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

Yvonne Katterle, Susanne Keipert, Jana Hof, and Susanne Klaus

German Institute of Human Nutrition in Potsdam-Rehbruecke, Group of Energy Metabolism, Nuthetal, Germany

Submitted 10 August 2007; accepted in final form 21 November 2007

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 % 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 from 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,
Germany). 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 anesthetized 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 = \( \frac{V_{\text{CO}_2}}{V_{\text{O}_2}} \)) 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 using the 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). Gene expression is calculated as

![Fig. 1. Development of body weight (A) and % body fat (B) of HSA-mUCP1 transgenic (tg) mice (open symbols) and littermate controls (closed symbols) on high carbohydrate/low fat (HCLF, squares), high carbohydrate/high fat (HCHF, circles), and low carbohydrate/high fat (LCHF, triangles) diet. Data are means ± SE, n = 8. ANOVA for body weight and % body fat revealed significant effects of diet, genotype, and diet × genotype interaction, respectively (all P < 0.001).

Table 1. Macronutrient composition of semisynthetic diets

<table>
<thead>
<tr>
<th></th>
<th>Control Diet (HCLF)</th>
<th>HCHF Diet</th>
<th>LCHF Diet</th>
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<tbody>
<tr>
<td>Casein, g/kg</td>
<td>410</td>
<td>180</td>
<td>500</td>
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<tr>
<td>Wheat starch, g/kg</td>
<td>370</td>
<td>430</td>
<td>80</td>
</tr>
<tr>
<td>Succharose, 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>Linseed 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>15.5</td>
<td>17.7</td>
<td>17.5</td>
</tr>
<tr>
<td>Protein, energy %</td>
<td>41.7</td>
<td>16.0</td>
<td>45.0</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>43.0</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.
ΔACT using 18S RNA as housekeeping gene and relative to the wild-type HCLF group normalized to a value of 1.

**Plasma analysis.** Free fatty acids (Wako NEFA C Kit; Wako Chemicals, Neuss, Germany), total cholesterol (Wako CHOL-H L-Type R1/R2 Chemicals), triglycerides, and glycerol (triglyceride and free glycerol reagent, SIGMA) were analyzed in triplicates on 96-well plates. Insulin was analyzed by an ultrasensitive ELISA assay (Insulin mouse ultrasensitive ELISA, DRG Instruments).

**Tissue triglyceride analysis.** Liver tissue was ground in liquid nitrogen to a homogenous powder. We homogenized 100 mg of tissue in 5 ml of 10 mM sodium phosphate buffer containing 1 mM EDTA and 1% polyoxylether 10 triglyceride using an Ultra-Turrax (IKA Werke) and centrifuged it 10 min at 23,000 g. Subsequently the supernatant was incubated at 70°C for 5 min and centrifuged again. Triglycerides (triglyceride and free glycerol reagent, SIGMA) and protein (DC protein assay, Bio-Rad) were analyzed in triplicates from the supernatant.

**Statistical analysis.** Results are given as means ± SE. Statistical significance was assessed by ANOVA (factorial or repeated measurements when appropriate) followed by Bonferroni post hoc test (SPSS 11.0 for Windows; SPSS, Chicago, IL). For plasma insulin levels, Mann-Whitney U-test was used to compare wild-type and transgenic mice. Differences at P < 0.05 were considered significant.

**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 carbohydrate 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 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 intervention. 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 groups.

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

<table>
<thead>
<tr>
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<th>HCLF</th>
<th>HCHF</th>
<th>LCHF</th>
<th>ANOVA</th>
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<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</td>
<td>37.9</td>
<td>39.4</td>
<td>1.0</td>
</tr>
<tr>
<td>EE, kJ/day</td>
<td>47.9</td>
<td>39.4</td>
<td>54.7</td>
<td>1.0</td>
</tr>
<tr>
<td>EE, kJ/day*g</td>
<td></td>
<td></td>
<td>1.17</td>
<td>0.026</td>
</tr>
<tr>
<td>RQ 23 h</td>
<td>0.96</td>
<td>0.009</td>
<td>0.97</td>
<td>0.012</td>
</tr>
<tr>
<td>RQ night (12 h)</td>
<td>1.00</td>
<td>0.007</td>
<td>1.01</td>
<td>0.014</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.
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 $\beta$-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 on the HCLF and HCHF diets compared with littermate controls, indicating increased insulin sensitivity (Fig. 4B). This was corroborated by an insulin tolerance test that revealed diet-independent increased insulin sensitivity in HSA-mUCP1 transgenic mice (Fig. 4A). Whereas 0.75 U/kg insulin was not sufficient to decrease glucose levels in wild-type mice, all transgenic groups displayed a highly significant decrease in blood glucose. The temporary increase in blood glucose levels in wild-type mice is probably due to a stress-induced glucose release from liver, since animals were not fasted before insulin injection.

**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 (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 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.

![Graph of Respiratory Quotient (RQ) over a 23 h period in HSA-mUCP1 tg mice (open symbols) and littermate controls (closed symbols) after 8 or 9 wk feeding different macronutrient diets. Black bar corresponds to night (lights off) period. Data are means ± SE, n = 8. For statistical analysis see Table 2.](image)

**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>
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<th>HCLF</th>
<th>HCHF</th>
<th>LCHF</th>
<th>ANOVA</th>
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<tr>
<td></td>
<td>wt</td>
<td>tg</td>
<td>wt</td>
<td>tg</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>1.923±0.088</td>
<td>1.248±0.029</td>
<td>2.23±0.267</td>
<td>1.303±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.
A linear increase in body fat accumulation. Interestingly, when diet high in fat and carbohydrates, HSA-mUCP1 mice showed effects on body fat accumulation because when exposed to a diet as evident from the insulin tolerance test and plasma insulin levels. Surprisingly, this was not linked to 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 good 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 skeletal muscle of HSA-mUCP1 mice than in wild type, especially in the LCHF group. Conversely, FAT/CD36 gene expression of insulin-sensitive glucose transporter Glut4 and retinol-binding protein 4 (RBP4) was significantly increased in WAT of transgenic animals under high protein conditions (HCLF, LCHF) compared with wild type.

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 insulin resistance independently of the macronutrient composition of the diet. 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. In transgenic animals, increased EI of HSA-mUCP1 mice on the HCHF diet is not 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 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.

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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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<tr>
<td></td>
<td>wt</td>
<td>tg</td>
<td>wt</td>
<td>tg</td>
</tr>
<tr>
<td>TG, μg/ml</td>
<td>477±77</td>
<td>391±49</td>
<td>937±124</td>
<td>845±209</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>
</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>
</tr>
<tr>
<td>Hydroxybutyrate, 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>
</tr>
<tr>
<td>Cholesterol, mg/dl</td>
<td>129±10</td>
<td>114±7</td>
<td>202±24</td>
<td>162±14</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>
</tr>
</tbody>
</table>

Data are means ± SE; n = 5 except blood glucose day 65 n = 8. TG, triglycerides; FFA, free fatty acids.
expression in liver was higher in wild type, again most pronounced in LCHF group. This points to a preferential channeling of fatty acids into skeletal muscle rather than liver in HSA-mUCP1 mice, explaining their lower liver triglyceride content and contributing to their resistance to increase body fat on the LCHF diet.

Using euclycemic-hyperinsulinemic clamps we found an increased insulin-stimulated glucose uptake in skeletal muscle and white fat of HSA-mUCP1 mice compared with wild type (S. Neschen, Y. Katterle, unpublished observations). This is reflected by an increased expression of GLUT4 in WAT but not in muscle in HSA-mUCP1 mice on the HCLF and LCHF diets. However, we cannot exclude the possibility that insulin-induced GLUT4 translation and/or translocation to the plasma membrane is increased in skeletal muscle of HSA-mUCP1 mice rather than GLUT4 gene transcription. Alternatively, there could be an insulin-independent glucose uptake caused by the skeletal muscle uncoupling (2). This needs to be evaluated in more detail.

Surprisingly, gene expression analysis points to an increased lipogenesis in white fat of HSA-mUCP1 mice compared with wild type when fed high-carbohydrate diets. This was confirmed in 24-wk-old male HSA-mUCP1 mice and their wild-type control in a higher number of individuals (n = 14–16 per group) fed a commercial high-fat/high-carbohydrate diet. Gene expression of FAS and SCD1 in white fat was almost double, and ACC approximately threefold increased in transgenic mice compared with wild type (Y. Katterle, unpublished results). 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.
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 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.

ACKNOWLEDGMENTS

We are grateful to Carola Plaue and Antje Sylvester for excellent technical assistance and thank Andrea Teichmann for plasma insulin determination.

GRANTS

This work was supported by Deutsche Forschungsgemeinschaft Grant KL613/14-1.
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