Mitochondrial uncoupling and lipid metabolism in adipocytes
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Abstract
Metabolism of white adipose tissue is involved in the control of body fat content. In vitro experiments indicated a dependence of lipogenesis on mitochondrial ATP production, as well as a reciprocal link between hormonal effects on metabolism and energetics of adipocytes. Therefore, mitochondrial uncoupling in adipocytes that results in stimulation of energy dissipation and depression of ATP synthesis may contribute to control of lipid metabolism and adiposity. This is supported by the expression of protonophoric proteins in adipocytes, e.g. uncoupling proteins (UCPs) 2 and 5, and some anion transporters, and induction of UCP1 and UCP3 in white fat by pharmacological treatments that reduce adiposity. Negative correlation between expression of UCPs in adipocytes and accumulation of white fat was also found. Expression of UCP1 from the adipose-specific promoter in aP2-UCP1 transgenic mice mitigated obesity induced by genetic or dietary factors. The obesity resistance, accompanied by mitochondrial uncoupling in adipocytes and increased energy expenditure, resulted from ectopic expression of UCP1 in white but not in brown fat.

Key words: adipose tissue, lipogenesis, lipolysis, obesity, uncoupling protein.
Abbreviations used: FA, fatty acid; GPDH, cytosolic sn-glycerol-3-phosphate dehydrogenase; UCP, uncoupling protein.
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Probably due to depression of ATP/ADP ratio in white fat of transgenic mice, both fatty acid synthesis and lipolytic action of noradrenaline in adipocytes were relatively low. These results support the role of protonophoric proteins in adipocytes in the control of adiposity. The main function of these proteins in white fat may be modulation of lipogenesis and intracellular hormone signalling. Augmentation of energy expenditure may be of relatively small importance, in accordance with the low oxidative capacity of white adipocytes.

Introduction
It is becoming evident that adipose-tissue metabolism contributes to the control of body fat content. Many of the candidate genes for obesity [1] have important functions in adipocytes [2]. Mice that are prone [3-7] or resistant [8-12] to obesity were created by transgenic modification of adipose tissue. Metabolic changes of white but not brown adipose tissue are mostly responsible for the altered accretion of body fat in these animals, highlighting the importance of lipid metabolism in adipocytes of white fat.

The amount of body fat reflects the balance between the rates of accumulation and breakdown of triacylglycerols occurring in adipocytes. The accumulation depends on the extraction of lipids from circulation mediated by lipoprotein lipase. In situ fatty acid (FA) synthesis from glucose is also important, namely in adipocytes of rats and other
animals, while the lipogenic capacity of adipose tissue in humans is believed to be much smaller than that of liver. However, even in humans, adipose tissue may account for up to 40% of whole-body lipogenesis [13,14]. High lipogenic rates in adipose tissue may contribute to the development of obesity [15] and may explain the high rate of relapse in patients treated by caloric restriction [16].

The breakdown of triacylglycerols is initiated by hormone-sensitive lipase. Released FAs may be re-esterified or oxidized in adipocytes, or they are exported to other tissues. FA oxidation is intense in brown fat, where FAs serve as fuel for thermogenesis, depending on the protonophoric activity of the uncoupling protein (UCP) 1, which controls proton leak in mitochondria [17]. Regulatable proton leak probably exists also in white fat cells, but its function is not clear (see below).

Lipid metabolism in adipocytes is under complex neurohormonal control, with insulin and catecholamines representing the two most important regulatory factors [18]. However, intracellular factors may also contribute. This paper aims to characterize the possible involvement of energy metabolism in the modulation of lipid metabolism in adipocytes with the main focus on the role of mitochondrial proton leak in such a process.

Energy metabolism in adipocytes and its links to lipid metabolism: in vitro studies

Specific metabolic rate and oxidative capacity of white fat are relatively low. The contribution of white fat to resting metabolic rate of a lean human subject is close to 5% [19], while thermogenesis in brown fat in rodents may account for more than 50% of total metabolic rate [20]. The low specific metabolic rate of white fat reflects ultrastructural features of uninuclear adipocytes that are filled with triacylglycerols, while all organelles including mitochondria are present in a thin periplasmic rim. In contrast, the cytoplasmic space in multinuclear brown fat cells is relatively large. Electron microscopy of adipose tissues of adult C57BL/6J mice (M. Rossmeisl, G. Barbatelli, P. Flachs, P. Brauner, M. C. Zingaretti, M. Marelli, S. Cinti and J. Kopecky, unpublished work) indicated that mitochondrial density in the cytoplasm was similar in uninuclear white and in multinuclear brown adipocytes (62 ± 7.2 versus 67 ± 3.7 mitochondria/100 μm² of cytoplasm). However, mitochondria in the former cells were about 4-fold smaller (0.16 ± 0.01 versus 0.69 ± 0.05 μm²). White-fat mitochondria are well equipped for oxidative phosphorylation, with pyruvate serving as the main source of energy for ATP synthesis. Due to low activity of carnitine palmitoyl transferase-1 in the inner mitochondrial membrane, oxidation of FAs is relatively slow and FAs are directed towards esterification [21,22], unless the transferase is activated by leptin [23]. This is in contrast with mitochondria in brown fat, where FAs serve as the main substrate for oxidation during activation of thermogenesis and activity of the transferase is not rate-limiting [21].

The enzymic machinery of both white and brown fat mitochondria promotes FA synthesis [21,22], which requires co-operation between mitochondrial and cytoplasmic enzymes (Figure 1). Mitochondrial uncoupling in adipocytes could affect in situ FA synthesis, as was suggested three decades ago by Rognstad and Katz [24,25], who studied the effect of 2,4-dinitrophenol, an uncoupler of oxidative phosphorylation, on glucose metabolism in epididymal fat from rats. Addition of 2,4-dinitrophenol resulted in depressed synthesis of FAs and increased production of lactate [24]. Inhibition of FA synthesis probably resulted from limited availability of intramitochondrial ATP for the carboxylation of pyruvate [24,26], while the level of both ATP and NADPH in the cytoplasm remained sufficiently high [24,27]. In response to changes of mitochondrial energetics in adipocytes, pyruvate carboxylase in mitochondrial matrix (Figure 1, enzyme 1) may modulate lipogenesis. The activity of this enzyme is 3-fold greater in white fat than in liver mitochondria, and the ATP/ADP ratio directly affects the enzyme activity [26,28].

Lipolysis also depends on the energy status of adipocytes [29]. A decrease of intracellular ATP, elicited in white adipocytes in vitro by uncouplers, inhibitors of the mitochondrial respiratory chain or ionophores counteracted the stimulation of lipolysis by catecholamines [29,30]. The intracellular ATP is also essential for insulin signalling, including the anti-lipolytic effect of this hormone [31,32]. In turn, incubation of isolated adipocytes with lipolytic hormones resulted in an up to 50% decrease of their intracellular ATP level [33] and inhibition of lipolysis itself [33]. Metabolism of glucose, lipogenesis and protein synthesis in adipocytes, as well as oxygen consumption, were also affected [34]. It has been suggested that these effects represented an artifact of the in vitro experiments [33].
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Co-operation between mitochondrial and cytoplasmic enzymes in FA synthesis

Formation of acetyl-CoA for FA synthesis proceeds via the 'pyruvate cycle', i.e., conversion of pyruvate into oxaloacetate and citrate in mitochondria, transport of citrate to the cytoplasm, and cleavage to acetyl-CoA and oxaloacetate [24,26,28]. NADH reduces cytosolic oxaloacetate to malate, and the malate is decarboxylated to pyruvate with generation of NADPH. Pyruvate returns to the mitochondrion. The cycle also contributes about half the NADPH required for FA synthesis, with the rest supplied by the pentose cycle [24]. ATP- and NADPH-dependent reactions are discriminated by different arrows, as shown. Enzymes: 1, pyruvate carboxylase; 2, ATP-citrate lyase; 3, acetyl-CoA carboxylase; 4, FA synthase; 5, fatty acyl-CoA synthase; 6, malic enzyme.

Mitochondrial uncoupling in vivo: aP2-Ucp1 mice

In order to test the hypothesis of whether enhanced energy dissipation in white fat mitochondria could reduce obesity, transgenic mice with a C57BL/6J background have been constructed in which the UCP1 gene is driven by the fat-specific aP2 promoter to achieve enhanced expression in both brown and white fat [8]. Mice with the aP2-Ucp1 transgene are partially resistant towards age-related obesity, induced by the Aβ gene [8], resulting from hypothalamic lesions after gold thioglucone injections (J. Kopecký and M. Rossmeisl, unpublished work) or induced by feeding a high-fat diet [9,35]. The resistance to obesity reflects lower accumulation of triacylglycerols in all fat depots, except for gonadal fat, which becomes relatively large [8,9,35]. Changes in adiposity induced by the transgene in various white-fat depots are closely paralleled by differences in total DNA content [35], whereas the diameter of white-fat cells decreases very little (M. Rossmeisl, G. Barbatelli, P. Flachs, P. Brauner, M. C. Zingaretti, M. Marelli, S. Cinti and J. Kopecký, unpublished work). Interestingly, reduction in total body weight becomes apparent when the animals are getting obese but not under standard conditions [8,9,35], similar to other models of obesity resistance induced by transgenic modification of adipose tissue [10–12] or muscle [37,38]. All these models indicate a strong systemic defence against weight loss in lean organisms that could not be compromised by metabolic changes in fat or muscle tissues. However, such metabolic changes may be sufficient for mitigation of obesity.

Transgenic UCP1 is present in both brown and white fat, while the expression of the UCP1 endogene in brown fat is greatly reduced [8,35]. However, the obesity resistance results only from the transgenic modification of white fat, because (i) transgenic mice exhibited atrophy of brown adipose tissue, as indicated by reduction of its size and DNA content [39]; (ii) the levels of transcriptional co-activator PGC-1 (peroxisomal proliferator-activated receptor-γ co-activator-1) mRNA,
the activator of mitochondrial biogenesis, were about 5-fold lower in interscapular brown fat of transgenic mice, while no such effect of the genotype was observed in white fat (P. Flachs and J. Kopecky, unpublished work); (iii) oxygen consumption by white-fat fragments from transgenic mice was higher than that from control mice, while an opposite effect of the transgene was observed in brown fat [35]; and (iv) the dose of the transgene was inversely correlated with the thermogenic response to noradrenaline (norepinephrine) injections or to cold [39]. The mechanism of brown-fat involution in the transgenic mice is not clear ([39]; see also below).

Consequences of the expression of transgenic UCPI in white fat, which induces the obesity resistance, have been studied in great detail. The transgenic UCPI is contained in all the unilocular adipocytes but it cannot induce conversion of these cells into multilocular adipocytes ([8] and M. Rossmeisl, G. Barbatelli, P. Brauner, M. Flachs, P. Brauner, M. C. Zingaretti, M. Marelli, S. Cinti and J. Kopecky, unpublished work). Expression of the transgene differs in various fat depots, with gonadal fat showing a relatively low expression ([40] and M. Rossmeisl, G. Barbatelli, P. Brauner, P. Flachs, M. C. Zingaretti, M. Marelli, P. Janovská, M. Horáková, I. Syrový, S. Cinti and J. Kopecky, unpublished work). This may partially explain the lack of the effect of the transgene on the accumulation of lipids in gonadal fat (see above). However, even in the gonadal fat, transgenic UCPI could decrease mitochondrial membrane potential in adipocytes [41] and elevate oxygen consumption 2-fold [35]. UCPI also induces mitochondrial biogenesis in unilocular adipocytes (M. Rossmeisl, G. Barbatelli, P. Brauner, P. Flachs, M. C. Zingaretti, M. Marelli, P. Janovská, M. Horáková, I. Syrový, S. Cinti and J. Kopecky, unpublished work), although the induction is much less than in adipocytes of mice treated with a β₂-adrenoceptor agonist [42]. The content of transgenic UCPI declines in all fat depots with age, reflecting changing activity of the aP2 promoter (M. Rossmeisl, G. Barbatelli, P. Brauner, P. Flachs, M. C. Zingaretti, M. Marelli, P. Janovská, M. Horáková, I. Syrový, S. Cinti and J. Kopecky, unpublished work). In adult mice, the total content of transgenic UCPI in white fat does not exceed 2% of the UCPI in interscapular brown fat [8]. Apparently, only minute amounts of ectopic UCPI in white fat mitochondria can uncouple oxidative phosphorylation [41] and reduce accumulation of fat.

In agreement with the low oxidative capacity of white fat, the 2-fold increase of oxygen consumption brought about by transgenic UCPI in this tissue (see above) results in only a marginal stimulation of the resting metabolic rate of mice [39]. The strong mitigation of obesity in aP2-Ucp1 mice, and the fact that UCPI acts locally to reduce adiposity (see above and [43]), indicate that the reduction results not only from increased energy expenditure but also from a differential modification of lipid metabolism in various fat depots. Indeed, a strong diminution of FA synthesis was found in subcutaneous but not in gonadal fat of transgenic mice. The diminution was up to 4-fold, reflecting the magnitude of UCPI expression and the decrease of adiposity in different fat depots [40], as well as the drop in ATP/ADP ratio, which was observed only in the subcutaneous and not in the gonadal fat of transgenic mice (K. Bardová and J. Kopecky, unpublished work). The decrease of FA synthesis was accompanied by down-regulation of acetyl-CoA carboxylase and FA synthase in white fat, reflecting the decrease of metabolic flux through the lipidogenic pathway [40].

Activity of the marker enzyme of triacylglycerol synthesis of GPDH activity was measured [65] in the cytosolic fraction isolated by centrifugation at 100,000 g from homogenates of subcutaneous (Sc-WF) and gonadal (Gon-WF) white fat of adult male control mice (open bars) or heterozygous aP2-Ucp1 mice (filled bars). Values are means±SEM (n = 6); *P < 0.03; statistically significant difference between genotypes.
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synthesis, cytosolic sn-glycerol-3-phosphate dehydrogenase (GPDH), was also reduced in those white-fat depots where FA synthesis was decreased (Figure 2).

It was also tested whether transgenic UCP1 could affect noradrenaline-induced lipolysis in adipocytes, as suggested by the in vitro experiments (see above). It was found that the maximum lipolytic effect of the catecholamine, measured as glycerol released from isolated adipocytes, was suppressed by 50%, in the subcutaneous but not in the gonadal fat. In parallel, UCP1 down-regulated the expression of hormone-sensitive lipase, lowered its activity and altered the expression of G-proteins in adipocytes (P. Flachs, K. Bardova and J. Kopecky, unpublished work).

Experiments on the aP2-Ucp1 mice indicate that mitochondrial uncoupling in white fat in vivo stimulates primarily oxidation of substrates and thermogenesis (Figure 3). When the ATP/ADP ratio in adipocytes drops, lipogenesis and noradrenaline-induced lipolysis are depressed. Changes in the oxidative and particularly in the lipogenic activity apparently override the diminution of noradrenaline-stimulated lipolysis, as far as the effect of UCP1 on fat accumulation is concerned. Whether mitochondrial uncoupling could eventually trigger apoptosis in adipocytes

Figure 3

Effects of mitochondrial uncoupling in adipocytes

Putative consequences of mitochondrial uncoupling in adipocytes as suggested by the phenotype of aP2-Ucp1 mice. At low levels of the transgene expression (in gonadal fat) both mitochondrial respiration and thermogenesis increase, while ATP/ADP ratio remains unchanged (mitochondrial membrane potential and ATP synthesis decreases very little due to increased respiration) and lipogenesis is not affected. Higher levels of UCP1 (in subcutaneous white fat) result in a progressive decrease of mitochondrial ATP synthesis and ATP/ADP ratio in adipocytes. Metabolic pathways that depend on ATP supply slow down, with lipogenesis showing probably a higher sensitivity to the decrease of ATP/ADP ratio than does noradrenaline-induced lipolysis. Apoptosis may account for the loss of adipocytes in brown fat and counteract hyperplasia of adipocytes in white fat of transgenic mice.
remains to be tested (Figure 3). Previously, apoptosis of white fat was induced by feeding a diet supplemented with conjugated linoleic acid, while fat mass decreased and UCP2 in adipocytes were up-regulated [44]. Interestingly, overexpression of UCP1 in white fat of aP2-Ucp1 mice results in up-regulation of UCP2 in white fat (M. Rossmeisl, P. Flachs and J. Kopecký, unpublished work).

Biological significance of mitochondrial uncoupling in white fat
Experiments of Brand and colleagues demonstrated mitochondrial proton leak in muscle and liver cells [45], suggesting a general occurrence of proton leak in mitochondria in vitro. Several candidate genes for increasing the leak are expressed in adipocytes, namely the genes for UCPS. In brown fat, UCP1, UCP2, UCP3 and UCP5 are expressed. In white fat, only UCP2 and UCP5 genes are normally active [17,46] and UCP2 antigen could be detected [47]; however, both UCP1 [42,43,48] and UCP3 [42,49] can also be induced by pharmacological treatments that reduce adiposity. Similarly to UCP1, UCP2 and UCP3 also probably enhance the proton leak and decrease ATP synthesis [17,50–52]. Moreover, some mitochondrial anion carriers such as the adenine nucleotide translocator [53,54] and 2-oxoglutarate carrier [55] could also mediate the proton leak.

The role of mitochondrial proton leak in mammals may be to adjust the metabolic rate to the demands of thermal homoeostasis [45]. This may be especially true for tissues with high oxidative capacity (such as brown fat, liver and muscle), while the function of regulatable proton leak in tissues with relatively low mitochondrial content may be different. Our experiments with aP2-Ucp1 mice suggest that the main function of mitochondrial protonophores in white fat is the modulation of lipogenesis and hormonal control of lipid metabolism, through the effect on ATP/ADP ratio in adipocytes. In accordance with the low oxidative capacity of white adipocytes, augmentation of energy expenditure may be of relatively little importance. It is to be inferred that energy expenditure in adipocytes is negatively correlated with in situ lipogenesis. A reciprocal link between FA oxidation and synthesis exists. In several cell types, including adipocytes [56], FA oxidation is depressed by malonyl-CoA (the first committed intermediate in FA synthesis), which inhibits the transfer of FAs into mitochondria.

Relatively low energy dissipation associated with obesity was demonstrated by direct measurement of production of heat by adipocytes isolated from subcutaneous white fat of humans [19]. In both mice [42,43,48,49,57] and humans [58,59] an inverse relationship between the expression of UCP1 [42,43,48,59], UCP2 [57–59] and UCP3 [42,49] in white fat depots and accumulation of fat has been observed. Nevertheless, the putative role of various mitochondrial protonophores in whitefat cells in the control of adiposity remains to be clarified. It should be investigated whether up-regulation of mitochondrial protonophoric proteins in adipocytes, and hence mitochondrial uncoupling, contribute to the depression of in situ FA synthesis during fasting [60,61], due to leptin [16,62] and dietary omega-3 polyunsaturated FAs [63], or during lactation [64].

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References

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