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Overexpression of GLUT4 in Mice Causes Up-Regulation of UCP3 mRNA in Skeletal Muscle

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Mitochondrial uncoupling protein 3 (UCP3) is expressed in skeletal muscles. We have hypothesized that increased glucose flux in skeletal muscles may lead to increased UCP3 expression. Male transgenic mice harboring insulin-responsive glucose transporter (GLUT4) minigenes with differing lengths of 5′-flanking sequence (−3237, −2000, −1000 and −442 bp) express different levels of GLUT4 protein in various skeletal muscles. Expression of the GLUT4 transgenes caused an increase in UCP3 mRNA that paralleled the increase of GLUT4 protein in gastrocnemius muscle. The effects of increased intracellular GLUT4 level on the expression of UCP1, UCP2 and UCP3 were compared in several tissues of male 4 month-old mice harboring the −1000 GLUT4 minigene transgene. In the −1000 GLUT4 transgenic mice, expression of GLUT4 mRNA and protein in skeletal muscles, brown adipose tissue (BAT), and white adipose tissue (WAT) was increased by 1.4 to 4.0-fold. Compared with nontransgenic littermates, the −1000 GLUT4 mice exhibited about 4- and 1.8-fold increases of UCP3 mRNA in skeletal muscle and WAT, respectively, and a 38% decrease of UCP1 mRNA in BAT. The transgenic mice had a 16% increase in oxygen consumption and a 14% decrease in blood glucose and a 68% increase in blood lactate, but no change in FFA or β-OHB levels. T3 and leptin concentrations were decreased in transgenic mice. Expression of UCP1 in BAT of the −442 GLUT4 mice, which did not overexpress GLUT4 in this tissue, was not altered. These findings indicate that overexpression of GLUT4 up-regulates UCP3 expression in skeletal muscle and down-regulates UCP1 expression in BAT, possibly by increasing the rate of glucose uptake into these tissues.

Uncoupling proteins (UCPs) are mitochondrial transporters that have the capacity to dissipate the proton gradient and thereby produce heat (1). UCP1 is expressed exclusively in brown adipose tissue (BAT) where it plays a pivotal role in the control of thermogenesis. In contrast to UCP1, UCP2 (2, 3) is expressed in multiple tissues, and UCP3 (4–6) is expressed at high levels in skeletal muscle as well as BAT. Skeletal muscle is an important site of regulated thermogenesis (7) where dissipation of the mitochondrial proton gradient, presumably involving UCP3, has been reported to contribute up to 50% of the resting metabolic rate (8). Whether UCP3 plays an important role in thermogenesis *in vivo,* however, has not been established. Since expression of UCP3 in skeletal muscle is upregulated in fasting, a state in which whole body energy is conserved and metabolism is switched to the utilization of free fatty acid (FFA), UCP3 may function as regulator of lipid as a fuel substrate rather than as mediator of thermogenesis (5, 9). Indeed, fatty acid loading by infusion of intralipid with heparin upregulates UCP3 mRNA in skeletal muscle *in vivo* (9).

Previously, we reported that exercise results in a marked (14- to 18-fold) increase of UCP3 mRNA in skeletal muscle (10). However, the UCP3 mRNA increase observed at 3 h after exercise was transient, and disappeared within 22-24 h. Because the utilization of FFA and glucose remains increased even after the increased ATP consumption used for muscle movement ceases at the completion of exercise, we have speculated that this continued utilization of lipid and/or carbohydrate by skeletal muscle may stimulate up-

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Abbreviations used: UCP, uncoupling protein; GLUT4, muscle/adipocyte insulin-responsive glucose transporter; FFA, free fatty acid; NIDDM, non-insulin-dependent diabetes mellitus; WAT, white adipose tissue; BAT, brown adipose tissue; FABP, fatty acid binding protein; PAGE, polyacrylamide gel electrophoresis; PSL, phosphostimulated luminescence; T3, triiodo-L-thyronine; β-OHB, β-hydroxybutyrate; VMA, vanillylmandelic acid; ANOVA, one-way analysis of variance; PLSD, protected least significant difference.

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regulation of UCP3 mRNA. Recently, in rat skeletal muscles, UCP3 and GLUT4 mRNAs increased two to three fold between 6 and 24 h of cold exposure and then decreased to 50% of the control value after 6 days in the cold (11). Furthermore, Krook *et al.* reported that the level of UCP3 mRNA in skeletal muscle of non-insulin-dependent diabetes mellitus (NIDDM) patients was lower than in control subjects (12). They also observed a positive correlation between UCP3 expression in skeletal muscles and whole-body insulin-mediated glucose utilization among NIDDM individuals. These observations suggest that increased glucose entry in muscle results in an increase in UCP3 expression which leads to an increase in energy expenditure.

The increased expression of GLUT4 in skeletal muscle (13, 14) and adipose tissue (15) results in increased intracellular glucose flux in these tissues and in improvement in whole body glucose tolerance. To determine whether the expression of UCP3 is affected by the level of GLUT4 (and presumably insulin-stimulated glucose uptake), UCP3 levels were measure in transgenic mice that overexpressed GLUT4 protein in insulin sensitive tissues.

EXPERIMENTAL PROCEDURES

GLUT4 transgenic mice. The 5′-deletion GLUT4 minigenes were derived from the 14 kb GLUT4 minigene containing 7395 bp of GLUT4 5′-flanking DNA (16) with a "TAG" of 281 bp of foreign DNA inserted into the exon encoding the 3′ untranslated region (17) and were constructed as previously described (18, 19). Minigene constructs were microinjected into C57BL/6 x SJL F2 hybrid mouse eggs. Identification of transgenic mice harboring the GLUT4 minigenes was accomplished by Southern blotting (20) using the 281 bp chloramphenicol acetyltransferase tag as probe as described previously (17–19). Transgenic mice were mated with C57BL/6J mice, and the third and fourth generations of heterozygote mice were used in this study. To avoid the unknown effects of hormonal changes on UCP expression due to the menstrual cycle, only male mice were used. Expression levels of the minigenes and endogenous GLUT4 by RNase protection assays in tissues was as described in our previous study (19). The mice were allowed free access to a standard laboratory diet (on a calorie basis, 11% fat, 60% carbohydrate, and 29% protein) and water and were maintained at a constant temperature of 22°C with fixed artificial light cycle (12 h light and 12 h dark).

Northern blots. Mice were sacrificed under feeding conditions with an intraperitoneal injection of pentobarbital (Abbot, North Chicago, IL) at 0.05 mg/g body weight. Skeletal muscles, epididymal white adipose tissue (WAT) and interscapular brown adipose tissue (BAT) were homogenized immediately in guanidine, and RNA was isolated from each tissue by the method of Chirgwin *et al.* (21). A portion of RNA (15 μg or 10 μg per lane) was denatured with glyoxal and dimethyl sulfoxide and analyzed by electrophoresis in 1% agarose gels. After transfer to nylon membranes (NEN, Buckinghamshire, England) and UV crosslinking, RNA blots were stained with methylene blue to locate 28S and 18S rRNAs and to ascertain the amount of loaded RNAs (22). The blots were hybridized overnight at 42°C with GLUT4, FABP, UCP1, UCP2 and UCP3 cDNA which had been labeled with ^{32}P-dCTP (NEN) by random prime labeling (23). Mouse fatty acid binding protein (FABP) cDNA clone containing the coding sequence was obtained by reverse transcription-PCR from mouse skeletal muscle RNA. For FABP (GenBank Accession X14961), forward and reverse primer sequences were 5′-TCATCGCACCATGGCGGA-3′ and 5′-ATTGACCTTGGAGCACCCT-3′, respectively. Mouse UCP1, human UCP2 and UCP3 cDNA clones containing the coding sequence were obtained by PCR amplification as described previously (10). The filters were washed several times with 1 X SSC, 0.1% SDS at room temperature, washed twice at 50°C (for UCPs and FABP) and washed twice further with 0.1 X SSC, 0.1% SDS at 65°C for 30 min (for GLUT4), and then exposed to X-ray film at −80°C. The amounts of UCPs, FABP and GLUT4 mRNAs were quantitated with an image analyzer (BAS 2000, Fuji Film, Tokyo, Japan) and expressed as the intensity of phosphostimulated luminescence (PSL).

Immunoblotting. Crude membrane fractions from various skeletal muscles, WAT and BAT were prepared by centrifugation of the tissue homogenates at 175,000 x g for 60 min as described previously (24). Proteins separated by SDS/polyacrylamide gel electrophoresis (PAGE) were electrophoretically transferred to Hybond-P (Amersham Life Science, Buckinghamshire, England) and immunoblotted with antibodies directed against the C-terminal amino acid sequence of GLUT4 and then ^{125}I-labeled protein A as described previously (24).

Oxygen consumption. Four-month-old male heterozygous, −1000 GLUT4 minigene transgenic mice and their control littermates (3 mice per cage) were placed in a metabolic chamber of the open-circuit oxygen consumption measuring system. Oxygen consumption was measured using a computerized system with a 2-liter chamber maintained at 22°C, air flow of 1 L/min, and oxygen consumption monitor (Osaka Microsystems, Osaka, Japan). Mice were unstrained and given free access to the experimental food and water. The oxygen consumption rate was monitored at night (8:00 p.m.–6:00 a.m.) and during the day (4:00 p.m.–6:30 p.m.) for 5 d, and is expressed as the average oxygen consumption per minute and per mouse during the day and at night.

Other analyses. Blood samples were obtained from the inferior vena cava for hormone and metabolite determination under feeding conditions. Immunoreactive insulin was measured by an insulin assay kit (Morinaga, Kanagawa, Japan); glucagon by a radioimmunoassay kit (Daiichi, Tokyo, Japan); triiodo-L-thyronine (T3) by T-3 RIA Bead (Dainabot, Tokyo, Japan); corticosterone by COAT-A-COUNT (DPC, Los Angeles, CA); FFA by NEFA C-test Wako (WAKO, Osaka, Japan); leptin by mouse leptin assay kit (Morinaga, Kanagawa, Japan); lactate by lactate reagent (Sigma Diagnostics, St. Louis, MO); β-hydroxybutyrate (β-OHB) by β-hydroxybutyrate reagent (Sigma Diagnostics, St. Louis, MO); urine catecholamines and VMA by high performance liquid chromatography (SRL, Tokyo, Japan).

Statistical analysis. Comparisons of data from two experimental groups were made by unpaired Student's t-test. Comparisons of data from multiple groups were made by one-way analysis of variance (ANOVA), and each group was compared with the others by Fisher's protected least significant difference (PLSD) test (Statview 4.0, Abacus Concepts). Statistical significance is defined as $P < 0.05$. Values are mean ± SE.

RESULTS

The effects of increased glucose flux in muscle on the expression of UCP3 were assessed in transgenic mice harboring GLUT4 minigenes with different lengths of 5′-flanking region, all exons and introns, and 1 kb of 3′-flanking sequence of the mouse GLUT4 gene. The mouse lines studied contained minigenes with −3237, −2000, −1000 or −442 bp of 5′-flanking sequence and express different levels of GLUT4 protein in skeletal muscles. Tissue specific expression levels of the mini-

genes and of endogenous GLUT4 of these lines were described in our previous study; −3237 in Fig. 2B, −2000 in Fig. 2C, −1000 in Fig. 2D and −442 in Fig. 2F of Tsunoda *et al.* (19). Of note, the levels of expression of minigene GLUT4 mRNA in WAT and BAT were very low in −442 mice. As in our previous study (19), all of these transgenic mice lines showed a marked improvement of glucose tolerance compared to non-transgenic mice, indicating that increased glucose uptake in peripheral tissues had occurred (data not shown). The levels of expression of GLUT4 protein and UCP3 mRNA in gastrocnemius muscle are presented in Fig. 1. Compared with non-transgenic mice, GLUT4 protein and UCP3 mRNA levels in skeletal muscle from these transgenic mice line were significantly increased. Furthermore, mice that expressed a higher GLUT4 protein level showed a higher expression level of UCP3 mRNA (Fig. 1C).

To examine the effect of the increased intracellular glucose flux on UCP1, UCP2 and UCP3 expression in different tissues, 4-month-old −1000 GLUT4 minigene transgenic mice were examined. Because the minigene GLUT4 has a 281 bp of "TAG" inserted into the 3′ untranslated region of the GLUT4 gene (17), the minigene transcript appears slightly larger than the 2.7 kb endogenous GLUT4 transcript on Northern blotting (Fig. 2). Male −1000 minigene mice had 3.1-, 2.5-, 1.6-, and 2.4-fold increases of GLUT4 mRNA levels in gastrocnemius, quadriceps, WAT and BAT, respectively. Parallel to GLUT4 mRNA levels, GLUT4 protein levels in gastrocnemius, WAT and BAT from −1000 transgenic mice was also increased approximately 2.6-, 1.4- and 4.0-fold, respectively, although the difference in GLUT4 protein level in WAT did not attain statistical significance ($p = 0.06$).

Expression of UCP1, UCP2 and UCP3 was also measured in the −1000 GLUT4 minigene mouse line. The −1000 GLUT4 minigene transgenic mice had 4.1-, 3.9- and 1.8-fold increases of UCP3 mRNA in gastrocnemius, quadriceps and WAT, respectively (Fig. 3). UCP3 mRNA was also increased in BAT; this difference was just above statistical significance ($p = 0.052$). UCP2 mRNA also increased in transgenic mice, although this increase achieved statistical significance only in quadriceps, where transgenic mice expressed a 2-fold higher level of UCP2. In contrast to UCP2 and UCP3, these mice exhibited a significant 38% decrease of UCP1 mRNA in BAT. FABP which plays an important role in cellular uptake of FFA (25), did not exhibit increases in its mRNA level of transgenic mice in gastrocnemius (non-transgenic, 652 ± 68 PSL, n = 8; transgenic, 771 ± 114 PSL, n = 7), quadriceps (non-transgenic, 569 ± 77 PSL, n = 4; transgenic, 417 ± 74 PSL, n = 3) and BAT (non-transgenic, 1160 ± 44 PSL, n = 7; transgenic, 722 ± 100 PSL, n = 7, $P < 0.01$).

Given the established importance of UCP1 and the proposed role of UCP2 and UCP3 (8) in the regulation of energy expenditure, oxygen consumption of −1000 GLUT4 minigene mice and control, non-transgenic mice, was measured. Compared with non-transgenic

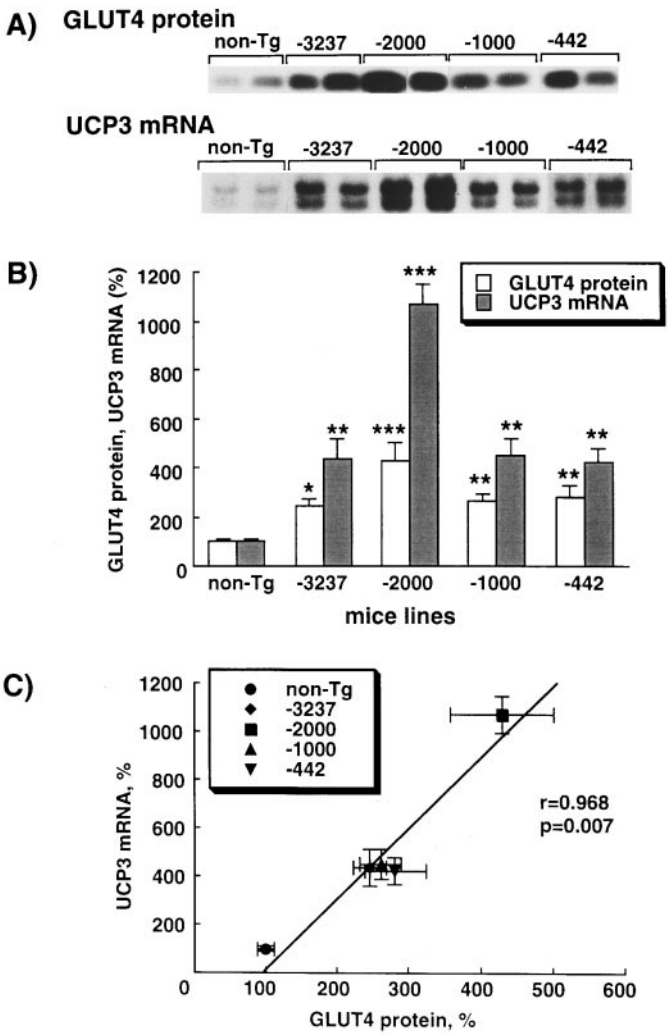

FIG. 1. Expression of GLUT4 protein and UCP3 mRNA in −3237, −2000, −1000 and −442 GLUT4 minigene transgenic mice. Gastrocnemius muscle was harvested from 2-3 month old male heterozygous transgenic mice and their control non-transgenic littermates. These mice harbored GLUT4 minigene transgenes containing different sizes of 5′-flanking region. Crude membrane fractions from gastrocnemius muscle (50 μg protein per lane) were subjected to SDS/PAGE. Transferred proteins were immunoblotted using an anti-GLUT4 antibody and detected using ^{125}I-labeled protein A. Ten μg of total RNA from gastrocnemius muscle was used for Northern blotting, and was probed using a ^{32}P-labeled human UCP3 cDNA. Panel A shows representative autoradiograms of GLUT4 protein and UCP3 mRNA in gastrocnemius muscle obtained by 3 h and 40 h exposure, respectively. In panel B, GLUT4 protein and UCP3 mRNA levels were quantitated using an image analyzer. Levels are expressed as the per cent of the level in non-transgenic mice. Each data point represents mean ± SE of 3-4 mice. Statistical differences are shown as $*P < 0.05$, $**P < 0.01$ and $***p < 0.001$ compared with control, non-transgenic mice by ANOVA and Fisher's PLSD test. In panel C, UCP3 mRNA level is plotted against the GLUT4 protein level for each mice line. The regression line for the relationship is shown; $r = 0.968$, $p = 0.007$.

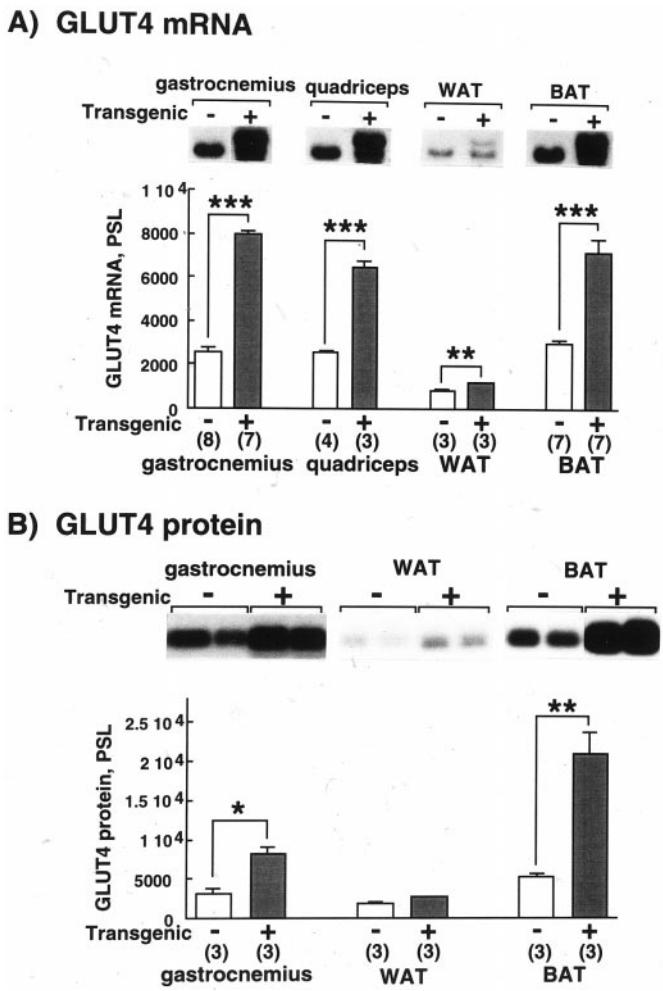

FIG. 2. Expression of GLUT4 mRNA (Panel A) and GLUT4 protein (Panel B) in −1000 GLUT4 minigene transgenic mice. Tissues from 4-month old male heterozygous, −1000 GLUT4 minigene transgenic mice and their control littermates were used for RNA (Panel A) or membrane protein (Panel B) preparation. In panel A, total RNA was extracted from gastrocnemius muscle, quadriceps muscle, WAT and BAT under feeding conditions and subjected to Northern Blotting, using a ^{32}P-labeled mouse GLUT4 cDNA as the probe. Since the minigene GLUT4 had a 281 bp "TAG" of foreign DNA inserted into the 3′ untranslated region of the GLUT4 gene (29), minigene transcripts appeared slightly larger than the 2.7 kb endogenous GLUT4 transcript. mRNA levels were quantified using an image analyzer. A typical autoradiogram and the quantification of GLUT4 mRNA levels in each tissue are shown. In panel B, crude membrane fractions from gastrocnemius (50 μg protein per lane), WAT (10 μg protein per lane) and BAT (10 μg protein per lane) were subjected to SDS/PAGE. GLUT4 protein was detected by immunoblotting and quantified. A representative autoradiogram and relative amount of GLUT4 protein levels are shown. Each data point represents mean ± SE of 3-8 mice (shown in parentheses). Statistical differences are shown as *P < 0.05, **P < 0.01 and ***p < 0.001, compared with control, non-transgenic mice by unpaired Student's t-test.

mice, the oxygen consumption of mice expressing the −1000 GLUT4 minigene both during the day and at night was increased by 19% ($p = 0.08$) and 16% ($p < 0.05$), respectively (Fig. 4). Body weights, however, of transgenic and non-transgenic mice did not differ (non-transgenic, 24.3 ± 1.0 g, n = 9; transgenic, 23.7 ± 1.1 g, n = 7). Plasma and urine metabolites which provide a

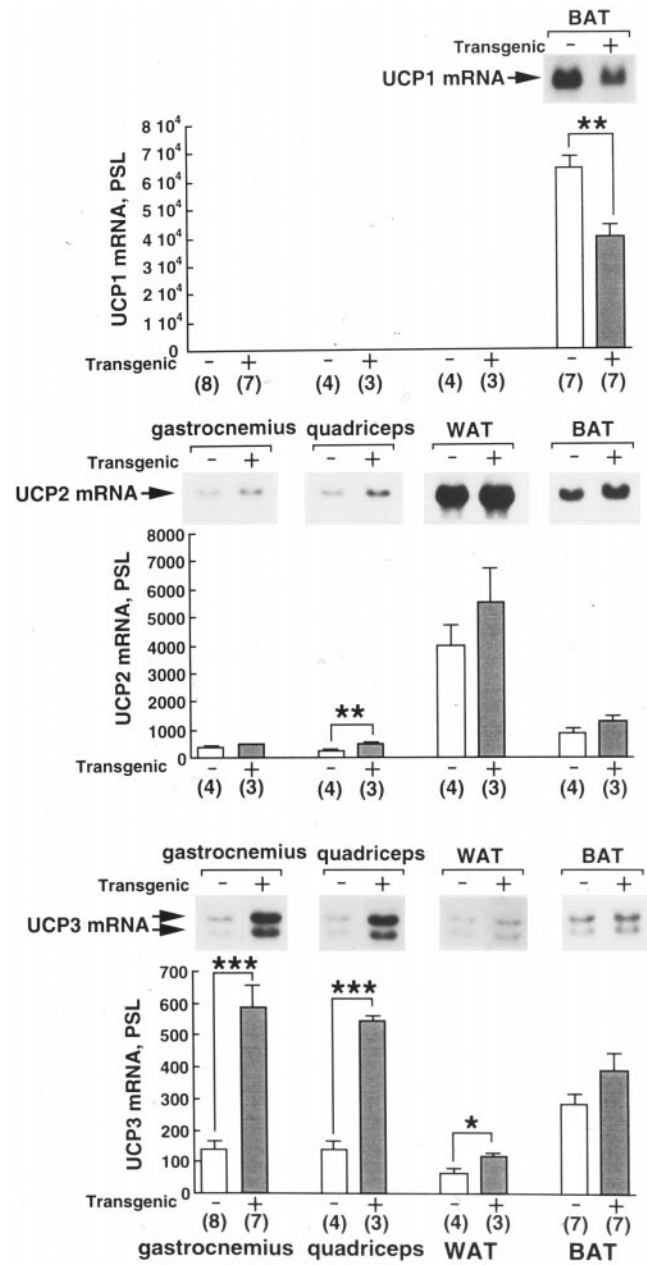

FIG. 3. Expression of UCP1, UCP2 and UCP3 mRNA in −1000 GLUT4 minigene transgenic mice. Tissues from 4-month old male heterozygous transgenic mice and their control littermates were used for preparation of total RNA. The same membrane sheets used in Fig. 2A were probed with ^{32}P-labeled mouse UCP1, human UCP2 and human UCP3 cDNAs. These mRNA levels were quantified using an image analyzer. A typical autoradiogram and quantification of UCP mRNAs from gastrocnemius muscle, quadriceps muscle, WAT and BAT are shown. Each data point represents mean ± SE of 3-8 mice (shown in parentheses). Statistical differences are shown as *P < 0.05, **P < 0.01 and ***p < 0.001, compared with control, non-transgenic mice by unpaired Student's t-test.

measure of the metabolic state in transgenic and non-transgenic mice were assessed under feeding conditions (Table 1). As a result of increased glucose uptake, blood glucose concentration was 14% lower and lactate concentration was 68% higher in transgenic mice than in non-transgenic mice. Blood insulin and glucagon concentrations did not differ between the two groups. The concentration of FFA, T3, corticosterone and leptin, which had been reported to up-regulate UCP3 mRNA in skeletal muscles, were not increased in transgenic mice. Rather, T3 and leptin levels were 16% and 43% lower in transgenic mice. No difference in β-OHB or FFA level was found, indicating similar levels of fatty acid oxidation in the two groups.

To determine whether the down-regulation of UCP1 observed in BAT from −1000 GLUT4 transgenic mice was due to overexpression of GLUT4 in BAT, UCP1 mRNA level in −442 GLUT4 transgenic mice was examined. It should be noted that this mouse line expressed minigene GLUT4 in skeletal muscle, but not in BAT or WAT (19). In contrast to the results with −1000 GLUT4 transgenic mice, UCP1 mRNA level in BAT from −442 GLUT4 transgenic mice did not differ from that of non-transgenic littermates (non-transgenic, 126972 ± 1681 PSL, n = 3; transgenic, 128541 ± 6791 PSL, n = 3).

TABLE 1

Comparison of Plasma Hormone and Metabolite and Urinary Catecholamine Levels between Non-transgenic and GLUT4 Minigene Transgenic Mice

	Nontransgenic	Transgenic
Glucose, mmol/l	8.37 ± 0.2 (n = 11)	7.22 ± 0.2 (n = 14)***
Lactate, mmol/l	2.43 ± 0.24 (n = 6)	4.08 ± 0.43 (n = 6)**
Insulin, pmol/l	0.24 ± 0.03 (n = 12)	0.22 ± 0.03 (n = 7)
Glucagon, ng/l	38.8 ± 4.5 (n = 5)	56.3 ± 6.7 (n = 3)
FFA, mEQ/l	0.58 ± 0.04 (n = 12)	0.60 ± 0.07 (n = 10)
β-OHB, μmol/l	1011 ± 151 (n = 6)	765 ± 102 (n = 5)
T3, mmol/l	0.98 ± 0.04 (n = 5)	0.82 ± 0.05 (n = 3)*
Corticosterone, ng/ml	159 ± 18 (n = 5)	190 ± 12 (n = 3)
Leptin, ng/ml	3.0 ± 0.5 (n = 6)	1.7 ± 0.3 (n = 6)*
Dopamine, ng/d	756 ± 101 (n = 5)	1039 ± 125 (n = 5)
Noradrenaline, ng/d	179 ± 26 (n = 5)	175 ± 20 (n = 5)
Adrenaline, ng/d	27.2 ± 4.7 (n = 5)	27.9 ± 4.2 (n = 5)
VMA, ng/d	332 ± 48 (n = 5)	395 ± 48 (n = 5)

Note. Values are means ± SE of values obtained in each of 3-14 mice of −1000 GLUT4 minigene male transgenic mice and their littermate, non-transgenic mice. Under feeding conditions, plasma T3 and corticosterone were measured at 9 wk; plasma glucose, lactate, insulin, glucagon, FFA, β-OHB and leptin at 13-15 wk. Urinary catecholamines were measured by collecting urine samples for 5 d in metabolic cages at 12-13 wk feeding. Thus, the standard error of urinary catecholamines excretion was from the variation of daily excretion, but not from that of the individual mouse. Statistical differences are shown as *P < 0.05, **P < 0.01 and ***P < 0.001 compared with non-transgenic mice by unpaired Student's t-test.

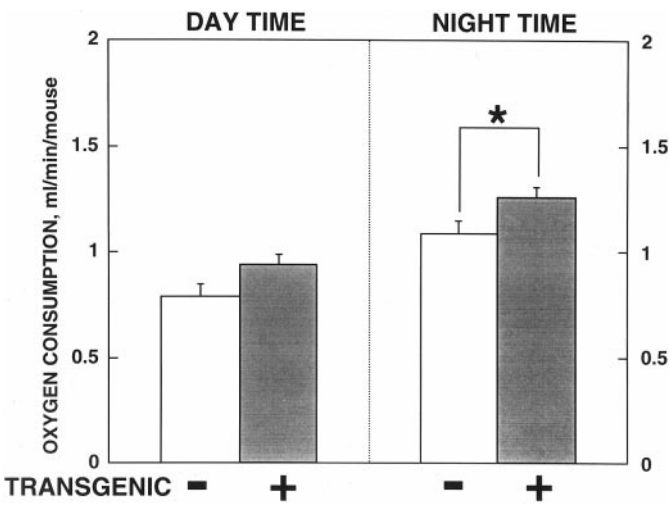

FIG. 4. Oxygen consumption. Oxygen consumption of 4-month old male heterozygous, −1000 GLUT4 minigene transgenic mice and of their control littermates was measured as described in "Experimental Procedures." The oxygen consumption rate was monitored at night (8:00 p.m.–6:00 am) and during the day (4:00 p.m.–6:30 p.m.) for 5 d, measured as the mean during each day and at night, and expressed as the average oxygen consumption per minute and per mouse. Each data point represents mean ± SE of the day. Thus, the standard error of oxygen consumption was from the variation of mean oxygen consumption of the day, but not that of individual mouse. Statistical differences are shown as *P < 0.05, compared with control, non-transgenic mice by unpaired Student's t-test.

DISCUSSION

In this paper we show that over-expression of GLUT4 in skeletal muscle and WAT of transgenic mice harboring a GLUT4 minigene leads to up-regulation of UCP3 in these tissues (Fig. 1). While FFA (9), T3 (5, 26), glucocorticoids (5) and leptin (27, 28) are known to increase UCP3 expression, changes in the levels of these factors in the bloodstream do not appear to be responsible for the increased expression of UCP3 observed in the present investigation. In fact, serum levels of T3 and leptin actually decreased in mice expressing the GLUT4 transgene (Table 1); moreover, serum levels of FFA and corticosterone were unaffected by overexpression of GLUT4 (Table 1). Rather, it appears that entry of glucose into skeletal myocytes and white adipocytes due to increased GLUT4 levels may be the cause of increased expression of UCP3.

Although results from other laboratories have shown that FFA's are potent inducers of UCP3 expression (5, 9), we believe that in the present investigation FFA is not the primary cause for the increased UCP3 expression found in transgenic mice expressing the GLUT4 minigene. Fasting, which leads to increased fatty acid oxidation, does produce a marked increase (about 19-fold) in UCP3 mRNA levels in skeletal muscle of C57BL/6J mice (n = 4; Tsuboyama-Kasaoka *et al.*, unpublished results). FFA turnover could be increased

in mice overexpressing GLUT4, despite the fact that the levels of FFA and β-OHB in the blood do not change. Thus, it is possible that such an increased FFA turnover caused the increased UCP3 expression in muscle. While FFA turnover studies have not been conducted in this exact context, the findings of other investigators suggest that increased FFA turnover is unlikely. Thus, it has been shown that transgenic mice, which overexpress human GLUT4 by 5- to 10-fold in insulin-sensitive tissues and which exhibit 50% and 63% increases in blood FFA and β-OHB, respectively (29), metabolize primarily carbohydrate (as measured by indirect calorimetry) during exercise and recovery (30). In the present study the transgenic mice overexpressing GLUT4 did not even exhibit increased FFA or β-OHB levels. Furthermore in a hyperinsulinemic, hyperglycemic clamp study (31) it was shown that increased glucose flux into skeletal muscle and WAT leads to an increased malonyl-CoA concentration, a known inhibitor of carnitine palmitoyl-transferase I and thereby of fatty acid oxidation (32). Taken together these investigations suggest that increased glucose flux into skeletal muscle and WAT, which slows fatty acid oxidation, may itself be responsible for the up-regulation of UCP3 expression.

The question remains, however, what might be the metabolic rationale for the up-regulation of UCP3 by increased glucose flux into skeletal muscle and WAT? The concentrations of ATP and ADP within the cell are tightly controlled, even when there are large changes in energy utilization. For example, a recent report examining ATP concentrations by ^{31}P magnetic resonance spectroscopy showed that the ATP concentration did not change despite large changes in ATP turnover rates induced by exercise (33). It is possible that the increased expression of UCP3 induced by an increased energy substrate supply (increased glucose flux) is another mechanism the cell uses to regulate cellular ATP levels by uncoupling oxidative phosphorylation. This mechanism would be another pathway to regulate cellular ATP levels in addition to the known effect of a decreased ADP concentration in the mitochondria to decrease the rate of respiration (34). Previously we speculated that the 14- to 18-fold increase of UCP3 expression in skeletal muscle observed 3 h after exercise is discontinued may have been caused by the increased FFA and glucose influx that would be occurring at that time (10). Thus, UCP3 expression may be stimulated when a large amount of glucose or FFA influx occurs in skeletal muscles, in order to prevent excessive production of ATP and to maintain ATP and ADP homeostasis.

Another important finding in this paper is that overexpression of GLUT4 in BAT causes down-regulation of UCP1. If GLUT4 is overexpressed only in muscle, as in the −442 GLUT4 transgenic mice, there is no change in UCP1 expression in BAT. Therefore, the decrease in UCP1 expression observed when GLUT4 is overexpressed in BAT must be due to the changes induced in the brown adipocyte by this increased GLUT4 expression, and not as a secondary effect of changes induced by overexpression of GLUT4 in muscle. These findings also indicate that the increase in UCP1 expression in BAT that is seen with cold exposure (35), noradrenaline administration (35), or T3 treatment (36) is not due to the concomitant increase in GLUT4 expression that also occurs. Rather, it is most likely that the increase in UCP1 and GLUT4 expression produced by cold exposure and noradrenaline administration might be mediated by cyclic AMP responsive elements in each gene, while T3 might increase these expression of these genes through thyroid hormone responsive element(s) in each gene (1, 37).

In conclusion, GLUT4 overexpression induces up-regulation of UCP3 mRNA in skeletal muscles. This finding indicates that in addition to fatty acids, increased glucose influx may up-regulate UCP3 mRNA and suggests that common metabolic changes of both substrates, such as increased tricarboxylic acid cycle and ATP production may be related to UCP3 mRNA up-regulation. We have also found that overexpression of GLUT4 in BAT decreases expression of UCP1. Further studies, including analysis of intracellular fatty acid and glucose metabolites in skeletal muscles and BAT will be necessary to identify the critical molecule which regulates UCP3 and UCP1 expression in these tissues.

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