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Skeletal muscle mitochondrial uncoupling, adaptive thermogenesis and energy expenditure

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Purpose of review

The prevalence of obesity is still increasing, despite obesity treatment strategies that aim at reducing energy intake. In addition to this, exercise programmes designed to increase energy expenditure have only a low efficiency and have generated mixed results. Therefore, strategies based on increasing energy expenditure via nonexercise means are currently under investigation. One novel strategy is the modulation of adaptive thermogenesis.

Recent findings

Among others, adaptive thermogenesis can be modulated by changing dietary composition, treatment with hormone mimetics as well as by cold exposure. In humans, a large part of the adaptive thermogenic response is, in addition to a putative role of brown adipose tissue, determined by the skeletal muscle mass via the process of mitochondrial uncoupling. Here, we describe the molecular processes involved in mitochondrial uncoupling, state-of-the-art techniques to measure mitochondrial uncoupling *in vitro* and *in vivo*, as well as the current strategies to mitochondrial uncoupling.

Summary

Data generated in rodents and humans implicate that increasing adaptive thermogenesis by increasing skeletal muscle mitochondrial uncoupling indeed elevates total energy expenditure and thus may provide a promising target for the treatment of obesity.

Keywords

adaptive thermogenesis, capsinoids, energy balance, uncoupling

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Introduction

In this review we set out to give an overview of the latest developments in the field of increasing whole-body energy expenditure via adaptive thermogenesis. In particular, we focus on the contribution of skeletal muscle mitochondrial uncoupling to whole-body energy expenditure and adaptive thermogenesis. In addition, we review the latest developments regarding the application of increasing adaptive thermogenesis to fight the obesity epidemic and the possibilities to achieve this goal by modulation of skeletal muscle mitochondrial uncoupling.

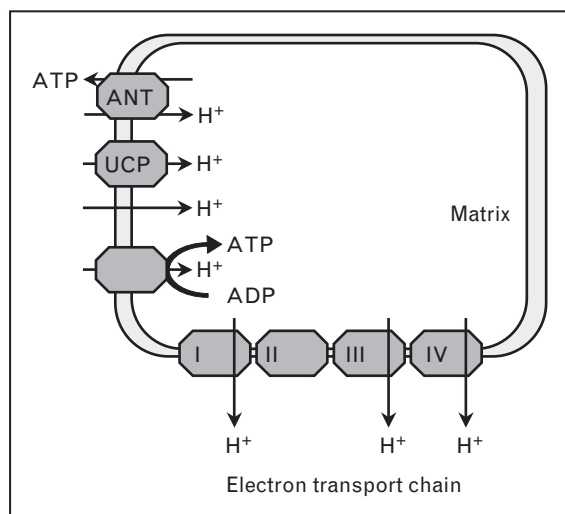
In the modern Western society, the prevalence of obesity and obesity-related disorders are rising. The development of obesity is the result of a prolonged imbalance between energy intake and energy expenditure, and classically focus has been on reducing energy intake to correct this imbalance. However, although successful in the short term, the success rate of reducing energy intake

in the long term is notoriously poor in humans, possibly due to the decrease in resting metabolic rate and exercise-related energy expenditure as well as by promoting an unfavourable energy intake pattern [1^{••},2[•],3^{••}]. The reduction in metabolic rate could be a risk factor for weight regain in itself, although the contribution of energy intake and expenditure to weight gain are still under debate [4[•]]. Attempts to improve energy balance by exercise programmes have generated unclear results. Structured physical activity has been reported to increase total energy expenditure rates [5] but was found not to affect energy expenditure in another study [6]. Interestingly, Turner *et al.* [7[•]] demonstrated that a 6-month progressive exercise programme does result in weight loss but the absolute reduction is less than expected due to an increase in energy intake. This stresses the importance to measure and report both sides of the energy balance. Thus, despite the obvious social and health benefits, the limited potency for physical exercise programmes to increase total energy expenditure may be due to the fact that the caloric cost per

time unit of common exercise types are typically low compared to the caloric intake [8]. An alternative, attractive approach would therefore be to induce sustained elevation of resting metabolic rate, especially because resting metabolic rate accounts for approximately 50–70% of total daily energy expenditure. Recent interest has focussed on adaptive thermogenesis, defined as ‘a greater than predicted change in energy expenditure in response to changes in energy balance [9]’, as a potential target for obesity therapy (reviewed in [10**]).

In addition to the classical adaptive thermogenic organ, the brown adipose tissue, skeletal muscle has been demonstrated to significantly contribute to adaptive thermogenesis. The capacity of skeletal muscle to contribute to whole-body energy expenditure can be related to the fact that muscle is composed of approximately 40% of the total body mass and to account for approximately 20–30% of the total resting oxygen uptake [11]. Furthermore, the contribution of proton leaks to resting metabolic rate can be as large as 20–50% [12], demonstrating the large potential of skeletal muscle to increase oxygen uptake. For example, the contribution of the skeletal muscle to diet-induced adaptive thermogenesis ranges between 35 and 67% [13]. In addition, up to 50% of adrenalin-induced thermogenesis was demonstrated to originate at the skeletal muscle level [14]. Mitochondrial uncoupling, the process whereby substrate oxidation is uncoupled from ATP production and directed towards heat loss (Fig. 1) is postulated to be an excellent target to

Figure 1 Schematic overview of skeletal muscle mitochondrial uncoupling



Oxygen consumption is uncoupled from ATP production by either a passive leak or UCP/ANT-mediated translocation of protons. ANT, adenine nucleotide translocator; ADP, adenosine diphosphate; ATP, adenosine triphosphate; UCP, uncoupling protein. Adapted with permission from [15].

Key points

- Increasing adaptive thermogenesis by elevating skeletal muscle mitochondrial uncoupling may provide a target for targeting obesity.
- An increase in energy intake may accompany the increase in energy expenditure upon increasing adaptive thermogenesis, stressing the importance to report changes on both sides of the energy balance.
- Nutritional induction of uncoupling, for example by capsinoids, are favourable in comparison to chemical uncoupling, for example using 2,4-dinitrophenol.

elevate whole-body energy expenditure [15]. This is mostly due to the high oxidative capacity of muscle, thereby allowing the upscaling of uncoupling-related energy expenditure. The other mechanisms that contribute to skeletal muscle adaptive thermogenesis (calcium cycling and protein turnover) may also provide potential targets, although the scaling possibilities are limited in comparison with uncoupling.

On the molecular level, mitochondrial uncoupling is the result of proton leak. In living cells, the metabolism of substrates such as fat, carbohydrate and proteins results in the production of reducing equivalents nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FADH₂), which can be oxidized to NAD⁺, FAD and H⁺ in a process called electron transfer or respiratory chain. The net transport of protons to the cytosolic side of the inner mitochondrial membrane generates a proton gradient across the mitochondrial membrane. When this proton gradient is high enough, protons flow back over the inner mitochondrial membrane through a so-called F₀F₁ complex and the energy thus generated is used by ATPase to transform ADP into ATP. In this way, substrate oxidation is coupled to the formation of ATP. However, the coupling between substrate oxidation and ATP formation is not 100% efficient; part of the generated proton gradient is lowered by proton leaks thereby dissipating energy as heat. It has been suggested that the contribution of proton leaks to resting metabolic rate can be as large as 20–50% [12]. The major protein involved in the process of skeletal muscle mitochondrial uncoupling is uncoupling protein 1 (UCP-1), which has its main function in brown adipose tissue [16*]. In other tissues, homologues of UCP-1 can be found (UCP-2 and UCP-3), although their function with regard to energy homeostasis is still under debate. Interestingly, whereas UCP-2 has been demonstrated to be expressed in a variety of tissues, UCP-3 expression is mainly limited to the skeletal muscle and is thought to be only indirectly involved in adaptive thermogenesis [17*].

Measurements of skeletal muscle mitochondrial uncoupling *in vivo* and *in vitro*

The process of skeletal muscle mitochondrial uncoupling can be measured *ex vivo* using high-resolution respirometry and *in vivo* using magnetic resonance spectroscopy. In-vivo measurements of skeletal muscle uncoupling are based on ratio between tricarboxylic acid (TCA) cycle flux and rates of ATP synthase flux. In short, TCA cycle flux is measured combining information from ^{31}P -MRS and NIRS to assess ATP production and oxygen consumption, respectively [18,19^{*}]. A second method is based on the combination of ^{13}C NMR (or MRS) and ^{31}P NMR (or MRS) [18]. This second approach is based on the ^{13}C NMR-based assessment of citric acid cycle activity and the ^{31}P NMR-based assessment of ATP synthesis. Both of these methods have been used extensively and have their own merits and drawbacks. One of the drawbacks is that the TCA:ATP ratio is taken as a measure of uncoupling but the ratio can be altered by both changes in TCA flux and/or changes in ATP flux. However, true mitochondrial uncoupling in a physiological sense is characterized by a lower efficiency of ATP production, but not a reduction (in other words, more substrate oxidation for the same amount of ATP production, as the latter is determined by demand, not by efficiency). Because the complete discussion of these techniques is beyond the scope of this review, we would like to refer the reviewer to the excellent and extensive review of these techniques [20^{**}].

High-resolution respirometry analysis involves the *in vitro* measurement of oxygen consumption of isolated mitochondria under various circumstances. Oxygen consumption in the presence of substrate, inorganic phosphate and ADP is referred to as state 3 respiration. Under state 3 conditions, oxygen consumption is coupled to

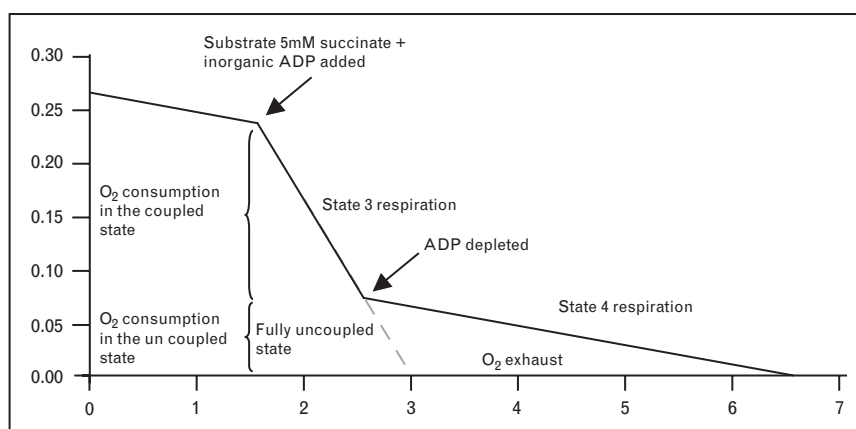
phosphorylation of ADP to ATP in addition to proton leak. On depletion of ADP (and thus without ATP synthesis), mitochondria maintain respiration at a much lower rate, referred to as state 4 respiration. In this case, respiration is uncoupled from ATP synthesis and the proton gradient is lowered by proton leak (Fig. 2) [21].

Using this method, it has been established that in humans skeletal muscle mitochondrial function is altered in the obese, insulin-resistant state, and thus that disturbances in skeletal muscle mitochondrial function may play a role in the propagation of these abnormalities [22,23]. In aged rats, it has been established that the degree of mitochondrial uncoupling was lower, and the optimal thermodynamic efficiency of the mitochondria was higher compared to young animals [24]. Interestingly, this may cause the more obesogenic phenotype of these animals, as elevated skeletal muscle mitochondrial proton leak has been indicated as a thermogenic mechanism favouring a lean phenotype [25]. Second, an obesogenic phenotype has been demonstrated to be associated with a low degree of mitochondrial coupling far before the development of obesity and/or insulin resistance in C57Bl/6 mice [26^{**}]. More specific, muscle mitochondrial uncoupling is higher in mice that have a high rate of energy intake which is insufficiently countered by an increase in energy expenditure. These data favour the hypothesis that increased mitochondrial oxidation, possibly through uncoupling, is a mechanism to protect the body from the deleterious effects of a positive energy balance.

The contribution of elevated skeletal muscle mitochondrial uncoupling to whole-body energy expenditure

As described before, the determination of skeletal muscle mitochondrial function can be performed *in vivo* or

Figure 2 Schematic representation of a respirometry analysis of mitochondria



Adapted with permission from [21].

ex vivo. Measurements of whole-body energy expenditure, however, can only be performed *in vivo*, as it involves the complex interplay of all organs and systems in the body. When data from skeletal muscle mitochondrial function are later correlated with whole-body energy expenditure data it is important to keep some key points in mind. For example, since the number of subjects involved in a study is often too small to use advanced statistics [27], indirect calorimetry data are often corrected for total body mass in animal studies. In contrast, the human studies are almost always corrected for fat-free mass. Since the error introduced by either methods is not equal [28[•]], the outcome of a study may be biased and comparison may not be completely valid.

The contribution of higher rates of skeletal muscle mitochondrial uncoupling to thermogenesis has been demonstrated in various mouse models. Evidence for beneficial effects of skeletal muscle uncoupling comes from UCP1 muscle-specific overexpressing mice [29^{••}]. UCP1 overexpressing mice have a higher degree of whole-body energy expenditure, skeletal muscle mitochondrial uncoupling and are protected against high-fat diet induced obesity as well as glucose intolerance. In addition, UCP3 overexpression protects mice from high-fat diet induced obesity and insulin resistance [30^{••}]. Although it has been suggested that the effects of UCP3 overexpression may be due to nonphysiological and nonregulated mitochondrial uncoupling [31], it still shows that the process of mitochondrial uncoupling lowers body weight.

In humans, convincing evidence for a role of skeletal muscle uncoupling to human energy expenditure comes from studies addressing the role of thyroid hormone. It is well known that thyroid hormone is involved in the regulation of human metabolic rate, and thyromimetics are potential therapeutical agents [32^{••}]. Interestingly, it has been demonstrated that skeletal muscle mitochondrial metabolism is affected by circulating thyroid hormone levels [33[•]]. In patients with high circulating levels of thyroid hormones, resting energy expenditure is significantly higher compared to controls. This was associated with high rates of muscle TCA cycle flux (>75%), but not ATP synthesis, indicating a low mitochondrial coupling index [34^{••}]. Similar results were found in triiodo-L-thyronine treated rats, in which energy coupling was decreased by approximately 60% after 10 days of treatment [35]. These data demonstrate that increasing skeletal muscle mitochondrial uncoupling is associated with resting energy expenditure. In addition to thyroid hormone, leptin has been demonstrated to regulate thermogenesis in muscle. Central leptin administration enhanced the postprandial response in muscle tissues in ovariectomized sheep, by increasing the amplitude and duration of the postprandial thermogenic response [36]. In part, this effect could be due to alterations in mitochondrial uncoupling,

since leptin has been demonstrated to increase UCP1 mRNA expression in brown adipose tissue [37] although data on muscle are lacking. Together, these data clearly demonstrate that endocrine stimuli can enhance skeletal muscle mitochondrial metabolism, and thereby increase whole-body energy expenditure.

In addition to endogenous signals, exogenous stimuli have been demonstrated to affect total energy expenditure as well as skeletal muscle mitochondrial metabolism. In healthy subjects nonshivering thermogenesis was induced by mild cold exposure (16°C). This induced total daily energy expenditure significantly by approximately 3% compared to subjects measured at room temperature. This increase in energy expenditure was linearly correlated with an increase in the rate of skeletal muscle mitochondrial uncoupling (state 4 respiration). No correlation was found between the increased energy expenditure and state 3 respiration, indicating that *in vivo*, the increase in energy expenditure was, at least in part, due to an increase in mitochondrial uncoupling [38]. Also, the postprandial processing of food has been demonstrated to induce energy expenditure, a process termed 'diet-induced thermogenesis'. In addition to total caloric intake [39,40], the magnitude of diet-induced thermogenesis has been determined to be dependent on the macronutrient composition of the diet [41] and previous obesity status [42]. Interestingly, it has been demonstrated that maternal energy restriction, as well as postnatal high-fat feeding, alters mitochondrial oxidative capacity and respiratory coupling ratio in sheep [43], suggesting that dietary composition may be able to directly alter muscle mitochondrial function.

Early experiments, involving the chemical uncoupler 2,4-dinitrophenol (DNP) [44], already demonstrated the potential benefits of enhanced mitochondrial uncoupling on energy balance [45[•]], which can (at least in part) be explained by increased skeletal muscle mitochondrial uncoupling [46]. It is important to mention, however, that DNP has proven to be lethal [47[•]] and is therefore marked as an illegal weight loss agent. However, mitochondrial uncoupling as a target for obesity treatment is still under close scrutiny and has found new input in the nutrition field. Capsinoids (including the subclasses capsiate, dihydrocapsiate, and nordihydrocapsiate) are substances naturally present in chilli peppers. Recently, it has been demonstrated that treatment of healthy participants with 10 mg of capsinoids results in an increased oxygen consumption and a lower respiratory quotient in healthy young men [48[•]]. However, another study using a similar set-up did not show this effect [49^{••}]. The main difference between the two studies was the exercise protocol and the time after ingestion of the capsinoid capsules at which respiratory gas exchange was measured. Galgani *et al.* [49^{••}] measured

resting metabolic rate 45 min prior and 120 after ingestion, whereas Josse *et al.* [48[•]] assessed metabolic substrate utilisation at various timepoints from 30 min prior up to 150 after ingestion. These data indicate that the effects of capsinoids on metabolism and may in part be dependent on the activity status of the participant. To prevent the potential confounding effects of nutritional and exercise status prolonged experiments are advisable. Prolonged treatment of obese patients with 6 mg/day capsinoids resulted in a significant increase in fatty acid oxidation and a decrease in abdominal adiposity [50^{••}]. In part, the effect of capsinoid treatment may be due to an increase in mitochondrial uncoupling, as a 2-week treatment increased the levels of UCP-1 protein and mRNA in brown adipose tissue [51]. In another study, a single dose of capsinoids raised UCP-1 mRNA in brown adipose tissue and UCP-3 mRNA in skeletal muscle [51]. Long-term treatment, however, demonstrates a marked reduction in skeletal muscle UCP-3 levels [52[•],53^{••}], although this may reflect the improved obesity and insulin resistance phenotype rather than increased mitochondrial metabolism. Clearly, more studies are needed to investigate if the effects of capsinoids on thermogenesis are due to increased muscle mitochondrial uncoupling.

Possible compensatory mechanisms to maintain energy balance upon increasing adaptive thermogenesis

One caveat, however, remains the fact that weight maintenance is a matter of energy balance. As soon as energy expenditure is raised, and the balance becomes negative, the body may respond to that by increasing energy intake, and thus lead to weight regain [54[•]]. The compensatory increase in energy intake has been demonstrated to be true, for example, in the described patients with high circulating levels of thyroid hormones. In addition to the increase in energy expenditure, energy intake was approximately 40% higher in patients compared to controls [34^{••}]. A second example is the UCP-1 overexpressing mouse. Body mass, as well as lean tissue mass, was lower but food intake was similar to wild-type controls. This indicates that, in addition to energy expenditure, food intake per gram of tissue mass was higher in UCP-1 overexpressing mice compared to wild-type controls [30^{••}]. Third, treatment of rats with thyroid hormone (T4) led to a decrease in body weight, despite an increase in food intake by almost 20% [55[•]]. It is therefore adamant that energy intake is controlled if adaptive thermogenesis is used to target obesity.

Conclusion

In conclusion, it is clear that skeletal muscle mitochondrial uncoupling plays a role in the process of adaptive

thermogenesis. Physiological endogenous as well as exogenous stimuli such as cold and leptin administration has been demonstrated to increase skeletal muscle uncoupling and to significantly affect total energy expenditure. In addition, because the upscaling possibility for skeletal muscle energy expenditure is large, increasing skeletal muscle mitochondrial uncoupling may be a potent target to fight the obesity epidemic.

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This paper is only one of many examples in which the elevation of energy expenditure is associated with an increase in energy intake, further stressing the need of the reparation of both sides of the energy balance in metabolic studies.