Expression of uncoupling protein 1 in skeletal muscle decreases muscle energy efficiency and affects thermoregulation and substrate oxidation

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Skeletal muscle uncoupling by ectopic expression of mitochondrial uncoupling protein 1 (UCP1) has been shown to result in a lean phenotype in mice characterized by increased energy expenditure (EE), resistance to diet-induced obesity, and improved glucose tolerance. Here, we investigated in detail the effect of ectopic UCP1 expression in skeletal muscle on thermoregulation in HSA-mUCP1 transgenic mice. Thermoneutrality was determined to be ~30°C for both wild-type (WT) and transgenic mice. EE, body temperature (Tb), activity, and respiratory quotient (RQ) were then measured over 24 h at ambient temperatures (Ta) of 30, 22, and 5°C. HSA-mUCP1 transgenic mice showed increased activity-related EE and heat loss but similar basal metabolic rate compared with WT. Tb at resting periods was progressively decreased with declining Ta in HSA-mUCP1 transgenic mice but not in WT. Compared with WT littermates, the transgenic HSA-mUCP1 mice displayed increased RQ levels during night time, indicative of increased overall glucose oxidation, and failed to decrease their RQ levels with declining Ta. Thus increased EE caused by skeletal muscle uncoupling is clearly due to a decreased muscle energy efficiency during activity combined with increased glucose oxidation and a compromised thermoregulation associated with increased overall heat loss. At Tb's below thermoneutrality, this puts increasing energy demands on the animals, whereas at thermoneutrality most differences in energy metabolism are not apparent any more.

Methods

Generation of HSA-mUCP1 transgenic mice. Transgenic mice were generated at DeveloGen AG (Göttingen, Germany) using standard techniques. Briefly, a 1-kb mouse UCP1 cDNA was cloned under control of a 2.2-kb human skeletal actin promoter (HSA) fragment (~1905−239), which confers striated muscle-specific gene expression (5, 7, 28). After removal of vector sequences, the linearized construct was microinjected into male pronuclei of fertilized C57Bl/6 × CBA one-cell mouse embryos. Injected embryos were transferred into pseudopregnant foster mice and allowed to develop to term. Transgenic mice were identified by PCR as described below.

Animal maintenance and experimental set up. Mice were maintained single, under standard conditions at 22°C and a 12:12-h dark-light cycle with food and water provided ad libitum. Animal maintenance and experiments were in accordance with the guidelines of the ethics committee of the Ministry of Agriculture and Environment (State Brandenburg, Germany). Mice were fed a standard rodent chow diet containing (wt/wt) 19% protein, 4% fat, and 50.5% carbohydrates (Altromin 1321, Lage, Germany) provided as pellets. Total energy content of standard chow as determined by bomb calorimetry was 17.1 kJ/g.

All experiments were performed with adult hemizygous transgenic mice obtained by mating of wild-type mice (C57Bl/6, Charles River Wiga GmbH, Germany) with hemizygous transgenic mice. Offspring was genotyped using genomic DNA isolated from tail tip and a HSA promoter primer (5′-ATATGGCTCGAGAAGGGCA-3′) as well as a murine UCP1 transgene primer (5′-TGATGTCTCCTAGGGACATCT-3′). This resulted in amplification of a 520-bp fragment in transgenic mice only. Wild-type littermates of HSA-mUCP1 transgenic mice were used as controls in all experiments.

For measurements of body composition and organ weights, five wild-type and five transgenic mice of both genders aged 12–14 mo...
were used. Male and female mice were analyzed separately. After measurement of body composition, animals were anesthetized and killed by cardiac puncture. Organs were removed, weighed, and frozen rapidly in liquid nitrogen and stored at −80°C.

In vivo experiments were performed in female mice aged 9–10 mo that were implanted with temperature-sensitive transponders as described below. Determination of the thermoneutral zone and measurements of energy metabolism at 30 (thermoneutrality), 22, and 5°C were performed in the same animals with at least 1-wk interval between the different measurements.

**Body composition.** Body composition was determined using quantitative magnetic resonance (QMR; Bruker’s Minispec MQ10, Houston, TX). This method was developed specifically for noninvasive, rapid determination of body composition in conscious mice. QMR analysis of fat content in mice was shown to detect values similar to dual-energy X-ray absorptiometry but offered an improved precision (29). Our own validation experiments showed a very high correlation between the QMR data for body fat and those obtained by chemical carcass analysis. Coefficients of correlation were 0.98 (P. Wiedmer and S. Klaus, unpublished data). All QMR measurements were performed in duplicates. Lean body mass was calculated by subtracting body fat mass values obtained by QMR from body weight obtained by weighing before QMR measurement.

**Determination of thermoneutral zone.** To determine basal metabolic rate (BMR), the zone of thermoneutrality was determined as described before (16). EE was measured in individual female mice placed individually in temperature-controlled chambers and provided with food and water ad libitum. The ambient temperature (Ta) was increased stepwise from 21 to 37°C. Each temperature step was maintained for at least 1 h and measurements were started at 8 AM. Animals were carefully observed during this time and measurements were performed every 6 min. The lowest three measurements of CO2 production at a given temperature (corresponding to inactivity periods) were averaged for each animal. Both transgenic and wild-type mice displayed lowest EE rates between 29 and 31°C. Therefore, subsequent determination of BMR was performed at 30°C in all animals.

**EE.** EE was measured by indirect calorimetry in individual female mice as described before (20, 21) using an open respirometric system (gas analyzers: Magnos 16 and Uras 14, Hartmann & Braun). Measurements were performed in modified standard cages, and animals were unrestrained throughout the measurements with free access to food and water. Respiratory quotient (RQ = VCO2/VO2) and EE (in kJ/day) were calculated according to Weir (31). Measurements were performed in 6-min intervals over a period of 23 h (12 AM to 11 AM the next day). Hourly means were calculated for every animal and averaged for visual presentation of data. For determination of resting EE (REE), the 10 lowest individual measurements were averaged for each animal. Activity EE (AEE) was calculated by subtraction of REE from total EE (TEE). EE for thermoregulatory thermogenesis (TT) was calculated by subtracting REE measured at 30°C (which was considered as corresponding to BMR) from REE at 22 and 5°C, respectively. Food was provided in special containers allowing the collection of spilled food. Food intake was determined by weighing before QMR measurement.

**Body temperature and activity.** Body core temperature (Tb) and activity were measured in 6-min intervals during indirect calorimetry measurements using passive telemetry transponders (series 4000, Mini Mitter, Sunriver, OR). At least 1 wk before the start of experiments, transponders were implanted into the abdominal cavity under anesthesia [80 mg/kg ketaminhydrochlorid and xylazin (16 mg/kg Rompun); ketamin: Gräub, A. Albrecht, Aulendorf, Germany; Rompun: BayerVital, Leverkusen, Germany] to allow recovery of animals. Data were recorded every 6 min and hourly means for each individual animal were calculated for visual presentation of data.

**Calculation of minimal and maximal body temperatures, the lowest and highest three values, respectively, were averaged for each animal.**

**Conductance.** The term thermal conductance (C) refers to the rate of heat transfer from an organism to the surroundings. When C is low, insulation is high. In fact, insulation is the reciprocal value of conductance (24). When exposed to cold, mammals increase heat production, i.e., EE to maintain body temperature. This increase is a function of C and the gradient between Tc and Tb is as follows (25):

\[
EE = C \times (T_b - T_c)
\]

Because we measured EE and Tb simultaneously, it was thus possible to calculate C by transformation of equation (1):

\[
C = EE/(T_b - T_c)
\]

**Gene expression.** Total RNA from individual tissues [epidymal or omental white adipose tissue (WAT), interscapular BAT, heart, skeletal muscle, kidney, lung, stomach] was extracted using a single-step acid phenol-guanidine method. Gene expression was analyzed by Northern blot analysis as described before (4). In brief, 10 μg of total RNA were separated by electrophoresis and blotted to a nylon membrane (Hybond N, Amersham Biosciences, Freiburg, Germany). The blots were probed with 32P-labeled cDNA probes. A cDNA probe for rat UCP1 was kindly provided by Prof. D. Ricquier (Centre National de la Recherche Scientifique, Paris, France). An Instant Imager (A202401, Canberra Packard GmbH, Dreieich, Germany) was used for analysis and quantification of radiolabeled signals.

**Statistical analysis.** Results are given as means ± SE. Differences between wild-type and HSA-mUCP1 transgenic mice were analyzed by unpaired Student’s t-test (Statview 4.5 for Apple Macintosh, Abacus Concepts, Berkley, CA). Differences at P < 0.05 were considered significant.

**RESULTS**

**mUCP1 transgene expression.** Northern blot analysis using RNA isolated from HSA-mUCP1 transgenic mice and littermate controls revealed ectopic mUCP1 expression in skeletal muscle but not in heart, stomach, and nonmuscle tissues (Fig. 1). Expression of the mUCP1 transgene in skeletal muscle was approximately five times lower than expression of endogenous UCP1 in muscle affects energy homeostasis.
mUCP1 in BAT as judged from Northern blot analysis. This was confirmed by quantitative RT-PCR (TaqMan analysis) performed on RNA isolated from BAT and muscle tissue, which showed an eightfold lower mUCP1 expression level in wild-type mice vs. 2.79 ± 0.52 g in transgenic mice at 22°C but higher in transgenic mice at 5°C (3.22 ± 0.14 g in wild-type mice vs. 3.98 ± 0.17 g in transgenic mice). Weight-specific energy intake as shown in Table 2 was significantly increased in HSA-mUCP1 transgenic mice at 22 and 5°C compared with wild-type controls.

### Energy metabolism

Figure 2 shows the determination of thermoneural zone in HSA-mUCP1 transgenic mice and wild-type controls. Both groups displayed almost identical weight-specific REE between 23 and 37°C and lowest metabolic rates between 28 and 32°C. Thus the thermoneural zone is not different between transgenic and control mice; 30°C therefore represents thermoneutrality for both control and transgenic mice.

#### Body composition and food intake

Male and female HSA-mUCP1 transgenic mice had a significantly reduced body weight as well as a reduced absolute and relative body fat content compared with wild-type littermate controls (Table 1). Accordingly, gonadal WAT depots and interscapular BAT were also reduced in HSA-mUCP1 transgenic mice. Lean body mass was also reduced in HSA-mUCP1 transgenic mice, a finding in agreement with previous reports on transgenic mice with UCP1 expression in muscle (8, 18). Heart and kidney weight was significantly increased in both male and female HSA-mUCP1 transgenic mice. Lean body mass was also reduced in HSA-mUCP1 transgenic mice compared with wild-type littermate controls (Table 1). Accordingly, gonadal WAT depots and interscapular BAT were also reduced in HSA-mUCP1 transgenic mice. Lean body mass was also reduced in HSA-mUCP1 transgenic mice, a finding in agreement with previous reports on transgenic mice with UCP1 expression in muscle (8, 18). Heart and kidney weight was significantly increased in both male and female HSA-mUCP1 transgenic mice. Lean body mass was also reduced in HSA-mUCP1 transgenic mice. Lean body mass was also reduced in HSA-mUCP1 transgenic mice, a finding in agreement with previous reports on transgenic mice with UCP1 expression in muscle (8, 18). Heart and kidney weight was significantly increased in both male and female HSA-mUCP1 transgenic mice.

#### Table 1. Body composition and organ weights of transgenic HSA-mUCP1 mice and WT litter mates

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
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<tbody>
<tr>
<td></td>
<td>WT</td>
<td>HSA-mUCP1</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>43.7 ± 0.52</td>
<td>27.0 ± 0.65</td>
</tr>
<tr>
<td>Body fat content, g</td>
<td>16.1 ± 0.46</td>
<td>6.6 ± 0.70</td>
</tr>
<tr>
<td>Body fat content, %</td>
<td>36.8 ± 0.94</td>
<td>24.4 ± 2.16</td>
</tr>
<tr>
<td>Lean body mass, g</td>
<td>27.6 ± 0.51</td>
<td>20.4 ± 0.47</td>
</tr>
<tr>
<td>Gonadal WAT, g</td>
<td>1.48 ± 0.23</td>
<td>0.69 ± 0.09</td>
</tr>
<tr>
<td>Interscapular BAT, mg</td>
<td>269 ± 27</td>
<td>124 ± 6</td>
</tr>
<tr>
<td>Heart, mg</td>
<td>175 ± 4</td>
<td>156 ± 3</td>
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<tr>
<td>Kidney, mg</td>
<td>229 ± 7</td>
<td>194 ± 9</td>
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</table>

Data are means ± SE, n = 5 per group. *Student’s t-test. WT, wild type; WAT, white adipose tissue; BAT, brown adipose tissue; ns, not significant.

#### Table 2. Parameters of energy metabolism in transgenic HSA-mUCP1 mice and WT litter mates measured at different ambient temperatures

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>HSA-mUCP1</th>
<th>P Value*</th>
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</thead>
<tbody>
<tr>
<td>Measurements at 30°C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total energy intake, kJ·day⁻¹·g⁻¹</td>
<td>1.31 ± 0.06</td>
<td>1.36 ± 0.15</td>
<td>ns</td>
</tr>
<tr>
<td>Body temperature, °C</td>
<td>36.99 ± 0.04</td>
<td>36.86 ± 0.06</td>
<td>ns</td>
</tr>
<tr>
<td>Activity, counts/6 min</td>
<td>14.8 ± 1.2</td>
<td>13.4 ± 2.0</td>
<td>ns</td>
</tr>
<tr>
<td>EE, kJ·day⁻¹·g⁻¹</td>
<td>1.00 ± 0.01</td>
<td>1.00 ± 0.01</td>
<td>ns</td>
</tr>
<tr>
<td>Conductance, kJ°C⁻¹·g⁻¹</td>
<td>0.926 ± 0.070</td>
<td>1.012 ± 0.044</td>
<td>ns</td>
</tr>
<tr>
<td>Measurements at 22°C</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Total energy intake, kJ·day⁻¹·g⁻¹</td>
<td>1.50 ± 0.22</td>
<td>2.34 ± 0.08</td>
<td>0.004</td>
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<tr>
<td>Body temperature, °C</td>
<td>36.85 ± 0.05</td>
<td>36.73 ± 0.11</td>
<td>0.002</td>
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<tr>
<td>Activity, counts/6 min</td>
<td>15.2 ± 1.4</td>
<td>11.0 ± 1.1</td>
<td>0.04</td>
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<tr>
<td>EE, kJ·day⁻¹·g⁻¹</td>
<td>0.94 ± 0.02</td>
<td>1.00 ± 0.01</td>
<td>0.02</td>
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<tr>
<td>Conductance, kJ°C⁻¹·g⁻¹</td>
<td>1.42 ± 0.03</td>
<td>1.60 ± 0.07</td>
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</tr>
<tr>
<td>Measurements at 5°C</td>
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<td></td>
<td></td>
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<tr>
<td>Total energy intake, kJ·day⁻¹·g⁻¹</td>
<td>2.09 ± 0.14</td>
<td>3.27 ± 0.15</td>
<td>0.005</td>
</tr>
<tr>
<td>Body temperature, °C</td>
<td>36.37 ± 0.04</td>
<td>35.95 ± 0.08</td>
<td>0.001</td>
</tr>
<tr>
<td>Activity, counts/6 min</td>
<td>10.3 ± 1.0</td>
<td>9.3 ± 1.3</td>
<td>ns</td>
</tr>
<tr>
<td>EE, kJ·day⁻¹·g⁻¹</td>
<td>0.92 ± 0.01</td>
<td>0.97 ± 0.01</td>
<td>0.004</td>
</tr>
<tr>
<td>Conductance, kJ°C⁻¹·g⁻¹</td>
<td>2.56 ± 0.09</td>
<td>2.93 ± 0.12</td>
<td>0.044</td>
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Data are means ± SE, n = 7, except for measurements at 5°C when n = 5. *Student’s t-test. RQ, respiratory quotient; EE, energy expenditure.
intake and EE but decreased mean body temperature and RQ. Thermal conductance was increased in HSA-mUCP1 transgenic mice at 30 and 22 but not at 5°C, and activity was decreased in HSA-mUCP1 transgenic mice at 22°C only (Table 2). Figure 3 shows that differences listed in Table 2 were mainly due to differences during specific periods; at 22°C Tb was decreased in HSA-mUCP1 transgenic mice during periods of low locomotor activity only, whereas the increased EE observed at 5°C was only evident during nighttime, i.e., periods of high locomotor activity.

Figure 4 shows a detailed analysis of Tb range (Fig. 4A) and different compartments of EE (Fig. 4B) at different Ta.s. Both control and transgenic mice showed similar maximal Tb at all temperatures but minimal Tb was significantly reduced in HSA-mUCP1 transgenic mice at 22 and 5°C compared with wild-type. In transgenic mice, Tb temporarily dropped to values below 33°C when mice were exposed to 5°C.

When the different compartments of EE were calculated, it became evident that weight-specific BMR and TT were not different between transgenic and wild-type mice. AEE, on the other hand, was increased in HSA-mUCP1 transgenic mice at all temperatures measured. Increased AEE was thus responsible for the increased overall EE observed at 22 and 5°C in transgenic mice.

Thermal conductance. Thermal conductance, i.e., the rate of heat transfer from an animal to the environment, is a function of EE and the difference between Tb and Ta. The time course of conductance at different temperatures is shown in Fig. 5. At 5°C, conductance was constant throughout the day and identical in wild-type and transgenic mice. With increasing temperature, conductance during the activity period increased and was higher in HSA-mUCP1 transgenic mice compared with wild-type controls (Table 2).

Substrate oxidation. RQ is indicative of the overall substrate oxidation: lipid oxidation results in a RQ close to 0.7, whereas carbohydrate oxidation has a RQ of 1.0 (12). Figure 6 shows the daily profile of RQ at different Ta.s. Transgenic mice showed a higher increase in RQ during the activity period than wild-type controls. Mean RQ values in control mice were reduced with decreasing temperature, whereas in transgenic mice this decrease was less pronounced, resulting in significantly increased mean RQ of HSA-mUCP1 transgenic mice at 22 and 5°C compared with WT. This suggests an increased overall carbohydrate oxidation.

DISCUSSION

It has been shown previously that skeletal muscle respiratory uncoupling achieved by ectopic expression of UCP1 in skeletal muscle resulted in a reduction in body weight and adiposity apparently caused by an increased EE (3, 8, 18). Here, we show that the increased EE in HSA-mUCP1 transgenic mice is caused by an increased activity-related EE. Furthermore, our results suggest important differential effects of Ta on energy metabolism and substrate oxidation in wild-type and transgenic mice, respectively.

Increased activity-related energy metabolism in HSA-mUCP1 transgenic mice. Our data show that the increased EE in HSA-mUCP1 transgenic mice is not due to an elevated REE or TT but clearly due to an increased AEE. Compared with littermate controls, AEE of HSA-mUCP1 transgenic mice was significantly increased at all temperatures studied, and most pronounced at 5°C. At this temperature, weight-specific AEE was over 50% higher in HSA-mUCP1 transgenic mice compared with wild-type controls. This was not due to an increased locomotor activity as activity counts were similar or even lower in HSA-mUCP1 mice. Thus our data strongly suggest a decreased muscle energy efficiency caused by the expression of UCP1 in skeletal muscle resulting in increased EE during activity. It should be pointed out that AEE as calculated here also includes diet-induced EE and that food intake was over-proportionally increased in HSA-mUCP1 mice at 5°C. However, at thermoneutrality (30°C) food intake was not different between the two groups, whereas AEE was still increased in HSA-mUCP1 transgenic mice, indicating that this difference is really activity related.

Impaired thermoregulation in HSA-mUCP1 transgenic mice. Previous studies on energy metabolism of mice with muscle-specific UCP1 expression (8, 18) were conducted under normal animal maintenance conditions where Ta is generally kept ~21°C. This represents a constant mild cold exposure as the thermoneutral zone of mice lies ~30°C as shown before (16) and again in this study. Furthermore, mice display marked reductions in food intake, metabolic rate, heart rate, and blood pressure when transferred to thermoneutrality (22). It has therefore been argued that mouse physiology studies should be performed under thermoneutral conditions (32). Because endogenous UCP1 in brown fat is only activated at temperatures below thermoneutrality (14), it was of special interest to study the effect of ectopic UCP1 expression in muscle not only at thermoneutrality but at different Ta.s. The activity of endogenous UCP1 in brown fat is tightly regulated and mainly under control of the sympathetic nervous system (6). Noradrenaline induced activation of BAT thermogenesis, i.e., UCP1 activation enables mice to maintain Tb under severe cold exposure even though BAT only constitutes few percent of body mass. It is thus conceivable that even a low constitutive uncoupling of muscle mitochondria by UCP1 could result in marked effects on thermoregulation considering the much higher muscle mass compared with BAT mass. Actually, the thermoneu-
tral range was not shifted by skeletal muscle uncoupling. Nevertheless, there were significant effects on Tb: with decreasing Ta, mean as well as minimum levels of Tb were reduced in HSA-mUCP1 transgenic mice. The episodes of decreased Tb coincided with episodes of inactivity, which could suggest that transgenic mice are not able to maintain normothermia at rest. On the other hand, through a decrease in Tb mice were able to minimize thermal conductance, i.e., heat loss, which is most important and also most prominent at 5°C. It is obvious that UCP1 expression in skeletal muscle does not improve cold-induced thermogenesis; on the contrary, HSA-mUCP1 transgenic mice seem to cope less well at 5°C than wild-type control mice. This is underscored by the finding that transgenic mice increased their food intake to a much higher extent when exposed to cold than wild-type mice. HSA-mUCP1 transgenic mice had a 2.4-fold higher energy intake at 5°C than at 30°C, in wild-type controls this increase was only 1.4-fold.

Taken together, our results suggest that the respiratory uncoupling of skeletal muscle led to an increased EE in muscle, a decreased muscle efficiency, and an increased overall heat loss due to an increased thermal conductance. Therefore, at temperatures below thermoneutrality these mice were forced to increase EE to a greater extent than wild-type controls to avoid hypothermia.

Altered substrate oxidation in HSA-mUCP1 transgenic mice. Simultaneous measurement of oxygen consumption and CO₂ production allows the calculation of RQ, which is indicative of the overall substrate oxidation. RQ values close to 1 indicate a preferential carbohydrate oxidation and values close to 0.7 a preferential fat oxidation (12). Therefore, RQ decreases during postabsorptive periods when carbohydrate

Fig. 3. Twenty-four-hour time course of activity (A), body temperature (B), and energy expenditure (EE; C) in WT (○) and HSA-mUCP1 transgenic mice (●) subjected to different ambient temperatures. Light off period was from 1800 to 0600. Data are means ± SE, n = 7 per group except for data at 4°C when n = 5.
stores are low or depleted and fatty acids liberated from adipose tissue are oxidized. This is reflected in the daily pattern of RQ as shown in Fig. 6. Mice are nocturnal animals and feed preferentially during nighttime. Because they were fed a standard chow diet high in carbohydrates and low in fat, RQ increased during night when exogenous carbohydrates were the main oxidative fuel. During daytime, RQ values were decreased when substrate oxidation relied also on endogenous lipid stores. As evident from Fig. 6, HSA-mUCP1 transgenic mice were able to decrease RQ to control levels during daytime, i.e., resting periods. However, during activity periods, they showed markedly increased RQ levels. This is similar to transgenic mice overexpressing GLUT4 in muscle and fat. These mice were found to show increased RQ during exercise (1), indicative of increased glucose oxidation in muscle. Taken together, this suggests a predominant glucose oxidation in skeletal muscle of HSA-mUCP1 transgenic mice during activity, muscle shivering, and high exogenous carbohydrate supply. This is consistent with data from Han et al. (11) who reported an increased skeletal muscle glucose transport in transgenic mice (UCP-H mice) with high levels of UCP1 expression in skeletal muscle. A preferential reliance on glucose as a fuel for muscle EE could explain the increased insulin sensitivity observed in other studies on transgenic mice with muscle-directed expression of UCP1 (3, 18).

In wild-type mice, mean RQ decreased with decreasing temperature. This is in line with earlier observations in rats (23) and indicative of increased lipid-fuelled thermogenesis. Although BAT is largely responsible for thermogenesis in mice, it should be noted that in our experimental set-up mice were not acclimated to cold. This means that they also had to rely on muscle shivering when acutely exposed to cold. The inability of HSA-mUCP1 transgenic mice to increase fat oxidation with decreasing Ta could either reflect a less efficient BAT thermogenesis or the inability to switch muscle fuel oxidation from glucose to fatty acids. The first explanation is not likely as the UCP1 gene expression in BAT was not affected in HSA-mUCP1 transgenic mice. Therefore, we suggest that the increased muscular glucose uptake and oxidation induced by UCP1 expression impair the ability of muscle cells to increase lipid oxidation.

The mechanisms leading to increased glucose metabolism through ectopic UCP1 expression in skeletal muscle are not yet clear. In transgenic UCP-H mice, the proximate cause of enhanced glucose transport was proposed to be an increase in GLUT4 (11). On the other hand, it has been shown that chemical uncoupling of oxidative phosphorylation led to an increased glucose uptake of L6 muscle cells apparently caused by an increased GLUT-1 protein biosynthesis and translocation (2).
Mice overexpressing human UCP3 in skeletal muscle are also lean and display improved glucose tolerance (7, 26, 27). In this respect, they are very similar to mice overexpressing UCP1 in skeletal muscle. But in contrast to UCP1-overexpressing mice, they were reported to display an increased fatty acid oxidation in skeletal muscle in vitro and no apparent increase in peripheral glucose uptake (30). This suggests that the similar physiological effects of ectopic UCP1 and UCP3 expression in skeletal muscle could be due to different molecular modes of action of these two proteins. Using UCP1 knockout mice, it was indeed demonstrated that the presence of UCP1 is necessary for cold-induced BAT thermogenesis as it could not be compensated by an increased expression of other UCPs (6). On the other hand, supraphysiological expression of UCP3 in muscle is apparently leading to mitochondrial uncoupling. It has been shown recently that overexpression of UCP3 led to an increased AMP-activated protein kinase (AMPK) activity in muscle, which could explain the improved glucose tolerance in these mice (26). AMPK in skeletal muscle is activated by contraction and results in increased glucose uptake (33). Further studies should thus investigate whether AMPK activity is increased in skeletal muscle of HSA-mUCP1 transgenic mice and whether this increase is influenced by $T_a$.

In conclusion, the increased EE caused by skeletal muscle uncoupling is clearly due to a decreased muscle energy efficiency during activity and a compromised thermoregulation that is associated with an increased overall heat loss. At $T_a$ below thermoneutrality, this puts increasing energy demands on the animals that apparently can be only partly compensated by increased dietary energy intake. Furthermore, UCP1 expression in skeletal muscle apparently leads to changes in muscle substrate oxidation, which is shifted toward glucose oxidation when the muscle is active. In this respect, it is interesting to note that most of the differences in energy metabolism between HSA-mUCP1 transgenic and wild-type mice were evident at temperatures below thermoneutrality only. When measured at thermoneutrality, differences in energy intake and expenditure, $T_b$, and substrate oxidation disappeared. This is of importance when considering the relevance of results from mouse studies with regard to human energy metabolism. It has been proposed that obesity could possibly be treated by promoting inefficient muscle metabolism (18). However, humans are very rarely in situations demanding increased TT as they rather rely on room heating and insulation through clothing. In mice, on the other hand, an $T_a$ of 21–23°C leads to a ~30% increase in EE compared with thermoneutrality thus imposing significant energetic constraints that do not exist for humans. Therefore, we suggest that studies related to energy metabolism in mice should take into account the effects of $T_a$ and that these studies should ideally be conducted at thermoneutral conditions.

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