Uncoupling protein 1 expression in murine skeletal muscle increases AMPK activation, glucose turnover, and insulin sensitivity in vivo


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Neschen S, Katterle Y, Richter J, Augustin R, Scherneck S, Mirhashemi F, Schümümmann A, Joost H-G, Klaus S. Uncoupling protein 1 expression in murine skeletal muscle increases AMPK activation, glucose turnover, and insulin sensitivity in vivo. Physiol Genomics 33: 333–340, 2008. First published March 18, 2008; doi:10.1152/physiolgenomics.00226.2007.—Uncoupling of oxidative phosphorylation represents a potential target for the treatment of hyperglycemia and insulin resistance in obesity and type 2 diabetes. The present study investigated whether the expression of uncoupling protein 1 in skeletal muscles of transgenic (mUCP1 TG) mice modulates insulin action in major insulin target tissues in vivo. Euglycemic-hyperinsulinemic clamps (17 pM/kg lean body mass min⁻¹·min⁻¹) were performed in 9-mo-old hemizygous male mUCP1 TG mice and wild-type (WT) littermates matched for body composition. mUCP1 TG mice exhibited fasting hypoglycemia and hypoinsulinaemia compared with WT mice, whereas fasting hepatic glucose production rates were comparable in both genotypes. mUCP1 TG mice were markedly more sensitive to insulin action compared with WT mice and displayed threefold higher glucose infusion rates, enhanced skeletal muscle and white adipose tissue glucose uptake, and whole body glycolysis rates. In the absence of alterations in plasma adiponectin concentrations, acceleration of insulin-stimulated glucose turnover in skeletal muscle of mUCP1 TG mice was accompanied by increased phosphorylated Akt-to-Akt and phosphorylated AMP-activated protein kinase (AMPK)-to-AMPK ratios compared with WT mice. UCP1-mediated uncoupling of oxidative phosphorylation in skeletal muscle was paralleled by AMPK activation and thereby stimulated insulin-mediated glucose uptake in skeletal muscle.

euglycemic-hyperinsulinemic clamp; glycolysis; lactate; white adipose tissue; triacylglycerol; adiponectin

THE WORLDWIDE RISING OBESITY PREVALENCE REPRESENTS A SERIOUS HEALTH PROBLEM AND IS ASSOCIATED WITH AN INCREASED RISK FOR TYPE 2 DIABETES. AN EFFECTIVE APPROACH TO COMBAT OBESITY MIGHT BE TO ALTER THE METABOLIC RATE BY INCREASING MITOCHONDRIAL PROTON CONDUCTANCE, LEADING TO A PARTIAL UNCOUPLING OF THE RESPIRATORY CHAIN (19). IN THE 1930S, THE CHEMICAL UNCOUPLER 2,4-DINITROPHENOL (DNP) WAS USED AS AN ANTI-OBESITY TREATMENT, CAUSING WEIGHT LOSS WITHOUT THE NEED TO REDUCE FOOD INTAKE. IN LOW DOSES NO ADVERSE EFFECTS SUCH AS RISE IN BLOOD PRESSURE OR HEART RATE WERE REPORTED, BUT SEVERAL DEATHS DUE TO OVERDOZING OCCURRED. THEREFORE, DNP HAS NOT BEEN USED IN THE THERAPY OF OBESITY SINCE 1938 (17).

An alternative approach to enhance mitochondrial uncoupling is to increase the expression of uncoupling proteins (UCP). UCP1 was suggested to function as the only “true” uncoupling protein in vivo (5). UCP1 is exclusively expressed in mitochondria of brown adipose tissue (BAT), the major thermogenic organ in small mammals, and plays a pivotal role in basal and regulatory thermogenesis, overall energy balance, and body weight regulation (22, 23). UCP1 dissipates the proton gradient across the inner mitochondrial membrane, thus allowing maximum activity of the respiratory chain uncoupled from ATP synthesis (24, 34). Although UCP1 ablation does not lead to the development of obesity (13), overexpression of UCP1 or hyperactivated BAT thermogenesis prevents obesity (23).

Enhancing uncoupling in tissues other than BAT has been suggested to exert long-term effects on body weight regulation and energy homeostasis. Several independent groups generated transgenic mouse models expressing ectopic UCP1 in skeletal muscles (9, 25, 28). In mice, expression of UCP1, or alternatively UCP3, in skeletal muscles and/or heart resulted in lower body weights, increased activity-related energy expenditure, compromised thermoregulation, fasting hypoglycaemia, resistance to high-fat diet-induced obesity, and increased nighttime respiratory quotients, the latter suggesting enhanced overall glucose utilization (8, 9, 16, 25, 28).

In humans, a decreased ability of skeletal muscle to respond adequately to normal circulating insulin concentrations appears to play an important role in the development of peripheral insulin resistance in type 2 diabetes (2, 12, 37). Thus skeletal muscle serves as an attractive site for targeting insulin resistance by altering its glucose turnover. This is in line with previous in vivo studies demonstrating increased glucose tolerance in transgenic mice with muscle-directed UCP1 expression (28).

However, it is not clear whether this resulted from enhanced muscle uncoupling per se or was rather a consequence of a relative increase in muscle mass or reduced body fat stores observed in these mice. Thus in the present study we assessed the consequences of ectopic UCP1 expression in skeletal muscle on both systemic and tissue-specific insulin action in vivo independent from differences in lean body mass.

MATERIALS AND METHODS

Animals and research design. Transgenic mice expressing UCP1 in skeletal muscles (mUCP1 TG) were generated by DeveloGen as previously described (Goettingen, Germany; Ref. 25). Hemizygous mUCP1 TG mice were obtained by mating wild-type (WT) mice (C57BL/6, Charles River WIGA, Sulzfeld, Germany) with hemizygous mUCP1 TG animals. At an age of 9 mo, male hemizygous mUCP1 TG mice and WT littermates were singly housed with unlimited access to a standard laboratory chow (V1534, Sniff, Soest, Germany). Resting rectal body temperature was measured with a thermocoupler (BAT-12R, Physiotemp Instruments, Clifton, NJ).
Body composition was measured as the distance between nose and anus. Body composition was determined by in vivo NMR-spectroscopy (Bruker-Mini-Spec-NMR-Analyzer mq10; Bruker Optics, Houston, TX). The term “lean body mass” refers to the muscle mass value given by the Bruker-NMR software. Individual resorption efficiencies were determined over a 70-h period \((n = 5\) genotype) and expressed as a relative fat and lean body mass as closely as possible and fasted for 16 ± 1 h. Mice were kept in accordance with National Institutes of Health guidelines for the care and use of laboratory animals, and all experiments were approved by the ethics committee of the Ministry of Agriculture, Nutrition, and Forestry (State of Brandenburg, Germany).

**Euglycemic-hypersulinemic clamps.** Permanent catheters were inserted in the left jugular vein under surgical anesthesia (ketamine-xylazine, 80 and 20 mg/kg body wt) as previously described (32, 33). On the sixth or seventh postoperative day relative fat and lean body mass-matched mUCP1 TG and WT littermates were fasted for 16 ± 1 h and placed in rodent restrainers set atop a heating blanket to prevent hypothermia. Euglycemic-hypersulinemic clamp experiments were carried out as previously described (32, 33), except for clamp calculations and insulin doses that were based on the animal’s own individual lean body mass. After a 120-min basal period, a primed-continuous insulin infusion \((17.4 \text{ pM}\cdot\text{kg lean body mass}^{-1}\cdot\text{min}^{-1}; \text{Humulin, Eli Lilly, Indianapolis, IN})\) increased plasma insulin concentrations within a physiological range. Ten-microliter blood samples were withdrawn from tail tips approximately every 10 min for measurement of plasma glucose concentrations. “Steady-state” \((\text{minutes 90–120})\) target glycemia in (mmol/L) 7.9 ± 0.4 in mUCP1 TG vs. 7.9 ± 0.2 in WT; \(P = 1.0\) was reached and maintained within 60–70 min by means of a variable 20% glucose infusion. For determination of basal and insulin-mediated whole body glucose fluxes a primed-continuous \([3-^3\text{H}]\)glucose infusion \((370-\text{KBo}u; 3.7 \text{KBo}u/\text{min})\) was started at the beginning of the basal period and maintained until the end of the clamp. For tissue-specific insulin-stimulated glucose uptake a single 2-deoxy-[\[^1-^{14}\text{C}\]]\)glucose \((2-^{14}\text{C}DG; 370 \text{KBo}u)\) was injected into the jugular vein catheter at minute 75 of the clamp. All radioisotopes were purchased from American Radiolabeled Chemicals (St. Louis, MO), and all infusions were performed with microdialysis pumps (CMA/Microdialysis, North Chelmsford, MA). \([3-^3\text{H}]\)Glucose and 2-[\[^1-^{14}\text{C}\]]DG specific activities, as well as plasma glucose, insulin, \(\beta\)-hydroxybutyrate, and lactate concentrations were measured at minute 110 of the basal period and/or at clamp minutes 80, 85, 90, 100, 110, and 120.

**Calculation of glucose fluxes.** Plasma \([3-^3\text{H}]\)glucose and 2-[\[^1-^{14}\text{C}\]]DG in deproteinized plasma samples (Somogyi filtrates) after \(\text{H}_2\text{O}\) was completely dried were determined in a LS 6500 Counter (Beckman Instruments, Fullerton, CA). Whole body glycosylation rates were calculated from the increase in plasma \([\text{H}_2\text{O}]\) concentration, the latter referring to the difference between \(\text{H}^1\) counts before and after drying, divided by the specific activity of plasma \([\text{H}]\)glucose and the plasma \([\text{H}_2\text{O}]\) concentration as previously described (32, 33). The rates of basal and insulin-stimulated whole body glucose turnover are given as the ratio of \([3-^3\text{H}]\)glucose infusion rate to plasma \([3-^3\text{H}]\)glucose specific activity at the end of the basal period or under steady-state conditions (defined as the last 30 min of the clamp). Hepatic \([3-^3\text{H}]\)glucose production (HGP) was determined by subtracting the steady-state glucose infusion rate (GIF) from the rate of whole body glucose turnover. 2-[\[^1-^{14}\text{C}\]]DG injected during steady state conditions resulted in the intracellular accumulation of 2-[\[^1-^{14}\text{C}\]]DG-6-phosphate (DG-6P) in tissue. 2-[\[^1-^{14}\text{C}\]]DG-6P in quadriceps muscle and epididymal white adipose tissue (epiWAT) homogenates was separated from 2-[\[^1-^{14}\text{C}\]]DG with ion-exchange columns (Poly-Prep no. 731-6211; Bio-Rad, Hercules, CA) according to a technique previously described (32, 33). 2-[\[^1-^{14}\text{C}\]]DG uptake was calculated from the plasma 2-[\[^1-^{14}\text{C}\]]DG area under the curve at minutes 80, 85, 90, 100, 110, to 120 and tissue 2-[\[^1-^{14}\text{C}\]]DG-6P content as described previously (32, 33).

**Tissue collection.** For tissue collection, mice were anesthetized at the end of clamp experiments (ketamine-xylazine iv) or after 16 ± 1 h of fasting (isoflurane). Musculus gastrocnemius including the adherent M. soleus, M. quadriceps, liver, epWAT, and M. tibialis anterior were immediately dissected in the described order, freeze-clamped, and stored at −80°C. To account for local differences in fiber type composition and/or metabolic properties all tissues were ground to fine powder in liquid nitrogen. Parameters most rapidly altered by metabolic changes due to prolonged anesthesia [e.g., 2-deoxyglucose (2-DOG) uptake, phosphorylation of AMP-activated protein kinase (AMPK) and Akt protein] were measured in gastrocnemius, a muscle with a mixed fiber type composition dissected first and immediately after the onset of surgical anesthesia. To avoid delays in freeze-clamping, the adherent soleus muscle was not removed. UCP1 protein and UCP2 and UCP3 gene expression were measured in quadriceps and triacylglycerol concentrations in tibialis anterior homogenates.

**Plasma analysis.** Glucose (Glucometer 3000, ABT, Radberg, Germany), lactate (Randox, Ardmore, UK), \(\beta\)-hydroxybutyrate, and immunoreactive insulin concentrations (ELISA, DRG Diagnostics, Marburg, Germany) were determined according to the instructions of the manufacturers.

**Tissue triacylglycerol content.** The tissue extraction procedure was adapted from methods described previously (4, 15, 31). Triacylglycerol concentrations were measured in duplicate after evaporation of the organic solvent by an enzymatic method (Randox).

**Subcellular fractionation and immunoblot analysis.** For UCP1 determination in mitochondria-enriched fractions, BAT and quadriceps samples were immersed in TES buffer (10 mM Tris·HCl pH 8.0, 10 mM EDTA, 1% SDS) containing protease inhibitors (Roche’s Complete Protease Inhibitor Cocktail Tablets; Roche Diagnostics, Mannheim, Germany) at 4°C, homogenized with a Potter-Elvehjem glass homogenizer polytetrafluoroethylene pestle, and centrifuged at 1,000 \(\text{g}\) for 10 min to remove cell debris and the nuclear fraction. The supernatant was centrifuged at 18,000 \(\text{g}\) for 15 min, and the mitochondria-containing pellet was resuspended in the same buffer with a syringe (BD Ultra-Fine Insulin Syringe, Becton Dickinson, Franklin Lakes, NJ). BAT (1.5 \(\mu\)g) or quadriceps (15 \(\mu\)g) protein fractions were separated by 10% SDS-PAGE. For all further immunohistochemical analyses whole tissue protein fractions (basal and postclamp quadriceps or gastrocnemius) were prepared and 15 \(\mu\)g of proteins were separated by 10% SDS-PAGE. After transfer onto polyvinylidene difluoride (PVDF) membranes (Amersham, Pittsburgh, PA), these were immunoblotted with primary antibodies (1:1,000 dilution) recognizing UCP1 (Abcam, Cambridge, UK), Akt1/2/3(Ser473), phosphorylated (p)Akt, AMPK, AMPKα1/2 Thr172, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (all Cell Signaling Technology, Beverly, MA).

For quantification of the denatured monomeric plasma adiponectin complex, 5-\(\mu\)l plasma samples were diluted with phosphate-buffered saline (PBS) and Laemmlı buffer containing \(\beta\)-mercaptoethanol, separated by 12% SDS-PAGE, and transferred onto PVDF membranes. For quantification of the high-molecular-weight (HMW) and medium-molecular-weight (MMW) adiponectin complex, 5-\(\mu\)l native plasma samples were diluted with PBS and Laemmlı buffer, separated by 8% SDS-PAGE, transferred onto nitrocellulose membranes, and immunoblotted with primary antibody recognizing adiponectin (Abcam).

To visualize proteins, immunoblots were incubated with secondary antibodies and protein bands were quantified with the ECL Western Blotting Detection System (Amersham Life Sciences, Little Chalfont, UK) and, except for adiponectin normalized for GAPDH, expressed as pAkt-to-Akt and pAMPK-to-AMPK ratios (UViProchmi, Biomebra; BioDocAnalyze 2.49.8.1).
Quantitative real-time-PCR gene expression analysis. RNA was isolated with a commercially available kit (Qiagen RNase-Free Kit, Qiagen, Valencia, CA) in combination with DNase digest treatment (Qiagen). Residual genomic DNA was removed with Turbo-DNA-free Kit (Ambion, Austin, TX). Synthesis of cDNA was performed from 2 μg of total RNA with the RevertAid H Minus First Strand cDNA Synthesis Kit (Fermentas, St. Leon-Rot, Germany). Quantitative real-time PCR was performed on the Applied Biosystems 7900 HT Sequence Detection System (Applied Biosystems, Foster City, CA). The PCR mix (5 μl) contained TaqMan Universal PCR Master Mix, No AmpErase UNG (Applied Biosystems), a cDNA amount corresponding to 5 ng of RNA used for cDNA synthesis, and gene-specific primer probe pairs for CD36: 5′-CCA AGC TAT TGC GAC ATG AT-3′ (F), 5′-ACA GCG TAG ATA GAC CTG CAA A-3′ (R), 5′-FAM-TGG CAC AGA CCG CTG CCT CT-3′; UCP1: 5′-AGT ACC CAA GCC TAC CAA GC-3′ (F), 5′-AGA AGC CAC AAA CCC TTT GA-3′ (R), 5′-FAM-AAG GGC GTC GGT CCT TCC TG-3′; UCP2: 5′-CAC TTT CCC CTC GGA TAC CG-3′ (F), 5′-GCC CTA GCC CTT GAC TCT CC-3′ (R), 5′-6-FAM-AGT GCC CGG CTG CAG ATC CAA-TAMRA-3′; UCP3 (AU: 1.88 vs. WT); and undetectable expression levels in WAT and liver in both genotypes (Fig. 1B). Ectopic expression of UCP1 in skeletal muscle was paralleled by increased expression of the UCP homologs UCP2 [arbitrary units (AU): 1.88 ± 0.30 in mUCP1 TG vs. 1.0 ± 0.26 in WT; P < 0.04, n = 5 per group] and UCP3 (AU: 1.60 ± 0.24 in mUCP1 TG vs. 1.0 ± 0.16 in WT; P < 0.06, n = 5 per group) compared with WT mice.

Reduced muscle mass and body weight in mUCP1 TG mice. Mice expressing the UCP1 transgene in skeletal muscle displayed increased weight-specific energy resorption paralleled by markedly decreased body length, body weight, and absolute fat and lean body mass compared with WT littermates (Table 1). When expressed relative to body weight, mUCP1 TG and WT mice subjected to euglycemic-hyperinsulinemic clamp experiments showed a comparable relative body composition (relative fat and lean body mass; Table 1). Expression of UCP1 in skeletal muscles markedly decreased relative weights of gastrocnemius and quadriceps muscles but did not affect relative liver weight (Table 1). Because of growth retardation all measured absolute organ weights were decreased in mUCP1 TG compared with WT mice (Table 1).

Decreased fasting plasma glucose and insulin concentrations in mUCP1 TG mice. Compared with WT littermates, mUCP1 TG mice exhibited significantly lower fasting plasma glucose and insulin concentrations paralleled by comparable HPGs, suggesting enhanced peripheral basal glucose turnover (Table 1). To directly assess whether expression of UCP1 in skeletal muscles enhanced insulin sensitivity by increasing insulin-stimulated glucose turnover, we next carried out euglycemic-hyperinsulinemic clamp analysis. As previously determined via Northern blot analysis, in this mutant mouse model UCP1 mRNA was solely expressed in skeletal muscle and not in WAT, heart, lung, kidney, or gut (25). Quantitative real-time PCR analysis confirmed skeletal muscle-specific UCP1 gene expression in mUCP1 TG mice and undetectable expression levels in WAT and liver in both genotypes (Fig. 1B).
intracellular triacylglycerol formation is discussed to potentially modulate insulin action, hepatic triacylglycerol concentrations were measured and found markedly reduced in mUCP1 TG mice compared with WT littermates (mmol/l: 4.5 ± 0.9 in mUCP1 TG vs. 8.5 ± 1.2 in WT; n = 8 per group, P < 0.02).

Increased glucose uptake and insulin sensitivity in skeletal muscle of mUCP1 TG mice. Improved whole body insulin sensitivity in mUCP1 TG mice could be attributed to approximately twofold higher skeletal muscle glucose uptake rates (Fig. 2D). In a next step, we investigated whether enhanced insulin-stimulated glucose uptake was paralleled by alterations in intracellular insulin signaling. Insulin receptor activation upon insulin binding initiates a complex cascade of events leading to phosphoinositide 3-kinase activation, formation of phosphatidylinositol 3,4,5-trisphosphate, and activation of Akt/protein kinase B. Immunoblot analysis revealed markedly increased ratios of activated and phosphorylated Akt-to-Akt protein ratios (Fig. 3) in mUCP1 TG mice compared with WT littermates.

In humans intramyocellular lipid concentrations have been correlated with insulin sensitivity (26). Therefore we determined skeletal muscle triacylglycerol concentrations (mmol/g tibialis anterior; 4.1 ± 1.2 in mUCP1 TG vs. 2.8 ± 0.7 in WT; n = 8 per group, P < 0.4), which did not differ between mUCP1 TG and WT mice.

Increased AMPK activation in skeletal muscle from mUCP1 TG mice. Next, we tested whether the increase in basal and insulin-stimulated glucose utilization in skeletal muscle was a consequence of UCP1-mediated AMPK activation. Activation of AMPK requires phosphorylation of threonine 172 within the catalytic subunit of AMPK by an upstream kinase (6). Under both basal and insulin-stimulated conditions protein levels of phosphorylated and activated AMPKα1/α2(Thr172) and pAMPK-to-AMPK ratios in skeletal muscle of mUCP1 TG mice were markedly higher than in WT littermates (Fig. 4).

Increased glucose uptake in white adipose tissue of mUCP1 TG mice. Under insulin insulin-stimulated conditions glucose uptake was markedly increased in epWAT in mUCP1 TG mice compared with WT littermates (Fig. 2E). In contrast to skeletal muscle, increased WAT glucose transport in mUCP1 TG mice was paralleled by neither Akt activation (pAkt-to-Akt protein ratios, AU: 53 ± 27 in mUCP1 TG vs. 64 ± 37 in WT; n = 4 per group, P < 0.04) nor AMPK activation (pAMPK-to-AMPK protein ratios, AU: 116 ± 58 in mUCP1 TG vs. 106 ± 53 in WT; n = 4 per group, P < 0.6) compared with WT mice.

No alterations in plasma adiponectin in mUCP1 TG mice. The adipokine adiponectin modulates whole body insulin sensitivity and is capable of activating AMPK. Therefore, we determined whether increased AMPK activation in skeletal muscle was caused by alterations in adiponectin metabolism. Adiponectin gene expression in adipocytes is induced by peroxisome proliferator-activated receptor-γ, a ligand-regulated transcription factor that also controls CD36 gene expression by binding to specific response elements within promoters (27). In epWAT, expression of both the adiponectin (AU: 0.60 ± 0.09 in mUCP1 TG vs. 1.0 ± 0.04 in WT; P < 0.004, n = 4 or 5 per group) and the CD36 (AU: 0.70 ± 0.10 in mUCP1 TG vs. 1.0 ± 0.08 in WT; P < 0.05, n = 4 or 5 per group) genes were decreased in mUCP1 TG mice compared with WT littermates. In contrast to adiponectin gene expression, in plasma total

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**Table 1. Physiological parameters from 9-month-old male mUCP1 TG and WT mice**

<table>
<thead>
<tr>
<th>Physiological and Plasma Parameters (basal)</th>
<th>WT</th>
<th>n</th>
<th>mUCP1 TG</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food intake, g·day⁻¹·g BW⁻¹</td>
<td>0.09±0.01</td>
<td>5</td>
<td>0.15±0.01</td>
<td>5</td>
</tr>
<tr>
<td>Energy resorption, J·g BW⁻¹·day⁻¹</td>
<td>1.109±0.02</td>
<td>5</td>
<td>1.921±0.2</td>
<td>5</td>
</tr>
<tr>
<td>Rectal body temperature (fasting), °C</td>
<td>36.0±0.2</td>
<td>5</td>
<td>35.9±0.2</td>
<td>5</td>
</tr>
<tr>
<td>Nose-to-anus body length, cm</td>
<td>10.0±0.1</td>
<td>22</td>
<td>10.5±0.1</td>
<td>36</td>
</tr>
<tr>
<td>BW, g</td>
<td>37±2.2</td>
<td>5</td>
<td>28.5±1.2</td>
<td>8</td>
</tr>
<tr>
<td>Lean body mass, g</td>
<td>25.3±0.7</td>
<td>5</td>
<td>18.0±0.6</td>
<td>8</td>
</tr>
<tr>
<td>Fat mass, g</td>
<td>10.0±1.0</td>
<td>5</td>
<td>5.7±0.8</td>
<td>5</td>
</tr>
<tr>
<td>Lean body mass, % BW</td>
<td>68.0±1.2</td>
<td>5</td>
<td>70.2±1.2</td>
<td>8</td>
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<tr>
<td>Fat mass, % BW</td>
<td>26.5±1.9</td>
<td>8</td>
<td>21.5±2.3</td>
<td>8</td>
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<tr>
<td>Epidymidal fat pad mass, g</td>
<td>1.11±0.14</td>
<td>8</td>
<td>0.54±0.13</td>
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<tr>
<td>M. gastrocnemius mass, g</td>
<td>0.16±0.01</td>
<td>8</td>
<td>0.09±0.01</td>
<td>8</td>
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<tr>
<td>M. quadriceps mass, g</td>
<td>0.17±0.01</td>
<td>8</td>
<td>0.08±0.01</td>
<td>8</td>
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<tr>
<td>Liver mass, g</td>
<td>1.40±0.09</td>
<td>8</td>
<td>0.99±0.08</td>
<td>8</td>
</tr>
<tr>
<td>Epidymidal fat pad mass, % BW</td>
<td>3.2±0.3</td>
<td>8</td>
<td>2.3±0.4</td>
<td>8</td>
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<tr>
<td>M. gastrocnemius mass, % BW</td>
<td>0.94±0.01</td>
<td>8</td>
<td>0.40±0.02</td>
<td>8</td>
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<tr>
<td>M. quadriceps mass, % BW</td>
<td>0.50±0.02</td>
<td>8</td>
<td>0.36±0.02</td>
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</tr>
<tr>
<td>Liver mass, % BW</td>
<td>4.1±0.2</td>
<td>8</td>
<td>4.3±0.2</td>
<td>8</td>
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<tr>
<td>Basal HGP, mmol·kg lean body mass⁻¹·min⁻¹</td>
<td>0.98±0.11</td>
<td>8</td>
<td>0.82±0.08</td>
<td>8</td>
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<tr>
<td>Glucose, mmol/l</td>
<td>7.80±0.48</td>
<td>8</td>
<td>6.34±0.35</td>
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<tr>
<td>Lactate, mmol/l</td>
<td>6.7±0.6</td>
<td>11</td>
<td>5.5±1.6</td>
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<tr>
<td>β-Hydroxybutyrate, mmol/l</td>
<td>1.26±0.21</td>
<td>13</td>
<td>1.62±0.22</td>
<td>10</td>
</tr>
<tr>
<td>Insulin, pm/ml</td>
<td>17.2±2.1</td>
<td>8</td>
<td>7.3±2.4</td>
<td>8</td>
</tr>
</tbody>
</table>

Results are means ± SE for n mice. Food intake, body length (nose to anus), body weight (BW), and body composition were measured in fed mice and all other parameters in 16-h fasting individuals. Mice expressing the uncoupling protein 1 (UCP1) transgene in skeletal muscle (mUCP1 TG) exhibited a 33% lower BW compared with wild-type (WT) littermates; and therefore body composition data and tissue weights are expressed as % BW. HGP, hepatic glucose production rate. *P < 0.05, †P < 0.005, ‡P < 0.0005 vs. WT.
(monomeric) adiponectin concentrations measured via immunoblot analysis did not differ between mUCP1 TG and WT mice (AU: 1,126 ± 1100 vs. 1,010 ± 210 in WT; P = 0.6, n = 4 or 5 per group; Fig. 5). Because circulating adiponectin forms trimeric, hexameric (MMW), and HMW oligomers that mediate different biological actions we next measured different adiponectin complexes. Immunoblot analysis did not reveal differences in the distribution of the HMW (AU: 13,479 ± 1,093 in mUCP1 TG vs. 11,894 ± 1,350 in WT; P = 0.4; n = 4 or 5 per group) and MMW (AU: 3,644 ± 301 in mUCP1 TG vs. 3,345 ± 257 in WT; P = 0.5, n = 4 or 5 per group) adiponectin oligomers in mUCP1 TG and WT mice (Fig. 5).

**DISCUSSION**

Insulin sensitivity and whole body substrate utilization in mice overexpressing UCP3 in glycolytic skeletal muscle (39) and muscle UCP3 gene expression in response to weight gain and fasting in rats are differently regulated in male and female animals (38). In hemizygous mUCP1 TG mice and their WT littermates no sexual dimorphisms regarding body length, plasma glucose, insulin, free fatty acids, and triacylglycerol concentrations were evident (data not shown). Therefore, and to rule out potential alterations in insulin sensitivity and glucose metabolism caused by estrous cycle-dependent variations in sex steroid hormones (30), we determined in vivo insulin action exclusively in male mice.

Skeletal muscle is the major site of insulin-stimulated glucose utilization. An effective strategy to augment whole body glucose turnover could be to increase skeletal muscle glucose oxidation by partial uncoupling of the respiratory chain via introduction of UCP1. UCP1 dimers serve as proton channels dissipating the electrochemical potential across the inner mitochondrial membrane. Results from the present study clearly demonstrate that UCP1 expression in skeletal muscles increased whole body insulin-stimulated glucose disposal due to enhanced glucose entry into skeletal muscle.

In mUCP1 TG mice increased insulin-stimulated glucose transport into skeletal muscle was paralleled by Akt activation, the latter a crucial component of the intracellular insulin signaling cascade. Akt is required for insulin-induced translo-
The expression of a UCP1 transgene in a similar mouse model predominantly affects muscles composed of fast fiber types (e.g., M. gastrocnemius), causing a transition from fast glycolytic type IIb to slow oxidative type IIa and IIx fibers. Type IIb fibers are characterized by low GLUT4 expression, glucose uptake, myoglobin concentrations, and, compared with other fiber types, low amounts of mitochondria. Thus in mUCP1 TG mice a potential upregulation of type IIa and IIx fibers, both rich in mitochondria and characterized by a high oxidative capacity, might in part account for the observed increase in insulin-stimulated whole body and skeletal muscle glucose turnover in mUCP1 TG mice.

The serine/threonine protein kinase AMPK acts as an intra-cellular energy sensor adjusting the rates of metabolic pathways that either consume or provide ATP (6). Regulation of AMPK activity involves its allosteric activation by AMP in response to cellular ATP depletion causing a rise in the intracellular [AMP]-to-[ATP] ratio (6). Others previously reported that overexpression of the UCP1 gene in skeletal muscles, WAT, and adipocytes markedly increased the intracellular [AMP]-to-[ATP] ratio, enhancing glucose uptake via AMPK activation under in vitro conditions (14, 16, 28). This is in line with results from the present in vivo study that suggest AMPK activation might have further amplified insulin-stimulated glucose uptake in skeletal muscle in the presence of UCP1, the latter increasing the cellular [AMP]-to-[ATP] ratio by enhancing uncoupling of the respiratory chain.

The adipocyte-derived protein adiponectin mediates insulin-sensitizing properties and is capable of activating AMPK (41). In the present study total plasma adiponectin concentrations were comparable in mUCP1 TG and WT mice, suggesting that AMPK activation was a consequence of alterations in the intracellular [AMP]-to-[ATP] ratio rather than modulations in circulating adiponectin. Circulating adiponectin exists in different oligomerization states, as a trimer, a hexamer, and a HMW oligomer, that activate different signal transduction pathways. In isolated rat skeletal muscle naturally formed trimeric adiponectin has been demonstrated to activate AMPK, whereas in contrast the hexameric and HMW oligomers failed to exert this effect (40). Therefore we determined MMW and HMW plasma adiponectin oligomers but found no differences in their plasma concentrations between mUCP1 TG and WT mice. Adiponectin gene expression in WAT was decreased in mUCP1 TG mice. Thus comparable plasma adiponectin concentrations in both genotypes might reflect either a lower adipocyte cell number or variations in posttranscriptional processing, secretion, or half-life of adiponectin in mUCP1 TG mice.
In addition to ATP synthesis by oxidative phosphorylation or cellular energy metabolism ([ADP]-to-[ATP] ratio) the generation of reactive oxygen species (ROS) has been linked to alterations in glucose homeostasis and the pathogenesis of insulin resistance. Insulin sensitivity increases when oxidative stress is reduced in cell systems and animal models (18). Because moderate uncoupling appears to counteract ROS production (19), it is possible that limitations in ROS formation due to the ectopic expression of UCP1 further improved skeletal muscle insulin action.

Surprisingly, expression of the UCP1 transgene in skeletal muscle was accompanied by an increase in insulin-stimulated glucose uptake in WAT but, in contrast to skeletal muscle, this was not paralleled by Akt activation. Recently published data by Katterle et al. (21) show an induction of GLUT4 gene expression in WAT of mUCP1 TG mice compared with WT animals under various dietary conditions. Thus alterations in GLUT4 expression or mechanisms located downstream of Akt could potentially explain the observed increase in WAT glucose uptake. Alternatively, these data might indicate cross talk between skeletal muscle and adipose tissue. The expression of UCP1 might affect the release of “myokines” such as interleukin-6 from skeletal muscle, the latter increasing lipid turnover or inhibiting the production of proinflammatory cytokines that have been implicated in the pathogenesis of muscle insulin resistance (35). Finally, a higher energy demand due to increases in uncoupling activity in skeletal muscle might potentially result in a systemic depletion of energy stores and thus indirectly affect adipocyte glucose utilization.

The observed increase in insulin-mediated glycolytic flux was paralleled by higher plasma lactate concentrations, suggesting a shift in the mode of intracellular ATP generation in mUCP1 TG mice. Similar adaptive changes have been reported in neuronal cells exhibiting increased UCP4 expression (29). Thus the increase in lactate production might represent a metabolic adaptation compensating for a lack in mitochondrial integrity and a decreased efficiency of mitochondrial ATP synthesis. Expression of UCP1 could also modulate mitochondrial substrate preference, and it has been suggested that UCPs facilitate a passive proton transport across the inner mitochondrial membrane. A role of UCP3 in a passive pyruvate shuttle ensuring a balance between oxidative phosphorylation and glycolysis has been proposed previously (10).

Human and animal studies found a strong relationship between the hepatic or intramyocellular triacylglycerol content and insulin sensitivity, and it is debated whether excess intracellular lipid deposition is a causal factor in the development of insulin resistance (7, 20, 26, 36). In UCP1 TG mice the degree of hepatic and skeletal muscle insulin sensitivity was uncoupled from the tissue triacylglycerol concentrations. Thus the present data are in line with a previous report showing that limiting hepatic triacylglycerol content failed to improve hepatic insulin sensitivity in mice (32).

Finally, expression of the UCP1 transgene in skeletal muscle was associated with an upregulation of UCP2 and UCP3 gene expression. This might potentially result from an increased intracellular energy demand due to uncoupling and mimic the intracellular metabolic states of food restriction and/or fasting, which have been shown to induce UCP2 and UCP3 in muscle (3).

In summary, the present study demonstrates that expression of a UCP1 transgene in skeletal muscles of mice substantially improved in vivo insulin action. Ectopic expression of UCP1 in skeletal muscle increased substrate flux through the glycolytic pathway and was paralleled by increased insulin-stimulated glucose uptake in skeletal muscle, presumably a result of AMPK activation. Modulation of respiratory uncoupling in skeletal muscle appears an efficient strategy to accelerate glucose utilization in both skeletal muscle and WAT, thereby protecting against the development of insulin resistance.

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