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REVIEW

Augmenting energy expenditure by mitochondrial uncoupling: a role of AMP-activated protein kinase

Susanne Klaus · Susanne Keipert · Martin Rossmeisl · Jan Kopecky

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Abstract Strategies to prevent and treat obesity aim to decrease energy intake and/or increase energy expenditure. Regarding the increase of energy expenditure, two key intracellular targets may be considered (1) mitochondrial oxidative phosphorylation, the major site of ATP production, and (2) AMP-activated protein kinase (AMPK), the master regulator of cellular energy homeostasis. Experiments performed mainly in transgenic mice revealed a possibility to ameliorate obesity and associated disorders by mitochondrial uncoupling in metabolically relevant tissues, especially in white adipose tissue (WAT), skeletal muscle (SM), and liver. Thus, ectopic expression of brown fat-specific mitochondrial uncoupling protein 1 (UCP1) elicited major metabolic effects both at the cellular/tissue level and at the whole-body level. In addition to expected increases in energy expenditure, surprisingly complex phenotypic effects were detected. The consequences of mitochondrial uncoupling in WAT and SM are not identical, showing robust and stable obesity resistance accompanied by improvement of lipid metabolism in the case of ectopic UCP1 in WAT, while preservation of insulin sensitivity in the context of high-fat feeding represents the major outcome of muscle UCP1 expression. These complex responses could be largely explained by tissue-specific activation of AMPK, triggered by a depression of cellular energy charge. Experimental data support the idea that (1) while being always activated in response to mitochondrial uncoupling and compromised intracellular energy status in general, AMPK could augment energy expenditure and mediate local as well as whole-body effects; and (2) activation of AMPK alone does not lead to induction of energy expenditure and weight reduction.

Keywords Adipose tissue · Mitochondria · Obesity · Skeletal muscle · Uncoupling protein · Transgenic mice · Insulin sensitivity

S. Klaus · S. Keipert
German Institute of Human Nutrition,
Arthur-Scheunert-Allee 114-116, 14558 Nuthetal, Germany

M. Rossmeisl · J. Kopecky (✉)
Department of Adipose Tissue Biology, Institute of Physiology
Academy of Sciences of the Czech Republic v.v.i.,
Videnska 1083, 14220 Prague, Czech Republic
e-mail: kopecky@biomed.cas.cz

Introduction

The prevalence of obesity has increased dramatically world wide. This constitutes a serious strain on health-care systems because of the obesity-associated disorders such as cardiovascular disease, diabetes, and other metabolic morbidities aggregated in the metabolic syndrome (Withrow and Alter 2010). Currently available pharmacological treatments of obesity mainly act on appetite and thus decrease energy intake. Increasing the other side of the energy balance equation, that is, energy expenditure, seems to be an attractive additional or alternative approach. However, so far there has been no success in developing agents that increase energy expenditure without activating sympathetic nervous system activity, which might result in side effects such as increased heart rate and elevated blood pressure.

Nevertheless, it should be recalled that an effective strategy for obesity treatment by increasing energy expenditure in peripheral tissues has been used in more than 100,000 patients in the USA alone during the 1930s (Parascandola 1974). This treatment was based on the use of 2,4-dinitrophenol (DNP), a compound known since 1885

to exert thermogenic effect (Cazeneuve and Lépine 1885). Unfortunately, the drug had to be withdrawn soon from clinical practice, due to a very narrow therapeutical window and overdosing, which even resulted in several fatalities. Later on, it was demonstrated that DNP acted as an uncoupler of oxidative phosphorylation, while facilitating proton transport across the inner mitochondrial membrane, thus allowing maximum activity of the respiratory chain uncoupled from ATP-synthesis and stimulation of energy expenditure [reviewed in (Parascandola 1974)].

The idea that increasing energy expenditure in mitochondria might be an attractive strategy for combating obesity [for a review, see (Himms-Hagen 1983)] was refreshed in the late 1970s when brown adipose tissue (BAT) was recognized as the effector of diet-induced thermogenesis (Rothwell and Stock 1979). BAT is the site of regulatable non-shivering thermogenesis that is important in a cold environment mainly for small and newborn mammals. This heat production is mediated by uncoupling protein 1 (UCP1), which can dissipate the proton gradient across the inner mitochondrial membrane (Klaus et al. 1991; Nicholls and Locke 1984; Nedergaard et al. 2005). The interest in BAT thermogenesis had subsequently waned because BAT was not considered to be important for energy metabolism in adult humans but lately, new studies have demonstrated the presence of active BAT in adult humans and its adaptive capacity in response to cold exposure (Zingaretti et al. 2009; Lichtenbelt et al. 2009; Cypess et al. 2009; Virtanen et al. 2009; Nedergaard and Cannon 2010).

As reviewed here, experiments using mouse models harboring ectopic expression of UCP1 either in white adipose tissue (WAT), skeletal muscle (SM), or liver verified the concept that mitochondrial uncoupling could counteract obesity and that the anti-obesity effect could be induced by activity of ectopic UCP1 in any of the metabolically relevant tissues. While restricting this review on the effects of ectopic expression of UCP1, the only member of the UCP family with undisputed uncoupling properties (Nedergaard et al. 2005), we present evidence that mitochondrial uncoupling in WAT, SM, and liver (1) affects whole-body energy metabolism, glucose homeostasis, and longevity depending in part of the tissue harboring ectopic UCP1 and (2) leads to activation of AMP-activated protein kinase (AMPK), a highly conserved serine/threonine kinase, which plays a key role as a master regulator of cellular energy homeostasis [for reviews, see (Hardie 2008; Zhang et al. 2009)]. In spite of a large number of studies focused on the mechanism and physiological role of AMPK, and of the interest in AMPK with respect to the prevention and treatment of metabolic syndrome, the role of AMPK in control of energy expenditure remains controversial. Therefore, we aim to review the evidence linking AMPK activation to mitochondrial uncoupling in selected tissues and evaluate the importance of such mechanism for whole-body energy homeostasis. A strategy, utilizing the above mechanisms for the treatment of obesity and associated metabolic disorders, will be also discussed.

UCP1 and energy metabolism

Identification of UCP1 (Ricquier and Kader 1976) represented the hallmark in the search for the mechanism underlying the BAT thermogenic function. UCP1 is the first identified, best studied, and name giving protein of a family of mitochondrial membrane proteins (Nedergaard et al. 2005). This protein was long thought to be unique to BAT mitochondria (Klaus et al. 1991), though recently it has also been identified in thymocytes (Adams et al. 2008). Although the exact mode of action of UCP1 is still under dispute, it is generally accepted that (1) purine nucleotides (in vivo probably ATP and ADP, while GDP is used frequently in experiments) inhibit proton conductance by UCP1 and (2) that its thermogenic activity is equivalent to a proton transporter (Nedergaard et al. 2005). Proton conductance activity in vivo is thought to be activated mainly by free fatty acids (FFA). The mechanism of BAT thermogenesis and UCP1 activity has been reviewed extensively (Nedergaard et al. 2005; Klaus 2004); therefore, only a brief overview will be presented here. BAT thermogenesis is subject to acute and long-term adaptive regulation. Acute activation is mediated by the sympathetic nervous system and its neurotransmitter norepinephrine (NE). NE not only activates acute thermogenesis but also induces the expression of genes required for the thermogenic function of BAT such as UCP1. It is important for the overall energy homeostasis that the thermogenic activity of BAT can be tightly regulated and is only manifest when extra heat production is required. NE released upon acute cold exposure binds to β-adrenergic receptors, finally leading to increased lipolysis and elevated levels of FFA. Thus, FFA have a dual function: they serve as a fuel for thermogenesis, and they also activate the proton conductance function of UCP1. Because ATP-synthesis is bypassed, the respiratory chain is uncoupled and can function at maximum speed, assuring a high oxidation rate of FFA and release of energy as heat (Nedergaard et al. 2005; Klaus 2004). Interestingly, UCP1 can also function as a transporter for chloride and some other anions, but the physiological role of this activity is unknown (Nicholls and Lindberg 1973; Kopecky et al. 1984).

Studies with UCP1-ablated mice have shown that UCP1 is essential for adaptive adrenergically regulated non-shivering thermogenesis, inducible by both cold and diet, and they suggested that both processes fully emanate from

UCP1 activity [for review, see (Feldmann et al. 2009)] and that UCP1 functions as the only "true" uncoupling protein in vivo (Nedergaard et al. 2005). UCP1-mediated thermogenesis plays a role not only in thermoregulation but also in the overall energy balance and body weight regulation (Rothwell and Stock 1979; Klaus 2004). UCP1 ablation leads to the development of obesity under thermoneutral conditions (Feldmann et al. 2009), and over-expression of UCP1 or hyper-activated BAT thermogenesis could prevent development of obesity in a number of different studies (Klaus 2004). However, it should be stressed that UCP1-independent non-shivering thermogenesis also exists (see "Treatments enhancing energy expenditure" section).

Role of AMPK in energy metabolism and glucose homeostasis

While neglected for many years after its first description in 1973 [for a review, see (Hardie et al. 1998)], AMPK has emerged recently as a key evolutionary conserved cellular energy sensor and a master regulator of glucose and lipid metabolism in different tissues. It is a serine/threonine kinase, which is activated by any metabolic stress, often in response to increased AMP/ATP ratio (Hardie 2008; Zhang et al. 2009). AMPK is a heterotrimeric protein with each of its three subunits (α, β, and γ, respectively) encoded by different genes, giving rise to a large variety of heterotrimeric combinations in different tissues. AMPK activation occurs through several mechanisms (Hardie 2008). Thus, binding of AMP to the γ subunit results in an allosteric activation and induces conformational changes exposing the α subunit to upstream kinases. Of these kinases, the most important is considered to be the tumor suppressor LKB1. Moreover, under the conditions of the increased AMP/ATP ratio, dephosphorylation of AMPK by protein phosphatase-2A is inhibited. To achieve an acute activation of AMPK, 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR) or A-769662 (6,7-dihydro-4-hydroxy-3-(2′-hydroxy[1,1′-biphenyl]-4-yl)-6-oxo-thieno[2,3-b]pyridine-5-carbonitrile) (so-called Abbot Compound) are frequently used. AICAR is a highly cell-permeable compound, which is converted to ZMP, an AMP analog that mimics its effects on AMPK. Whereas LKB1 is required for the activation of AMPK in response to AMP, calmodulin-dependent protein kinase kinase (CaMKK) activates AMPK by phosphorylating the same residue (Thr-172) of the α subunit as LKB1 when activated by increased intracellular Ca^{2+}, independent of AMP (Hardie 2008; McGee and Hargreaves 2010). As described in BAT (Qi et al. 2008), AMPK is negatively regulated by cell-death-inducing like effector A (Cidea), inducing ubiquitination of the β subunit of AMPK and degradation of the enzyme. Also protein kinase A-mediated phosphorylation of α1-AMPK subunit at Ser-173 prevents activation of AMPK by LKB1-mediated phosphorylation of the α1-AMPK subunit at Thr-172, as found recently in white adipocytes (Djouder et al. 2010).

Different metabolic stresses that either inhibit ATP production such as hypoxia and hypoglycemia or increase ATP consumption (e.g., muscle contraction) lead to an activation of AMPK, which then turns on glucose uptake and various catabolic pathways (namely glycolysis and fatty acid oxidation) and switches off biosynthetic pathways (synthesis of glycogen, protein, and fatty acids). It thus activates pathways resulting in ATP production while turning off energy-consuming pathways both at a single cell and the whole-body level (Hardie 2008; Zhang et al. 2009). Eventually, AMPK contributes to an increase in mitochondrial biogenesis in response to chronic energy depletion (Reznick and Shulman 2006). AMPK is also involved in the regulation of food intake through its action on the expression of hypothalamic neuropeptides, with an activation of AMPK leading to increased food intake (Lim et al. 2010). Effects of AMPK activation further include modulation of cell cycle, tumor growth suppression, inhibition of proteosynthesis, stimulation of autophagy, ion channel regulation, and many other effects, which are out of the scope of this review.

On a molecular level, a close connection exists between AMPK and the histone/protein deacetylase SIRT1, a molecule supposed to be involved in the genetic changes that mediate the increase in longevity induced by caloric restriction (Canto et al. 2010). AMPK and SIRT1 apparently regulate each other and share common target molecules. AMPK could be upstream of SIRT1, that is, regulate SIRT1 activity (Canto et al. 2010; Um et al. 2010), thus controlling SIRT1-mediated effects on aging and health. In addition, due to its interaction with cryptochrome 1, a component of circadian clock in peripheral tissues, AMPK contributes to metabolic entrainment of peripheral clocks (Lamia et al. 2009).

In line with its function as a cellular energy sensor, AMPK has been involved in the metabolic effects of hormones such as leptin, ghrelin, adiponectin, glucocorticoids, insulin, glucagon as well as cannabinoids—all these hormones being involved in regulation of energy metabolism (Lim et al. 2010). Although AMPK is apparently present in all tissues (Hardie 2008; Zhang et al. 2009), its activation and mode of action seem to be regulated in a tissue-specific manner. For example, leptin activates AMPK in adipose tissue, SM, and liver but inhibits it in hypothalamus (Lim et al. 2010), while adiponectin and anti-diabetic drugs primarily activate the AMPK in the peripheral tissues. Furthermore, there is a number of natural food components

believed to exert beneficial effects on health in association with activation of AMPK like plant polyphenols (Um et al. 2010) or *n*-3 long-chain polyunsaturated fatty acids (*n*-3 PUFA) (Jelenik et al. 2010; Kopecky et al. 2009). The role of AMPK in energy metabolism has been most intensively studied in SM, heart, adipose tissue, liver, beta cells, and hypothalamus, that is, tissues that are highly sensitive to changes in energy balance and also actively involved in its regulation. Here, we will briefly summarize the role of AMPK in liver, SM, and adipose tissue, while focusing on the role of AMPK in the control of lipid and glucose metabolism.

AMPK function in liver

Hepatic AMPK has a central role in the metabolic adaptation to acute and chronic nutritional stress (Berglund et al. 2009). AMPK influences hepatic lipid metabolism, where its activation leads to a reduction in lipogenesis and cholesterol synthesis and a simultaneous increase in fatty acid oxidation leading to a decreased hepatic lipid deposition (Hardie 2008; Zhang et al. 2009). AMPK is believed to control liver glucose homeostasis, due to downregulation of key gluconeogenic genes. However, recent studies suggest that AMPK rather supports than inhibits hepatic gluconeogenesis by improving hepatic energy status during fasting (Berglund et al. 2009), also in accordance with the notion that metformin, a glucose-lowering drug, inhibits hepatic gluconeogenesis independently of LKB1/AMPK pathway and independently of the transcriptional repression of gluconeogenesis, via a decrease in hepatic energy state (Foretz et al. 2010). Thus, the role of AMPK in the control of hepatic glucose metabolism by thiazolidinediones (TZDs), and also by adiponectin, an insulin-sensitizing adipokine (Zhang et al. 2009; Yamauchi et al. 2002), should be reconsidered. Functional adiponectin-AMPK regulatory pathway is required for the beneficial effects of both TZDs (Kubota et al. 2006) and *n*-3 PUFA (Jelenik et al. 2010) on hepatic glucose metabolism and its sensitivity to insulin.

AMPK function in SM

Muscle AMPK is involved in the coordinated transcription of genes important for lipid and glucose metabolism during exercise and for acute control of metabolic fluxes, namely the switch from carbohydrate to lipid oxidation (Hardie 2008; Zhang et al. 2009; McGee and Hargreaves 2010; Canto et al. 2010; Viollet et al. 2009). Thus, AMPK is activated by muscle contraction, which depletes ATP leading to an increase in the AMP/ATP ratio. It restores cellular energy balance by increasing ATP through an increase in fatty acid uptake and lipid oxidation. AMPK also increases SM glucose uptake by increasing the glucose transporter GLUT4 in the sarcolemma, a mechanism, which is distinct from the action of insulin that also stimulates GLUT4-mediated glucose uptake in muscle cells (Karagounis and Hawley 2009). Endurance exercise increases mitochondrial activity and content, while insulin resistance is associated with defects in mitochondrial lipid catabolism and accumulation of ectopic lipids. AMPK was found to directly phosphorylate and thus activate PGC-1α (Jager et al. 2007), which has a crucial role in enhancing mitochondrial biogenesis in SM and BAT, and up-regulate expression of the PGC-1α gene (Irrcher et al. 2009), in association with a shift of SM fibers toward a more oxidative phenotype (McGee and Hargreaves 2010). Involvement of AMPK in the stimulation of lipid catabolism in SM by leptin (Minokoshi et al. 2002) is discussed below (see "Treatments enhancing energy expenditure" section).

AMPK function in adipose tissue

In WAT, AMPK can be activated in a fat depot-specific manner by both fasting (Sponarova et al. 2005) and exercise (Park et al. 2002), that is, situations of increased adipose tissue lipolysis. Furthermore, the adipokines leptin and adiponectin (Orci et al. 2004; Wu et al. 2003) and dietary *n*-3 PUFA (Kopecky et al. 2009) activate AMPK in WAT. It has been found in rodent adipocytes that lipolytic and cAMP-elevating stimuli increase AMPK activity, while its activation by AICAR or phenformin strongly reduces isoproterenol-induced lipolysis and fatty acid release (Daval et al. 2005). In human adipocytes, β-adrenergic stimulation of lipolysis was found to be down-regulated by concomitant activation of AMPK through TZDs (Bourron et al. 2010). Overall, acute AMPK activation in adipose tissue leads to an inhibition of both lipogenesis and lipolysis, whereas it increases fatty acid oxidation and glucose transport (Viollet et al. 2009; Daval et al. 2006). It seems counter-intuitive (Daval et al. 2006) that AMPK inhibits lipolysis, although it is itself activated in situations of increased lipolysis. It could be speculated (Djouder et al. 2010; Sponarova et al. 2005; Daval et al. 2006; Gauthier et al. 2008) that AMPK-induced reduction of lipolysis could be a feed back mechanism limiting the cellular energy drain, because part of the fatty acids released can be re-activated to acyl-CoA and re-esterified which requires ATP and generates AMP. Also, accumulation of large amounts of fatty acids could be deleterious for the cells.

There are relatively few studies on AMPK function and regulation in BAT considering the important role of this tissue in energy metabolism. AMPK protein amount and activity was found to be two to threefold higher in BAT

than in liver (Mulligan et al. 2007). Chronic cold exposure of mice led to a progressive increase in AMPK activity in BAT (Mulligan et al. 2007) and prolonged lack of adrenergic stimulation led to decreased AMPK activity, which was secondary to reduction in both α1-and α2-AMPK subunits levels (Pulinilkunnil et al. 2011). On the other hand, acute blockage of α-adrenergic signaling resulted in stimulation of AMPK activity, mediated by alteration in AMPK α Thr^{172} phosphorylation, which could be secondary to diminished oxygen consumption in BAT (Pulinilkunnil et al. 2011). Acute stimulation of β-adrenergic signaling in mice also resulted in increased AMPK activity in BAT, leading to increased glucose uptake with both effects abolished in UCP1 knock-out mice (Inokuma et al. 2005). In brown adipocytes differentiated in vitro, β-adrenergic stimulation was also found to increase AMPK phosphorylation and β-adrenergically mediated glucose uptake required AMPK. But in contrast to the former study, this stimulation was also found in brown adipocytes from UCP1-ablated mice, thus independent of the presence of UCP1 (Hutchinson et al. 2005). Thus, the mechanistic link between UCP1 and AMPK in BAT, as well as the role of AMPK in the BAT thermogenic function, remains to be clarified. Emerging evidence suggests that α2-but not α1-AMPK subunit might be required for thermogenesis in BAT (Pulinilkunnil et al. 2011; Bauwens et al. 2011). In addition to its potential role to regulate BAT metabolism, AMPK-mTOR signaling may promote differentiation of BAT cells (Vila-Bedmar et al. 2010).

The above data indicate that AMPK functions as the key component of a complex signaling cascade, to maintain stable intracellular energy state. Mitochondrial uncoupling induced by ectopic UCP1 in various tissues represents a unique approach to further understand and delineate AMPK biology.

Mouse models of ectopic UCP1 expression

The search for pharmacological tools that counteract obesity, based on the induction of BAT thermogenesis, led to the identification of the UCP1 gene in both rat (Bouillaud et al. 1988) and mouse (Kozak et al. 1988) (and later on also in other species). The subsequent investigation of the regulatory regions of the rodent genes demonstrated a complex interplay of transcription factors including cAMP- and thyroid hormone-response elements in the BAT-specific induction of UCP1 (Cassard-Doulcier et al. 1993; Kozak et al. 1994; Rabelo et al. 1995). However, so far, all these efforts have not resulted in any practical application as far as obesity treatment in human patients is concerned. On the other hand, the identification of UCP1 gene structure and the well-documented thermogenic role of UCP1 and its effect on energy metabolism provided a unique handle to study physiological consequences of mitochondrial uncoupling in tissues other than BAT, using various transgenic mouse models.

White adipose tissue

That mitochondrial uncoupling in WAT could reduce obesity was found in 1995 by Kopecky et al. (1995) using aP2-*Ucp1* transgenic mice and explored systematically later on (Kopecky et al. 1996a, b; Stefl et al. 1998; Baumruk et al. 1999; Rossmeisl et al. 2000, 2002, 2005; Flachs et al. 2002; Matejkova et al. 2004). In these mice, the fat-specific aP2 promoter was used to drive expression of UCP1 from the whole genomic DNA sequence, resulting in enhanced expression of UCP1 in both WAT and BAT of mice with C57BL/6 J background. Only one line of the aP2-*Ucp1* mice has been studied, mostly animals heterozygous for the transgene. These mice show atrophy of BAT, resulting presumably from excessive amounts of UCP1, which collapse energy metabolism in BAT cells, with the most pronounced phenotype in the homozygous transgenic mice (Stefl et al. 1998). More transgenic UCP1 is detected in subcutaneous than in gonadal WAT, and the expression declines during aging (Rossmeisl et al. 2002), suggesting a posttranscriptional control of the transgene expression or possibly an elimination of UCP1-expressing adipocytes with time. About 2–10% of UCP1/membranous protein is present in WAT of heterozygous adult mice as compared with BAT of wild-type warm-acclimated mice (Kopecky et al. 1995).

Transient UCP1 expression in epididymal fat has been achieved using an adenoviral vector, resulting in ~20-fold lower UCP1 levels than in BAT (Yamada et al. 2006). There are also reports on the forced expression of UCP1 in 3T3-L1 cells, a murine preadipocyte cell line (Yamada et al. 2006; Si et al. 2007, 2009; Senocak et al. 2007).

Skeletal muscle

Ectopic expression of UCP1 in SM has been achieved by three independent groups, using different promoters in transgenic constructs (Li et al. 2000; Couplan et al. 2002; Klaus et al. 2005). Several transgenic lines were generated and investigated (Li et al. 2000; Couplan et al. 2002). Studies of these transgenic mice, all of them containing UCP1 cDNA, provide the unique opportunity to analyze the "true" effects of ectopic UCP1 in SM independent of mouse background strain, animal maintenance, or possible artifacts due to the insertion site of the transgene.

In the model first described by Li et al. (2000), UCP1 expression was driven by the rat myosin light chain 2 promoter resulting in two independent lines of mice on a

C57Bl/6 × CBA hybrid background: Ucp-L and Ucp-H, with low and high expression of UCP1 gene in SM but no other tissues. Low expression corresponded to ~1% of UCP1 content in muscle mitochondria compared to BAT mitochondria in heterozygous mice. Couplan et al. (2002) used rat UCP1 cDNA driven by the mouse muscle creatine kinase (MCK) promoter creating several lines of transgenic mice of which the MCK-UCP1-13 and MCK-UCP1-20 lines (Bl6D2 F1 background) were further characterized. Expression of UCP1 in SM was similar in both lines, but MCK-UCP1-13 showed the highest expression in heart, whereas MCK-UCP1-20 showed relatively low cardiac expression. UCP1 mRNA was not detected in brain or liver. Content of UCP1 in muscle mitochondria of heterozygous transgenic mice was similar to that in BAT (Couplan et al. 2002). Heterozygous HSA-mUCP1 transgenic mice (C57Bl/6 × CBA) were generated using mouse UCP1 cDNA under control of a human SM actin promoter (HSA) fragment, which confers striated muscle-specific gene expression. UCP1 gene expression could be detected in SM but not in heart, stomach, and non-muscle tissues (Klaus et al. 2005), resulting in ~ tenfold lower UCP1 content in SM than in BAT on a whole tissue level (S. Keipert, unpublished data).

Liver

Ishigaki et al. (2005), using an adenoviral vector, induced a transient UCP1 gene expression in the liver of mice rendered obese and insulin resistant by prior feeding of a high-fat diet. UCP1 content in liver mitochondria peaked at 3–7 days after the adenovirus injection, followed by a decline in the expression. However, no data are provided on expression levels compared to BAT or on the tissue specificity of the expression. Gonzalez-Muniesa and coworkers used hydrodynamic delivery, a liver-specific technique, to obtain UCP1 gene expression in liver mitochondria of FVB mice, leading to a transient expression, which lasted for ~7 days (Gonzalez-Muniesa et al. 2006). There are no reports on transgenic mouse models with permanent hepatic UCP1 expression.

Functionality of ectopic UCP1

Similarly as in humans (see "Introduction" section), also in mice, a general increase in proton conductance of the inner mitochondrial membrane in response to a chemical uncoupler such as DNP (Nedergaard et al. 2005) has been shown to reduce obesity, while improving insulin sensitivity and increasing lifespan (Caldeira da Silva et al. 2008). However, in contrast to the effect of DNP, transgenic expression of UCP1 was expected to increase mitochondrial proton conductance in a tissue-specific and regulatable manner. Therefore, the functionality of UCP1 expressed ectopically in various tissues was examined (Table 1). Ectopic UCP1 decreased mitochondrial membrane potential in adipocytes isolated from gonadal fat of aP2-*Ucp1* mice and rendered it sensitive to fatty acids and purine nucleotides (Baumruk et al. 1999). Full uncoupling activity was achieved in the presence of ~15-fold less UCP1 than in BAT mitochondria of control mice (Kopecky et al. 1996b), resulting in the molar UCP1/respiratory chain ratio ~1, while this ratio is between 5 and 11 in BAT (Baumruk et al. 1999). Increased AMP/ATP ratio was found in WAT of aP2-*Ucp1* mice, suggesting a decrease in ATP production in vivo (Matejkova et al. 2004). There are no data on the functionality of UCP1 expressed transiently in epididymal fat of mice using the adenoviral vector (Yamada et al. 2006); however, adenoviral UCP1 expression in 3T3-L1 cells decreased intracellular ATP concentrations (Si et al. 2009).

Mitochondria from heart and SM of MCK-UCP1 mice showed an increased proton leak, which could be completely inhibited to wild-type levels by addition of albumin (which binds free fatty acids) and GDP (Couplan et al. 2002). Interestingly, ectopic UCP1 in heart mitochondria had no apparent detrimental effects under normal conditions, suggesting that in heart UCP1 would be either inhibited or without any influence on mitochondrial energetics. Furthermore, phosphocreatine/ATP ratio in leg muscles of MCK-UCP1 mice was decreased, while total creatine levels were unchanged, suggesting a decrease in ATP production. UCP1 expression in mitochondria from rats subjected to injection of UCP1-cDNA into the tibialis muscle resulted in a decrease of mitochondrial membrane potential, and mitochondrial uncoupling, which could be counteracted by addition of GDP (Larrarte et al. 2002). Keipert et al. (2010) showed in isolated muscle mitochondria from HSA-mUCP1 mice that the activity of ectopic UCP1 could be fully inhibited by GDP and reactivated by fatty acids. Additionally, this study is the first to demonstrate a significant reduction (by ~76% compared to wild type) in mitochondrial superoxide production during mitochondrial resting state due to UCP1.

The effects of ectopic UCP1 in liver mitochondria were studied either in vitro, using HepG2 cells (Gonzalez-Muniesa et al. 2005), or using liver mitochondria from mice subjected to hydrodynamic-based transfection of rat UCP1 (Gonzalez-Muniesa et al. 2006). In both cases, increased proton leakage and decreased ATP production were described. However, the functionality of UCP1 with regard to its regulation by purine nucleotides and fatty acids was not assessed.

Together, these data document that ectopic UCP1 was inserted correctly into the inner mitochondrial membrane,

Table 1 Modulation of cellular energy metabolism by ectopic UCP1 expression in various tissues

	White fat cells	Muscle cells	Liver cells
Mitochondrial uncoupling regulated by purine nucleotides and fatty acids	Yes (Baumruk et al. 1999)	Yes (Keipert et al. 2010)	ND
Maximum mitochondrial respiration rates	ND	Unchanged (Si et al. 2007) Decreased (Ishigaki et al. 2005)	ND
Mitochondrial biogenesis and/or content	Increased (Rossmeisl et al. 2002)	Content decreased (Han et al. 2004)	ND
Mitochondrial ROS production	ND	Reduced (Keipert et al. 2010)	ND
Cellular ATP levels	Decreased (Flachs et al. 2002; Yamada et al. 2006); increased AMP/ATP ratio (Matejkova et al. 2004)	Unchanged (Li et al. 2000) or decreased (Li et al. 2000; Couplan et al. 2002; Han et al. 2004) depending on expression level	Decreased (Gonzalez-Muniesa et al. 2006; Gonzalez-Muniesa et al. 2005)
AMPK phosphorylation	Increased (Matejkova et al. 2004)	Increased (Gates et al. 2007) Increased basal and insulin stimulated (Neschen et al. 2008)	Increased (Ishigaki et al. 2005)
Cellular fat accumulation	Decreased (Yamada et al. 2006; Si et al. 2007; Si et al. 2009)	Unchanged (76) or increased (75)	Decreased (Ishigaki et al. 2005)

ND not determined, *ROS* reactive oxygen species

where it was functional and regulated in the context of intracellular environment in various tissues, unlike in the case of a permanent modulation of mitochondrial function by chemical uncouplers. However, it remains to be established whether the activity of ectopic UCP1 is really changing in response to fluctuations of intracellular fatty acid concentrations, that is, increased under the conditions of increased supply of dietary lipids, or when levels of fatty acids in adipocytes and in plasma are elevated during fasting (see "Whole-body effects of ectopic UCP1 on energy and substrate metabolism" section). The results also suggest that UCP1 exhibits the maximum uncoupling activity even when present in much lower amounts in mitochondria as compared with BAT, in accordance with the notion that the capacity of UCP1 in BAT mitochondria exceeds several fold the proton-pumping activity of the respiratory chain (Lin and Klingenberg 1982).

Metabolic effects of ectopic UCP1 at cellular and tissue levels

In response to ectopic UCP1 expression, complex metabolic changes were found in cells and tissues harboring UCP1 expression. The most extensive characterization was performed in the case of WAT of aP2-*Ucp1* mice showing increased activity of lipoprotein lipase (LPL) especially in epididymal fat, which correlated with the hypolipidemic effect of the transgene (Kopecky et al. 1996b; Rossmeisl et al. 2005), decreased fatty acid synthesis (Rossmeisl et al. 2002; Kopecky et al. 2001) associated with a down-regulation of fatty acid re-esterification (Rossmeisl et al. 2005), inhibition of norepinephrine-stimulated lipolysis associated with reduced cAMP levels and activity of hormone sensitive lipase (HSL) (Flachs et al. 2002), increased in situ fatty acid oxidation (Matejkova et al. 2004; Rossmeisl et al. 2005), reflected by elevated oxygen consumption measured ex vivo in WAT (Kopecky et al. 1996b), and increase in mitochondrial content in adipocytes (Rossmeisl et al. 2002; see also Table 1). Except for the effect on LPL activity, all the metabolic changes observed in the aP2-*Ucp1* mice were more pronounced in subcutaneous than in epididymal fat, in accordance with a higher content of UCP1 (Rossmeisl et al. 2002) and markedly lower weight (Kopecky et al. 1996a) of the subcutaneous fat. Furthermore, expression of ectopic UCP1 resulted in reduced expression of genes characteristic of differentiated energy-storing adipocytes, such as peroxisome proliferator-activated receptor gamma (PPARγ) and aP2 (Matejkova et al. 2004), in analogy with the induction of "fat burning adipocytes" in response to leptin treatment in rats (Zhou et al. 1999). In accordance, mitochondrial uncoupling in adipocytes in vitro led to lower intracellular ATP concentrations (Yamada et al. 2006; Si et al. 2009), up-regulation of glycolysis and mitochondrial biogenesis, and down-regulation of energy-consuming pathways such as fatty acid synthesis (Rossmeisl et al. 2000; Si et al. 2009; Senocak et al. 2007), while lactate production was increased (Rossmeisl et al. 2000; Si

et al. 2009). The analysis of gene expression (M. Rossmeisl et al., unpublished) suggested an upregulation of lipid catabolism in SM of high-fat diet-fed mice in response to the transgenic UCP1 expression in WAT, which is also associated with hypolipidemic effects, especially under fasted conditions.

The effect of UCP1 expression on SM phenotype was studied in detail by Couplan et al. (2002), who reported a fiber type switch from fast glycolytic fibers to slow oxidative fibers in gastrocnemius and plantaris muscle accompanied by a reduced muscle mass. A reduced mass of gastrocnemius and quadriceps muscle was also found in HSA-UCP1 mice (Neschen et al. 2008). High levels of UCP1 in SM seem to compromise muscle function by causing a severe mitochondrial myopathy with increased intramuscular lipid accumulation (Han et al. 2004). UCP1 expression in SM was accompanied by decreased accumulation of hepatic lipids especially after high-fat feeding (Katterle et al. 2008). Interestingly, UCP1 expression in SM affected lipid metabolism in WAT, where increased expression of lipogenic as well as lipolytic genes in association with an increase in insulin stimulated glucose uptake was observed, pointing to the induction of a futile cycle of lipogenesis and lipolysis (Katterle et al. 2008). The mechanisms of the tissue cross-talks from WAT to muscle, and from muscle to WAT, respectively, remain to be explained.

Whole-body effects of ectopic UCP1 on energy and substrate metabolism

Table 2 summarizes the effects of ectopic UCP1 expression on phenotypes of energy and substrate metabolism. It is noteworthy that these effects depend in part on the site of ectopic UCP1 expression. As data on phenotype of mice with liver-specific expression of UCP1 are rather limited (see "Mouse models of ectopic UCP1 expression" section), we will focus on mice with transgenic expression of UCP1 in WAT and SM, respectively.

Body composition and obesity

Ectopic UCP1 expression in WAT of aP2-*Ucp1* transgenic mice did not affect body weight under basal conditions, that is, when the animals were fed a standard chow (Kopecky et al. 1995, 1996a). However, transgenic mice were protected against both genetic obesity, as shown in a cross of aP2-*Ucp1* mice with lethal yellow (A^y/a) mice (Kopecky et al. 1995), as well as against high-fat diet-induced obesity (Kopecky et al. 1996a; Stefl et al. 1998), independent of gender (Kopecky et al. 1996a). The reduction in body weight gain of ~50% of heterozygous aP2-*Ucp1* transgenic mice under high-fat feeding was associated with a decreased adiposity but not with changes in lean body mass (Kopecky et al. 1996a), although homozygous transgenic mice, which were almost completely resistant to obesity, tended to be smaller (Stefl et al. 1998). Importantly, the weight of subcutaneous but not gonadal WAT of the transgenic mice was reduced, in accordance with the higher expression of the transgene in the subcutaneous fat (Kopecky et al. 1995, 1996a), suggesting the importance of metabolic changes induced in situ by ectopic UCP1 for reduction of WAT mass. The anti-obesity effect was very robust, observed even in 13-month-old A^y/a mice (Kopecky et al. 1995), and during the whole life in transgenic mice with C57BL/6 J background fed high-fat diet (S. Kopecky et al., unpublished), in spite of the decline in the content of ectopic UCP1 in WAT during aging (Rossmeisl et al. 2002). Thus, obesity resistance represented a dominant feature induced by ectopic UCP1 in WAT (Kopecky et al. 1995, 1996a; Stefl et al. 1998). In mice with the transient adenoviral UCP1 expression in epididymal fat, no significant changes in body weight and the weight of epididymal fat were observed, but the diameter of UCP1-expressing adipocytes was significantly reduced (Yamada et al. 2006). Forced UCP1 expression in 3T3-L1 adipocytes also led to a reduction in lipid accumulation (Si et al. 2007, 2009).

In all models of UCP1 expression in SM, a decrease in body weight was observed, which resulted both from a decreased lean and fat mass (Li et al. 2000; Couplan et al. 2002; Klaus et al. 2005). The reduction in lean body mass could result from reduced SM mass as well as from reduced bone mineral content (Han et al. 2004). Overall growth was also affected in both HSA-mUCP1 mice (Katterle et al. 2008) and UCP-H mice (Han et al. 2004), which showed reduced body length compared to wild type. However, in other model, no changes in body length were observed (Li et al. 2000; Bernal-Mizrachi et al. 2002). The reduction in body fat mass suggested a resistance to diet-induced obesity in mice with SM UCP1 expression, which was indeed observed in the very first transgenic model by Li et al. (2000). However, it was found later that in elderly (1-year-old) HSA-UCP1 mice, long-term feeding of high-fat diet did induce considerable accumulation of body fat (Katterle et al. 2008). Indeed, HSA-UCP1 mice showed a delayed development of obesity on a high-fat diet but reached comparable maximum adiposity compared to WT (Keipert et al. 2011).

Energy metabolism

Although obesity resistant, the aP2-*Ucp1* mice exhibit BAT atrophy associated with decreased norepinephrine-induced respiration at the whole-body level, cold-sensitivity of homozygous (but not heterozygous) transgenic

Table 2 Physiology of transgenic mice with ectopic expression of UCP1 in WAT, SM, or liver

	UCP1 in white fat	UCP1 in muscle	UCP1 in liver
Body composition			
Body weight	Unchanged on STD, reduced on HFD (Kopecky et al. 1996a; Stefl et al. 1998)	Reduced (Li et al. 2000; Couplan et al. 2002; Klaus et al. 2005)	Reduced on HFD (Ishigaki et al. 2005)
Lean body mass	Unchanged (Kopecky et al. 1996a)	Reduced (Li et al. 2000; Couplan et al. 2002; Klaus et al. 2005)	ND
Muscle mass	ND	Reduced (Li et al. 2000; Couplan et al. 2002; Neschen et al. 2008)	ND
Fat mass	Reduced on HFD (Kopecky et al. 1996a)	Reduced (Li et al. 2000; Couplan et al. 2002; Klaus et al. 2005)	Reduced on HFD (Ishigaki et al. 2005)
Body length	ND	Reduced (Klaus et al. 2005; Han et al. 2004) Unchanged (Li et al. 2000; Bernal-Mizrachi et al. 2002)	ND
Blood pressure	ND	Reduced on a genetically obese background (Bernal-Mizrachi et al. 2002; Gates et al. 2007)	ND
Energy metabolism			
Energy expenditure	Increased (Stefl et al. 1998)	Increased (Li et al. 2000; Couplan et al. 2002; Klaus et al. 2005)	Increased on HFD (Ishigaki et al. 2005)
Energy intake	Cumulative energy intake decreased or unchanged (Kopecky et al. 1996a; Stefl et al. 1998)	Increased (Couplan et al. 2002; Klaus et al. 2005)	Reduced per animal on HFD (Ishigaki et al. 2005)
Body temperature	Reduced in homozygous mice (Stefl et al. 1998)	Increased (Gates et al. 2007) Unchanged (Li et al. 2000) Reduced in cold (Klaus et al. 2005)	Unchanged (Ishigaki et al. 2005)
High-fat diet-induced obesity	Reduced (Kopecky et al. 1996a; Stefl et al. 1998)	Reduced (Li et al. 2000) Delayed (Katterle et al. 2008)	Reduced (Ishigaki et al. 2005)
Genetic obesity	Reduced (Kopecky et al. 1995)	Reduced (Bernal-Mizrachi et al. 2002; Gates et al. 2007)	ND
Glucose and lipid metabolism			
Plasma insulin	Reduced on HFD (Kopecky et al. 1996a)	Reduced on HFD (Li et al. 2000; Katterle et al. 2008)	Reduced on HFD (Ishigaki et al. 2005)
Plasma glucose	Elevated on STD, reduced on HFD (Kopecky et al. 1996a)	Unchanged (Couplan et al. 2002; Klaus et al. 2005; Katterle et al. 2008) Reduced (Li et al. 2000; Neschen et al. 2008)	Reduced (fed state) (Ishigaki et al. 2005)
Glucose tolerance	Increased on HFD (Kopecky et al. 1996a)	Increased (Li et al. 2000)	Increased on HFD (Ishigaki et al. 2005)
Insulin sensitivity	ND (unchanged after 3 week of HFD—unpublished)	Increased on HFD only (Li et al. 2000) Increased independent of diet (Katterle et al. 2008)	Increased on HFD (Ishigaki et al. 2005)
Glucose oxidation	ND	Increased (Neschen et al. 2008)	ND
Plasma cholesterol	Reduced on HFD (Kopecky et al. 1996a)	Reduced (Li et al. 2000; Katterle et al. 2008) Unchanged (Couplan et al. 2002)	Reduced on HFD (Ishigaki et al. 2005)
Plasma TG	Reduced, interaction of HFD and transgene dosage (Kopecky et al. 1996a; Rossmeisl et al. 2005)	Reduced (Li et al. 2000) Unchanged (Couplan et al. 2002)	Reduced on HFD (Ishigaki et al. 2005)
Plasma NEFA	Mostly reduced, effect of transgene dosage (Rossmeisl et al. 2005 and unpublished)	Reduced (Katterle et al. 2008) Unchanged (Li et al. 2000) Increased (Han et al. 2004)	Reduced on HFD (Ishigaki et al. 2005)

Table 2 continued

	UCP1 in white fat	UCP1 in muscle	UCP1 in liver
Longevity			
Median lifespan	Marginally increased on HFD (M. Rossmeisl and J. Kopecky, unpublished)	Increased by 10% on chow diet (Gates et al. 2007)	ND
		Increased by 33% on HFD (Keipert et al. 2011)	
Maximum lifespan	ND	Increased by 10% (Keipert et al. 2011)	ND

BW body weight, *HFD* high-fat diet, *ND* not determined, *NEFA* non-esterified fatty acids, *STD* standard chow, *TG* triglycerides

mice (Stefl et al. 1998), and a decreased oxygen consumption in BAT (Kopecky et al. 1996b). These data are in accordance with the idea that an increase in endogenous heat production, not mediated by BAT, prevents development of obesity in these mice, as also supported by the measurement of minimum metabolic rate (MMR). It is evaluated as oxygen consumption in an anaesthetized animals under thermoneutral conditions (Denckla and Marcum 1973), and represents the activity of the core mechanisms underlying basal metabolic rate and regulatable solely by thyroid hormones, independent on muscle tone and sympathetic activity (Girardier and Stock 1983). Indeed, MMR was found to be slightly but significantly increased in aP2-*Ucp1* transgenic mice when measured at several time points during aging, independent on body weight (Stefl et al. 1998). In addition, aP2-*Ucp1* mice reared at thermoneutrality (30°C) show a similar degree of obesity resistance compared to their counterparts raised at 22°C (M. Rossmeisl et al., unpublished data). Interestingly, BAT deficiency in the aP2-*Ucp1* mice was associated with only slightly reduced food intake (Kopecky et al. 1996a) or no changes in food intake at all (Stefl et al. 1998).

In contrast to aP2-*Ucp1* mice, no effect of adenoviral UCP1-overexpression in epididymal fat on resting oxygen consumption was found (Yamada et al. 2006), possibly because a subtle increase of energy expenditure in response to a transient UCP1 overexpression limited to epididymal fat was undetectable at the whole-body level. In analogy with aP2-*Ucp1* mice, food intake was rather decreased than increased in the presence of UCP1 in WAT (Yamada et al. 2006). Importantly, this effect was found to be linked to increased hypothalamic leptin sensitivity via afferent-nerve signals emanating from intra-abdominal fat tissue, associated with decreased leptinemia. These results suggested a novel type of signaling from WAT, dependent on energy metabolism in adipocytes (Yamada et al. 2006). Thus, most probably, such mechanism also contributes to obesity resistance of aP2-*Ucp1* transgenic mice, and it may be instrumental for novel treatment strategies for obesity.

As anticipated, UCP1 expression in SM led to increases in energy expenditure in all different mouse models studied, which was partly compensated by an increased energy intake (Couplan et al. 2002; Klaus et al. 2005). The most detailed characterization of energy metabolism was performed in HSA-UCP1 mice, which displayed increased energy expenditure and increased heat loss during activity only, suggesting a decreased activity-related muscle energy efficiency (Klaus et al. 2005). Indirect calorimetry indicated increased overall oxidation of glucose (Klaus et al. 2005; Katterle et al. 2008). However, in the different models, there is a discrepancy with regard to body temperature, which was reported to be increased (Gates et al. 2007), decreased during activity and in cold (Klaus et al. 2005) or unchanged (Li et al. 2000), compared to wild type. The reason for this is not quite clear but might be related to different measurement methods and maintenance conditions. Spontaneous physical activity levels do not seem to be affected in this model (Klaus et al. 2005; Keipert et al. 2011), but grip strength was reduced in mice with high levels of UCP1 in SM (Han et al. 2004), and voluntary wheel running activity was also found to be slightly decreased (S. Keipert and S. Klaus, unpublished data).

Whole-body substrate metabolism and glucose homeostasis

Profound effects of ectopic UCP1 in either WAT or SM on systemic lipid metabolism and glucose homeostasis were observed. Results suggest that the profound systemic effects of transgenic UCP1 on lipid metabolism of the aP2-*Ucp1* mice (Kopecky et al. 1996a; Rossmeisl et al. 2005) originate from changes in lipid handling by WAT cells (see "Metabolic effects of ectopic UCP1 at cellular and tissue level" section), resulting in a relatively low release of fatty acids from adipocytes into the circulation. In accordance with the idea that the activity of transgenic UCP1 is stimulated by intracellular fatty acids in adipocytes, this activity should be increased under the conditions of increased lipolysis during fasting. In fact, most data regarding systemic levels of lipid metabolites in aP2-*Ucp1* transgenic mice were obtained in animals subjected to

overnight fasting. The effects of ectopic UCP1 in WAT on lipid metabolism were much less pronounced when the animals were analyzed under fed conditions (M. Rossmeisl et al., unpublished data). Thus, fasted plasma levels of triglycerides were reduced, while these changes were potentiated by feeding animals a high-fat diet (Kopecky et al. 1996a; Rossmeisl et al. 2005). In this respect, transgene dosage and high-fat diet interacted in amplifying the effect of transgenic UCP1 (Rossmeisl et al. 2005). The effect of ectopic UCP1 in WAT was less consistent with respect to plasma FFA levels, which tended to decrease in mice fed standard chow and was either unchanged or slightly decreased in mice fed a high-fat diet (Rossmeisl et al. 2005 and M. Rossmeisl et al., unpublished data). Cholesterol levels were decreased in male transgenic mice fed a high-fat diet (Kopecky et al. 1996a), but otherwise unchanged. The increased fatty acid utilization of WAT during fasting could also affect lipid deposition and metabolism in other tissues. Indeed, a physiological induction of hepatic triglyceride accumulation during fasting was blunted in aP2-*Ucp1* mice, while hepatic lipid content was similar in fed transgenic and wild-type mice (M. Rossmeisl et al., unpublished data).

Glucose levels were elevated in overnight fasted aP2-*Ucp1* transgenic mice fed a standard chow (Kopecky et al. 1996a), similarly to transgenic mice lacking HSL (Voshol et al. 2003), although hepatic glucose production was unchanged in aP2-*Ucp1* transgenic (M. Rossmeisl et al., unpublished data) as well as in HSL knock-out mice (Voshol et al. 2003). In both transgenic models, however, a decrease in plasma insulin levels could be responsible for this phenomenon (Kopecky et al. 1996a; Voshol et al. 2003). On the other hand, in aP2-*Ucp1* transgenic mice challenged by a high-fat diet, glucose tolerance was significantly improved, while their plasma insulin levels were decreased (Kopecky et al. 1996a), suggesting improved insulin sensitivity associated with a markedly reduced body fat content. Also the transient UCP1 overexpression in WAT improved glucose tolerance and insulin sensitivity, while decreasing plasma triglycerides and FFA, even in the absence of any effect on body weight (Cassard-Doulcier et al. 1993).

In contrast to ectopic UCP1 in WAT, data on plasma lipid levels in different models of transgenic mice with UCP1 gene expression in SM are not conclusive. Indirect calorimetry suggests a decreased overall lipid oxidation (Klaus et al. 2005) but also suggests a higher metabolic flexibility, that is, a more rapid switch between glucose and fat oxidation under feeding and fasting conditions, respectively (Katterle et al. 2008). However, no direct measurements of fatty acid uptake and oxidation have been published so far, although an increased gene expression of the fatty acid transporter CD36 in transgenic mice may point to increased fatty acid uptake in SM upon expression of UCP1.

A very consistent phenotype of all mouse models with UCP1 expression in SM is an improved glucose homeostasis, that is, increased insulin sensitivity accompanied by decreased insulin levels, especially under the conditions of high-fat diet feeding (Li et al. 2000; Klaus et al. 2005; Neschen et al. 2008; Katterle et al. 2008), which was found even in the monogenetic obesity model (Bernal-Mizrachi et al. 2002). Actually, glucose homeostasis is the best studied metabolic phenotype in this model using different methodological approaches such as euglycemic-hyperinsulinemic clamps (Neschen et al. 2008), glucose and insulin tolerance tests (Li et al. 2000; Katterle et al. 2008; Bernal-Mizrachi et al. 2002), and evaluation of fuel partitioning using indirect calorimetry (Klaus et al. 2005). Interestingly, although mice with UCP1 expression in SM have been found to develop late onset of dietary obesity, insulin sensitivity was increased independent of the diet and body fat accumulation in comparison to wild type suggesting a dissociation of obesity and insulin resistance in this model (Katterle et al. 2008; Keipert et al. 2011).

Longevity

In view of the whole-body beneficial metabolic effects induced by ectopic UCP1 in WAT or SM, a positive influence of the ectopic UCP1 on survival and lifespan should be expected. Indeed, in heterozygous aP2-*Ucp1* mice fed both chow or high-fat diet, a tendency for a longer lifespan was observed (J. Kopecky et al., unpublished), while a significantly increased median and maximum survival was found in mice expressing UCP1 in SM (Keipert et al. 2011; Gates et al. 2007). Remarkably, UCP1 expression in SM could almost completely abolish the reduction in lifespan caused by chronic high-fat diet feeding (Keipert et al. 2011).

Major differences in phenotypes induced by ectopic UCP1

The tissue as well as whole-body effects of ectopic UCP1 depend substantially on the tissue harboring UCP1 expression as summarized in Fig. 1. That induction of energy expenditure by mitochondrial uncoupling in WAT has a more pronounced effect on obesity and lipid metabolism as compared with ectopic UCP1 in SM seems paradoxical, regarding the difference in the weight-adjusted oxidative capacity of these two tissues, with WAT and SM contributing 5–10% and 20–30%, respectively, to basal metabolic rate (Tataranni and Ravussin 1995). However, the measurements of mitochondrial cytochrome c oxidase

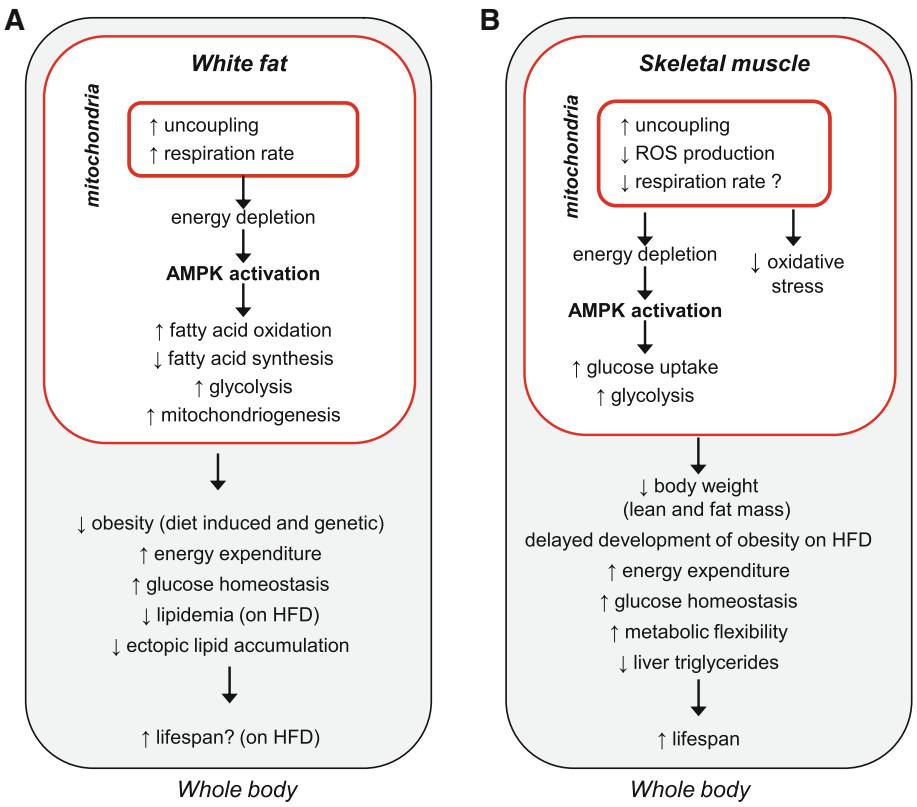

Fig. 1 Differential and common phenotypes induced by ectopic UCP1 in WAT (**a**) and SM (**b**) and possible involvement of AMPK. Mice with ectopic UCP1 expression in WAT are characterized by obesity resistance, which is apparent throughout the whole adult life, associated with minimal changes in lean body mass, while mice with ectopic UCP1 in SM are resistant to obesity only during the first 8 months of life. In both models, glucose homeostasis (evaluated as a tolerance to glucose load and/or as fasted glycemia) is permanently improved, suggesting better insulin sensitivity. However, a dissociation between the effects of ectopic UCP1 in SM on glucose homeostasis and obesity was observed. A relatively strong hypolipidemic effect of ectopic UCP1 in WAT, which is most pronounced under obesogenic conditions, is probably absent in mice expressing UCP1 in SM. Many of the observed phenotypes could be attributed to activation of AMPK induced by partial energy depletion caused by the uncoupling of respiratory chain. However, different mechanisms may be involved in the beneficial effects on glucose homeostasis exerted by mitochondrial uncoupling in WAT and SM, respectively. Thus, metabolic changes induced by ectopic UCP1 in adipocytes counteract release of fatty acids from WAT into circulation and prevent accumulation of ectopic lipids in liver as well as in SM, while preserving insulin sensitivity of these two organs, namely under obesogenic conditions. On the other hand, the beneficial effects of ectopic UCP1 in SM on muscle as well as whole-body insulin sensitivity, and on metabolic flexibility (i.e., a more rapid switch between glucose and fat oxidation under feeding and fasting conditions), are clearly unrelated to the prevention of lipotoxic accumulation of muscle lipids (Han et al. 2004) and must be mediated by another mechanism. A decreased mitochondrial ROS production in SM UCP1 expressing mice could possibly lead to decreased oxidative damage caused by detrimental diets and aging

(COX) activity in mice maintained at 20°C suggest that total COX activity of WAT represents ~30 to 50% of the total COX activity in BAT (Kopecky et al. 1996b), providing therefore a substantial capacity for energy expenditure. Moreover, both human and animal studies suggest that the energy cost of fat mass in vivo is greater than expected on a basis of its intrinsic metabolic rate and that body fat mass is a major determinant of metabolic rate (Kaiyala et al. 2010). This may depend on leptin (Kaiyala et al. 2010), as well as on other afferent signaling from WAT (Yamada et al. 2006). However, irrespective of the nature of this signaling, this further supports the original idea (Kopecky et al. 1995) that metabolism of WAT represents a promising target for obesity treatment, as also discussed more recently (Rossmeisl et al. 2004; Maassen et al. 2007). On the other hand, the robust improvement of glucose homeostasis, independent of the anti-obesity effect, is the dominant phenotype induced by ectopic UCP1 in SM (Li et al. 2000; Couplan et al. 2002; Klaus et al. 2005).

Involvement of AMPK in consequences of mitochondrial uncoupling in WAT and SM

As described above, metabolic effects of ectopically expressed UCP1 in WAT and SM are surprisingly complex. To explain all these phenotypic changes, involvement of key cellular energy sensors, and especially AMPK, must be

considered (see "Role of AMPK in energy metabolism and glucose homeostasis" section; see Table 1). Indeed, stimulation of AMPK activity in WAT of aP2-*Ucp1* mice was detected (Matejkova et al. 2004). The activity of α1 AMPK complex, a more abundant form of AMPK in WAT as compared with α2 AMPK (Daval et al. 2005), was increased more in subcutaneous than in epididymal WAT (Matejkova et al. 2004), reflecting the UCP1 content in WAT and the change in the cellular AMP/ATP ratio (Rossmeisl et al. 2002). The activation of AMPK in response to mitochondrial uncoupling could explain fat depot-specific depression of lipogenesis (Rossmeisl et al. 2000) and lipolysis (Flachs et al. 2002), increase in fatty acid oxidation (Matejkova et al. 2004) and mitochondrial content (Rossmeisl et al. 2002), as well as down-regulation of PPARγ and its target gene aP2, documenting suppression of the adipogenic potential of the tissue (Matejkova et al. 2004). Thus, AMPK possibly represents the major regulatory intracellular pathway responsible for the array of metabolic changes induced by UCP1 in white adipocytes. In addition to the inhibitory effect of AMPK on lipolysis (see "Role of AMPK in energy metabolism and glucose homeostasis" section), the suppression of lipolytic activity could be augmented by lactate (Ahmed et al. 2010) produced at a higher rate in response to mitochondrial uncoupling (Rossmeisl et al. 2000; Si et al. 2009), while increased in situ fatty acid oxidation would limit further release of fatty acids into circulation. The inhibition of de novo fatty acid synthesis in WAT of aP2-*Ucp1* mice could also reflect changes in the extramitochondrial acetyl-CoA and NADPH levels (Rossmeisl et al. 2000). In fact, both the activation of fatty acid oxidation and inhibition of lipogenesis by AMPK in response to mitochondrial uncoupling would lower fat accumulation.

Mitochondrial uncoupling induced by ectopic UCP1 in SM was also associated with activation of the AMPK signaling (Neschen et al. 2008; Han et al. 2004). However, oxidative capacity of the muscle is rather decreased than increased and muscle lipid accumulation enhanced, reflecting possibly an impairment of mitochondrial metabolism by unphysiologically high levels of mitochondrial uncoupling. In spite of this undesired effect of UCP1, muscle insulin sensitivity was increased (Neschen et al. 2008; Han et al. 2004), which could be explained by AMPK-stimulated glucose uptake into the muscle, also consistent with the induction of whole-body carbohydrate oxidation in the transgenic mice (Klaus et al. 2005). In addition, mitigation of mitochondrial superoxide production in response to mitochondrial uncoupling may contribute to the beneficial effect on insulin sensitivity (Keipert et al. 2010). Furthermore, muscle AMPK could be involved in the regulation of muscle energy efficiency based on regulatable substrate cycling between de novo lipogenesis and lipid oxidation.

Treatments enhancing energy expenditure

It is frequently assumed that increased capacity for lipid catabolism and increased activity of fatty acid oxidation result inevitably in increased energy expenditure and reduction of body fat stores. In fact, this simplified view has motivated in part the whole AMPK research. However, as it was also stressed recently by Cooney and colleagues (Hoehn et al. 2010), thermodynamically, the only way to decrease body fat in the absence of changes in physical activity is to decrease the efficiency of energy conversion or to decrease food intake (Hoehn et al. 2010). Accordingly, stimulation of whole-body lipid oxidation in mice either in response to activation of AMPK following 10-day-lasting AICAR treatment or to genetic disruption of acetyl-CoA carboxylase 2 gene had no effect on adiposity (Hoehn et al. 2010). That prolonged treatment of mice by the AMPK activator AICAR resulted in reduction of adiposity (Giri et al. 2006) could perhaps be explained by a stimulation of energy expenditure linked to AMPK-independent mechanisms of AICAR action, well described to occur in liver (Foretz et al. 2010). Alternatively, AICAR treatment could induce thermogenic brown adipocytes containing endogenous UCP1 by affecting AMPK-mTOR signaling (Vila-Bedmar et al. 2010). In this respect, further studies using animals or cells with inactivated AMPK are required. Therefore, AMPK may augment the weight-reducing effect of mitochondrial uncoupling by increasing the capacity for lipid catabolism and redirecting metabolic fluxes, but its activation in SM, WAT, or liver cells per se should not induce weight loss.

Evidently, any intervention counteracting obesity, based on modulation of metabolism rather than affecting food intake or physical activity, must primarily reduce the efficiency of energy conversion. Concerning the induction of energy expenditure in WAT, several physiologically relevant treatments in mice have been demonstrated to induce a phenotype similar to that of aP2-*Ucp1* mice (Rossmeisl et al. 2004 for review). Thus, treatment using leptin (Orci et al. 2004), PPARα agonists (Ribet et al. 2010), adrenoreceptor agonists and lipolytic agents (Granneman et al. 2003; Yehuda-Shnaidman et al. 2010), dietary *n*-3 polyunsaturated fatty acids (Jelenik et al. 2010; Kopecky et al. 2009; Flachs et al. 2005), and thiazolidinediones acting as specific agonists of PPARγ [TZD; (Tiraby et al. 2003; Wilson-Fritch et al. 2004; Petrovic et al. 2010)] resulted in reduced adiposity and metabolic disturbances related to obesity, observed mostly under the conditions of high-fat feeding in obesity-prone C57BL/6 mice. Importantly, lipid catabolism in WAT was always up-regulated, and in situ lipogenesis in WAT was suppressed (Orci et al. 2004; Ribet et al. 2010; Flachs et al. 2005; Tiraby et al. 2003; Wilson-Fritch et al. 2004), suggesting activation of AMPK

in response to decreased energy status in WAT. Indeed, when studied under the conditions of the above treatments, AMPK in WAT and other tissues was found to be activated (Jelenik et al. 2010; Kopecky et al. 2009; Orci et al. 2004; Ye et al. 2006). Concerning the mechanism of energy expenditure induced by the treatments, mitochondrial uncoupling mediated by UCP1 in WAT adipocytes (Orci et al. 2004; Tiraby et al. 2003; Petrovic et al. 2010; Collins et al. 1997; Guerra et al. 1998; Himms-Hagen et al. 2000) could be involved in most of the cases. Further studies are required to learn more about the mechanisms underlying the induction of energy dissipating adipocytes in response to treatments inducing UCP1 in adipose tissue. It needs to be determined whether the induction of BAT precursor cells, trans-differentiation of mature white adipocytes into their brown counterparts (Himms-Hagen et al. 2000; Cinti 2002), or the formation of brown-adipocyte-like cells, a new adipocyte subtype inducible by TZD [i.e., 'brite'cells (Petrovic et al. 2010)], is responsible for the induction of UCP1-containing adipocytes in white fat.

However, it was also demonstrated that a lean phenotype could be induced in mice by augmenting energy expenditure in white adipocytes in the absence of UCP1, by (1) mitochondrial uncoupling elicited by increased intracellular levels of free fatty acids (Maassen et al. 2007; Yehuda-Shnaidman et al. 2010), (2) futile cycling between fatty acid synthesis and re-esterification (Ribet et al. 2010), or (3) other mechanisms (Granneman et al. 2003; Ukropec et al. 2006; Anunciado-Koza et al. 2008). In this respect, 15-deoxy-delta 12,15-prostaglandin J2, an anti-inflammatory mediator and potent endogenous PPARγ agonist (Forman et al. 1995; Kliewer et al. 1995), that is, endogenous analog of TZD was synergistically induced in response to the combination treatment using *n*-3 polyunsaturated fatty acids and calorie restriction in association with stimulation of mitochondrial biogenesis and fatty acid oxidation in WAT in the absence of UCP1 (Flachs et al. 2011). These results suggest a new promising approach for the treatment of obesity and metabolic syndrome.

In resting muscle, energy expenditure is stimulated by leptin (Minokoshi et al. 2002), depending possibly on both (1) AMPK-induced mitochondrial fatty acid oxidation (Minokoshi et al. 2002) and (2) increased insulin-induced fatty acid synthesis, resulting in a substrate cycling between de novo lipogenesis and lipid oxidation, which is possibly the mechanism for increased energy expenditure. This dual mechanism [for review, see (Summermatter et al. 2008)] may control changes in the efficiency of muscle energy metabolism during the fasting-feeding transition and may also contribute to genetically determined differences in propensity to obesity (Kus et al. 2008).

Concluding remarks

Experiments performed mainly in transgenic mice revealed a possibility to ameliorate obesity and associated metabolic disorders by mitochondrial uncoupling in metabolically relevant tissues, particularly in WAT, SM, and liver. Thus, ectopic expression of UCP1 has been shown to elicit major metabolic effects both at the cellular/tissue level and at the whole-body level. In addition to the expected increase in energy expenditure, surprisingly complex metabolic effects were detected, depending in part on the tissue harboring ectopic UCP1. This complex response could be largely explained by activation of AMPK, triggered by the depression of cellular energy charge, as found in both WAT and SM. Experimental data support the idea that (1) while being always activated in response to mitochondrial uncoupling and to a compromised intracellular energy status in general, AMPK could augment and mediate local as well as whole-body effects of changes in intracellular energy status; and that (2) activation of AMPK alone does not lead directly to an induction of energy expenditure. It is apparent that activation of AMPK may improve insulin sensitivity in various tissues (namely SM and liver), reflecting its effect on fuel partitioning, independent on the reduction of adiposity.

Concerning the importance of the site of ectopic UCP1 expression in the whole-body effects of mitochondrial uncoupling, surprising differences exist between the UCP1 expression in WAT and SM, while the role of hepatic UCP1 expression is much less characterized. Thus, in spite of a relatively small oxidative capacity of WAT, mitochondrial uncoupling in this tissue exerted a robust and persistent anti-obesity effect, while also preserving systemic lipid and glucose metabolism under obesogenic conditions. Data suggest that in addition to increased lipid catabolism in adipocytes, also depression of in situ fatty acid synthesis is involved in lowering of adiposity, and that this metabolic switch is activated by AMPK. Importantly, in addition to the metabolic changes in fat cells induced by mitochondrial uncoupling, also afferent signaling from WAT may contribute to the weight-reducing effect in response to the modulation of adipose tissue metabolism. This mechanism may even outweigh the importance of energy expenditure induced by ectopic UCP1 in WAT regarding its anti-obesity effects. Thus, efficacy of various treatments to reduce weight gain may depend on both, the modulation of WAT metabolism and the associated changes in regulation of energy homeostasis.

In contrast to pronounced anti-obesity effects of ectopic UCP1 in WAT, mitochondrial uncoupling in SM resulted only in a delay of body fat accumulation in mice exposed to an obesogenic environment. However, independent on its effect on obesity, ectopic UCP1 in SM strongly improved

tissue and whole-body insulin sensitivity, reflecting the role of SM as a major site of glucose catabolism and activation of muscle AMPK by ectopic expression of UCP1. That also insulin sensitivity of WAT was augmented by ectopic UCP1 in SM suggests important inter-organ communication, which remains to be characterized.

Basic mechanisms of energy conversion and control of metabolic fluxes, that is, mitochondrial oxidative phosphorylation and AMPK, represent key targets for treatment of obesity and associated metabolic disorders. Experiments in mouse models strongly suggest that decreasing efficiency of energy conversion may elicit differential effects depending on the tissue with altered energy metabolism. Thus, novel treatment strategies may be explored aimed at increasing by separate mechanisms energy dissipation as well as AMPK activity in metabolically relevant tissues to achieve synergistic anti-obesity and insulin-sensitizing effects.

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