Uncoupling protein 3 and physical activity: the role of uncoupling protein 3 in energy metabolism revisited

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Uncoupling protein 3 and physical activity: the role of uncoupling protein 3 in energy metabolism revisited

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Physical activity influences energy metabolism in human subjects by increasing activity-induced energy expenditure and resting metabolic rate for several hours after exercise. On the other hand, physical activity increases mechanical energy efficiency, suggesting that trained subjects would need less energy for daily activities. The underlying mechanism by which physical activity influences energy metabolism is largely unknown. The skeletal muscle-specific homologue of uncoupling protein (UCP) 1, UCP3, could possibly play a major role in energy expenditure. UCP3 is, like UCP1, able to uncouple respiration from ATP production. A strong link or association between the *UCP3* gene and energy metabolism was found. Furthermore, UCP3 mRNA expression is related to sleeping metabolic rate, and thyroid hormone, a powerful stimulator of energy expenditure, up regulates UCP3. Finally, mice overexpressing UCP3 are hyperphagic but lean. These findings indicated that UCP3 is related to energy metabolism and that UCP3 could have a role in the effect of physical activity on energy expenditure. Thus, acute exercise up regulates UCP3, whereas endurance training results in the down-regulation of UCP3 protein content. Only a minimal amount of physical activity is needed for down-regulation of UCP3. Moreover, there is very strong evidence that UCP3 is negatively related to mechanical energy efficiency, suggesting that the down-regulation of UCP3 with training increases mechanical energy efficiency. Taken together, although the exact function of UCP3 is still unknown, exercise and training studies clearly show that under certain circumstances UCP3 is strongly related to human energy metabolism, possibly as a secondary effect of its (yet) unknown primary function.

Uncoupling protein 3: Endurance training: Energy expenditure

Regular physical activity is often prescribed in the prevention and treatment of obesity (Schrauwen & Westerterp, 2000). The development of obesity is characterized by an imbalance between energy intake and energy expenditure, and physical activity increases the latter. Daily energy expenditure can be divided into three main components: resting metabolic rate (RMR), diet-induced thermogenesis, energy expenditure for activity. Of these three components, energy expenditure for activity varies most and is by definition directly influenced by physical activity. However, in most human subjects the contribution of activity-induced energy expenditure to total daily energy expenditure accounts for only approximately 20–40 % (Westerterp, 1998). On the other hand, RMR is the largest component of daily energy expenditure, accounting for 50–70 % of all energy expended during 24 h (Ravussin *et al.* 1986). Thus, it is clear that this component has a major effect on energy balance. In elegant studies done in Pima Indians, it has indeed been shown that inter-individual variations in RMR can influence the development of obesity. In 126 Pima Indians energy expenditure before and after a 4-year follow-up period was measured in a respiration chamber. At the end of the 4-year follow-up period, metabolic rate of subjects who had gained > 10 kg body weight was compared with that of subjects who did not gain body weight. After adjusting for fat-free mass, fat mass, age and sex, RMR was significantly lower in the weight-gainers (+15·7 kg/4 years) as compared with the subjects not gaining weight (+0·1 kg/4 years; Ravussin *et al.* 1988). However, the baseline difference in RMR was only 290 kJ (70 kcal)/d, indicating the enormous impact of small differences in RMR on the susceptibility to obesity. The reason for the inter-individual variation in resting energy needs is not yet clear, but studies of twins have indicated

Abbreviations: FFA, non-esterified fatty acids; RMR, resting metabolic rate; UCP, uncoupling protein; V_{O_2}, O_2 consumption.

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that up to 40 % of the unexplained variance in RMR might be explained by genetic factors (Fontaine *et al*. 1985).

Physical activity and energy metabolism

Since energy expenditure has an important impact on the overall energy balance, many researchers have studied the effect of physical activity on the energy expenditure component of energy balance. Physical activity will increase activity-induced energy expenditure. Westerterp (1998) re-evaluated the available literature on the effect of exercise interventions on total daily energy expenditure, as measured with doubly-labelled water. He concluded that although exercise did not increase spontaneous physical activity, the exercise interventions resulted in an increase in 24 h energy expenditure. Remarkably, however, the increase in total energy expenditure was calculated to be twice the workload of the training regimen, indicating that the increase in energy expenditure was not fully accounted for by an increase in activity-induced exercise (Westerterp, 1998).

Apart from an effect of physical activity on total energy expenditure, many researchers have examined whether regular physical activity (or training) can influence RMR. Since the major determinant of RMR is the amount of fat-free mass, any training-induced increase in the latter will therefore also increase RMR. However, whether regular physical activity also increases RMR independent of changes in fat-free mass is less clear. Some studies have found a positive effect of physical activity on RMR (Tremblay *et al*. 1986; Poehlman *et al*. 1988, 1994; Pratley *et al*. 1994; Wilmore *et al*. 1998), whereas other studies have found no effect (Davis *et al*. 1983; Meijer *et al*. 1991; Schulz *et al*. 1991; Broeder *et al*. 1992; Westerterp *et al*. 1994). Part of this controversy can be explained by the residual effect of the exercise bout preceding the measurement of RMR. There is compelling evidence that acute exercise leads to an increase in energy expenditure after exercise (also referred to as excess post-exercise O_2 consumption (V_{O_2})), and this effect can last for several hours and might still affect RMR or sleeping metabolic rate on the following day. For example, the effect of cycling exercise on energy metabolism was studied using a respiration chamber, and sleeping metabolic rate measured during the second night (after exercise) was found to be approximately 7 % higher than during the first night (before exercise; WH Saris and P Schrauwen, unpublished results). This finding is in agreement with results obtained by other researchers, who showed an elevated RMR (or sleeping metabolic rate) after exercise (Bielinski *et al*. 1985; Bahr *et al*. 1987). Thus, the positive effect of training on RMR reported in some studies might be explained by the residual effect of the last exercise bout on RMR. In this context Tremblay *et al*. (1988) found that RMR is reduced by 6·6 % compared with the baseline measurement (acutely after the last exercise bout) when highly-trained subjects suspend their training programme for 3 d, indicating that endurance training has no long-lasting effect on RMR. Similar findings have been found in endurance-trained females when measurements are done 87 h post-exercise (Herring *et al*. 1992). Thus, apart from the short-term exercise-induced increase in RMR, there is little evidence for a long-lasting effect of endurance training on RMR. This finding indicates that physical activity has to be performed on a regular basis in order to maintain its positive effect on energy balance.

Moreover, it has even been suggested that, in order to be able to maintain energy balance, the human body increases its energy efficiency in response to frequent endurance training. Such an increase in energy efficiency would be a beneficial adaptation in relation to exercise performance, but would diminish the positive effect of regular training on the prevention and treatment of obesity. Furthermore, the improved energy efficiency would make the body more susceptible to reaching a positive energy balance when the training programme is discontinued, further indicating that physical activity will only positively affect energy balance as long as it is performed on a regular and continued basis. The energy efficiency of the human body becomes apparent during exercise, when 10–30 % of the energy expended can be used for external work (defined as mechanical energy efficiency), whereas the remaining ATP production is used for homeostasis or dissipated as heat. Endurance training has indeed been shown to improve mechanical energy efficiency (Gaesser & Brooks, 1975; Gissane *et al*. 1991), indicating that training indeed decreases energy needs for the same level of physical activity. Furthermore, there are some reports showing that mechanical energy efficiency is related to body-weight regulation (Freyschuss & Melcher, 1978; Lammert & Hansen, 1982) and the rapid body-weight gain observed in some elite athletes after their professional career also suggests that efficiency might be enhanced. However, scientific data relating to the latter effect is lacking and more work in this field is necessary.

Uncoupling protein 3: a human homologue of uncoupling protein 1?

The mechanisms by which training could influence energy expenditure and mechanical energy efficiency are largely unknown. In human subjects skeletal muscle seems to play a major role in determining energy expenditure. For example, it has been shown that 40–50 % of the adrenalin-induced thermogenesis in human subjects can be attributed to skeletal muscle (Astrup *et al*. 1985; Simonsen *et al*. 1993). In 1997 a muscle-specific uncoupling protein (UCP), UCP3, was discovered that might well be involved in the regulation of energy expenditure.

In living cells ATP is continuously resynthesised from ADP, by the metabolism of substrates such as fat, carbohydrate and proteins, resulting in the production of NADH and $FADH_2$. Subsequently, NADH and $FADH_2$ can be oxidized to NAD^+, FAD and H^+ in the respiratory chain. According to the Nobel prize-winning chemi-osmotic hypothesis of Mitchell (1966), the protons are transported to the cytosolic side of the inner mitochondrial membrane by a series of reactions. Thus, a proton gradient across the inner mitochondrial membrane is generated, which causes the protons to flow back across the inner mitochondrial membrane through the so-called F_0–F_1 complex. In tightly-coupled mitochondria the energy thus generated is used by ATPase to transform ADP into ATP. However, build up of the proton gradient can be diminished by the action of UCP. These proteins transport either protons or fatty acid anions across the inner mitochondrial membrane, thereby lowering

the proton gradient and thus uncoupling mitochondrial respiration from ATP production. This process will result in lower energy efficiency and possibly increased energy expenditure.

In rodents such a UCP (UCP1) is responsible for the well-known thermogenic activity of brown adipose tissue (Nicholls & Locke, 1984). In 1997 two human homologues of UCP1, UCP2 (Fleury *et al*. 1997; Gimeno *et al*. 1997), which is present in a wide variety of tissues, and UCP3 (Boss *et al*. 1997; Vidal-Puig *et al*. 1997), expression of which is restricted to skeletal muscle, were discovered. Since UCP3 expression is restricted to skeletal muscle this particular protein was considered to be of importance in skeletal muscle energy metabolism. Similar to UCP1, UCP3 has been shown to lower the proton gradient across the inner mitochondrial membrane (Gong *et al*. 1997). Furthermore, mitochondria isolated from mice lacking UCP3 show a decreased state 4 respiration (rate of O_2 consumption after all ADP in the mitochondria has been phosphorylated to form ATP), indicating improved coupling (Vidal-Puig *et al*. 2000), whereas mitochondria isolated from mice overexpressing UCP3 show an increased state 4 respiration (Clapham *et al*. 2000). Also, *in vivo*, there are indications that UCP3 indeed is able to uncouple respiration from ATP production. The rate of ATP synthesis:tricarboxylic acid cycle flux, measured using ^{31}P NMR, was found to be increased in mice lacking UCP3, indicating a 2–4-fold higher coupling of oxidative phosphorylation (Cline *et al*. 2001).

Uncoupling protein 3 is related to energy expenditure in human subjects

The finding that UCP3 can uncouple respiration from ATP production suggests that it might be involved in the regulation of human energy metabolism. First evidence for a relationship between UCP3 and energy metabolism came from genetic studies. Bouchard *et al*. (1997) found that markers in the vicinity of the *UCP2* and *UCP3* genes (which are only 7 kb apart) were very strongly linked ($P = 0{\cdot}000002$) to RMR. Direct screening of the *UCP2* and *UCP3* genes revealed several polymorphisms, which were used to examine the association between UCP2 and UCP3 and energy metabolism. It was found that in Pima Indians an alanine→valine substitution in exon 4 and a 45 bp insertion/deletion in exon 8 of the *UCP2* gene were associated with sleeping metabolic rate and 24 h energy expenditure, as measured in a respiration chamber (Walder *et al*. 1998). This polymorphism has also been associated with 24 h energy expenditure in a Danish population (Astrup *et al*. 1999), as well as with exercise efficiency (Buemann *et al*. 2001). Due to the close genetic mapping of UCP2 and UCP3, the association between energy expenditure and UCP2 might also reflect an association between UCP3 and energy metabolism.

A polymorphism in exon 3 of the *UCP3* gene was found to be related to RMR, both before and after a period of overfeeding, but not with overfeeding-induced changes in RMR (Ukkola *et al*. 2001). Recently, Kimm *et al*. (2002) found that a C→T substitution in exon 5 was associated with RMR in African–American women, but not in white women, suggesting that this variant might explain (part of) the racial differences in resting energy expenditure. However, negative associations between polymorphisms in the *UCP2* and *UCP3* genes and energy metabolism have also been found (Klannemark *et al*. 1998; Lentes *et al*. 1999; Yanovski *et al*. 2000). Thus, together these studies indicate, but do not conclusively prove, that there is evidence for a role of UCP2 and UCP3 in energy metabolism. A more direct assessment of a role for UCP3 in energy metabolism comes from studies in which UCP3 mRNA expression and/or protein content is measured and directly related to energy expenditure. For example, in Pima Indians it was found that the mRNA expression of UCP3 was positively correlated with sleeping metabolic rate (which accounts for approximately 60 % of total energy expenditure in human subjects; Schrauwen *et al*. 1999*b*). Furthermore, weight reduction, which reduces RMR, also leads to a reduction in UCP3 mRNA expression and protein content (Schrauwen *et al*. 2000; Vidal-Puig *et al*. 1999). During cold exposure, extra heat is produced to maintain body temperature. In accordance with a role for UCP3 in energy metabolism, acute cold exposure in mice resulted in a 3-fold increase in UCP3 protein content, accompanied by a pronounced lowering of mitochondrial membrane potential and dissipation of energy as heat (Simonyan *et al*. 2001). It was shown recently that in human subjects exposed to mild cold for 60 h UCP3 protein content is not (yet) changed, but is related to sleeping metabolic rate and 24 h energy expenditure. However, UCP3 mRNA expression is down regulated, suggesting that prolonged cold exposure also decreases UCP3 protein content. Thus, this result indicates that, although basal UCP3 protein content is related to energy metabolism, the function of UCP3 is not in the adaptation to cold (Schrauwen *et al*. 2002*c*). Recently, de Lange *et al*. (2001) reported evidence of a role for UCP3 in thyroid hormone-induced thermogenesis. In hypothyroid rats skeletal muscle UCP3 protein content and RMR, measured for 144 h after thyroid hormone injection, both peaked 65 h after thyroid hormone injection. Moreover, mitochondria showed increased uncoupling activity when UCP3 protein content was high (de Lange *et al*. 2001). In human subjects Lebon *et al*. (2001) showed that thyroid hormone treatment increased tricarboxylic acid cycle flux in combination with unchanged ATP synthesis, indicative of increased mitochondrial uncoupling. Although UCP3 was not measured, it is tempting to suggest that increased UCP3 content was responsible for the increased mitochondrial uncoupling. Finally, in mice overexpressing UCP3, an increased metabolic rate has been found (Clapham *et al*. 2000). Together, these studies indicate that high levels of UCP3 can lead to elevated energy expenditure.

However, there is also evidence that the primary physiological function of UCP3 is not the regulation of energy expenditure! Mice lacking UCP3 have normal energy expenditure, even though their mitochondria are more tightly coupled (Gong *et al*. 2000; Vidal-Puig *et al*. 2000). Furthermore, fasting up regulates UCP3, while in this condition energy conservation is observed (Millet *et al*. 1997). As mentioned earlier, the finding that prolonged cold exposure decreases UCP3 expression but increases energy metabolism is also in contrast with a major role for UCP3 in the regulation of energy metabolism (Schrauwen *et al*. 2002*c*). Thus, based on the available literature, there is clear

evidence that UCP3 is indeed related to energy metabolism, and that high levels of UCP3 could even contribute to increased energy expenditure by uncoupling mitochondria. However, the uncoupling function of UCP3 is most likely not primarily the regulation of energy expenditure, but has another, yet to be determined, physiological function. It has, for example, been shown that a high proton gradient across the mitochondrial membrane results in the production of reactive oxygen species (Skulachev, 1998), and by lowering this proton gradient UCP3 could prevent the production of reactive oxygen species. Alternatively, the observation that UCP3 is up regulated in the fasted state (Boss *et al*. 1998*b*) and that plasma non-esterified fatty acids (FFA) levels are able to up regulate UCP3 mRNA (Khalfallah *et al*. 2000) has led to the suggestion that UCP3 might be involved in fatty acid metabolism (for a more extensive review of the putative functions of UCP3, see Dulloo & Samec, 2001; Hagen & Vidal-Puig, 2002; Schrauwen & Hesselink, 2002).

Uncoupling protein 3, energy metabolism and physical activity

Based on the previously mentioned evidence that UCP3 is at least related to energy metabolism, it is a likely candidate for explaining the effect of acute exercise and long-term endurance training on energy expenditure and efficiency. The first evidence of a role for UCP3 in training adaptation again came from genetic studies. Lanouette *et al*. (2001) showed that a polymorphism in the *UCP3* gene (a micro-satellite located in intron 6) was associated with the changes in BMI and percentage body fat induced by a 20-week endurance training programme. Although it is not known whether this polymorphism in intron 6, which obviously does not alter the amino acid sequence of UCP3, does influence UCP3 mRNA, protein content or uncoupling activity, this study suggests that UCP3 might indeed be involved in training-induced adaptations in energy metabolism. Furthermore, an alanine→valine substitution in exon 4 of the *UCP2* gene was associated with 24 h energy expenditure; subjects with the val/val genotype had lower 24 h energy expenditure compared with subjects with the ala/val and ala/ala genotype. However, surprisingly, 24 h spontaneous physical activity was approximately 20 % higher in subjects with the val/val genotype, thereby compensating for the lower resting 24 h energy expenditure in this genotype (Astrup *et al*. 1999). Interestingly, in another report the same group showed that subjects with the val/val genotype were characterized by a higher mechanical energy efficiency, determined at three different workloads (Buemann *et al*. 2001). It is tempting to speculate that the polymorphism in the *UCP2* gene, close to the *UCP3* gene, is associated with lower UCP2 and/or UCP3 content, thereby explaining the lower resting energy expenditure and the increased mechanical energy efficiency. Alternatively, it is possible that the increased spontaneous physical activity (for any unknown reason) is the primary effect and that the lower resting energy expenditure and improved mechanical energy efficiency are compensatory adaptations to the increased activity.

At the mRNA level, the effect of physical activity on UCP3 was studied by Hjeltnes *et al*. (1999). They found a 4·1-fold higher level of UCP3 mRNA in skeletal muscle of subjects with a complete chronic lesion of the cervical spinal cord compared with healthy subjects, indicating that muscle inactivity leads to a pronounced up-regulation of UCP3. Interestingly, when these tetraplegic subjects were exercise trained for 8 weeks, using electrically-stimulated leg cycling, UCP3 mRNA expression was down regulated by approximately 50 %. Also in rats, denervation (and thus inactivation) resulted in a 331 % increase in UCP3 mRNA expression in gastrocnemius muscle, although opposite effects were found in mouse (Cortright *et al*. 1999).

Several studies have examined whether UCP3 is involved in the (endurance) training-induced adaptation of energy expenditure. Boss *et al*. (1998*a*) showed that the mRNA expression of UCP3 was significantly lower ($P < 0{\cdot}05$) after 8 weeks of endurance training in rats, when measured 24–30 h after the last exercise bout. To examine whether a similar effect is found in human subjects, UCP3 mRNA expression was compared in endurance-trained athletes and untrained subjects (Schrauwen *et al*. 1999*a*). It was found that UCP3 mRNA expression was significantly lower in endurance-trained athletes ($P = 0{\cdot}028$) and that the level of UCP3 mRNA expression was very strongly and negatively correlated with aerobic capacity (maximal V_{O_2}; r –0·61, $P = 0{\cdot}009$). This correlation remained significant when only untrained subjects were considered (r –0·86, $P = 0{\cdot}028$), illustrating that the level of physical fitness is related to UCP3 mRNA expression. Furthermore, mechanical energy efficiency was determined in all subjects and it was found that UCP3 mRNA expression was negatively correlated (r –0·56, $P = 0{\cdot}019$) with this energy efficiency, suggesting that the reduction in UCP3 with training might be responsible for the improvement in efficiency. Similar findings were reported recently by Russell *et al*. (2002). They found that the lower level of UCP3 mRNA expression in their endurance-trained athletes was positively correlated with the slow component of V_{O_2} kinetics. Thus, in healthy individuals exercising at an intensity above the lactate threshold, V_{O_2} gradually increases without an increase in workload. This increase in V_{O_2} is termed the slow component of V_{O_2}, and it suggests a possible decrease in mechanical energy efficiency. However, these studies only examined UCP3 mRNA expression and therefore, in collaboration with Russell's group, it was confirmed recently that the lower level of UCP3 mRNA in endurance-trained athletes can be extended to the protein levels (Russell *et al*. 2003). Thus, UCP3 protein was 46 % lower in endurance-trained individuals compared with untrained subjects. Together, these studies show that endurance training down regulates UCP3 in human subjects, and that this down-regulation of UCP3 coincides with improved mechanical energy efficiency. To confirm the relationship between UCP3 protein content and physical fitness (maximal V_{O_2}) and mechanical energy efficiency, baseline data from a recent study was re-analysed (Schrauwen *et al*. 2002*a*). In this study seven untrained men (age 22·7 (SE 0·6) years, BMI 23·8 (SE 1·0) kg/m^2; maximal V_{O_2} 3852 (SE 211) ml/min) exercised at 50 % maximal V_{O_2} for 2 h and a muscle biopsy was taken before the exercise bout. Energy expenditure during exercise was determined by measuring V_{O_2} and CO_2 production and using this energy expenditure to calculate

mechanical energy efficiency. In a separate test, 1 week before sampling of the muscle biopsy, maximal V_{O_2} was determined (Schrauwen *et al*. 2002*a*). It was found that the level of UCP3 (after an overnight fast) was very strongly and negatively correlated with maximal V_{O_2} adjusted for body weight (r −0·94, P=0·0018; Fig. 1(a)), as well as with mechanical energy efficiency (r −0·97, P=0·0002; Fig. 1(b)). These findings indicate that even in a group of untrained subjects UCP3 protein content is very strongly related to the level of physical fitness and to the subject's energy efficiency! Interestingly, it was found recently that a mild activity programme for 3 months (three times per week cycling exercise at 40 % maximal workload for approximately 2 h/week) in sedentary middle-aged subjects decreased UCP3 protein content by approximately 30 % (Schrauwen *et al*. 2001; 2002*b*), suggesting that 'training'-induced reductions in UCP3 can occur rapidly and with a minimal increase in physical activity. In accordance with this possibility, it was found recently that UCP3 protein content tended to be lower (P=0·08) and mechanical energy efficiency higher (P=0·08) after only 2 weeks of training in untrained young subjects (P Schrauwen and MKC Hesselink, unpublished results). Together these studies clearly show that endurance training and physical activity rapidly down regulate UCP3, and there are very strong indications that this down-regulation of UCP3 affects energy efficiency. Very recently, direct evidence for the latter has come from mice overexpressing UCP3. During a series of isometric tetani in muscle fibres isolated from these mice, heat production was found to be increased, illustrating that more energy was dissipated as heat and indicating lower energy efficiency (Curtin *et al*. 2002).

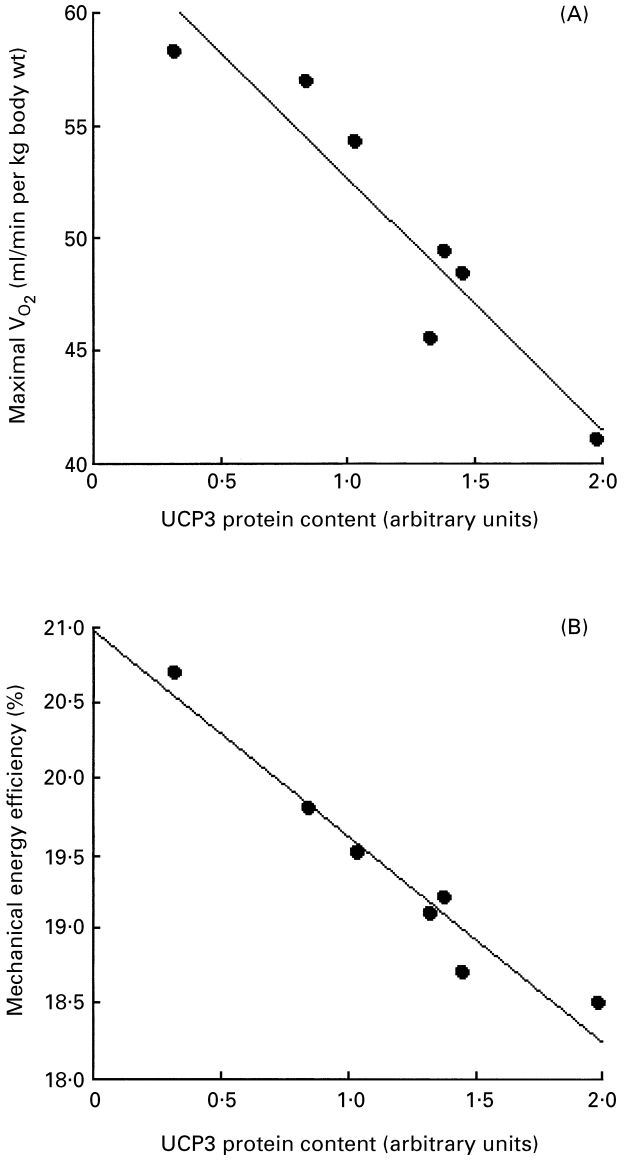

Fig. 1. Relationship between skeletal muscle uncoupling protein (UCP) 3 content and (a) maximal oxygen consumption (V_{O_2}) relative to body weight (r −0·94, P=0·0018) and (b) energy efficiency during exercise (r −0·97, P=0·0002) in seven untrained men (age 22·7 (SE 0·6) years, BMI 23·8 (SE 1·0) kg/m^2; maximal V_{O_2} 3852 (SE 211) ml/min) who exercised at 50 % maximal V_{O_2} for 2 h and from whom a muscle biopsy was taken before the exercise bout.

Although these studies all suggest reduced levels of UCP3 after training, there are also contrasting findings. For example, chronic exercise for 9 weeks did not alter UCP3 mRNA expression in the rat (Cortright *et al*. 1999). An explanation for the lack of effect of training on UCP3 in the latter study could relate to the time interval between the last exercise bout and muscle sampling, which was only a few hours in this study, whereas in the other studies there was at least 24 h between the last exercise bout and UCP3 determination. It was shown that after 2 weeks of swimming training, UCP3 mRNA expression in skeletal muscle was up regulated approximately 14–18-fold 3 h after the last exercise bout, but was not different from pre-training levels when measured 22 h after the last exercise bout (Tsuboyama-Kasaoka *et al*. 1998). Furthermore, 1 week of treadmill running resulted in a 4·7-fold up-regulation of UCP3 mRNA 3 h after exercise, but reduced levels when measured 44 h post-exercise (Tsuboyama-Kasaoka *et al*. 1998). Thus, these results clearly indicate that sufficient time is needed between the last bout of exercise and the determination of UCP3 expression in order to find an effect of endurance training rather than from acute exercise on UCP3 level. Furthermore, the findings suggest that acute exercise and endurance training might have opposite effects on UCP3 mRNA expression.

Effect of acute exercise on uncoupling protein 3

The suggested up-regulation of UCP3 with acute exercise encouraged researchers to examine whether UCP3 plays a role in the elevated post-exercise energy expenditure (excess post-exercise V_{O_2}), which can be sustained for several hours. In rodents UCP3 mRNA expression was significantly (P<0·05) up regulated 1–3 h after an acute exercise bout (Tsuboyama-Kasaoka *et al*. 1998; Cortright *et al*. 1999). This up-regulation of UCP3 after acute exercise seems to be highly specific; the very pronounced up-regulation of UCP3 mRNA 200 min after either running or swimming exercise was not accompanied by changes in other mitochondrial genes (Zhou *et al*. 2000). Since during exercise plasma FFA levels are increased and AMP-activated protein kinase,

a key regulator of enzymes involved in energy and substrate metabolism, is activated under situations of elevated FFA levels (Ruderman *et al*. 1999), it was suggested that activation of AMP-activated protein kinase is necessary for the up-regulation of UCP3 mRNA expression. To test this hypothesis 5-aminoimidazole-4-carboxamide 1-β-D-ribofuranoside, which mimics the effects of AMP on activation of AMP-activated protein kinase, was administered and it was found that this treatment also resulted in up-regulation of UCP3. Similar results were obtained by Pedersen *et al*. (2001), who showed that electrical stimulation of skeletal muscle *in vitro* resulted in an up-regulation of UCP3 mRNA, and that this up-regulation could be mimicked by 5-aminoimidazole-4-carboxamide 1-β-D-ribofuranoside. In human subjects, also, it was found that UCP3 mRNA expression is up regulated 3–6-fold 4 h after an acute exercise bout (Pilegaard *et al*. 2000). Again, this effect might have been due to elevated plasma FFA levels during exercise. To examine whether the up-regulation of UCP3 after acute exercise in human subjects is related to the increased energy expenditure, or is simply an effect of elevated plasma FFA levels, the effect of acute exercise (2 h at 50 % maximal V_{O_2}) on UCP3 mRNA expression 0, 1 and 4 h post-exercise was tested once in the fasted state and once in the glucose-fed state (Schrauwen *et al*. 2002*a*). It was observed that energy expenditure during and after exercise was not influenced by glucose administration, whereas glucose drinks inhibited plasma FFA levels and fat oxidation. UCP3 mRNA expression was up regulated 4 h after exercise, but only in the fasted situation. This result indicated that the up-regulation of UCP3 with acute exercise was more likely to be linked to changes in fatty acid metabolism than to changes in energy expenditure. These findings were confirmed recently by Pilegaard *et al*. (2002), who examined the effect of glycogen content on up-regulation of UCP3 mRNA after exercise. Subjects performed a 1 h cycling exercise bout at 70 % maximal workload followed by a 1 h two-arm cycling exercise bout to lower glycogen levels. Subsequently, subjects consumed either a low-carbohydrate (resulting in low glycogen content) or a high-carbohydrate diet (resulting in high glycogen content). On the following day subjects performed a 3 h exercise bout to examine the effect of glycogen content on UCP3 mRNA expression. It was found that UCP3 mRNA expression was significantly increased ($P < 0{\cdot}05$) 2 h after exercise, but only in the low glycogen condition. Since plasma FFA levels were also significantly higher ($P < 0{\cdot}05$) in the low glycogen trial, these results again suggest that changes in plasma FFA levels might be responsible for the up-regulation of UCP3 after acute exercise (Pilegaard *et al*. 2002). Whether in human subjects, also, activation of AMPK is involved still needs to be examined. Taken together, there is little evidence that the up-regulation of UCP3 after acute exercise is triggered by elevated energy expenditure. Rather, the up-regulation of UCP3 after acute exercise seems to be more related to changes in fatty acid metabolism. Recently, it was postulated that the primary function of UCP3 would be in the handling of those fatty acids that cannot be oxidized (Schrauwen *et al*. 2001). After acute exercise in the fasted state more fatty acids are released from the adipose tissue than can be oxidized, thus explaining the up-regulation of UCP3. However, with endurance training the capacity to oxidize fatty acids would increase, reducing the need for high levels of UCP3. As a secondary effect of the reduced UCP3 content with training, mechanical energy efficiency would be improved. However, more research is needed to test this hypothesis and to examine the physiological function of UCP3 in human skeletal muscle.

Conclusion

Physical activity can affect energy metabolism in human subjects. Apart from increasing the daily energy requirement for physical activity, it has also been suggested that physical activity might improve energy efficiency. The human skeletal muscle-specific UCP3 might play a role in this activity-induced alteration in energy metabolism. UCP3 is down regulated by endurance training and strongly related to physical fitness. Furthermore, there is strong evidence that UCP3 protein content is a determinant of mechanical energy efficiency. Although at present there is evidence that the primary physiological function of UCP3 is not the regulation of energy expenditure, there is also very strong evidence that UCP3, as a secondary effect of its physiological function, influences energy metabolism. Thus, UCP3 can still be considered as a potential target for the elevation of energy expenditure in the treatment and prevention of obesity and diabetes; however, first it is necessary to learn more about the exact physiological function of UCP3.

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