Dissociation of obesity and insulin resistance in transgenic mice with skeletal muscle expression of uncoupling protein 1

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Katterle Y, Keipert S, Hof J, Klaus S. Dissociation of obesity and insulin resistance in transgenic mice with skeletal muscle expression of uncoupling protein 1. Physiol Genomics 32: 352–359, 2008. First published November 27, 2007; doi:10.1152/physiolgenomics.00194.2007.—We evaluated the effect of skeletal muscle mitochondrial uncoupling on energy and glucose metabolism under different diets. For 3 mo, transgenic HSA-mUCP1 mice with ectopic expression of uncoupling protein 1 in skeletal muscle and wild-type littermates were fed semisynthetic diets with varying macronutrient ratios (energy %: carbohydrate-protein-fat): HCLF (41:42:17), HCHF (41:16:43); LCHF (11:45:44). Body composition, energy metabolism, and insulin resistance were assessed by NMR, indirect calorimetry, and insulin tolerance test, respectively. Gene expression in different organs was determined by real-time PCR. In wild type, both high-fat diets led to an increase in body weight and fat. HSA-mUCP1 mice considerably increased body fat on HCHF but stayed lean on the other diets. Irrespective of differences in body fat content, HSA-mUCP1 mice showed higher insulin sensitivity and decreased plasma insulin and liver triglycerides. Respiratory quotient and gene expression indicated overall increased carbohydrate oxidation of HSA-mUCP1 but a preferential channeling of fatty acids into muscle rather than liver with high-fat diets. Evidence for increased lipogenesis in white fat of HSA-mUCP1 mice suggests increased energy dissipating substrate cycling. Retinol binding protein 4 expression in white fat was increased in HSA-mUCP1 mice despite increased insulin sensitivity, excluding a causal role in the development of insulin resistance. We conclude that skeletal muscle mitochondrial uncoupling does not protect from the development of obesity in all circumstances. Rather it can lead to a “healthy” obese phenotype by preserving insulin sensitivity and a high metabolic flexibility, thus protecting from the development of obesity associated disturbances of glucose homeostasis.

UCP1; thermogenesis; insulin resistance; adipose tissue gene expression; retinol binding protein 4

PERSISTENT POSITIVE ENERGY BALANCE and especially overconsumption of diets with a high fat and glucose content lead to obesity and impaired glucose tolerance increasing the risk for the development of Type 2 diabetes (7, 24). Since the development of obesity and the metabolic syndrome is a major world-wide human health problem, numerous strategies for prevention ranging from dietary intervention with different macronutrient compositions (19, 29) to enhancement of metabolic rate by either increased physical activity or pharmacological intervention (8) have been considered.

In animal studies diet-induced obesity and insulin resistance have been described mainly in C57BL/6 mice (3, 23, 25, 31), focusing on the role of dietary fat. A more detailed insight into the development of the metabolic syndrome is provided by studies on the influence of the protein/carbohydrate ratio on energy metabolism, body composition, and glycemic control in rodents. An increased protein/carbohydrate ratio in the diet leads to a lower body weight and body fat content, improving glucose homeostasis in rats (4, 17), and delays adiposity, resulting in higher insulin sensitivity in high fat-fed mice (16). Rats fed a high-protein diet over a long time show increased tissue-specific expression of uncoupling protein 1 (UCP1), suggesting a role in thermogenesis (26). UCP1 is expressed in brown adipose tissue (BAT), where it dissipates the proton gradient across the inner mitochondrial membrane, uncoupling the respiratory chain from ATP synthesis (14, 20). Activation of uncoupling in tissues other than BAT to increase energy expenditure is a promising approach to control body weight and energy homeostasis. Independently, different transgenic mouse models have been generated expressing ectopic UCP1 in skeletal muscles (9, 15, 18). It was demonstrated that UCP1 was fully active in the muscle inner mitochondrial membrane (9). In these mouse models numerous phenotypic changes could be observed, ranging from lower body weight and resistance against diet-induced obesity over increased energy expenditure, fasting hypoglycemia, and improved glucose tolerance to higher night-time respiratory quotients, indicating a higher overall glucose turnover.

Common standard and high-fat rodent diets have high carbohydrate contents and low protein/carbohydrate ratios, thus meeting the metabolic preferences of UCP1 transgenic mice for utilizing glucose as an energy substrate. In a previous study we have shown that UCP1 transgenic mice are unable to increase cold-induced fat oxidation to the same extent as wild-type controls (15). Together this raises the question of how UCP1 transgenic mice cope with a different macronutrient supply, especially a high-fat diet low in carbohydrates. It is not clear yet if skeletal muscle uncoupling leads to a general protection against diet-induced obesity and if it increases insulin sensitivity irrespective of dietary challenge. Therefore, the aim of this study was to investigate the impact of dietary challenge on the development of obesity, energy metabolism, and insulin sensitivity in UCP1 transgenic mice by feeding two isocaloric semisynthetic high-fat diets with different protein/carbohydrate ratios compared with a low-fat diet containing the same ingredients.

MATERIALS AND METHODS

Animal maintenance and experimental set up. Experiments were performed in adult (9–10 mo old) male hemizygous HSA-mUCP1 transgenic mice and their wild-type littermates obtained as previously described (15). Mice were maintained singly at 22°C and a 12 h:12 h dark-light cycle with food and water provided ad libitum. Animal maintenance and experiments were approved by the ethics committee of the Ministry of Agriculture and Environment (State Brandenburg, Allee 114-116, 14558 Nuthetal, Germany (e-mail: Klaus@dife.de).


Before the experiments all mice were fed ad libitum with standard rodent chow diet containing (wt/wt) 19% protein, 4% fat, and 50.5% carbohydrates (Altromin 1321, Lage, Germany) provided as pellets. At start of the experiment mice were divided randomly into six experimental groups (two genotypes, three diets) with eight mice each and assigned to the different macronutrient diets as described below. Food was provided ad libitum in special containers allowing for the collection of spilled food. Body weight and food intake were determined two times per week. Mice were anaesthetized and killed by cardiac puncture after 97 days. Organs were removed, weighed, frozen in liquid nitrogen, and stored at −80°C.

**Diets.** During the intervention mice were fed semisynthetic diet with different macronutrient composition (Table 1). Dietary components were as described before (10, 16), and diets were provided as pellets ad libitum. Metabolizable energy density of diets was calculated according to following macronutrient energy content: casein, 15.7 kJ/g; carbohydrates, 16 kJ/g; and fat, 38 kJ/g. The two high-fat diets [low carbohydrate/high fat (LCHF); high carbohydrate/high fat (HCHF)] had a similar fat content and energy density. The control diet was a low-fat diet with carbohydrate content (energy %) matched to the HCHF diet (HCLF).

**Body composition and blood glucose.** Body composition was determined before the start of experiment (day 0) and at 23, 45, and 70 days of dietary intervention using quantitative magnetic resonance (QMR) (Bruker’s Minispec MQ10, Houston, TX) as described (15). Lean body mass was calculated by subtracting body fat values obtained by QMR from body weight obtained by weighing prior to QMR measurement. Postabsorptive blood glucose levels were determined between 0800 and 1000 h, 2–4 h after food withdrawal in whole blood from the tail using a common blood glucose sensor (Medisense Q.I.D.; Abbott Laboratories).

**Insulin tolerance test.** Insulin sensitivity was tested after 10 wk of dietary intervention by intraperitoneal injection of insulin (0.75 U/kg body wt Actrapid; Novonordisk) as described before (22). Glucose concentration was determined in tail blood at 0, 15, 30, and 60 min after insulin injection.

**Energy expenditure.** Energy expenditure (EE) was measured by indirect calorimetry in individual mice as described before (15, 21) using an open respirometric system (gas analyzers: Magnos 16 and Uras 14, Hartmann & Braun). Measurements were performed in the 8th or 9th wk of intervention. Mice were unrestrained and had free access to food and water during the measurement. Respiratory quotient (RQ = V\textsubscript{CO2}/V\textsubscript{O2}) and EE (in kJ/day) were calculated as described before (26). Measurements were performed in 6 min intervals over a period of 23 h.

**Quantitative real-time PCR.** RNA was isolated from tissue as described before (5) with modifications as described by Weber et al. (33). Residual genomic DNA was removed using Turbo-DNA-free Kit (Ambion). Synthesis of cDNA was performed from 2 μg total RNA with RevertAid H Minus First Strand cDNA Synthesis Kit (Fermentas). Quantitative real-time PCR was performed on the Applied Biosystems 7900 HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA). The PCR mix (5 μl) contained TaqMan(R) Universal PCR Master Mix, No AmpErase(R) UNG (Applied Biosystems), and a cDNA amount corresponding to 5 ng RNA was used for cDNA synthesis and gene-specific primer probe pairs (online appendix Table 1).1 Gene expression is calculated as

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**Table 1. Macronutrient composition of semisynthetic diets**

<table>
<thead>
<tr>
<th>Component</th>
<th>Control Diet (HCLF)</th>
<th>HCHF Diet</th>
<th>LCHF Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein, g/kg</td>
<td>410</td>
<td>180</td>
<td>500</td>
</tr>
<tr>
<td>Wheat starch, g/kg</td>
<td>370</td>
<td>430</td>
<td>80</td>
</tr>
<tr>
<td>Saccharose, g/kg</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Palm kernel fat, g/kg (45% lauric acid, 21% myristic acid, 9% palmitic acid)</td>
<td>50</td>
<td>180</td>
<td>180</td>
</tr>
<tr>
<td>Thistle oil, g/kg (76% linoleic acid, 12% oleic acid)</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Linoel oil, g/kg (58% linolenic acid, 18% oleic acid, 14% linoleic acid)</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Cellulose, g/kg</td>
<td>30</td>
<td>70</td>
<td>100</td>
</tr>
<tr>
<td>Mineral mixture, g/kg</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Vitamin mixture, g/kg</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Metabolizable energy, kJ/g</td>
<td>15.7</td>
<td>17.7</td>
<td>17.5</td>
</tr>
<tr>
<td>Protein, energy %</td>
<td>41.7</td>
<td>41.0</td>
<td>41.5</td>
</tr>
<tr>
<td>Carbohydrates, energy %</td>
<td>41.1</td>
<td>41.0</td>
<td>11.5</td>
</tr>
<tr>
<td>Fat, energy %</td>
<td>17.2</td>
<td>43.2</td>
<td>43.6</td>
</tr>
</tbody>
</table>

HCLF, high carbohydrate/low fat; HCHF, high carbohydrate/high fat; LCHF, low carbohydrate/high fat.

1 The online version of this article contains supplemental material.
Body fat (38 LCHF mice, respectively. In wild-type mice both high-fat diets 19.9
in transgenic animals. Lean body mass did not change due to an
experiment, which was significantly lower than wild type with
mice had a body weight of 23.6
5. Exclusivity of mUCP1 expression in BAT and skeletal
control and not affected by the dietary intervention (see Table 2).

RESULTS

mUCP1 transgene expression. Quantitative real-time PCR
analysis using RNA isolated from skeletal muscle of HSA-
mUCP1 transgenic mice confirmed ectopic mUCP1 expression
compared with wild-type littermate controls in all groups.
UCP1 expression levels in skeletal muscle were found to be ~50–100 times higher in transgenic than in wild-type
control and not affected by the dietary intervention (see Table 5). Exclusivity of mUCP1 expression in BAT and skeletal
muscle of HSA-mUCP1 transgenic mice has been documented previously by Northern blot analysis (15).

Body composition and food intake. HSA-mUCP1 transgenic mice had a body weight of 23.6 ± 0.3 g at the start of the
experiment, which was significantly lower than wild type with
a mean body weight of 38.4 ± 0.6 g. These differences were
due to an ~25% lower lean body mass as well as to a lower fat
mass in transgenic animals. Lean body mass did not change
significantly during feeding in any of the groups. In wild type
it was 25.4 ± 0.5, 26.5 ± 0.4, and 25.0 ± 0.7 g in HCLF, HCHF, and LCHF mice, respectively, after 60 days of feeding.
In transgenic lean body mass after 60 days of feeding was
19.9 ± 0.5, 18.3 ± 0.4, and 19.5 ± 0.6 g in HCLF, HCHF, and
LCHF mice, respectively. In wild-type mice both high-fat diets
caused a significant increase in body weight (see Table 3) and
body fat (38 ± 1% in HCLF vs. 44 ± 1% in both HCHF and
LCHF), whereas in HSA-mUCP1 mice only the HCHF diet led
to an increase in body weight (Table 3) and body fat (20 ± 2% and 21 ± 2% in HCLF and LCHF vs. 38+/−3% in HCHF)
(Fig. 1). Interestingly, although final body weight still differed
significantly between HSA-mUCP1 mice and wild type (Table
3), the relative fat content of 44 ± 1% in wild-type and 38 ± 3%
in transgenic mice was comparable (P > 0.05) after 70
days of dietary intervention in the HCHF groups (Fig. 1B).
Changes in body weight were mainly due to increased food
intake of both control and transgenic mice fed the high-fat diets
(Table 2). Total energy intake (EI) as well as EE were lower in
HSA-mUCP1 mice compared with wild type, due to the lower
body weight. Weight-specific EE was increased in HSA-
mUCP1 mice as reported previously (18), but there was no
influence of diet in either wild-type or transgenic mice (Table 2).

Substrate oxidation. Figure 2 shows a 23 h time course of
the RQ, which is a marker of overall substrate oxidation.
Values near 1.0 account for carbohydrate oxidation, values
near 0.7 for lipid oxidation, and values higher than 1.0 are
indicative for lipid synthesis from carbohydrates (13). Mean
daily RQs reflect the different dietary carbohydrate/fat ratios
decreasing significantly with increasing fat and reduced carbo-
hydrate content (Table 2). Genotype-related differences could
not be detected at this level. However, when day and night
were analyzed separately, a significant effect of genotype could
be observed with HSA-mUCP1 mice showing higher RQ
levels at night, i.e., during their activity period (Table 2).
This was apparent in the carbohydrate-rich diets and most
pronounced in the HCHF diet (Fig. 2) and is indicative for
preferential carbohydrate utilization or in case of the HCLF
diet with a mean RQ slightly above 1.0 even for lipid synthesis
from carbohydrates. Interestingly, a pronounced RQ decrease
was observed compared with wild-type mice in the last 2 h of
the dark phase and the first hours of the light phase pointing to
increased fat oxidation of HSA-mUCP1 mice at this time of the
day.

Body and organ weights after 3 mo of dietary intervention.
Body weight of HSA-mUCP1 mice was still significantly
lower compared with wild type after 3 mo of dietary interven-
tion. Accordingly, liver weights were also lower (Table 3).
Also, liver triglyceride content was substantially decreased
in transgenic compared with control mice, which showed more
than two times higher liver triglyceride concentrations in all
dietary groups (Fig. 3). Interestingly, both brown and white fat
depots were largely increased in HCHF-fed HSA-mUCP1 mice
compared with the other diet groups. Epididymal WAT weight
was not different any more between transgenic and control

Table 2. Parameters of energy metabolism in HSA-mUCP1 transgenic mice and littermate controls fed different
macronutrient diets

<table>
<thead>
<tr>
<th></th>
<th>HCLF</th>
<th>HCHF</th>
<th>LCHF</th>
<th>ANOVA</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>wt</td>
<td>tg</td>
<td>wt</td>
<td>tg</td>
</tr>
<tr>
<td>EI, kJ/day</td>
<td>45.3 ± 1.17</td>
<td>37.9 ± 1.00</td>
<td>56.6 ± 1.33</td>
<td>48.0 ± 1.72</td>
</tr>
<tr>
<td>EE, kJ/day</td>
<td>47.9 ± 1.23</td>
<td>39.4 ± 1.00</td>
<td>54.3 ± 1.42</td>
<td>43.5 ± 1.50</td>
</tr>
<tr>
<td>EE, kJ/day*g</td>
<td>1.17 ± 0.026</td>
<td>1.64 ± 0.057</td>
<td>1.16 ± 0.026</td>
<td>1.54 ± 0.082</td>
</tr>
<tr>
<td>RQ 23h</td>
<td>0.96 ± 0.009</td>
<td>0.97 ± 0.012</td>
<td>0.93 ± 0.005</td>
<td>0.95 ± 0.011</td>
</tr>
<tr>
<td>RQ night (12h)</td>
<td>1.00 ± 0.007</td>
<td>1.01 ± 0.014</td>
<td>0.95 ± 0.006</td>
<td>0.97 ± 0.007</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 8. EI is the average daily intake over 70 days of dietary intervention. EE was measured over a 23 h period in the 8th or 9th wk
of dietary intervention. wt, Wild type; tg, transgenic; EI, energy intake; EE, energy expenditure; RQ, respiratory quotient; ns, not significant.

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mice in the HCHF groups (Table 3). Together with the data on total body fat, this shows the development of considerable obesity in HSA-mUCP1 mice on the HCHF diet.

**Plasma parameters and insulin sensitivity.** High-fat feeding significantly increased plasma triglyceride levels in both HSA-mUCP1 transgenic and wild-type control mice, whereas the increase in plasma cholesterol was more pronounced in the HCHF group and control animals (Table 4). Free fatty acids as well as glycerol were increased in HSA-mUCP1 mice only in the HCHF group and were significantly lower in the HCLF and LCHF diet compared with wild-type littermates, suggesting lower rates of lipolysis in HSA-mUCP1 mice compared with wild type. No significant changes in β-hydroxybutyrate levels could be detected, indicating no ketogenic metabolism even in the LCHF group.

Blood glucose levels were dependent on the macronutrient composition of the high-fat diets but did not differ between the genotypes. However, plasma insulin levels were significantly lower in HSA-mUCP1 transgenic mice compared with LCHF diet controls. However, HSL was not affected by diet in transgenic mice, whereas wild-type animals had a markedly reduced expression of HSL in the HCHF group. UCP2 gene expression was increased in HSA-mUCP1 mice on the HCHF diet. The diet-independent regulation of the expression of fatty acid transport/translocation such as FAT/CD36 and CPT1b (carnitine-palmitoyl-transferase-1b) was increased under the LCHF diet in transgenic mice, indicating a higher fatty acid turnover when carbohydrate supply is limited (Table 5). Interestingly, most genotype effects on gene expression were found in white fat [white adipose tissue (WAT)]. Acetyl-CoA carboxylase (ACC), FAS, SCD1, and hormone-sensitive lipase (HSL) gene expression was generally increased in HSA-mUCP1 mice as FAT/CD36, fatty acid synthase (FAS), and SCD1 in HSA-mUCP1 mice is in concordance with the reduction in liver triglycerides in all diet groups. In muscle there was little dietary influence on overall gene expression, only the expression of genes involved in fatty acid transport/translocation such as FAT/CD36 and CPT1b (carbonyl-palmitoyl-transferase-1b) was increased under the LCHF diet in transgenic mice, indicating a higher fatty acid turnover when carbohydrate supply is limited (Table 5). Interestingly, most genotype effects on gene expression were found in white fat [white adipose tissue (WAT)]. Acety-CoA carboxylase (ACC), FAS, SCD1, and hormone-sensitive lipase (HSL) gene expression was generally increased in HSA-mUCP1 transgenic compared with littermate controls. However, HSL was not affected by diet in transgenic mice, whereas wild-type animals had a markedly reduced expression of HSL in the HCHF group. UCP2 gene expression on the other hand was decreased in HSA-mUCP1 mice compared with littermate controls. However, HSL was not affected by diet in transgenic mice, whereas wild-type animals had a markedly reduced expression of HSL in the HCHF group. UCP2 gene expression on the other hand was decreased in HSA-mUCP1 mice compared with littermate controls.

**Table 3. Body and organ weights of HSA-mUCP1 tg mice and littermate controls fed different macronutrient diets after 3 mo of dietary intervention**

<table>
<thead>
<tr>
<th></th>
<th>HCLF</th>
<th>HCHF</th>
<th>LCHF</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt</td>
<td>tg</td>
<td>wt</td>
<td>tg</td>
<td></td>
</tr>
<tr>
<td>Body weight</td>
<td>39.88±0.96</td>
<td>24.64±0.38</td>
<td>48.52±0.97</td>
<td>33.60±0.70</td>
</tr>
<tr>
<td>Liver</td>
<td>1.92±0.088</td>
<td>1.24±0.029</td>
<td>2.23±0.267</td>
<td>1.30±0.031</td>
</tr>
<tr>
<td>WAT</td>
<td>1.42±0.20</td>
<td>0.63±0.08</td>
<td>1.98±0.23</td>
<td>2.14±0.13</td>
</tr>
<tr>
<td>BAT</td>
<td>0.213±0.026</td>
<td>0.090±0.008</td>
<td>0.370±0.024</td>
<td>0.269±0.007</td>
</tr>
</tbody>
</table>

Data are means ± SE in g; n = 5. BAT, interscapular brown adipose tissue; WAT, epididymal white adipose tissue.

**Gene expression analysis.** Overall gene expression in liver was highly affected by diet but very little by genotype (Table 5). Gene expression of key gluconeogenic enzymes (PEPCK and G6Pase) and glycolytic genes (GK and PK) were generally upregulated under high-fat conditions. Glucokinase (GK), phosphoenolpyruvate-carboxy-kinase (PEPCK), stearoyl-CoA desaturase-1 (SCD1), and fatty acid translocase (FAT/CD36) showed increased expression only in the control group fed the LCHF diet. The diet-independent regulation of the expression of FAT/CD36, fatty acid synthase (FAS), and SCD1 in HSA-mUCP1 mice is in concordance with the reduction in liver triglycerides in all diet groups. In muscle there was little dietary influence on overall gene expression, only the expression of genes involved in fatty acid transport/translocation such as FAT/CD36 and CPT1b (carbonyl-palmitoyl-transferase-1b) was increased under the LCHF diet in transgenic mice, indicating a higher fatty acid turnover when carbohydrate supply is limited (Table 5). Interestingly, most genotype effects on gene expression were found in white fat [white adipose tissue (WAT)]. Acetyl-CoA carboxylase (ACC), FAS, SCD1, and hormone-sensitive lipase (HSL) gene expression was generally increased in HSA-mUCP1 transgenic compared with littermate controls. However, HSL was not affected by diet in transgenic mice, whereas wild-type animals had a markedly reduced expression of HSL in the HCHF group. UCP2 gene expression on the other hand was decreased in HSA-mUCP1 mice compared with littermate controls.
diet high in fat and carbohydrates, HSA-mUCP1 mice showed increased overall glucose oxidation during activity (15). Here we explored the effect of different dietary challenges on glucose homostasis in this model. In wild-type mice, feeding a diet high in fat and carbohydrates leads to development of obesity and insulin resistance (16), but skeletal muscle mitochondrial uncoupling clearly protected them from development of insulin sensitivity and thus an increased oxidation of carbohydrates from the RQ measurements. Because their muscle energy efficiency is diminished (15), they have a higher demand for energy supply, which in this turn would result in increased food intake on the HCHF diet to obtain sufficient essential amino acids such as leucine.

**DISCUSSION**

Challenging HSA-mUCP1 mice with different macronutrient diets resulted in two major findings: Firstly, skeletal muscle uncoupling does not provide a protection against the development of obesity in all circumstances, but, secondly, it protects from the obesity-associated deterioration in glucose metabolism.

We have shown previously that under a standard low-fat diet HSA-mUCP1 mice show an increased RQ indicative of increased overall glucose oxidation during activity (15). Here we explored the effect of different dietary challenges on glucose homostasis in this model. In wild-type mice, feeding a diet high in fat and carbohydrates leads to development of obesity and insulin resistance (16), but skeletal muscle mitochondrial uncoupling clearly protected them from development of insulin resistance independently of the macronutrient composition of the diet as evident from the insulin tolerance test and plasma insulin levels. Surprisingly, this was not linked to dietary effects on body fat accumulation because when exposed to a diet high in fat and carbohydrates, HSA-mUCP1 mice showed a linear increase in body fat accumulation. Interestingly, when dietary carbohydrates were reduced and partly replaced by protein, body fat was not increased. HSA-mUCP1 mice thus respond differently to macronutrient challenges compared with wild-type mice.

When fed a high-carbohydrate/high-fat diet HSA-mUCP1 transgenic mice showed a marked increase in EI apparently not matched by a corresponding increase in EE (Table 2). However, it is difficult to directly relate EI and EE because EI was measured over an intervention period of 70 days, whereas EE represents a 23 h measurement performed in a single day after 8–9 wk of feeding. Still it is interesting that, in transgenic mice on the HCHF diet, EI largely exceeded EE, indicating that these mice were still in a positive energy balance, i.e., gaining weight, which is in agreement with the linear increase of body weight and body fat of these mice. All other groups seem to have reached a sort of plateau after 70 days. Apparently, HSA-mUCP1 mice were not able to increase their EE high enough to compensate for the increased EI. The reason for the increased EI of HSA-mUCP1 mice on the HCHF diet is not clear but could be related to the lower protein content (16% of energy) of this diet compared with the other two diets with a high protein content (>40% of energy). HSA-mUCP1 mice show consistently a lower lean body mass than wild type (15) as again confirmed in the present study. This could possibly be related to changes in protein and amino acid metabolism leading to a higher dietary protein requirement, which in turn would result in increased food intake on the HCHF diet to obtain sufficient essential amino acids such as leucine.

RQ measurements suggest that HSA-mUCP1 transgenic mice show a preferential oxidation of carbohydrates during their activity period that was most evident after feeding an HCHF diet. It seems that the increased skeletal muscle mitochondrial uncoupling led permanently to increased insulin sensitivity and thus an increased oxidation of carbohydrates during muscle activity. This in turn resulted in a sparing of fat leading to an increased body fat deposition when both carbohydrate and fat content in the diet was high. On the other hand, when dietary carbohydrates were limited (LCHF diet), HSA-mUCP1 mice were able to switch to fat oxidation as evident from the RQ measurements. Because their muscle energy efficiency is diminished (15), they have a higher demand for energy supply, which in this dietary condition was apparently accomplished by increasing muscle fatty acid uptake.

A key protein involved in moving fatty acids across the plasma membrane is FAT/CD36 (1, 6), which was more highly expressed in transgenic mice as again confirmed in the present study. This could possibly be related to changes in protein and amino acid metabolism leading to a higher dietary protein requirement, which in turn would result in increased food intake on the HCHF diet to obtain sufficient essential amino acids such as leucine.

**Table 4. Plasma parameters of HSA-mUCP1 tg mice and littermate controls fed different macronutrient diets after 3 mo of dietary intervention**

<table>
<thead>
<tr>
<th></th>
<th>HCLF</th>
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<th>LCHF</th>
<th></th>
<th>GENOTYPE × DIET</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>wt</td>
<td>tg</td>
<td>wt</td>
<td>tg</td>
<td>wt</td>
<td>tg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TG, μg/ml</td>
<td>477±77</td>
<td>391±49</td>
<td>937±124</td>
<td>845±209</td>
<td>1297±441</td>
<td>753±118</td>
<td>ns</td>
<td>0.043 ns</td>
</tr>
<tr>
<td>FFA, mM</td>
<td>0.80±0.06</td>
<td>0.58±0.09</td>
<td>0.88±0.08</td>
<td>0.83±0.10</td>
<td>0.86±0.06</td>
<td>0.58±0.03</td>
<td>0.007</td>
<td>0.082 ns</td>
</tr>
<tr>
<td>Free glycerol, μg/ml</td>
<td>39.4±0.9</td>
<td>31.1±3.1</td>
<td>50.4±2.9</td>
<td>57.4±8.2</td>
<td>55.9±1.6</td>
<td>29.2±1.8</td>
<td>0.012</td>
<td>0.001 0.002</td>
</tr>
<tr>
<td>Hydroxbutyrate, mg/dl</td>
<td>1.28±0.77</td>
<td>0.68±0.12</td>
<td>0.45±0.03</td>
<td>0.61±0.09</td>
<td>0.54±0.07</td>
<td>0.20±0.03</td>
<td>ns</td>
<td>ns ns ns</td>
</tr>
<tr>
<td>Cholesterol, mg/dl</td>
<td>129±10</td>
<td>114±7</td>
<td>202±24</td>
<td>162±14</td>
<td>144±15</td>
<td>110±5</td>
<td>0.018</td>
<td>&lt;0.001 ns</td>
</tr>
<tr>
<td>Blood glucose day 65, mg/dl</td>
<td>116±17</td>
<td>126±5</td>
<td>135±7</td>
<td>147±9</td>
<td>102±8</td>
<td>119±11</td>
<td>ns</td>
<td>0.013 ns</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 5 except blood glucose day 65 n = 8. TG, triglycerides; FFA, free fatty acids.
Mean values; controls, open symbols HSA-mUCP1 tg mice, horizontal bars represent after 97 days of dietary intervention. Closed symbols represent littermate differences between wt and tg mice in the HCLF (\( P \leq 0.03 \)) and genotype (\( P \leq 0.02 \)) groups. This evidence for increased lipogenesis is supported by the RQ measurements that showed values close to and even above 1.0, which is indicative of de novo lipogenesis from carbohydrates. In the HCHF group this increased white fat lipogenesis could have contributed to the linear increase in body fat accumulation. However, in the LCHF group the increased lipogenesis did not lead to increased lipid accumulation, presumably because of the increased oxidation of fatty acids in skeletal muscle. Substrate cycling between lipogenesis and lipid oxidation in skeletal muscle has been proposed as a thermogenic mechanism against skeletal muscle lipotoxicity and glucotoxicity (11). Possibly, such an energy dissipating substrate cycling also occurs between adipose tissue and skeletal muscle in HSA-mUCP1 mice, as suggested by increased HSL expression in white fat. On the HCHF diet this proposed substrate cycling could be either reduced or no longer sufficient to compensate for the increased EI.

Interestingly, HSA-mUCP1 mice showed a very rapid drop in RQ at the end of the night (i.e., activity period), most pronounced in the HCLF group, resulting in lower RQ values during the early morning hours compared with wild type. This indicates a very rapid transition from carbohydrate to fat oxidation, which could reconcile the seemingly paradoxical evidence for increased overall glucose oxidation and increased channeling of fatty acids into skeletal muscle: during activity, there was a preferential oxidation of glucose in skeletal muscle, whereas in times of inactivity fatty acid oxidation was higher in HSA-mUCP1, which obviously showed a more rapid switch between carbohydrate and fat oxidation than control mice. An impaired substrate switching from carbohydrate to fat oxidation, i.e., a metabolic inflexibility, has been hypothesized to contribute to insulin resistance in humans (30). It is therefore possible that a relative metabolic inflexibility in wild-type mice contributed to the development of insulin resistance because an overall decreased muscle mitochondrial fatty acid oxidative capacity is not considered to be causal in rodents (31).

Skeletal muscle uncoupling obviously affects adipose tissue metabolism. We investigated the expression of RBP4 because it is an adipokine secreted from adipose tissue that was recently reported to play a causal role in the development of skeletal muscle insulin resistance in mice (34). In marked contrast to this report, RBP4 expression was significantly increased in WAT of HSA-mUCP1 mice compared with wild type in the HCLF and LCHF group. One possible explanation is that the increase in RBP4 expression could be a compensatory reaction to the increased peripheral glucose utilization in HSA-mUCP1 mice, as suggested by increased HSL expression in white fat. On the HCHF diet this proposed substrate cycling could be either reduced or no longer sufficient to compensate for the increased EI.
mice to avoid hypoglycemia. RBP4 was found to stimulate glucose output from hepatoma cells in vitro and to activate hepatic gluconeogenic enzymes in vivo including PEPCk (34). This would be in line with the tendency for increased PEPCk expression in the HCLF and LCHF groups of HSA-mUCP1 mice. Interestingly, the highly correlated expression of RBP4 and GLUT4 in white fat mirrors recent findings in humans (12). This similarity of HSA-mUCP1 mice to humans with respect to RBP4 regulation might make them an interesting model to study the physiological function of RBP4 in glucose homeostasis. In any case, although RBP4 expression in white fat seems to be connected to glucose metabolism, we can exclude the possibility that RBP4 plays a causal role in the development of insulin resistance in this mouse model.

It should be noted that RBP4 and GLUT4 expression in white fat was elevated in HSA-mUCP1 mice in the two groups fed a diet high in protein (≈40% energy). It is known that dietary proteins and amino acids are potent modulators of glucose metabolism (28) and that high-protein diets lead to pronounced changes in plasma amino acid composition, such as an increase in leucine/threonine ratio as well as an increased amino acid oxidation (27). We have demonstrated that an increase in dietary protein/carbohydrate ratio delayed development of adiposity and improved glucose homeostasis in normal mice (16). As shown recently, an increase of dietary leucine supply had similar effects apparently via multimechanisms (35). If the decreased lean body weight of HSA-mUCP1 mice is related to changes in protein and amino acid metabolism, it would be interesting to investigate if this is causally related to the observed changes in glucose metabolism.

Taken together, gene expression analyses suggest that an increase in skeletal muscle uncoupling led to profound changes in glucose and lipid metabolism in other organs such as liver and most importantly white fat. Whether this is caused indirectly by the altered energy metabolism in muscle or directly by muscle-derived signals remains to be established. Also, the contributions of the different pathways involved in the complex regulation of energy metabolism need to be further elucidated by studies of signal transduction and enzyme activities.

**Conclusions**

It can be concluded that skeletal muscle mitochondrial uncoupling does not protect from the development of obesity in general; rather, it leads to a “healthy” obese phenotype by preserving insulin sensitivity and thus protecting from the development of obesity associated disturbances of glucose homeostasis.

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**GRANTS**

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