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Energy Metabolism of Adipose Tissue – Physiological Aspects and Target in Obesity Treatment

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Summary

Body fat content is controlled, at least in part, by energy charge of adipocytes. *In vitro* studies indicated that lipogenesis as well as lipolysis depend on cellular ATP levels. Respiratory uncoupling may, through the depression of ATP synthesis, control lipid metabolism of adipose cells. Expression of some uncoupling proteins (UCP2 and UCP5) as well as other protonophoric transporters can be detected in the adipose tissue. Expression of other UCPs (UCP1 and UCP3) can be induced by pharmacological treatments that reduce adiposity. A negative correlation between the accumulation of fat and the expression of UCP2 in adipocytes was also found. Ectopic expression of UCP1 in the white fat of aP2-*Ucp1* transgenic mice mitigated obesity induced by genetic or dietary factors. In these mice, changes in lipid metabolism of adipocytes were associated with the depression of intracellular energy charge. Recent data show that AMP-activated protein kinase may be involved in the complex changes elicited by respiratory uncoupling in adipocytes. Changes in energy metabolism of adipose tissue may mediate effects of treatments directed against adiposity, dyslipidemia, and insulin resistance.

Key words

Uncoupling protein • Adipose tissue • Lipogenesis • Lipolysis • Obesity

Introduction

Excessive accumulation of adipose tissue – obesity – implies a health risk. Even though obesity per se is not a disease, it leads to various chronic morbidities, including type II diabetes, dyslipidemia, cardiovascular disease (together designated as metabolic syndrome), and certain forms of cancer. Prevalence of obesity has taken on epidemic proportions and the economic costs of obesity range from 2 to 7 % of total health-care budget in industrialized countries (Kopelman 2000). In order to preserve energy stores of body fat during periods of negative energy balance, various regulatory mechanisms have evolved, i.e. central control of behavior and thermogenesis mediated by neuroendocrine system, control of fluxes of metabolites among organs, and control of energy metabolism within individual tissues. Due to the complex nature of the control of body fat content and numerous compensatory mechanisms involved in this control, the treatment of obesity is very difficult.

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Many pieces of evidence suggest that body fat content is controlled, at least partially, by the metabolism of adipose tissue itself. First, most of the candidate genes for obesity have important roles in adipocytes (Arner 2000). Second, mice that are prone or resistant to obesity were created by transgenic modification of adipose tissue (for review see Kopecký *et al.* 2001). In these transgenic models, metabolic changes in white but not in brown adipose tissue are mostly responsible for the altered accretion of body fat, highlighting the importance of lipid metabolism in adipocytes of the white fat. Evidently, treatment strategies for obesity and metabolic syndrome should include specific modifications of the metabolism of white adipose tissue.

The characteristic feature associated with obesity and the pivotal factor in the pathogenesis of type II diabetes is insulin resistance. Reduced ability of peripheral tissues, in particular skeletal muscle and liver, to respond to insulin stimulation may be detected many years before the clinical onset of hyperglycemia. In obese subjects, insulin resistance most likely results from increased accumulation of lipids in peripheral tissues due to enhanced release of fatty acid (FA) from hypertrophic fat cells (Perseghin *et al.* 2003). In fact, hypertrophic adipocytes themselves become resistant to insulin, which results in lower clearance of plasma triacylglycerols and higher FA release from the adipose tissue. Interestingly, it was observed that insulin resistance also develops as a consequence of the lack of white adipose tissue (Gavrilova *et al.* 2000, Moitra *et al.* 1998). Under these circumstances, levels of circulating FA are elevated due to insufficient "buffering" capacity of adipocytes for FA. In consequence, excess lipids are deposited in other organs. In addition to FA, also various adipocyte-secreted proteins, like leptin, adiponectin, tumor necrosis factor α, and interleukin-6 modulate sensitivity of other tissues to insulin and may be involved in the induction of systemic insulin resistance (Fasshauer and Paschke 2003). Impaired insulin sensitivity associated with the excess of lipids in the liver, skeletal muscle, and pancreas has the consequences typical for the metabolic syndrome (Frayn and Summers 1998). Thus, metabolism of adipose tissue together with adipocyte-derived factors is involved in the control of systemic insulin sensitivity.

Only few laboratories have focused their research effort on the links between energy and lipid metabolism in cells of white adipose tissue. Department of Adipose Tissue Biology at the Institute of Physiology of the Academy of Sciences of the Czech Republic represents one of the laboratories in this field, namely due to studies on the mechanism by which mitochondrial uncoupling protein 1 (UCP1), expressed in white fat of transgenic mice (aP2-*Ucp1*), protects against obesity. These studies clarified several aspects of the modulation of adipose tissue metabolism by the intracellular energy charge, as well as the role of AMP-activated protein kinase (AMPK) in the integration of various signals affecting metabolism of white fat. The results are relevant for developing novel strategies for prevention and treatment of obesity based on the links between energy and lipid metabolism in adipocytes.

Links between energy and lipid metabolism in adipocytes

As in other tissues, mitochondria represent the main source of ATP in the white fat. Efficiency of ATP synthesis during oxidative phosphorylation and hence the rate of ATP synthesis depends on the proton leak through the inner mitochondrial membrane. Experiments of Brand and colleagues (1999) suggest a general occurrence of basal proton leak in mitochondria *in vivo*. Several candidate genes encoding for proteins that could enable a regulatable proton leak are expressed in adipocytes, namely the genes for uncoupling proteins. In the brown fat, UCP1, UCP2, UCP3, and UCP5 genes are expressed. In the white fat, only UCP2 and UCP5 genes are normally active (Pecqueur *et al.* 2001, Ricquier and Bouillaud 2000). Similarly to UCP1, UCP2 and UCP3 might also enhance the proton leak, induce respiratory uncoupling, and decrease ATP synthesis (Echtay *et al.* 2001, Ricquier and Bouillaud 2000). It is well known that UCP1-mediated respiratory uncoupling in brown fat is involved in thermogenesis and in the control of energy balance (Ricquier and Bouillaud 2000). On the other hand, the physiological role of respiratory uncoupling and UCPs in the white fat requires further clarification. It may be hypothesized that uncoupling in white fat would increase energy expenditure and thermogenesis. However, the effect on energy balance may be relatively small, because contribution of the white fat to resting metabolic rate in humans is only about 5 % (Bottcher and Furst 1997). Alternatively, depression of ATP synthesis due to respiratory uncoupling in the white fat with the consequent rise in ADP and AMP levels may result in allosteric effects on the activities of key regulatory enzymes of carbohydrate and lipid metabolism or in modulation of intracellular regulatory pathways (see

below). *In vitro* experiments support the inhibition of FA synthesis and enhancement of glycolysis due to respiratory uncoupling in adipocytes (Rognstad and Katz 1969). Oxidation of FA in mitochondria is expected to increase due to removal of the inhibition of FA transport to mitochondria by malonyl-CoA (the first committed intermediate in FA synthesis) (Saggerson and Carpenter 1983) and also due to the activation of mitochondrial biogenesis (Li *et al.* 1999). Lipolysis may be depressed (Fassina *et al.* 1974). Without complementary evidence from *in vivo* studies, significance of the above findings in the link between energy and lipid metabolism in adipocytes would be limited. Therefore, the following paragraphs will focus on the relevant studies in humans and experimental animals. The main focus will be on understanding the role of mitochondria, respiratory uncoupling, and intracellular energy charge in the control of lipid metabolism in adipocytes and in the mitigation of obesity.

Reduced accumulation of fat due to respiratory uncoupling in white adipose tissue of aP2-*Ucp1* transgenic mice

The mechanism by which respiratory uncoupling may reduce accumulation of fat can be analyzed in the aP2-*Ucp1* transgenic mice, in which the UCP1 gene is driven by the fat-specific aP2 promoter to achieve enhanced expression in both brown and white fat (Kopecký *et al.* 1995). This transgenic mouse model was constructed well before the discoveries of UCP2 and UCP3. The animals are partially resistant to obesity related to age, induced by genetic background (Kopecký *et al.* 1995) or by a high-fat diet (Kopecký *et al.* 1996a,b). The resistance to obesity reflects lower accumulation of triacylglycerols in all fat depots, except for gonadal fat, which becomes relatively large (Kopecký *et al.* 1995, 1996a,b). Interestingly, reduction in total body weight becomes apparent only under obesity-promoting conditions such as feeding the high-fat diet (Kopecký *et al.* 1995, 1996a,b), similarly to other models of obesity resistance induced by transgenic modifications of adipose tissue or skeletal muscle. Transgenic UCP1 is present in both brown and white fat, however, the expression of endogenous UCP1 in brown fat is greatly reduced (Kopecký *et al.* 1995). Importantly, obesity resistance of the aP2-*Ucp1* mice results from the transgenic modification of white fat only (Kopecký *et al.* 2001), since brown fat of the transgenic mice is greatly atrophied (Štefl *et al.* 1998). The origin of this atrophy is not clear.

Consequences of the expression of transgenic UCP1 in the white fat have been studied in great detail. Transgenic UCP1 is contained in all unilocular adipocytes (Kopecký *et al.* 1995, 2002). Expression of the transgene differs in various fat depots with gonadal fat showing a relatively low expression (Rossmeisl *et al.* 2000). This may explain in part the lack of effect of the transgene on lipid accumulation in gonadal fat (see above). However, even in gonadal fat transgenic UCP1 is capable of decreasing mitochondrial membrane potential in adipocytes (Baumruk *et al.* 1999) and elevating oxygen consumption two-fold (Kopecký *et al.* 1996b). UCP1 also induces mitochondrial biogenesis in unilocular adipocytes, probably due to up-regulation of the transcription factor NRF-1 (Rossmeisl *et al.* 2002). In adult mice, the total content of transgenic UCP1 in white fat does not exceed 2 % of the total UCP1 found in interscapular brown fat (Kopecký *et al.* 1995). Apparently, only minute amounts of ectopic UCP1 in unilocular adipocytes of white fat can uncouple oxidative phosphorylation (Baumruk *et al.* 1999, Xu *et al.* 1991) and reduce the accumulation of fat.

In agreement with the low oxidative capacity of white fat, the two-fold increase in oxygen consumption brought about by transgenic UCP1 (see above) results in only a marginal stimulation of the resting metabolic rate in the transgenic mice (Štefl *et al.* 1998). This suggests that in addition to increased energy expenditure there must be another way how transgenic UCP1 reduces adiposity. Indeed, a strong diminution of FA synthesis was found in greatly reduced subcutaneous fat depots of transgenic mice, while the changes in gonadal fat were less obvious (Rossmeisl *et al.* 2000). It likely reflects the magnitude of UCP1 expression, which was the highest in subcutaneous fat (Kopecký *et al.* 1995, Rossmeisl *et al.* 2000), as well as the drop in ATP/ADP ratio. The latter was observed only in the subcutaneous and not in gonadal fat of transgenic mice (Flachs *et al.* 2002). The decrease in FA synthesis was accompanied by down-regulation of acetyl-CoA carboxylase, fatty acid synthase (Rossmeisl *et al.* 2000), and peroxisome proliferator-activated receptor γ (Kopecký *et al.*, unpublished results) in the white fat. Transgenic UCP1 lowered FA synthesis by means of respiratory uncoupling, as confirmed by *in vitro* experiments (Rognstad and Katz 1969, Rossmeisl *et al.* 2000). Ectopic UCP1 in the white fat also affected lipolysis and activity of adipose tissue lipoprotein lipase

(LPL). Maximum lipolytic effect of noradrenaline was suppressed by 50 % in the subcutaneous but not gonadal fat of the transgenics. In parallel, UCP1 transgene caused down-regulation of the expression of hormone-sensitive lipase, lowered its activity and altered the expression of G-proteins in adipocytes (Flachs *et al.* 2002). Activity of LPL was higher in transgenic than in control mice, especially when the animals were fed a high-fat diet. These data suggest that respiratory uncoupling in adipocytes stimulates LPL-mediated clearance of triacylglycerols by adipose tissue. In agreement with this, it was found that plasma triacylglycerol levels were also lower in transgenic than in control mice (Kopecký *et al.* 1996a) with a clear-cut dose-dependent effect of the transgene. Plasma nonesterified FA followed a similar trend as triacylglycerols (Rossmeisl *et al.*, unpublished results). The phenotype of the aP2-*Ucp1* mice suggests that the main function of respiratory uncoupling and UCPs in the white fat might be the modulation of lipogenesis, oxidation of substrates, lipolysis, and hormonal control of lipid metabolism.

AMP-activated protein kinase

The broad range of effects of the ectopic UCP1 on the metabolic properties of white adipose tissue is difficult to explain and requires further clarification. In the white fat, as in other tissues, lipid and carbohydrate metabolism is modulated by AMP-activated protein kinase (AMPK), which serves as a metabolic master switch (Winder and Hardie 1999). In response to the fall in the intracellular ATP/AMP ratio, AMPK becomes activated through phosphorylation of its α-subunit by an upstream kinase. Activated AMPK then phosphorylates and inactivates a number of enzymes involved in biosynthetic pathways, thus preventing further ATP utilization (for review see Winder and Hardie 1999). In the skeletal muscle, AMPK also up-regulates NRF-1, which induces mitochondrial biogenesis (Bergeron *et al.* 2001). In adipocytes, AMPK is known to inhibit both lipolysis and lipogenesis by regulating directly enzymes engaged in lipid metabolism (Sullivan *et al.* 1994). In the liver, the inhibitory effect of AMPK on lipogenesis is indirect, being mediated by transcription factor SREBP-1 (Zhou *et al.* 2001), which up-regulates genes engaged in lipogenesis (Kim *et al.* 1998). All the effects of transgenic UCP1 on biochemical properties of the white fat in the aP2-*Ucp1* mice are in accordance with the activation of AMPK (see above). Indeed, it was observed that UCP1-induced depression of the ATP/ADP ratio in subcutaneous white fat of the aP2-*Ucp1* mice (Flachs *et al.* 2002) was associated with reduction in the ATP/AMP ratio and increased activity of AMPK. No such changes were observed in the gonadal fat (Šponarová *et al.*, unpublished results). Other studies indicate that leptin can affect lipid metabolism of skeletal muscle through the activation of AMPK (Minokoshi *et al.* 2002) and that mutual links between AMPK activity and UCP3 expression exist in this tissue (Pedersen *et al.* 2001, Zhou *et al.* 2000). Therefore, some effects of leptin on tissue metabolism may involve respiratory uncoupling and AMPK may represent the link between respiratory uncoupling and lipid metabolism (see below).

Physiological relevance of energy metabolism in white adipocytes and new perspectives for the treatment of obesity and metabolic syndrome

Substantial amount of evidence indicates the involvement of mitochondrial UCPs, and presumably intracellular energy charge, in white adipocytes in the control of adiposity. Even in adult humans, relatively low levels of the UCP1 transcript can be detected in various fat depots. In abdominal fat, UCP1 mRNA levels are inversely related to the level of obesity (Oberkofler *et al.* 1997), similarly to UCP2 (Oberkofler *et al.* 1998). A common polymorphism in the promoter region of the UCP2 gene is associated with a decreased risk of obesity in the middle-aged humans (Esterbauer *et al.* 2001). A negative correlation between heat production in adipocytes and body fat content has also been found (Bottcher and Furst 1997).

It is necessary to assess whether the changes in energy metabolism and intracellular energy charge occur in adipocytes during treatments that affect adiposity, insulin resistance and other components of the metabolic syndrome. The effects of several typical treatments like administration of leptin (Ceddia *et al.* 2000, Rustan *et al.* 1998, Soukas *et al.* 2000, Zhou *et al.* 1999), bezafibrate (Cabrero *et al.* 1999, 2001), adrenoreceptor agonists (Angel *et al.* 1971, Gong *et al.* 1997, Himms-Hagen *et al.* 2000, Ho *et al.* 1970, Pontecorvi and Robbins 1986, Yoshitomi *et al.* 1998), dietary n-3 polyunsaturated fatty acids (PUFAs) (Benhizia *et al.* 1994, Clarke 2001, Hun *et al.* 1999) or fasting (Ho *et al.* 1970, Iritani *et al.* 1996, Kalderon *et al.* 2000, Millet *et al.* 1997) can be compared with the phenotype of aP2-*Ucp1* transgenic mice

(Kopecký *et al.* 1996a,b, Rossmeisl *et al.* 2000). In all these situations, fat accumulation is reduced and disturbances related to the metabolic syndrome are improved. In most cases, expression of various UCPs is up-regulated, FA oxidation is increased, *in situ* lipogenesis is suppressed, and LPL-mediated clearance of triacylglycerols in the white fat is augmented. Opposite changes in the activity of FA oxidation and lipogenesis probably reflect the elimination of inhibitory effect of malonyl-CoA on the transport of FA into mitochondrial matrix mediated by carnitine palmitoyl transferase-1 (Saggerson and Carpenter 1983). Due to the low activity of this transferase in the white fat, oxidation of FA is relatively slow and FA are directed towards esterification (Martin and Denton 1970), unless the oxidation is activated by leptin (Wang *et al.* 1999) or perhaps by respiratory uncoupling. The alteration of energy charge in adipocytes, as revealed by changes in the intracellular content of ATP and/or ATP/ADP and ATP/AMP ratios, was detected not only in the aP2-*Ucp1* mice (Flachs *et al.* 2002) but also during fasting (Ho *et al.* 1970) and after the administration of adrenergic agonists (Angel *et al.* 1971, Ho *et al.* 1970). Experiments in the aP2-*Ucp1* mice (see above) thus strongly support the role of AMPK in adipocytes under the conditions reducing adiposity. The involvement of AMPK in the effects of leptin on lipid metabolism in the skeletal muscle has recently been found (see above). Antidiabetic drugs such as thiazolidinediones stimulate glucose uptake and insulin sensitivity in adipocytes. Under these conditions, parallel induction of glycerol kinase (Guan *et al.* 2002) and PEPCK (Tordjman *et al.* 2003) presumably leads to futile cycling of FA in adipocytes. In the skeletal muscle cells, thiazolidinediones stimulate AMPK by decreasing ATP/AMP ratio (Fryer *et al.* 2002). Therefore, it is essential to learn whether AMPK activity in adipocytes could also be modulated by thiazolidinediones. In support of this idea, adipocyte-derived hormone adiponectin with insulin-sensitizing properties increases glucose uptake into adipocytes by inducing AMPK (Wu *et al.* 2003). It is also assumed that changes in energy metabolism of adipocytes may affect secretion of biologically active substances from adipose tissue (e.g. leptin and other adipokines) and, hence, systemic insulin sensitivity.

Conclusions

Metabolism of adipose tissue as well as its secretory functions are deeply involved in the patho-physiology of the metabolic syndrome. It is becoming apparent that the intracellular energy charge of fat cells has a major role in controlling metabolism of adipose tissue. Therefore, energy metabolism of adipocytes is becoming a promising target for the treatment strategies in obesity and metabolic syndrome, including dietary manipulations and pharmacological approaches. Future studies should further elucidate how adipocytes respond to the above treatments with respect to changes in their intracellular energy charge and the mechanism of their metabolic adaptations.

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Abbreviations

AMPK, AMP-activated protein kinase; aP2-*Ucp1* transgenic mouse, mouse with the expression of *UCP1* from the fat-specific aP2 gene promoter; DNP, 2,4-dinitrophenol; FA, fatty acid; HSL, hormone-sensitive lipase; LPL, lipoprotein lipase; PPAR, peroxisome proliferator-activated receptor; PGC-1, PPAR γ coactivator-1; PUFA, polyunsaturated fatty acid; UCP, mitochondrial uncoupling protein.

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