Glucose and fat metabolism in adipose tissue of acetyl-CoA carboxylase 2 knockout mice

WonKeun Oh*, Lutfi Abu-Elheiga*, Parichher Kordari, Zeiwei Gu, Tattym Shaikenov, Subrahmanyam S. Chirala, and Salih J. Wakil†

Verna and Marrs McLean Department of Biochemistry and Molecular Biology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030

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***Acc2*$^{-/-}$ mutant mice, when fed a high-fat/high-carbohydrate (HF/HC) diet, were protected against diet-induced obesity and diabetes. To investigate the role of acetyl-CoA carboxylase 2 (ACC2) in the regulation of energy metabolism in adipose tissues, we studied fatty acid and glucose oxidation in primary cultures of adipocytes isolated from wild-type and *Acc2*$^{-/-}$ mutant mice fed either normal chow or a HF/HC diet. When fed normal chow, oxidation of [^{14}C]palmitate in adipocytes of *Acc2*$^{-/-}$ mutant mice was ≈80% higher than in adipocytes of WT mice, and it remained significantly higher in the presence of insulin. Interestingly, in addition to increased fatty acid oxidation, we also observed increased glucose oxidation in adipocytes of *Acc2*$^{-/-}$ mutant mice compared with that of WT mice. When fed a HF/HC diet for 4–5 months, adipocytes of *Acc2*$^{-/-}$ mutant mice maintained a 25% higher palmitate oxidation and a 2-fold higher glucose oxidation than WT mice. The mRNA level of glucose transporter 4 (GLUT4) decreased several fold in the adipose tissue of WT mice fed a HF/HC diet; however, in the adipose tissue of *Acc2*$^{-/-}$ mutant mice, it was 7-fold higher. Moreover, lipolysis activity was higher in adipocytes of *Acc2*$^{-/-}$ mutant mice compared with that in WT mice. These findings suggest that continuous fatty acid oxidation in the adipocytes of *Acc2*$^{-/-}$ mutant mice, combined with a higher level of glucose oxidation and a higher rate of lipolysis, are major factors leading to efficient maintenance of insulin sensitivity and leaner *Acc2*$^{-/-}$ mutant mice.**

Acc2$^{-/-}$ mutant | acetyl-CoA | carboxylase2 | malonyl-CoA

Acetyl-CoA carboxylase (ACC) catalyzes the ATP-dependent carboxylation of ACC to malonyl-CoA, the key intermediate in fatty acid synthesis (1–3). In animals, including humans, two carboxylase isoforms (ACC1 and ACC2) display distinct tissue distribution and are encoded by separate genes. ACC1 ($M_r \approx 265{,}000$) is the predominant isoform in lipogenic tissues such as liver, adipose tissue, and lactating mammary gland, although ACC2 ($M_r \approx 280{,}000$) is highly expressed in the skeletal muscle, heart, and, to a considerable degree, the liver (4–6). In lipogenic tissues, malonyl-CoA is the source of the C_2 units used for the synthesis of fatty acids by the fatty acid synthase. Malonyl-CoA is also considered a key regulator of fatty acid oxidation and energy metabolism, because it inhibits carnitine palmitoyltransferase I (CPT I) and hence mitochondrial fatty acid oxidation (7). Our studies provided the evidence that ACC2 is localized at the mitochondrial membranes of the heart, muscle, and liver; hence, the malonyl-CoA it synthesizes controls the transfer of the cytosolic long-chain fatty acyl-CoA to the mitochondria by an allosteric regulation of the membrane-bound CPT I (6, 7).

Recent studies suggest that a decrease in the level of malonyl-CoA during starvation or exercise is accompanied by increased fatty acid oxidation; however, in the fed state, increased malonyl-CoA concentration results in lower fatty acid oxidation (27). The sustained elevations of malonyl-CoA concentration bring about insulin resistance (less insulin sensitivity) in the muscle tissues of a wide variety of hyperglycemic and/or hyperinsulinemic animals such as the *fa/fa* rat and KKA^y mouse (8, 9). A high concentration of malonyl-CoA results in elevated levels of triglyceride (TG), diacylglycerol, and long-chain fatty acyl-CoA (10). It is suggested that the increased intracellular levels of these fatty acid derivatives cause a change in the distribution and/or activity of the PKC isoforms that result in a decrease in the translocation of glucose transporters (GLUTs) from the cytoplasm to the cellular membrane (10–12).

The primary role of adipose tissue is to store fuel lipids in the form of TG when energy intake exceeds expenditure. In addition, adipose tissue is a complex endocrine gland in which a variety of adipocytes and preadipocytes-derived proteins (adipokines) act both locally and distally to regulate fat cell differentiation and to adjust systemic energy balance (13). Leptin, among several adipokines, plays important roles in regulating food intake and energy expenditure. Recently, it has been shown that when leptin is induced in the adipose tissue of normal rats by adenovirus gene transfer, rapid depletion of fat and the down-regulation of lipogenic enzymes occur. On the other hand, the enzymes related to fatty acid oxidation, such as UCP1 and UCP2, normally expressed at low levels in adipose tissue, are highly increased in leptin-induced rats (14).

We have recently reported that *Acc2*$^{-/-}$ mutant mice continuously oxidize fatty acids and accumulate less fat than WT mice (15). *Acc2*$^{-/-}$ mutant mice, fed a HF/HC diet, weighed less than their WT cohorts, accumulated less fat, and maintained normal levels of insulin and glucose, whereas WT mice developed hyperglycemic and hyperinsulinemic conditions (16). To explore the role of ACC2 in adipose tissues in glucose homeostasis and fatty acid metabolism, we isolated adipocytes from *Acc2*$^{-/-}$ mutant mice and their WT cohorts and measured the oxidation of fatty acid and glucose. We also determined and compared the levels of mRNAs related to the oxidation of glucose and fatty acids, fatty acid transporters, and GLUTs by using real-time quantitative PCR.

Materials and Methods

Maintenance of *Acc2*-Deficient Mice. The mice were maintained on a 12-hr light/dark cycle at 25°C and had ad libitum access to normal chow (Purina) or a HF/HC diet (59% of calories derived from fat and 24% from carbohydrate, Bioserv, Frenchtown, NJ). Male *Acc2*$^{-/-}$ mutant mice and WT cohorts, fed a HF/HC diet for 4 mo, were used. Animal experiments were conducted in accordance with the Baylor College of Medicine's Animal Care and Use Guidelines.

Blood Constituents. Male *Acc2*$^{-/-}$ mutant mice and WT cohorts were fasted for 8 h, blood samples collected from their tails, and serum samples kept at −80°C until assayed. The levels of very low-density lipoprotein, TG, glucose, and cholesterol were mea-

Abbreviations: ACC, acetyl-CoA carboxylase; TG, triglyceride; GLUT, glucose transporter; HF/HC, high fat/high carbohydrate; KRB, Krebs–Ringer bicarbonate buffer.

*W.O. and L.A.-E. contributed equally to this work.

†To whom correspondence should be addressed. E-mail: swakil@bcm.tmc.edu.

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Table 1. Primers used for real-time quantitative PCR

Gene	Forward primer (5′–3′)	Reverse primer (5′–3′)
GLUT1	CTTCTCTGTCGGCCTCTTTGT	ACAGCTCCAAGATGGTGACCT
GLUT2	CTATGACGTCAATGGCACAGA	AGAGCAGTAGCAGACACTGCA
GLUT4	TCGTCATTGGCATTCTGGTTG	AGCTCGTTCTACTAAGAGCAC
ATP synthase	GTCCCGGGCTATTGCTGAGTTG	TCCCCATGTGACCCGTGAAGA
ACO	AAACCTTCAGGCCCAAGTGAG	GGTGGACCTCTGTCTTGTTCA
L-CPT	GGGCTGCACTCCTGGAAGAAGAA	CTCGGCCCCGCAGGTAGATGTA
LCACD	GAAGATGTCCGATTGCCAGCT	GCTGTCACTGACGATCTGTCT
PPAR-γ	CCAACTTCGGAACTCAGCTCTG	AACCTGATGGCATTGTGAGACA
FABP	GTGATGCCTTTGTGGGAACCT	ACTCTTGTGGAAGTCGCCT
HSL	ACGGATACCGTAGTTTGGTGC	TCCAGAAGTGCACATCCAGGT
β-actin	GACATGGAGAAGATCTGGCA	GGTCTTTACGGATGTCAACGT

ACO, acyl-CoA oxidase; L-CPT, liver type carnitine palmitoyltransferase; LCACD, long-chain acyl-CoA dehydrogenase; PPAR-γ, peroxisome proliferator-activated receptor γ; FABP, fatty acid-binding protein; HSL, hormone-sensitive lipase.

sured by the Comparative Pathology Laboratory at Baylor College of Medicine by using standard procedures.

Concentration of Malonyl-CoA in Adipose Tissue. The animals were killed by cervical dislocation, and tissues were removed and pulverized under liquid nitrogen. The tissues were then homogenized with 6% $HClO_4$ (5 ml/g tissue) and the extracts neutralized to pH 6.0 with 2 M KOH. The malonyl-CoA content was measured by incorporating [^{3}H]acetyl-CoA to [^{3}H]palmitate in the presence of NADPH and purified chicken fatty acid synthase (15).

Determination of ACC Activity and Its Phosphorylation Status. The adipose tissue was homogenized in lysis buffer (100 mM Tris buffer, pH 7.4/1 mM EDTA/100 mM NaCl/2 mM Na_3VO_4/10 mM β-glycerophophate/10 mM NaF/1 mM DTT/2 mM PMSF/5 μg/ml aprotinin/5 μg/ml leupeptin). The homogenate was centrifuged for 15 min at 12,000 $\times$ *g*, and the supernatant solution was fractionated with 30% ammonium sulfate. The pellets were dissolved in 0.1 M Tris·HCl buffer (pH 7.5) containing 5% glycerol, 0.5 M NaCl, and protease inhibitors and used for ACC assay and Western blot analysis. ACC activity was assayed by measuring the incorporation of [^{14}C]HCO_3^- into malonyl-CoA (17). The immunoblot analysis for the phosphorylation status of the ACCs was carried out by using antiphospho-ACC (Ser-79, Upstate Biotechnology) as the primary antibody and horseradish peroxidase goat anti-rabbit as the secondary antibody.

Adipocytes Isolation and Culture. Epididymal fat pads (three to four) from each of the *Acc2*$^{-/-}$ mutant mice and WT cohorts were removed under sterile conditions, washed with saline solution, minced, and incubated at 37°C with shaking for 45 min in Krebs–Ringer Hepes buffer containing 1 mg/ml collagenase (C-6885, Sigma) and 2% BSA. The digested tissues were filtered through a sterile 200-μm nylon mesh and washed three times with Krebs–Ringer bicarbonate buffer (KRB). The adipocytes (2×10^5 cells per well) were resuspended in 1 ml of KRB and maintained for 30 min in sterile polypropylene tube before the start of the assays, as described (18).

Fatty Acid Oxidation in Adipocytes. To determine fatty acid oxidation, the media of adipocytes were replaced with 1 ml of KRB containing 1.0 mM [^{14}C]palmitic acid [specific acivity 1 mCi/mmol (1 Ci = 37 GBq)] and gassed for 30 sec under humidified 95/5% O_2/CO_2. The polypropylene tube was capped with a rubber stopper having a centered well with a loosely folded filter paper moistened with 0.2 ml of benzethonium solution. After incubation for 4 h at 37°C, the reaction was stopped by injecting 0.3 ml of H_2SO_4 (4 M), and the radioactivity trapped in the filter paper was determined (19, 20).

Lipolysis Measurements. Freshly isolated adipocytes (2×10^5 cells per tube) were incubated at 37°C for 0–180 min with gentle shaking in 0.5 ml of KRB containing 2 units/ml adenosine deaminase (P-9876, Sigma) in the absence or presence of isoproterenol (10 μM). Aliquots of the reaction mixture (50 μl) were withdrawn after 1, 2, and 3 h of incubation and placed on ice. Glycerol levels were measured by the enzymatic method by using the GPO-Tinder kit (Sigma) (21).

Glucose Oxidation in Adipocytes. Adipocytes (2×10^5 cells per well) were incubated in a polypropylene tube with 1 ml of KRB containing 5.6 mM [^{14}U]-glucose (0.5 μCi). After 4 h of incubation at 37°C, 0.3 ml of H_2SO_4 (4 M) was added to stop the reaction, and the radioactivity trapped in the filter paper was measured (18, 19).

Real-Time Quantitative PCR. Total RNA was isolated from the epididymal fat pads with TRIzol reagent (Invitrogen). The cDNA was synthesized in 20-μl volume containing 5 μg of RNA, 0.25 μg of random hexamer, 200 μM dNTPs, 10 mM DTT, 20 units of RNase inhibitor, and 0.5 μl of SuperScript II enzyme (Invitrogen). Real-time PCR was performed with the DNA Engine Opticon 2 system by using Dynamo SYBR Green I Master Mix reagent (MJ Research, Cambridge, MA). Specific primers used for synthesis and amplification of the cDNA of interest are shown in Table 1. RNA samples were normalized for comparison by determining β-actin levels in real-time quantitative PCR. The relative quantification of each gene was calculated by using the $2^{-\Delta CT}$ formula, in which $\Delta\Delta C_T$ equals the difference between C_T values of mRNA from *Acc2*$^{-/-}$ mutant mice and WT cohorts (22).

Northern Blot Analysis. Total RNA (6–8 μg) was subjected to 1% agarose gel in the presence of formalin. The fractionated RNA was transferred to Hybond N (Amersham Pharmacia Biotech), and the filters were hybridized with ^{32}P-labeled cDNA probes for GLUT1, GLUT2, and GLUT4, as well as β-actin RNA to ensure equal loading.

Results

ACC Activity and Malonyl-CoA Levels in White Adipose Tissues. We reported previously that the concentration of malonyl-CoA is greatly reduced in the tissues of *Acc2*$^{-/-}$ mutant mice where ACC2 is the dominant isoform, such as in the heart and skeletal

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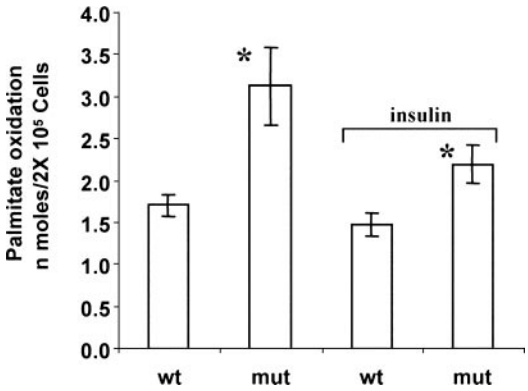

Fig. 1. Fatty acid oxidation in adipocytes prepared from epididymal fat pads of WT and *Acc2*$^{-/-}$ mutant mice. Adipocytes (2×10^5 cells) were cultured with shaking in sterile polypropylene tube. Fatty acid oxidation was measured in KRB with 1.0 mM [^{14}C]palmitic acid [specific activity 1 mCi/mmol] complexed with defatted BSA. Data are expressed as means ± SD of three determinations. *, $P < 0.01$, WT vs. mutant.

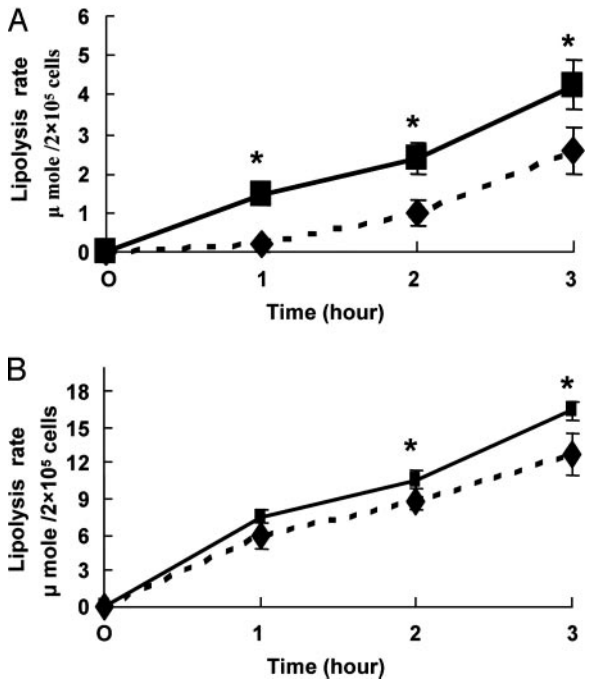

Fig. 2. Determination of lipolysis rate on adipocytes prepared from epididymal fat pads of WT and *Acc2*$^{-/-}$ mutant mice. Freshly isolated adipocytes (2×10^5 cells per tube) were incubated for 0–180 min in the absence (*A*) or presence (*B*) of isoproterenol. The rate of lipolysis was determined by measurement of infranatants. Data are expressed as means ± SD of three determinations. *, $P <$ 0.01, WT vs. mutant.

muscle; however, there was little change of its concentration in the liver (15). To decipher the roles of ACC1 and ACC2 in adipose tissue and their respective contributions to the overall level of malonyl-CoA, we measured the malonyl-CoA levels in the adipose tissues of the mutant and WT mice fed a normal diet. There was no significant difference (1.00 ± 0.34 nmol/g and 1.05 ± 0.30 nmol/g, respectively). These results confirm that, as in the liver, ACC1 is the dominant isoform in white adipose tissue and the major contributor of malonyl-CoA levels in this tissue. In addition, we found that the total ACC activities in extracts of epididymal fat pads from *Acc2*$^{-/-}$ mutant and WT mice were also similar (4.63 ± 1.09 and 4.20 ± 1.23 nmol/min per mg of protein, respectively). We also did not detect any significant changes in the level of the ACC1 protein or its phosphorylation status, suggesting that ACC1 is the major contributor to the overall enzymatic activity and to the malonyl-CoA level in the adipose tissue (data not shown).

Fatty Acid Oxidation in Adipocytes. Although the main role of adipose tissue in lipid metabolism is lipid synthesis and storage, the presence of ACC2 enzyme in this tissue suggests that it plays a role in regulating fatty acid oxidation. To assess that role, we cultured adipocytes from WT and *Acc2*$^{-/-}$ mutant mice fed a normal chow or a HF/HC diet for 4–5 mo. When fed a normal chow, as shown in Fig. 1, the oxidation of [^{14}C]-palmitate in cultured adipocytes of *Acc2*$^{-/-}$ mutant mice was ≈80% higher than that of WTs (3.12 ± 0.46 nmol vs. 1.71 ± 0.12 nmol/2 × 10^5 cells, respectively). The incubation of cultured adipocytes with 0.2 μM of insulin for 4 h reduced palmitate oxidation in both mutant and WT cell types; however, the oxidation level in mutant adipocytes remained ≈50% higher than in the WT cells (2.20 ± 0.23 vs. and 1.47 ± 0.14 nmol/2 × 10^5 cells, respectively; Fig. 1). These results imply that, despite the very low level of ACC2 and its contribution to the overall amount of intracellular malonyl-CoA, ACC2 does regulate fatty acid oxidation in the epididymal fat pads.

Lipolysis Levels. Triacylglyceride, stored in adipose tissue, plays a major role in providing energy to most tissues during fasting or increased energy demand situations. That the adipocytes of mutant mice have a higher fatty acid oxidation may warrant an increased demand for fat stored in adipose tissues and a determination of the lipolysis rate in adipocytes isolated from the epididymal fat pads of *Acc2*$^{-/-}$ mutant and WT mice. The lipolysis rate in the adipocytes of *Acc2*$^{-/-}$ mutant mice after 2-h cultivation was ≈2.4 times higher than in WT cells (1.00 ± 0.33 vs. 2.38 ± 0.38 μmol/2 × 10^5 cells, respectively). Addition of isoprenalin, a β-agonist, for 2 h, to the adipocytes of WT and *Acc2*$^{-/-}$ mutant mice increased the rate of lipolysis (8.78 ± 0.56 vs. 10.60 ± 0.81 μmol/2 × 10^5 cells, respectively, as shown in Fig. 2).

Glucose Oxidation in Adipocytes. Glucose and fatty acid metabolism are interrelated through their common intermediate, acetyl-CoA. Under normal physiological conditions, the oxidation of fatty acids causes significant inhibition of glucose oxidation (Randle cycle) (23). *Acc2*$^{-/-}$ mutant mice fed a HF/HC diet not only accumulated less fat but also had a significantly lower blood glucose level than that of their WT cohorts. We determined the impact of increased fatty acid oxidation in the adipocytes of *Acc2*$^{-/-}$ mutant mice on glucose oxidation and compared it with that in the adipocytes of WT mice. Oxidation of [^{14}C]-glucose in the adipocytes of *Acc2*$^{-/-}$ mutant mice was ≈50% higher than that of WTs (16.72 ± 3.75 vs. 11.23 ± 1.26 nmol/2 × 10^5, respectively, as seen in Fig. 3). Addition of 0.2 μM insulin increased glucose oxidation in the adipocytes of WT and *Acc2*$^{-/-}$ mutant mice by ≈40% (15.53 ± 0.67 and 24.05 ± 5.41 nmol/2 × 10^5 cells, respectively), as was expected. These results clearly showed that, despite a significant increase in fatty acid oxidation in adipocytes of *Acc2*$^{-/-}$ mutant mice, glucose oxidation was maintained at a higher level than in WT mice.

The Effect of Continuous Oxidation on *Acc2*$^{-/-}$ Mutant Mice Fed a HF/HC Diet. We further investigated the physiological changes of *Acc2*$^{-/-}$ mutant and WT mice fed a HF/HC diet. As expected, *Acc2*$^{-/-}$ mutant mice weighed ≈20% less than WT mice, and had 50% less serum glucose levels (Table 2). Their TG blood level was also significantly lower than their cohorts' (94.6 ± 22.7 and 169 ± 24.9 mg/dl, respectively), as was their very low-density

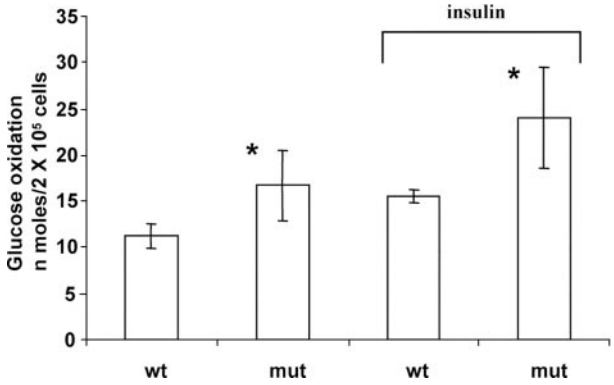

Fig. 3. Glucose oxidation in adipocytes prepared from epididymal fat pads of WT and *Acc2*$^{-/-}$ mutant mice. Glucose oxidation was measured by using [^{14}C]-U-glucose. The radioactivity trapped in the filter paper was calculated as a result of glucose oxidation. The data are shown as means ± SD of three determinations. *, $P < 0.03$, WT vs. mutant.

lipoprotein concentration (18.9 ± 4.6 and 33.8 ± 11.1 mg/dl, respectively). However, levels of serum cholesterol were similar or slightly increased (175.4 ± 27.2 mg/dl and 200.4 ± 0.1 mg/dl for WT and *Acc2*$^{-/-}$ mutant mice, respectively). In adipocyte isolated from *Acc2*$^{-/-}$ mutant mice fed a HF/HC diet, palmitate oxidation was ≈25% higher than that of WTs (1.69 ± 0.09 vs. 1.35 ± 0.11 nmol/2 × 10^5 cells, respectively; Fig. 4*A*). The insulin had little effect on fatty acid oxidation in the adipocytes isolated from mice fed a HF/HC diet; however, oxidation of fatty acid by adipocytes from *Acc2*$^{-/-}$ mutant mice remained ≈30% higher than that by adipocytes from WT mice (1.74 ± 0.15 vs. 1.30 ± 0.05 nmol/2 × 10^5 cells, respectively). Interestingly, glucose oxidation in the adipocytes of *Acc2*$^{-/-}$ mutant mice was ≈2-fold higher than that of WT mice (8.30 ± 1.15 vs. 4.10 ± 0.87 nmol/2 × 10^5 cells), and the addition of insulin increased glucose oxidation slightly in both adipocytes (8.99 ± 0.96 and 4.77 ± 0.36 nmol/2 × 10^5 cells, respectively, as Fig. 4*B* shows).

The Expression Levels of Genes Involved with Energy Metabolism in Adipose Tissue. To study the effect of increased fatty acid oxidation in adipose tissue on the expression levels of genes related to obesity and diabetes, we isolated RNA from the white adipose tissue of *Acc2*$^{-/-}$ mutant and WT mice fed either normal chow or a HF/HC diet. We used real-time quantitative PCR and Northern blot analysis to compare the expression levels of genes such as GLUTs and several others related to the carbohydrate and fat metabolic pathways. There was up-regulation of genes related to energy metabolisms such as ATP synthase (1.76 ± 0.4-fold) and the uncoupling protein 2 (UCP2) (1.71 ± 0.1-fold,

Table 2. Serum constituents of WT and *Acc2*$^{-/-}$ mutant mice fed HF/HC diet

	WT (n = 9)	Mutant (n = 8)
Weight, g	35.8 ± 4.9	29.1 ± 3.2^{+++}
Adipose weight, g	1.6 ± 0.4	0.5 ± 0.2^{+++}
Plasma VLDL, mg/dl	33.8 ± 11.1	18.9 ± 4.6^{+}
Plasma TG, mg/dl	169 ± 24.9	94.6 ± 22.7^{++}
Plasma glucose, mg/dl	98.8 ± 14.0	66.7 ± 4.8^{+}
Plasma cholesterol, mg/dl	175.4 ± 27.2	200.4 ± 0.1

Blood was collected from tail veins, and serum was collected after separation from blood. Glucose, TG, very low-density lipoprotein (VLDL), and cholesterol were determined as described in *Materials and Methods*. The data are shown as mean ± SD. +, $P < 0.001$; ++, $P < 0.005$; +++, $P < 0.05$, WT vs. mutant.

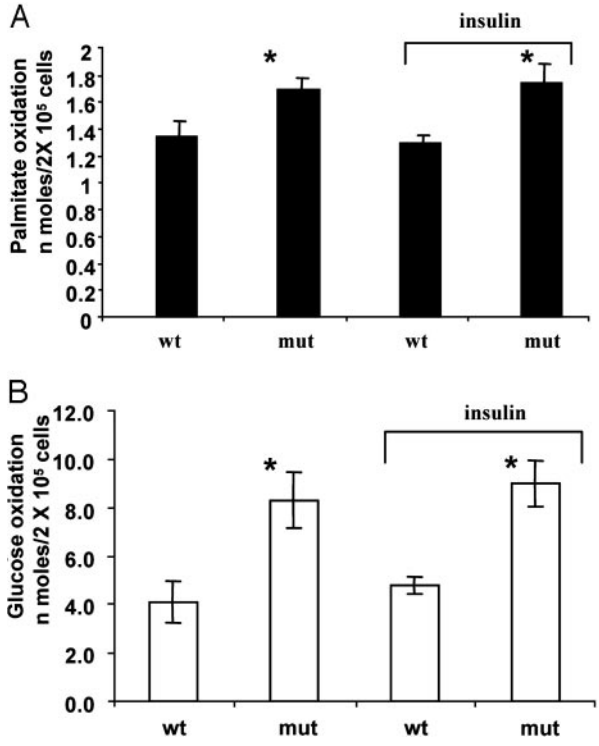

Fig. 4. Fatty acid and glucose oxidation in adipocytes prepared from epididymal fat pads of WT and *Acc2*$^{-/-}$ mutant mice fed a HF/HC diet. The adipocytes were isolated from 5- to 6-mo-old *Acc2*$^{-/-}$ mutant mice and WT cohorts fed a HF/HC diet for 4 mo. The data are shown as means ± SD of two determinations. *, $P < 0.01$; +, $P < 0.05$, WT vs. mutant.

data not shown). The lower glucose levels in the blood of *Acc2*$^{-/-}$ mutant mice, compared with those found in WT mice fed a HF/HC diet, prompted us to determine the levels of GLUT genes. We measured the mRNA levels of genes such as GLUT1, GLUT2, and GLUT4 in adipose tissues of WT and *Acc2*$^{-/-}$ mutant mice. mRNA levels of GLUT1 and GLUT2 were similar in WT and *Acc2*$^{-/-}$ mutant mice fed a normal chow (Table 3). Interestingly, the mRNA level of GLUT4 was ≈7-fold higher in the adipose tissue of *Acc2*$^{-/-}$ mutant mice fed a HF/HC diet

Table 3. Expression levels of various genes in epididymal fat pads of WT and *Acc2*$^{-/-}$ mutant mice

	mRNA levels ($2^{-\Delta\Delta CT}$)	
	Normal chow	HF/HC diet
GLUT1	0.96 ± 0.05	1.04 ± 0.02
GLUT2	0.91 ± 0.16	1.29 ± 0.03
GLUT4	1.30 ± 0.04*	7.70 ± 0.67*
ATP synthase	1.24 ± 0.02	1.76 ± 0.05*
ACO	1.10 ± 0.02	0.92 ± 0.07
L-CPT	0.98 ± 0.13	0.96 ± 0.03
LCACD	1.30 ± 0.02*	2.22 ± 0.08*
PPAR-γ	1.20 ± 0.16	1.29 ± 0.27
FABP	0.86 ± 0.05	1.39 ± 0.05
HSL	0.94 ± 0.03	1.02 ± 0.04

The ratio of mRNA encoding each gene of interest to that of β-actin was calculated and normalized. Each mRNA was isolated from three mice of WT and *Acc2*$^{-/-}$ mutant origins, and they were measured by real-time quantitative-PCR. Relative quantification of each gene was calculated by using the $2^{-\Delta\Delta CT}$ formula. Data are expressed as means ± SD of three determinations. *, $P < 0.05$.

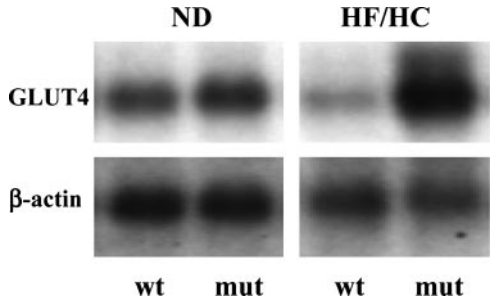

Fig. 5. Expression of GLUT4 in adipose tissues of WT and *Acc2*$^{-/-}$ mutant mice. Total RNA (5–6 μg) was prepared from white adipose tissues of WT and *Acc2*$^{-/-}$ mutant mice fed either normal or HF/HC diet. RNA was subjected to 1% agarose electrophoresis in the presence of formalin and transferred to Hybond N filters. The filters were hybridized with ^{32}P-labeled cDNA probes of GLUT4 and β-actin RNA.

than for WT mice fed the same diet. These results were further confirmed by Northern blot analysis (Fig. 5).

Discussion

Acc2$^{-/-}$ mutant mice fed a normal chow had a higher food intake, weighed less, and had ≈50% fewer epididymal fat pads than their WT cohorts. In addition, they were resistant to obesity and diabetes when fed a HF/HC diet, and they maintained normal glucose and insulin levels compared with WT mice, which became obese and developed type 2 diabetes (15, 16). Reduction in the mass of adipose tissue of the *Acc2*$^{-/-}$ mutant mice can be attributed to (*i*) increase of fatty acid oxidation in adipose tissue as a result of the absence of ACC2; (*ii*) increased lipolysis and transport of fatty acids for oxidation in muscle, heart, and other tissues; and (*iii*) decreased mobilization of lipids from the liver to be stored in adipose tissues. Adipocytes isolated from the fat pads of *Acc2*$^{-/-}$ mutant mice had significantly higher palmitate oxidation than that of WT mice. Interestingly, the overall ACC enzymatic activity, ACC1 protein levels, their phosphorylation status, and levels of malonyl-CoA were similar in white adipose tissues from WT and *Acc2*$^{-/-}$ mutant mice. These results strongly suggest that ACC1, which remained intact in the mutant, is the main contributor of malonyl-CoA for fatty acid synthesis in this lipogenic tissue, as is the case in the liver (15). On the other hand, there was a 780% increase in fatty acid oxidation in the adipocytes of *Acc2*$^{-/-}$ mutant mice, most likely due to the relief of carnitine palmitoyltransferase I activity as the absence of ACC2-generated malonyl-CoA. Hence, in adipose tissue, ACC1 and ACC2 play major roles in regulating fatty acid synthesis and oxidation rates with regard to energy homeostasis. Diet, hormones, and other physiological factors regulate the activities of both ACCs. Regulation can be at the level of gene expression or by modulation of enzymatic activities, either through allosteric activation by citrate or by the phosphorylation/dephosphorylation of specific serine residues by the AMP-activated protein kinase (24–26). Insulin activates ACCs by promoting their dephosphorylation, whereas glucagon and epinephrine inactivate the enzymes by promoting phosphorylation (17, 24, 26). Addition of insulin to their cultured adipocytes decreased fatty acid oxidation in both *Acc2*$^{-/-}$ mutant and WT mice; however, the level of oxidation remained 50% higher in mutant adipocytes than in WT mice (Fig. 1). Recently, the Unger laboratory reported (14) that overexpression of leptin in white adipose tissue of rats transformed the animals into fat-oxidizing machines. The study reported the main reason for the increased fatty acid oxidation was an increase in AMP-activated protein kinase that phosphorylated and consequently inactivated both ACC1 and ACC2. Another major player in the regulation of fatty acid metabolism is malonyl-CoA decarboxylase (MCD), which degrades malonyl-CoA. Ruderman *et al.* (27) reported that after exercise, there was a rapid decrease in the malonyl-CoA concentration of white adipose tissue. This decrease was due to the activation of AMP kinase that resulted in the inhibition of ACC1 and ACC2 activities and 2-fold activation of MCD. We believe the increase of fatty acid oxidation in the adipocytes of *Acc2*$^{-/-}$ mutant mice is mainly due to a lack of inhibition of carnitine palmitoyltransferase I by malonyl-CoA that would normally be generated by ACC2 in the outer membrane of the mitochondria. Hence, these results confirm the different roles of ACC1 and ACC2 and the compartmentalization of malonyl-CoA within the cell.

Another factor that may contribute to the lean phenotype of the *Acc2*$^{-/-}$ mutant mice is the decrease in fatty acid mobilization from the liver to the adipose tissue as a result of higher fatty acid oxidation in the liver and a higher rate of lipolysis to provide fuel needs to the heart, muscle, and brain. TG, stored in the adipose tissue, plays a major role in providing energy to most tissues in times of fasting or for increased energy demands. We determined the lipolysis ratio in adipocytes isolated from epididymal fat pads of *Acc2*$^{-/-}$ mutant and WT mice. The lipolysis rate in cultured adipocytes was significantly higher in *Acc2*$^{-/-}$ mutant mice, in both the absence and presence of the β-agonist, isoprenalin. In the fasting state and in the presence of a higher demand for energy (under exercise), fatty acids are predominantly generated within the adipose tissue by the action of hormone-sensitive lipase on stored TG. These fatty acids are then mobilized out of the adipose tissue into the systemic circulation for delivery to other tissues, such as skeletal muscle, heart, and liver. The increased lipolysis rate in the adipocytes of *Acc2*$^{-/-}$ mutant mice suggests that the white adipose tissue in these mice is well adapted to respond to a higher demand for energy supply by other tissues, such as the muscle and heart.

The interrelationship between glucose and free fatty acid metabolism, as proposed by Randle *et al*. (23), is that essentially an increased availability of one substrate would suppress oxidation of the other. Increased FFA availability results in increased acetyl-CoA, which inhibits pyruvate dehydrogenase. The increased availability of carbohydrate increases glucose oxidation, and this results in the increased synthesis of malonyl-CoA by ACC1 and ACC2. Glucose and insulin also decrease fatty acid oxidation by controlling the rate of fatty acid entry into the mitochondria. Insulin stimulates glucose uptake in tissues such as muscle and adipose, activates ACC1 and ACC2, promotes fatty acid synthesis, and inhibits fatty acid oxidation. In the adipocytes of *Acc2*$^{-/-}$ mutant mice, there was a simultaneous increase in glucose and fatty acid oxidations. These results suggest that, due to the lack of malonyl-CoA by ACC2 in the mutants, the adipose tissue of *Acc2*$^{-/-}$ mutant mice continued to synthesize fatty acids without inhibiting oxidation in contrast to the adipose tissue of WT mice where both ACC1 and ACC2 were activated by glucose and insulin (28).

We determined the mRNA levels of genes related to GLUTs such as GLUT1, GLUT2, and GLUT4 in the adipose tissues of WT and *Acc2*$^{-/-}$ mutant mice. The mRNA levels of GLUT1 and GLUT2 were similar, but the mRNA level of GLUT4 was significantly higher (7.70 ± 0.67-fold) in the adipose tissue of *Acc2*$^{-/-}$ mutant mice fed a HF/HC diet (Table 3). These results are in agreement with the previous finding that showed the mRNA level of GLUT4 to be significantly down-regulated in mice that are chronically fed a HF/HC diet (29). In addition to the changes in GLUTs, we have identified several genes that are up- or down-regulated as a result of the ACC2 null mutation in mice fed normal chow or a HF/HC diet. There was up-regulation of genes in fatty acid oxidation pathways such as the long-chain acyl-CoA dehydrogenase, ATP synthase, and UCP2. The level of hormone-sensitive lipase (HSL) was similar in the adipose tissues of WT and *Acc2*$^{-/-}$ mutant mice, suggesting that the

higher lipolytic activity that we observed in the mutant adipocytes may be due to a higher activity of HSL caused by phosphorylation of the enzyme by AMP-dependent PKA (30).

Taken together, the ACC2-knockout mutant studies reported here and elsewhere (15, 16) strongly suggest it is not the overall reduction of malonyl-CoA levels but a reduction in the ACC2-produced malonyl-CoA levels that is critical in increasing fatty acid oxidation. These results strongly support our conclusion that ACC2 would be a useful target in the treatment of obesity and related metabolic diseases such as hyperlipidemia, insulin resistance, and diabetes.

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