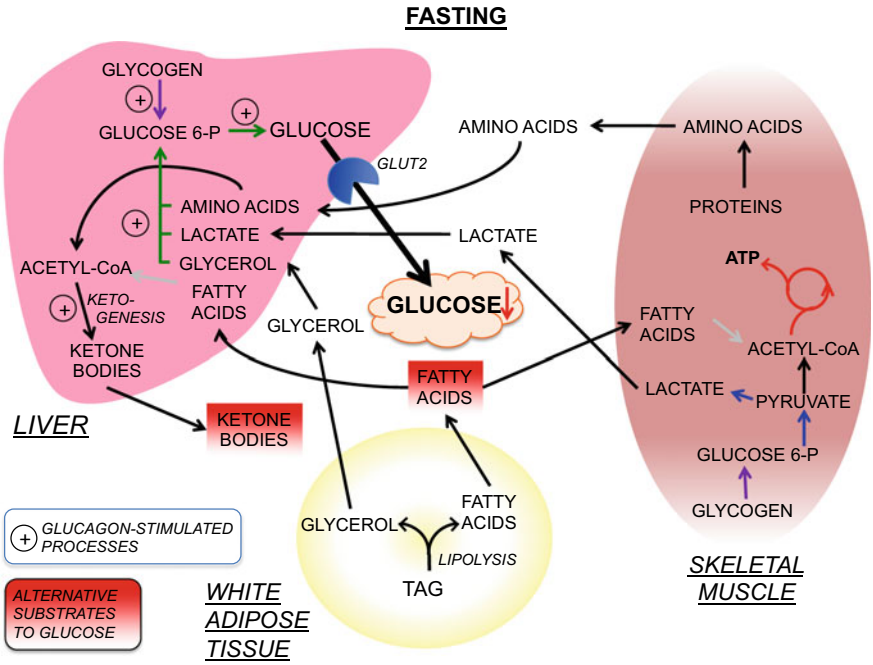

Fig. 3 Interplay between the liver, skeletal muscle and white adipose tissue in the fasting state. The colors of the pathways depicted here correspond to the same pathways in Fig. 1

to maintain blood glucose levels. However, as gluconeogenesis is stimulated in the liver, which uses TCA cycle intermediates as substrates, the activity of the TCA cycle is reduced, and acetyl-CoA builds up. The accumulated acetyl-CoA can be condensed into acetoacetate and β-hydroxy-butirate, known as ketone bodies. In prolonged fasting, as β-hydroxy-butirate concentration elevates, this ketone body induces the expression of its own metabolizing enzyme, β-hydroxy-butirate dehydrogenase, in the brain, which adapts this way its metabolism to use ketone bodies as alternative substrates to glucose. However, the increased ketogenesis leads to metabolic acidosis, which in part is compensated by the induction of proton-consuming gluconeogenesis in the kidneys, as the gluconeogenic enzymes there are induced by a curious mechanism in which the stability of their mRNA increases in low pH.

By this finely concerted interplay of tissues through metabolic signaling, humans are able to endure many weeks in fast, giving testimony to the selective pressures that prevailed in our dire evolutionary history of scarcity, rather than plenty. It is only very recently that plenty has become the norm, rather than the still sad exception, in most human societies. And so, as a species we have not had time to adapt to this excess food availability, thus explaining the sharp increase in metabolic and obesity-related diseases that will be considered in other chapters of this book [38, 39].

5 Pathway Crosstalk in the Regulation of Metabolism

As we have seen so far, cell metabolism is both subject to intrinsic signals in each cell itself that are derived from its own nutrient supply and to extracellular chemical mediators that integrate and signal the overall metabolic state of the organism. As expected, both these levels in signaling interact, as they may share common signaling proteins, into what is known as pathway crosstalk. Figure 4 is a crude attempt to show a very incomplete account of the possibilities of crosstalk in some of the pathways we have discussed in this chapter. The complexity of this network is evident, which could be seen maybe as an impediment to comprehension and research in this field. However, it is also a display of the robustness and sensitivity of metabolic signaling, a clear evidence of the evolutionary advance and sheer reliability it offers. With the outstanding advance in computational power, as well as the growing interdisciplinary interest that metabolism draws, systems biology approaches are steadfastly increasing our comprehension of these interactive levels of control and complexity [9, 10].

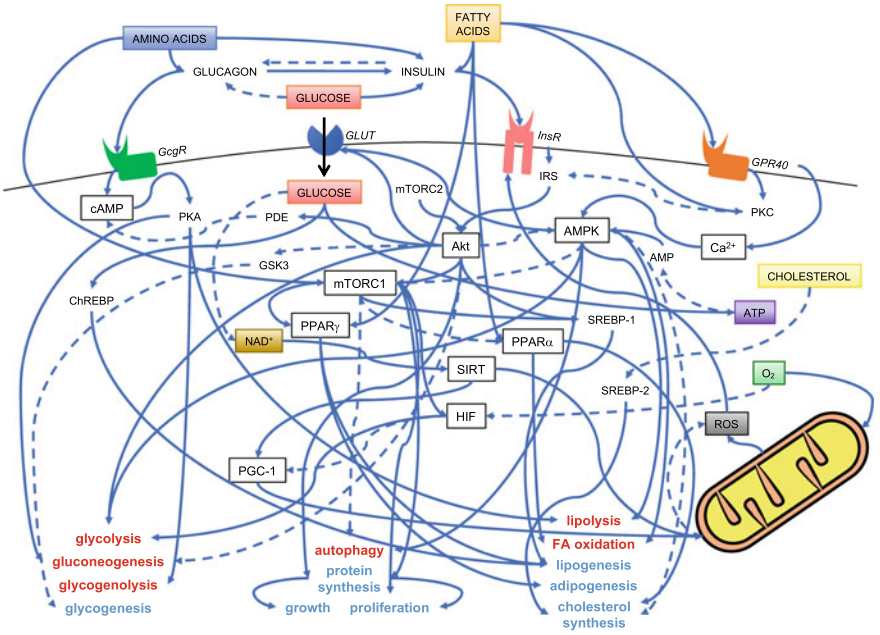

Fig. 4 Crosstalk between pathways in metabolic signaling. Nutrients and other signals that are sensed appear in colored boxes. The main signaling hubs are depicted in white boxes. Anabolic processes are written in blue boldface whereas catabolic processes are in red boldface. Solid arrows depict stimulatory interactions, whereas broken arrows represent inhibitory interactions

References

1. Frayn KN (2010) Metabolic regulation: a human perspective, 3rd edn. Wiley-Blackwell, Oxford
2. Cantó C, Menzies KJ, Auwerx J (2015) NAD^+ Metabolism and the control of energy homeostasis: a balancing act between mitochondria and the nucleus. Cell Metab 22:31–53
3. Monod J, Changeux J-P, Jacob F (1963) Allosteric proteins and cellular control systems. J Mol Biol 6:306–329
4. Monod J, Wyman J, Changeux J-P (1965) On the nature of allosteric transitions: a plausible model. J Mol Biol 12:88–118
5. Randle PJ, Garland PB, Hales CN, Newsholme EA (1963) The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. Lancet 281(7285):785–789
6. Husted AS, Trauelsen M, Rudenko O, Hjorth SA, Schwartz TW (2017) GPCR-mediated signaling of metabolites. Cell Metab 25:777–796
7. Haro D, Marrero PF, Relat J (2019) Nutritional regulation of gene expression: carbohydrate-, fat- and amino acid-dependent modulation of transcriptional activity. Int J Molec Sci 20:1386
8. Sugden MC, Caton PW, Holness MJ (2010) PPAR control: it's SIRTainly as easy as PGC. J Endocrinol 204:93–104
9. Thirupathi A, Souza CT (2017) Multi-regulatory network of ROS: the interconnection of ROS, PGC-1 alpha, and AMPK-SIRT1 during exercise. J Physiol Biochem 73:487–494
10. Watson E, Yilmaz LS, Walhout AJM (2015) Understanding metabolic regulation at a systems level: metabolite sensing, mathematical predictions, and model organisms. Annu Rev Genet 49:553–575
11. Burkewitz K, Zhang Y, Mair WB (2014) AMPK at the nexus of energetics and aging. Cell Metab 20:10–25
12. Carling D, Viollet B (2015) Beyond energy homeostasis: the expanding role of AMP-activated protein kinase in regulating metabolism. Cell Metab 21:799–804
13. Hardie DG (2014) AMP-activated protein kinase: a key regulator of energy balance with many roles in human disease. J Intern Med 276:543–559
14. Hardie DG (2014) AMPK-sensing energy while talking to other signaling pathways. Cell Metab 20:939–951
15. Hindupur SK, González A, Hall MN (2015) The opposing actions of target of rapamycin and AMP-activated protein kinase in cell growth control. Cold Spring Harb Perspect Biol 7:a019141
16. Boutant M, Canto C (2014) SIRT1 metabolic actions: integrating recent advances from mouse models. Mol Metab 3:5–18
17. Lin S-C, Hardie DH (2018) AMPK: sensing glucose as well as cellular energy status. Cell Metab 27:299–313
18. Schwer B, Verdin E (2008) Conserved metabolic regulatory functions of sirtuins. Cell Metab 7:104–112
19. Yu Q, Dong L, Li Y, Liu G (2018) SIRT1 and HIF1a signaling in metabolism and immune responses. Cancer Lett 418:20e26
20. Choudhry H, Harris AL (2018) Advances in hypoxia-inducible factor biology. Cell Metab 27:281–298
21. Metzen E, Ratcliffe PJ (2004) HIF hydroxylation and cellular oxygen sensing. Biol Chem 385:223–230
22. Semenza GL (2004) Hydroxylation of HIF-1: oxygen sensing at the molecular level. Physiology 19:176–182
23. Shoag J, Arany Z (2010) Regulation of hypoxia-inducible genes by PGC-1α. Arterioscler Thromb Vasc Biol 30:662–666
24. Vercesi AE, Castilho RF, Kowaltowski AJ, Oliveira HCF, Souza-Pinto NC, Figueira TR, Busanello ENB (2018) Mitochondrial calcium transport and the redox nature of the calcium-induced membrane permeability transition. Free Radic Biol Med 129:1–24
25. Ang Z, Xiong D, Wu M, Ding JL (2018) FFAR2-FFAR3 receptor heteromerization modulates short-chain fatty acid sensing. FASEB J 32:289–303

26. Kimura I, Ichimura A, Ohue-Kitano R, Igarashi M (2020) Free fatty acid receptors in health and disease. Physiol Rev 100:171–210
27. Charo IF (2007) Macrophage polarization and insulin resistance: PPARg in control. Cell Metab 6:96–98
28. Dubois V, Eeckhoute J, Lefebvre P, Staels B (2017) Distinct but complementary contributions of PPAR isotypes to energy homeostasis. J Clin Invest 127(4):1202–1214
29. Hong F, Pan S, Guo Y, Xu P, Zhai Y (2019) PPARs as nuclear receptors for nutrient and energy metabolism. Molecules 24:2545
30. Zechner R, Zimmermann R, Eichmann TO, Kohlwein SD, Haemmerle G, Lass A, Madeo F (2012) FAT SIGNALS—lipases and lipolysis in lipid metabolism and signaling. Cell Metab 15:279–291
31. Besseiche A, Riveline J-P, Gautier J-F, Bréant B, Blondeau B (2015) Metabolic roles of PGC-1α and its implications for type 2 diabetes. Diabetes Metab 41(5):347–357
32. Martínez-Redondo V, Petterson AT, Ruas JL (2015) The hitchhiker's guide to PGC-1α isoform structure and biological functions. Diabetologia 58:1969–1977
33. Supruniuk E, Mikłosz A, Chabowski A (2017) The implication of PGC-1a on fatty acid transport across plasma and mitochondrial membranes in the insulin sensitive tissues. Front Physiol 8:923
34. Shao W, Espenshade P (2012) Expanding roles for SREBP in metabolism. Cell Metab 16:414–419
35. Lamming DW, Sabatini DM (2013) A central role for mTOR in lipid homeostasis. Cell Metab 18:465–469
36. Liu GY, Sabatini DM (2020) mTOR at the nexus of nutrition, growth, ageing and disease. Nat Rev Mol Cell Biol 21(4):183–203
37. Singh R, Cuervo AM (2011) Autophagy in the cellular energetic balance. Cell Metab. 13:495–504
38. Baker RG, Hayden MS, Ghosh S (2011) NF-κB, inflammation, and metabolic disease. Cell Metab 13:11–22
39. Vitale I, Manic G, Coussens LM, Kroemer G, Galluzzi L (2019) Macrophages and metabolism in the tumor microenvironment. Cell Metab 30:36–50