Effects of the presence, absence, and overexpression of uncoupling protein-3 on adiposity and fuel metabolism in congenic mice

Sheila R. Costford, Shehla N. Chaudhry, Mahmoud Salkhordeh, and Mary-Ellen Harper

Department of Biochemistry, Microbiology, and Immunology, Faculty of Medicine, University of Ottawa, Ottawa, Ontario, Canada

Submitted 24 August 2005; accepted in final form 18 January 2006

Costford, Sheila R., Shehla N. Chaudhry, Mahmoud Salkhordeh, and Mary-Ellen Harper. Effects of the presence, absence, and overexpression of uncoupling protein-3 on adiposity and fuel metabolism in congenic mice. Am J Physiol Endocrinol Metab 290: E1304–E1312, 2006. First published January 24, 2006; doi:10.1152/ajpendo.00401.2005.—Uncoupling protein-3 (UCP3) is a poorly understood mitochondrial inner membrane protein expressed predominantly in skeletal muscle. The aim of this study was to examine the effects of the absence or constitutive physiological overexpression of UCP3 on whole body energy metabolism, glucose tolerance, and muscle triglyceride content. Congenic male UCP3 knockout mice (Ucp3−/−), wild-type, and transgenic UCP3 overexpressing (UCP3Tg) mice were fed a 10% fat diet for 4 or 8 mo after they were weaned. UCP3Tg mice had lower body weights and were less metabolically efficient than wild-type or Ucp3−/− mice, but they were not hyperphagic. UCP3Tg mice had smaller epididymal white adipose tissue and brown adipose tissue (BAT) depots; however, there were no differences in muscle weights. Glucose and insulin tolerance tests revealed that both UCP3Tg and Ucp3−/− mice were protected from development of impaired glucose tolerance and were more sensitive to insulin. 2-Deoxy-d-[1-14C]glucose tracer studies showed increased uptake of glucose into BAT and increased storage of liver glycogen in Ucp3−/− mice. Assessments of intramuscular triglyceride (IMTG) revealed that both UCP3Tg and Ucp3−/− mice were protected from increased accumulation of IMTG compared with wild-type mice, which in turn had greater IMTG than UCP3Tg mice. Results are consistent with a role for UCP3 in preventing accumulation of triglyceride in both adipose tissue and muscle.

intramuscular triglyceride; obesity; glucose tolerance; insulin resistance; skeletal muscle

LIPOTOXICITY is the DETRIMENTAL ACCUMULATION of lipids and lipid metabolites in tissues other than adipose tissue (e.g., liver, heart, or skeletal muscle). The accumulation of triglyceride in muscle [intramuscular triglyceride (IMTG)] correlates well with impaired insulin-stimulated glucose uptake (27, 28). Although IMTG is not related to body mass index (BMI), age, fasting plasma triglycerides, nonesterified fatty acids, glucose, or insulin, it is a determinant of insulin resistance (21). Insulin resistance is the best predictor of type 2 diabetes mellitus, and therefore, there is a keen interest in understanding processes that are related to the accumulation of lipid in muscle.

There is evidence of a potential link between uncoupling protein-3 (UCP3) in muscle and protection against the development of type 2 diabetes. F1 generation mice overexpressing human UCP3 20-fold in skeletal muscle showed reduced fasting glucose and insulin levels as well as increased glucose tolerance in response to an oral glucose tolerance test (9, 10). Interestingly, these mice were also reported to be hyperphagic despite being protected from the development of obesity. Patients with type 2 diabetes have been shown to have decreased UCP3 expression in vastus lateralis (30), and although initial studies revealed a negative correlation between UCP3 mRNA levels and BMI (31), later studies showed no association between UCP3 protein levels and BMI (30).

UCP3 is a mitochondrial inner membrane protein of unknown function expressed predominantly in skeletal muscle (6). Ever since its identification in 1997, it has also been found to be expressed in brown adipose tissue (BAT), at low levels in the heart, and in certain parts of the brain (34, 35). UCP3 was named because of its 57% sequence homology to UCP1 (6, 34), which is expressed exclusively in BAT and serves to uncouple oxidative phosphorylation, resulting in the dissipation of energy as heat in response to cold or overfeeding (26). It was originally hypothesized that UCP3 would act to uncouple oxidative phosphorylation in muscle, as UCP1 does in BAT (13), and in fact, overexpression studies of UCP3 in yeast yielded increased uncoupling (20, 38). However, it has since been demonstrated that this observed uncoupling may have been artifactual due to very high levels of protein expression in the mitochondrial inner membrane (16, 32).

Physiologically, UCP3 expression is increased in response to fasting (3, 5, 8, 24), acute exercise (11, 33), and elevation of plasma lipids via a high-fat diet (14) or infusion of Intralipid (37), conditions where reliance on fat oxidation is increased regardless of any increases or decreases in energy expenditure. Moreover, unlike UCP1, UCP3 does not increase in response to cold adaptation (5). These findings suggest that UCP3 is not likely involved in dissipating energy through uncoupling of oxidative phosphorylation, which would be detrimental to the organism during dietary energy deficits but may indicate that UCP3 is involved in facilitating fat oxidation. A mechanism through which UCP3 could facilitate fatty acid oxidation was proposed (19) to have involved a mitochondrial thioesterase functioning in tandem with UCP3. The former liberates the free CoA, which is in high demand during fatty acid oxidation, and the latter exports excess fatty acid anions. Although several reports (4, 23, 25, 36) support such a role, the molecular mechanism remains to be proved. Moreover, there is strong support for a role for UCP3 in the protection from reactive oxygen species (7, 23). Altogether, findings are consistent with the possibility that low levels of UCP3 could impair fat oxidation and therefore result in the accumulation of lipid metabolites in tissues other than adipose tissue (e.g., liver, heart, or skeletal muscle). The accumulation of triglyceride in muscle [intramuscular triglyceride (IMTG)] correlates well with impaired insulin-stimulated glucose uptake (27, 28). Although IMTG is not related to body mass index (BMI), age, fasting plasma triglycerides, nonesterified fatty acids, glucose, or insulin, it is a determinant of insulin resistance (21). Insulin resistance is the best predictor of type 2 diabetes mellitus, and therefore, there is a keen interest in understanding processes that are related to the accumulation of lipid in muscle.

There is evidence of a potential link between uncoupling protein-3 (UCP3) in muscle and protection against the development of type 2 diabetes. F1 generation mice overexpressing human UCP3 20-fold in skeletal muscle showed reduced fasting glucose and insulin levels as well as increased glucose tolerance in response to an oral glucose tolerance test (9, 10). Interestingly, these mice were also reported to be hyperphagic despite being protected from the development of obesity. Patients with type 2 diabetes have been shown to have decreased UCP3 expression in vastus lateralis (30), and although initial studies revealed a negative correlation between UCP3 mRNA levels and BMI (31), later studies showed no association between UCP3 protein levels and BMI (30).

UCP3 is a mitochondrial inner membrane protein of unknown function expressed predominantly in skeletal muscle (6). Ever since its identification in 1997, it has also been found to be expressed in brown adipose tissue (BAT), at low levels in the heart, and in certain parts of the brain (34, 35). UCP3 was named because of its 57% sequence homology to UCP1 (6, 34), which is expressed exclusively in BAT and serves to uncouple oxidative phosphorylation, resulting in the dissipation of energy as heat in response to cold or overfeeding (26). It was originally hypothesized that UCP3 would act to uncouple oxidative phosphorylation in muscle, as UCP1 does in BAT (13), and in fact, overexpression studies of UCP3 in yeast yielded increased uncoupling (20, 38). However, it has since been demonstrated that this observed uncoupling may have been artifactual due to very high levels of protein expression in the mitochondrial inner membrane (16, 32).

Physiologically, UCP3 expression is increased in response to fasting (3, 5, 8, 24), acute exercise (11, 33), and elevation of plasma lipids via a high-fat diet (14) or infusion of Intralipid (37), conditions where reliance on fat oxidation is increased regardless of any increases or decreases in energy expenditure. Moreover, unlike UCP1, UCP3 does not increase in response to cold adaptation (5). These findings suggest that UCP3 is not likely involved in dissipating energy through uncoupling of oxidative phosphorylation, which would be detrimental to the organism during dietary energy deficits but may indicate that UCP3 is involved in facilitating fat oxidation. A mechanism through which UCP3 could facilitate fatty acid oxidation was proposed (19) to have involved a mitochondrial thioesterase functioning in tandem with UCP3. The former liberates the free CoA, which is in high demand during fatty acid oxidation, and the latter exports excess fatty acid anions. Although several reports (4, 23, 25, 36) support such a role, the molecular mechanism remains to be proved. Moreover, there is strong support for a role for UCP3 in the protection from reactive oxygen species (7, 23). Altogether, findings are consistent with the possibility that low levels of UCP3 could impair fat oxidation and therefore result in the accumulation of lipid metabolites in tissues other than adipose tissue (e.g., liver, heart, or skeletal muscle). The accumulation of triglyceride in muscle [intramuscular triglyceride (IMTG)] correlates well with impaired insulin-stimulated glucose uptake (27, 28). Although IMTG is not related to body mass index (BMI), age, fasting plasma triglycerides, nonesterified fatty acids, glucose, or insulin, it is a determinant of insulin resistance (21). Insulin resistance is the best predictor of type 2 diabetes mellitus, and therefore, there is a keen interest in understanding processes that are related to the accumulation of lipid in muscle.

There is evidence of a potential link between uncoupling protein-3 (UCP3) in muscle and protection against the development of type 2 diabetes. F1 generation mice overexpressing human UCP3 20-fold in skeletal muscle showed reduced fasting glucose and insulin levels as well as increased glucose
lipid in muscle, the onset of insulin resistance, and the development of type 2 diabetes.

Our purpose here was to assess the metabolic impacts of the absence, presence, and physiological levels of overexpression of UCP3 in mice. We hypothesized that transgenic UCP3 overexpressing mice (UCP3Tg) would be protected from the development of insulin resistance, whereas UCP3 knockout mice (Ucp3−/−) would be predisposed to the development of insulin resistance. Strengths of this study include the use of congenic lines of mice, a defined diet, and levels of UCP3 overexpression that are physiological.

MATERIALS AND METHODS

Treatment of animals. Congenic male Ucp3−/−, wild-type (WT), and UCP3Tg C57BL/6 mice (n = 20 per group) were housed individually and fed a defined rodent diet with 10% kcal fat (D12450C; Research Diets, New Brunswick, NJ) ad libitum from being weaned. An additional group of congenic male Ucp3−/−, WT, and UCP3Tg mice (n = 5 per group) were fed a 45% kcal fat diet (D12451; Research Diets) ad libitum from being weaned for further IMTG studies. The D12450C diet consisted of 20% protein, 70% carbohydrate, and 10% fat, whereas the D12451 diet consisted of 20% protein, 35% carbohydrate, and 45% fat by kcal. Protein from both carbohydrate, and 10% fat, whereas the D12451 diet consisted of 20% protein, 70% carbohydrate, and 10% fat, whereas the D12451 diet consisted of 20% protein, 35% carbohydrate, and 45% fat by kcal. Protein from both diets was from casein and l-cysteine, carbohydrate was from corn starch, maltodextrin, and sucrose, and fat was from soybean oil and lard. Food was given to mice inside the clear polycarbonate cages where they were housed. UCP3Tg mice, which overexpress human UCP3 in skeletal muscle (~66-fold increase in mRNA) and at low levels in BAT (50% increase in mRNA), were provided originally by Dr. John Clapham at GlaxoSmithKline (Harlow, UK), the F1 generation of which has been previously described (10). Ucp3−/− mice on a mixed genetic background have also been previously described (15). Mice used in the present study had been backcrossed 10 generations into the C57BL/6 background to minimize effects of genetic background. Overexpressing mice express UCP3 protein levels in skeletal muscle that are 2- to 2.5-fold of WT levels (4). Mice were maintained at 23°C with light from 0600 to 1800 and were studied from being weaned to 4 or 8 mo on the defined diets. Animals were cared for in accordance with the principles and guidelines of the Canadian Council on Animal Care and the Institute of Laboratory Animal Resources (National Research Council). The study was approved by the Animal Care Committee of the University of Ottawa.

Whole body analyses. Food intake was measured and mice were weighed semiweekly from weaning to death. Food intake was calculated by dividing the total amount of energy ingested over the lifespan of the mouse by the number of days that the mouse consumed the defined diet (4 or 8 mo). The results are expressed as mean kcal ingested per day. Metabolic efficiency was calculated as total body weight gained divided by total amount of food consumed over 4 or 8 mo and is expressed as milligrams body weight gained per kilocalorie ingested. Naso-anal length and rectal body temperature were measured before death at the same time every day (Model TH-8; Physitemp Thermalert, Clifton, NJ).

Glucose and insulin tolerance tests. Glucose and insulin tolerance tests were carried out (1 wk apart to allow mice to recover) after 1, 3, 4, and 8 mo on the diet. Mice were fasted for 16 h with water ad libitum before the test. Fasting blood glucose was determined by bleeding mice from the saphenous vein onto a glucometer (One Touch Basic; LifeScan, Burnaby, BC, Canada). Mice were given an intraperitoneal injection of 1 mg of glucose (10% dextrose in sterile saline) or 1 × 10−4 units of human insulin (Humalog Rapid Acting in sterile saline; Eli Lilly) per g body wt. Blood glucose was assessed again at 10, 20, 30, and 120 min postinjection.

Indirect calorimetry. Oxygen consumption (VO2) and carbon dioxide production (VCO2) of mice were measured using a customized four-chamber Oxymax system with automatic temperature and light controls (Columbus Instruments, Columbus, OH). Temperature was maintained at 24°C, and lights were on from 0600 to 1800. System settings included a flow rate of 0.5 L/min, a sample line-purge time of 2 min, and a measurement period of 60 s every 12 min. Mice were placed in separate 2.5-liter calorimetry chambers with ad libitum access to the defined diet and water. Twenty-four-hour energy expenditure was expressed as percent relative cumulative frequencies of all points collected throughout a 24-h period, as described previously (2, 29). Respiratory exchange ratios (RERs) were calculated as the ratio of VCO2 divided by VO2.

Analysis of 2-deoxy-D-[1-13C]glucose 6-phosphate in tissue. This procedure was carried out as detailed in Wang et al. (36), with minor modifications. At 4 mo, one-half of the mice were fasted for 16 h. Mice were given an intraperitoneal injection of 0.5 μCi 2-deoxy-D-[1-13C]glucose mixed with 10% dextrose to provide a fixed specific activity of 1 g glucose/kg body wt. Mice were decapitated 70 min postinjection, and quadriceps, gastrocnemius, diaphragm, interscapular BAT, epididymal white adipose tissue (EWAT), liver, pancreas, and heart were dissected and flash-frozen in liquid nitrogen. Thawed tissues were homogenized in 2% HClO4 (10 μl/mg tissue) and left at 4°C overnight. Homogenates were centrifuged for 10 min at 2,000 g, and supernatants were neutralized to pH 7.4 with 2 M KOH. Total 3H radioactivity was measured in one-half the volume of the supernatant, and the other half of the supernatant was added to a column filled with anion exchange resin (Ag1-X8; Bio-Rad, Mississauga, ON, Canada). Free 2-deoxy-D-glucose was eluted with 12 ml of water and 2-deoxy-D-glucose 6-phosphate was eluted with 10 ml of 0.4 M ammonium acetate-0.5 M formic acid. 3H radioactivity was measured in 2-deoxy-D-glucose and 2-deoxy-D-glucose 6-phosphate eluants. Tissue glucose uptake (pmol·g−1·min−1·wt tissue) was calculated by dividing tissue 2-deoxy-D-[1-13C]glucose 6-phosphate activity by the glucose specific activity.

Analysis of 2-deoxy-D-[1-13C]glucose 6-phosphate in liver glycogen. This procedure was carried out as detailed in Wang et al. (36), with minor modifications. Liver samples were homogenized, extracted, and neutralized as tissues undergoing 2-deoxy-D-[1-13C]glucose 6-phosphate analysis. Five hundred microliters KOH containing 2.5% oyster glycogen and 100 μl of 0.2% Na2SO4 were added to 500 μl of neutralized supernatant. Glycogen was precipitated with 1 ml of ethanol and centrifuged for 15 min at 850 g at 4°C, and the pellet was washed with 66% ethanol before resuspension in 1 ml of distilled water and counted for 3H radioactivity.

IMTG determination. After 8 mo, quadriceps muscles from mice fed the 10%-fat diet and mice fed the 45%-fat diet were fixed in 10% buffered neutral formalin. Tissues were trimmed no thicker than 4 mm, rinsed in distilled H2O2, and left in 0.17 M potassium dichromate-0.08 M osmium tetroxide solution for 8 h (1). Tissues were left under running H2O2 for 2 h and then processed in an automatic tissue processor, embedded in paraffin, and cut in 5-μm sections. Tissues were mounted on glass slides and then incubated at 42°C overnight. Imaging software (Northern Eclipse) was used to analyze tissues at ×10 magnification with an Axioskop light microscope (Axioport; Zeiss, Oberkochen, Germany). Images from fields in 9–12 sections per mouse were selected and converted into 8-bit grayscale. The number of sections per mouse depended on the amount of quadriceps muscle available for histological staining, and therefore it varied somewhat. Images (fields of view) consisted of the entire section (this was consistent between mice). Threshold was set at 0 to 99. IMTG was measured as a proportion of surface area of each field.

BAT histology. BAT was fixed in 10% buffered neutral formalin and subjected to standard histological methods for staining with hematoxylin and eosin.
RESULTS

Whole body. UCP3Tg mice had lower body weights than both WT and Ucp3−/− mice at both 4 and 8 mo. However, there were no differences in linear growth, as assessed by naso-anal length (Table 1). At 8 mo, UCP3Tg mice were less metabolically efficient than both WT and Ucp3−/− mice. This trend was also present at 4 mo but did not reach statistical significance. There were no differences in food intake or body temperature between genotypes at either 4 or 8 mo (Table 1). UCP3Tg mice had lower quadriceps weights than WT mice at 4 mo, but this difference disappeared by 8 mo (Table 2). UCP3Tg mice had lower diaphragm weights than WT and Ucp3−/− mice at 4 mo, but these differences also disappeared by 8 mo (Table 2). UCP3Tg mice had lower EWAT and BAT weights than both WT and Ucp3−/− mice at both 4 and 8 mo (Table 2). UCP3Tg mice also had lower liver weights than WT mice at both 4 and 8 mo (Table 2).

Indirect calorimetry. There were no differences in RERs between genotypes at either 4 or 8 mo (Fig. 1, A and B), indicating that there was no shift in fuel preference at the level of the whole body in either UCP3Tg or Ucp3−/− mice. There were no differences in VO2 between genotypes after either 4 or 8 mo on the diet (Fig. 2, A and B), indicating that energy expenditure was similar between genotypes.

Glucose tolerance. Surprisingly, both UCP3Tg and Ucp3−/− mice showed protection from the development of glucose intolerance, as measured by glucose tolerance test (Fig. 3). Initially, after 1 mo on the diet, only Ucp3−/− showed...
better glucose tolerance than WT mice (Fig. 3A). At 3 mo, however, both UCP3Tg and Ucp3−/− mice showed better glucose tolerance than the WT mice (Fig. 3B). At 4 mo the differences in glucose tolerance between the genotypes narrowed, but statistical significance was maintained, and both UCP3Tg and Ucp3−/− mice had better glucose tolerance than WT mice (Fig. 3C). After 8 mo on the diet both UCP3Tg and Ucp3−/− mice still showed protection from the development of glucose intolerance. However, Ucp3−/− mice were more glucose tolerant than both WT and UCP3Tg mice (Fig. 3D). These results were unexpected and point to possible compensatory mechanisms in the Ucp3−/− mouse.

Insulin tolerance. Both UCP3Tg and Ucp3−/− mice showed some protection from the development of insulin resistance, as measured by insulin tolerance test (Fig. 4). After 1 mo on the diet, UCP3Tg mice were more sensitive to insulin in the early part of the curve in the first ~20-min postinjection (Fig. 4A). At 3 mo this difference disappeared (Fig. 4B), but it reappeared after 4 mo on the diet; both UCP3Tg and Ucp3−/− mice were more sensitive to insulin than WT mice in the first ~20 min postinjection (Fig. 4C). After 8 mo on the diet, there was a trend toward both UCP3Tg and Ucp3−/− mice to be more insulin sensitive than WT mice, but this did not reach statistical significance (Fig. 4D). These results were also surprising and again suggest that the Ucp3−/− mice might have developed mechanisms to compensate for the lack of UCP3.

2-Deoxy-d-[1-3H]glucose uptake. After 4 mo on the diet, Ucp3−/− mice took up and phosphorylated more 2-deoxy-d-[1-3H]glucose in BAT (Fig. 5A). Histological studies indicated that BAT from UCP3Tg mice was more metabolically active and had smaller lipid droplets than BAT from WT and Ucp3−/− mice (Fig. 6). Ucp3−/− also incorporated more 2-deoxy-d-[1-3H]glucose into liver glycogen compared with UCP3Tg mice (Fig. 5B).

IMTG. After 8 mo on the diet, quadriceps from UCP3Tg mice had less IMTG than quadriceps from both WT and Ucp3−/− mice (Fig. 7, A and C). In humans, UCP3 protein is expressed most abundantly in type IIB fibers, less abundantly in type IIA fibers, and at even lower levels in type I fibers (18). In mice, UCP3 protein is highest in gastrocnemius (1/3 type I, 1/3 type IIA, and 1/3 type IIB), lowest in soleus (90% type I), and intermediate in tibialis anterior (2/3 type IIA and 1/3 type IIB) (12). Quadriceps muscle was assessed in our present study, because we hypothesized that the effects of UCP3 would be more apparent in muscles with a higher ratio of glycolytic-to-oxidative fibers. There was a trend for Ucp3−/− mice to store more triglyceride in their quadriceps than WT mice; however, this did not reach statistical significance. To further challenge the genotypes with respect to IMTG accumulation, mice were fed a 45%-fat diet for 8 mo. Under these dietary conditions, UCP3Tg mice were still protected from the accumulation of IMTG; however, Ucp3−/− mice had higher levels of IMTG than both UCP3Tg and WT mice (Fig. 7, B and D).
Fig. 3. Glucose tolerance tests. Measurements taken at 1 (A), 3 (B), 4 (C), and 8 mo (D). Values are means ± SE, n = 20 (A-C), n = 10 (D). Two-way ANOVA with Bonferroni correction. *P < 0.05; **P < 0.01; ***P < 0.001, UCP3Tg vs. WT mice; †P < 0.05; ‡P < 0.01; ‡‡P < 0.001, WT vs. Ucp3−/−. §P < 0.01 UCP3Tg vs. Ucp3−/−. Both UCP3Tg and Ucp3−/− mice are protected from the development of impaired glucose tolerance.

Fig. 4. Insulin tolerance tests. Measurements taken at 1 (A), 3 (B), 4 (C), and 8 mo (D). Values are means ± SE, n = 19–20 (A), n = 20 (B and C), n = 10 (D). Two-way ANOVA with Bonferroni correction. **P < 0.01; ***P < 0.001, UCP3Tg vs. WT; ‡‡P < 0.001, WT vs. Ucp3−/−. Both UCP3Tg and Ucp3−/− mice show some protection from the development of insulin resistance.
Physiological overexpression of UCP3 protected against the accumulation of triglyceride in muscle, whereas the lack of UCP3 promoted accumulation of IMTG.

**DISCUSSION**

Contrary to our initial hypothesis, both UCP3Tg and Ucp3⁻/⁻ mice demonstrated protection from the development of glucose intolerance and insulin resistance. However, UCP3Tg mice showed decreased overall adiposity, whereas Ucp3⁻/⁻ mice showed increased lipid storage specifically in skeletal muscle.

Strengths of this study include the use of congenic transgenic and knockout mice backcrossed 10 generations into the C57BL/6 background. When identifying phenotypic differences between mice, comparing mice that have identical (or nearly identical) genetic backgrounds increases confidence that identified differences are due to the gene being studied and not to random genetic variance (17). The use of a defined diet is also extremely important in metabolic studies such as these, because nutrient composition and macromolecule sources are consistent in such diets. Notably, the level of UCP3 overexpression in these transgenic mice was 2- to 2.5-fold compared with WT levels (4) and represents a physiological increase. Very high expression levels of uncoupling proteins can lead to artifactual uncoupling of oxidative phosphorylation (16, 32), which would mask the true metabolic effects of physiological increases of the protein in vivo.

Compared with WT mice, UCP3Tg mice had lower body weights, lower fat pad and liver weights, less IMTG, and lower metabolic efficiencies. However, they showed no difference in food intake and no difference in metabolic rate or fatty acid oxidation (as measured by indirect calorimetry). Because there were no differences in food intake and no differences in energy expenditure, it appears as though neither one can account for the clear differences in metabolic efficiency observed. The explanation may lie, in part, in the ~5% error associated with indirect calorimetry (22). In addition, whereas food intake and metabolic efficiency determinations were conducted over long periods of time (4 or 8 mo), indirect calorimetry was assessed over only a 24-h period. Thus small, but meaningful, differences in energy expenditure may not have been detected during this shorter time frame. Conversely, Ucp3⁻/⁻ mice did not show any reduction in body weight, fat pad weight, liver weight, or IMTG compared with WT mice; however, they showed increased 2-deoxy-D-glucose uptake in BAT. Ucp3⁻/⁻ mice showed no differences in food intake, metabolic rate, or respiratory exchange ratio.

Interestingly, glucose and insulin tolerance tests demonstrated that both UCP3Tg and Ucp3⁻/⁻ mice were protected from the development of glucose intolerance and insulin resistance. These findings run counter to our original hypothesis.

Fig. 5. 2-Deoxy-D-[1-³H]glucose uptake into tissues (A) and incorporation into liver glycogen (B) at 4 mo; n = 10. *P < 0.05, UCP3Tg vs. Ucp3⁻/⁻. One-way ANOVA with Tukey’s posttest. Ucp3⁻/⁻ take up and phosphorylate more 2-deoxy-D-[1-³H]glucose into brown adipose tissue (BAT) than UCP3Tg. Less glucose is incorporated into glycogen in UCP3Tg than in Ucp3⁻/⁻ mice.

Fig. 6. BAT stained with hematoxylin and eosin.

UCP3Tg

WT

UCP3⁻/⁻
that, whereas transgenic mice would be protected from glucose and insulin intolerance, knockout mice would be predisposed to the latter compared with WT mice. In hindsight, given that these are germline genetic modifications and given the complexities of intertissue mechanisms involved in the development of glucose intolerance and insulin resistance, it could be considered somewhat naïve to expect that overexpressors and knockouts would have phenotypes mirror imaging one another (17). Compensatory mechanisms are likely present. One possible explanation for the increased glucose and insulin tolerance in the UCP3Tg mice may be that these mice are leaner than the WT and $\text{Ucp3}^{-/-}$ mice. In contrast, this cannot explain why the $\text{Ucp3}^{-/-}$ mice also show increased glucose and insulin tolerance, because they have a similar degree of adiposity to WT mice. Further studies are underway in our laboratory to elucidate these mechanisms. Determining which genes are upregulated to compensate for UCP3 in its absence will hopefully be instructive with regard to the function of this protein.

In the examination of results from the various experimental approaches used herein, and in light of additional findings from our laboratory and others, a number of consistencies emerge. In these two experimental groups of mice it appears that improved glucose tolerance and insulin tolerance occur through different mechanisms. In the transgenic mice there are decreases in muscle and BAT triglyceride contents and decreases in the weights of fat depots that coincide with the improved glucose and insulin tolerance. These findings are consistent with the ideas that the protection afforded to the mice occurs as a result of lipotoxicity prevention. Importantly, there were no differences identified in total (whole body) energy expenditure and no differences in food intake compared with WT controls.

UCP3Tg mice had lower liver weights as well as a decreased incorporation of 2-deoxy-D-glucose into liver glycogen, thus identifying some important secondary effects of the muscle-specific changes in UCP3 content on liver fuel metabolism. In $\text{Ucp3}^{-/-}$ mice there were no changes in fat depot weights or skeletal muscle, and yet these mice still had better glucose control overall than WT mice. The latter, in conjunction with the results from overexpressors, is consistent with the conclusion that UCP3 has little or no role in regulation of adiposity but does play some role in the control of fuel substrate oxidation (glucose vs. fatty acids) in some tissues where it is expressed (e.g., BAT). Again, consistent with the results from overexpressors, there were no differences in total energy expenditure or in food intake compared with WT mice. The absence of UCP3 appears to have a significant effect on fuel metabolism in BAT. In $\text{Ucp3}^{-/-}$ mice, BAT took up and
phosphorylated more glucose than did the BAT of the other two groups of mice. This could explain the improved glucose tolerance and insulin tolerance in these mice compared with the other two groups. The latter hypothetically occurs as a result of some impaired capacity to take up and/or oxidize fatty acids in BAT, leading to an increased reliance on glucose for fuel. Indeed in another series of studies conducted, we found that UCP3 played an important role in facilitating fatty acid oxidation in muscle (4). However, the paradox remains as to why the absence of UCP3 in muscle does not lead to lipotoxicity in muscle or even an increased reliance on glucose there, as observed in BAT. Further research into this paradox is actively underway in our laboratory. Findings from this series of studies also identify the need for studies in conditional knockout and overexpressing mouse models to avoid the issues of compensation that are associated with germline models.

ACKNOWLEDGMENTS

We are very grateful to Linda Jui, Nicole Sabet, Mitra Abaeian, Kim Yates, and Eileen Franklin for their assistance with histology and care of the mice.

GRANTS

This research was supported by funding from the Canadian Institutes of Health Research: Institute of Nutrition, Metabolism, and Diabetes (M-E. Harper).

REFERENCES

19. Schrauwen P, Hesselink MK, Blaak EE, Borghouts LB, Schaart G, Saris WH, and Keizer HA. Uncoupling protein 3 content is decreased in...