

Induced Adiposity and Adipocyte Hypertrophy in Mice Lacking the AMP-Activated Protein Kinase- α 2 Subunit

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AMP-activated protein kinase (AMPK) is considered as a cellular energy sensor that regulates glucose and lipid metabolism by phosphorylating key regulatory enzymes. Despite the major role of adipose tissue in regulating energy partitioning in the organism, the role of AMPK in this tissue has not been addressed. In the present study, we subjected AMPK α 2 knockout (KO) mice to a high-fat diet to examine the effect of AMPK on adipose tissue formation. Compared with the wild type, AMPK α 2 KO mice exhibited increased body weight and fat mass. The increase in adipose tissue mass was due to the enlargement of the preexisting adipocytes with increased lipid accumulation. However, we did not observe any changes in adipocyte marker expression, such as peroxisome proliferator-activated receptor- γ , CCAAT/enhancer-binding protein α (C/EBP α) and adipocyte fatty acid-binding protein (aFABP/aP2), or total cell number. Unlike impaired glucose homeostasis observed on normal diet feeding, when fed a high-fat diet AMPK α 2 KO mice did not show differences in glucose tolerance and insulin sensitivity compared with wild-type mice. Our results suggest that the increase in lipid storage in adipose tissue in AMPK α 2 KO mice may have protected these mice from further impairment of glucose homeostasis that normally accompanies high-fat feeding. Our study also demonstrates that lack of AMPK α 2 subunit may be a factor contributing to the development of obesity. *Diabetes* 53:2242–2249, 2004

AMP-activated protein kinase (AMPK) belongs to a family of serine/threonine kinases that are regulated by metabolic and nutritional stresses that result in ATP depletion, including heat shock, hypoxia, hypoglycemia, or prolonged exercise (1,2). Mammalian AMPK is composed of three types of subunits, α , β , and γ . The α -subunit contains the kinase domain as well as an inhibitory domain that inhibits kinase activity in the absence of AMP. The precise role of the β -

and γ -subunits remains unclear, but coexpression of all three subunits and formation of the heterotrimeric complex are required for AMPK activity. Two to three isoforms for each subunit (α 1, α 2, β 1, β 2, γ 1, γ 2, and γ 3), encoded by different genes, are known. As for the catalytic α -subunit, the α 1 isoform is widely distributed, whereas the α 2 isoform is highly expressed in muscle and liver but also found in adipose tissue (3,4).

AMPK has been proposed to act as a "fuel gauge" that monitors the energy status of the cell. In situations of energy depletion, a decrease in the cellular ATP-to-AMP ratio activates a system of protein kinases involving an AMPK kinase and its downstream target AMPK. Activation of AMPK is achieved not only via phosphorylation by the AMPK kinase, recently identified as LKB1 (5), but is also activated allosterically by AMP. It has also been described that AMPK activity is regulated by the phosphocreatine-to-creatine ratio, as well as by changes in the intracellular pH (6). Moreover, studies on leptin signaling suggest that AMPK can be activated by neural signals via the α -adrenergic pathways (7), and the G(q)-coupled receptor has been implicated in the mediation of AMPK activation by catecholamines (8). The overall result of AMPK activation is the inhibition of energy-consuming biosynthetic pathways, such as fatty acid and sterol synthesis, and activation of ATP-producing catabolic pathways, such as fatty acid oxidation.

Adipose tissue is the major organ for storage of energy in the form of triglycerides. Adipose tissue also exerts an important role in energy homeostasis through the secretion of various molecules, including leptin and adiponectin (9,10). Altered energy balance in the organism can cause dramatic change in adipose tissue mass, leading to either obesity or lipoatrophy, disorders that are associated with pathologies such as diabetes or cardiovascular diseases. Many factors, such as genetic background, diet, physical activity, and hormonal balance, are involved in the control of fat mass (11,12). The expression of AMPK subunits in adipose tissue has been well documented. However, despite the importance of fat tissue in energy homeostasis, relatively little attention has been paid to the role of AMPK in adipose tissue metabolism and development. It is well known that AMPK regulates lipogenesis, mainly by phosphorylating acetyl CoA carboxylase (ACC), as well as lipolysis (13–15). In addition to its role on adipocyte metabolism, it has been reported (16) that the treatment of 3T3-L1 preadipocytes with an AMPK activator, 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR), inhibits their differentiation into adipocytes. Since energy balance

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ACC, acetyl CoA carboxylase; AICAR, 5-aminoimidazole-4-carboxamide ribonucleoside; AMPK, AMP-activated protein kinase; DGAT, diacylglycerol acyltransferase; FFA, free fatty acid; GPAT, glycerol-3-phosphate acyltransferase; HSL, hormone-sensitive lipase; KO, knockout; PPAR, peroxisome proliferator-activated receptor.

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in the organism is the major determinant of adipose tissue mass and AMPK is considered as a fuel gauge, AMPK may be involved in the control of adipose function, regulating both its metabolic activity and development. It has been hypothesized that alterations in AMPK activity could be a factor that predisposes the organism to the development of obesity. Despite this, to date, there is no *in vivo* evidence that demonstrates a link between dysregulation of AMPK activity and increased fat mass or adipogenesis.

Here, we examined if AMPK, particularly AMPK complex containing the $\alpha 2$ subunit, regulates adipose tissue development. We report that AMPK $\alpha 2$ knockout (KO) mice exhibit higher body weight, with a specific increase in adipose tissue mass. However, expression of genes that control adipogenesis, including C/EBP α and peroxisome proliferator-activated receptor (PPAR) γ , and other adipocyte markers, such as adipocyte fatty acid-binding protein (aFABP/aP2), is not changed, suggesting that AMPK $\alpha 2$ does not regulate adipocyte differentiation *in vivo*. We show that the augmentation in adipose tissue mass in AMPK $\alpha 2$ KO mice results from the enlargement of preexisting adipocytes, and the adipocyte hypertrophy is due to an increase in triglyceride accumulation. Despite the increased adiposity, AMPK $\alpha 2$ KO mice on a high-fat diet show glucose tolerance and insulin sensitivity similar to those of wild-type mice.

RESEARCH DESIGN AND METHODS

AMPK $\alpha 2$ KO mice on a mixed 129/Sv and C57BL/6 background were generated as described (17). After weaning, wild-type and homozygous AMPK $\alpha 2$ KO male mice were fed *ad libitum* a high-fat diet (45 kcal% fat, 35 kcal% carbohydrate, 20 kcal% protein) (Research Diets, New Brunswick, NJ) for 13 weeks. At 16 weeks of age, animals were killed and tissues removed, weighed, and stored for subsequent analysis. All procedures were performed in accordance with the experimental guidelines for animal care and use at the University of California at Berkeley and those established by the European Convention for the Protection of Laboratory Animals.

Food-intake measurement. For food-intake measurement, mice were individually housed with food and water *ad libitum*. Food consumption was monitored every 2 days during an 8-day period.

Histology and cell-size measurement. Freshly isolated gonadal white adipose tissue from three wild-type and three AMPK $\alpha 2$ KO male mice was fixed overnight in 10% formalin, dehydrated, and embedded in paraffin for subsequent sectioning. Sections (8 μ m) were stained with hematoxylin and eosin, and cell size was analyzed using ImageJ (National Institutes of Health, Bethesda, MD) software. At least 300 cells from each animal were measured.

RNA extraction, Northern blot analysis, and real-time PCR. Total RNA was extracted from gonadal and inguinal white adipose tissue and interscapular brown adipose tissue using RNA-Plus (Qbiogene, Illkirch, France) according to the manufacturer's instruction. For Northern blot analysis, 15 μ g of total RNA were electrophoresed on a 1.2% agarose-formaldehyde gel and transferred onto nylon membranes (Hybond N+; Amersham Biosciences, Piscataway, NJ). Blot hybridization was carried out in ExpressHyb solution (Clontech, Palo Alto, CA) using specific cDNA probes for C/EBP α , PPAR γ , aFABP/aP2, adipocyte-specific secretory factor (ADSF)/resistin, and β -actin. After hybridization, blots were exposed onto a film, and the signal was quantified by densitometry. Mitochondrial glycerol-3-phosphate acyltransferase (GPAT) and diacylglycerol acyltransferase (DGAT) mRNA were quantified by real-time PCR using the ABI Prism 7700 Sequence Detection System (Perkin-Elmer Applied Biosystems, Foster City, CA) and the following primers: DGAT1 forward, 5'-TTCCGCCTCTGGGCATT-3'; DGAT1 reverse, 5'-AGAATCGGCCACAATCCA-3'; GPAT forward, 5'-ATTGACACCTGCTGCTT TGA-3'; and GPAT reverse, 5'-CCTACCCACTACAACAGAG-3'.

Tissue triglyceride content. Lipids were extracted by the Folch method in a mixture of 2:1 chloroform/methanol (vol/vol) (18). The extract was washed with 0.2 volumes of saline (NaCl 0.9%) and centrifuged at 2,000 rpm for 10 min. The organic phase was then recovered, and triglyceride content was determined using Infinity triglyceride reagent (Sigma, St. Louis, MO).

Metabolic measurements. Blood metabolites from wild-type and AMPK $\alpha 2$ KO mice fed or fasted for 5 h were analyzed. Blood glucose was measured

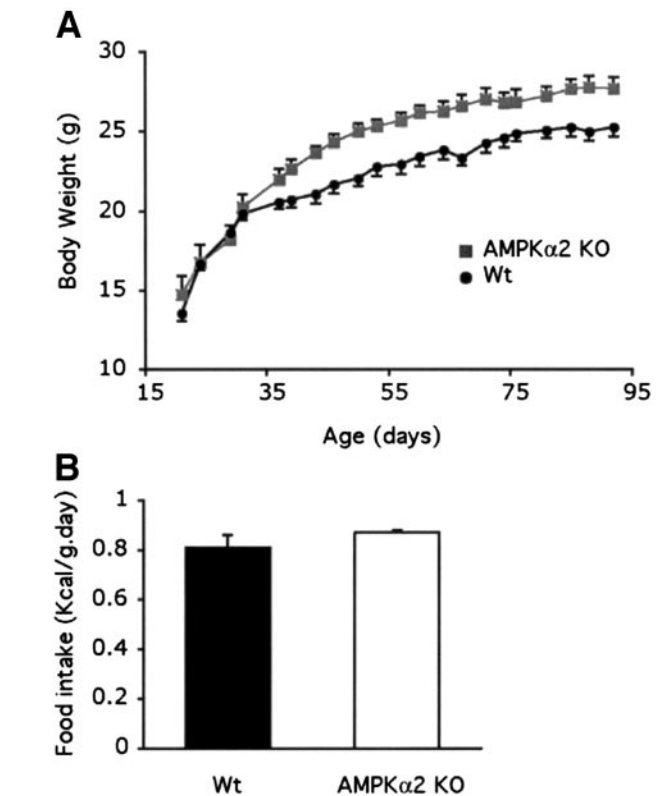


FIG. 1. Body weight and food intake in AMPK $\alpha 2$ KO mice. **A:** AMPK $\alpha 2$ KO mice ($n = 10$) exhibited higher body weight than wild-type mice ($n = 12$) over a 13-week period of being fed a high-fat diet. **B:** Food intake in AMPK $\alpha 2$ KO and wild-type mice was measured for an 8-day period in which mice were given food and water *ad libitum*. Results are shown as mean \pm SE.

with a glucometer (AccuChek; Roche Diagnostics, Indianapolis, IN). Serum triglycerides were analyzed with the Infinity triglyceride reagent (Sigma). Serum free fatty acids (FFAs) were determined with the NEFA C kit (Wako, Richmond, VA). Serum insulin and leptin concentrations were determined using a rat insulin and mouse leptin enzyme-linked immunosorbent assay kits (Crystal Chem, Downers Grove, IL), respectively. Adiponectin in serum was also determined using a rat/mouse adiponectin enzyme-linked immunosorbent assay kit (B-Bridge, Sunnyvale, CA).

Glucose and insulin tolerance tests. A glucose tolerance test was performed on mice fasted for 12 h. Blood glucose levels were determined at 0, 20, 40, 60, 80, 100, and 120 min after an intraperitoneal injection of glucose (2 g/kg body wt). For the insulin tolerance test, animals fasted for 5 h were injected intraperitoneally with 0.75 units insulin/kg body wt and glucose levels were measured at 0, 15, 30, 60, and 90 min postinjection.

Statistical analysis. Data are expressed as means \pm SE. The statistical significance of differences in mean values between transgenic and wild-type littermates was assessed by Student's *t* test.

RESULTS

AMPK $\alpha 2$ KO mice exhibit increased body weight and adipose tissue mass. Compared with a normal diet, a hypercaloric diet is known to have exacerbated effects on body weight and adipose tissue mass. In the present study, mice were fed a high-fat diet to document the potential effects of lack of AMPK $\alpha 2$ subunit on adipose tissue mass and development. At weaning, there was no difference in body weight between wild-type and AMPK $\alpha 2$ KO mice (Fig. 1A). However, after 3 weeks under a high-fat regime, KO mice started to gain body weight faster than wild-type mice. At 6 weeks of age, AMPK $\alpha 2$ KO mice showed a significantly higher body weight than wild-type mice (23.6 ± 0.4 g, $n = 10$ vs. 21.1 ± 0.6 g, $n = 12$, respectively,

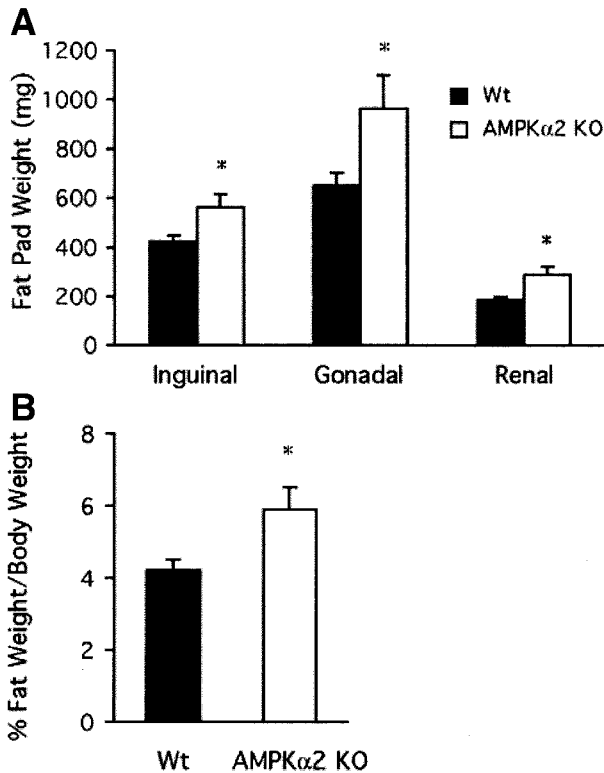


FIG. 2. Increased adipose tissue mass in AMPK α 2 KO mice. **A:** White adipose tissue depot weights from 16-week-old mice ($n = 7-9$ per group). **B:** Combined weight of major white adipose tissue depots (sum of inguinal, gonadal, and renal depot weight) expressed as a percentage of total body weight in AMPK α 2 KO and wild-type mice. Statistically significant differences between the groups: * $P < 0.05$.

$P < 0.01$). These differences in body weight were sustained for up to 16 weeks of age, the last time point examined (Fig. 1A). The higher body weight gain observed in AMPK α 2 KO mice was not due to a differential energy input because no difference in food intake between wild-type and AMPK α 2 KO mice was observed (Fig. 1B).

Because changes in body weight are frequently associated with alterations in adipose tissue mass, we next examined the weight of major white adipose tissue depots: inguinal, gonadal, and retroperitoneal. When compared with wild-type mice, a significantly higher mass for all white adipose depots, the differences ranging from 25 to 35.5%, was observed in AMPK α 2 KO male mice (Fig. 2A). When expressed as a percentage of total body weight, we observed a 40% increase in the combined weight of inguinal, gonadal, and retroperitoneal fat depots of AMPK α 2 KO mice compared with wild-type mice (Fig. 2B). We did not observe any change in the weight of other organs, including liver, heart, or muscle, tissues where the α 2 subunit of AMPK is highly expressed (data not shown). Neither did we find any significant differences in the weight of mouse carcasses. These results indicate that the higher body weight detected in AMPK α 2 KO mice was due to an increase in adipose tissue mass.

Increased adipocyte size without changes in cell number or adipogenesis in AMPK α 2 KO mice. Increase in adipose tissue mass can be the result of an increase in adipose cell size (hypertrophy), an increase in cell number (hyperplasia), or a combination of both of these processes. No changes in total DNA content in the

fat depots of the two experimental groups were found (Fig. 3A), indicating that the increase in the mass of the various fat pads of AMPK α 2 KO mice was not due to an increase in cell number. In contrast, microscopic analysis of gonadal adipose tissue from wild-type and AMPK α 2 KO mice revealed that KO mice had larger adipocytes than wild-type mice (Fig. 3B). In addition, a significantly higher triglyceride accumulation was observed in adipose depots of AMPK α 2 KO mice compared with wild-type mice (Fig. 4). To determine whether the increase in adipose tissue mass was accompanied by an enhancement of the adipocyte differentiation process, expression levels for several adipocyte markers were monitored by Northern blot analysis. As shown in Fig. 5A, the increase in adipose tissue mass observed in AMPK α 2 KO mice was not reflected in the change in the expression of adipocyte marker genes such as C/EBP α , PPAR γ , and aFABP/aP2. Neither did we find any difference in the mRNA levels for DGAT or mitochondrial GPAT, two enzymes involved in the synthesis of triglycerides (Fig. 5B). Circulating levels of leptin and adiponectin, hormones synthesized and secreted by mature adipocytes, were not changed by the ablation of the AMPK α 2 subunit (Table 1). Interestingly, however, we found a considerable reduction, up to 50%, in the expression of ADSF/resistin in adipose tissue of the AMPK α 2 KO

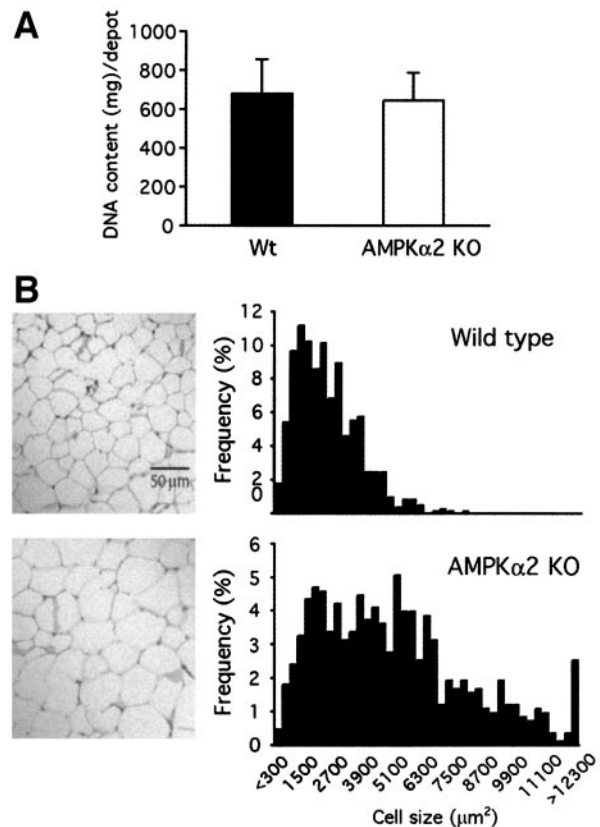


FIG. 3. Total DNA content and adipocyte size in AMPK α 2 KO mice. **A:** White adipose tissue from AMPK α 2 KO ($n = 6$) and wild-type ($n = 6$) mice was digested with proteinase K, and DNA was extracted with phenol:chloroform. DNA content was measured by spectrophotometry ($\lambda = 260$ nm). **B:** Paraffin-embedded sections of gonadal white adipose tissue from 16-week-old mice were stained with hematoxylin and eosin (left). Distribution of adipocyte size in wild-type and AMPK α 2 KO mice is shown at right. The size of at least 300 cells per sample from three different mice per group was determined using Image J software (National Institutes of Health).

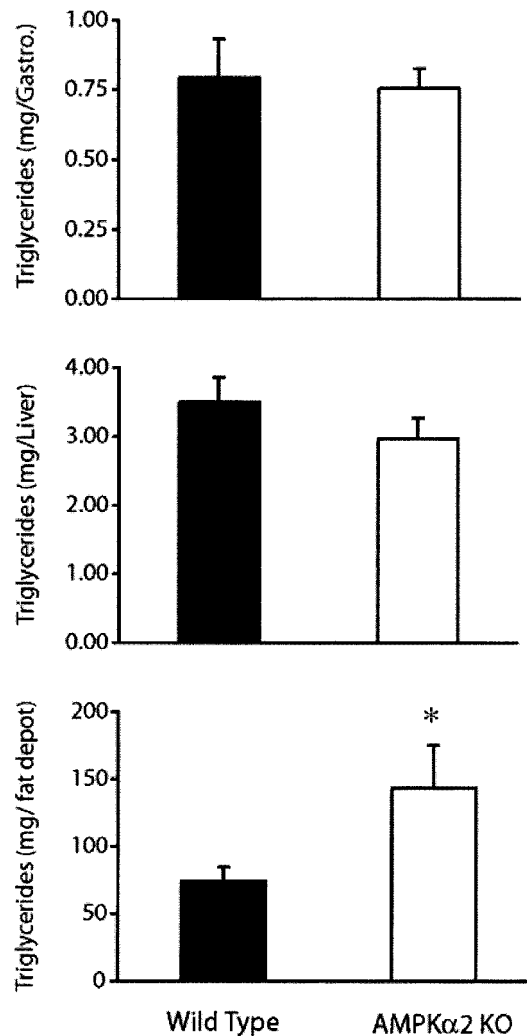


FIG. 4. Triglyceride content in muscle, liver, and white adipose tissue of wild-type and AMPK α 2 KO mice. Total lipids were extracted by the Folch method, and triglycerides were measured using the Infinity triglyceride reagent (Sigma). Results are presented as total triglycerides (in milligrams) per organ and the results as the means \pm SE of seven to nine animals in each experimental group. * $P < 0.05$.

mice. Overall, these results demonstrate that the increase in adipose tissue mass observed in AMPK α 2 KO mice is not due to changes in cell number or adipocyte differentiation but due to adipocyte hypertrophy.

AMPK α 2 KO mice exhibit similar glucose tolerance and insulin sensitivity to wild-type mice despite an increase in adipose tissue mass. It is well documented that changes in adipose tissue mass are frequently associated with alterations in glucose and insulin homeostasis. Therefore, we next examined whether the increase in lipid accumulation in adipose tissue of AMPK α 2 KO mice on a high-fat diet had any effect on glucose homeostasis. In both fasting and fed conditions, we observed no differences in basal glucose levels between AMPK α 2 KO and wild-type mice on a high-fat diet (Table 1). We next performed a glucose tolerance test on overnight-fasted mice. Wild-type mice on a high-fat diet, as expected, had an impaired glucose disposal compared with wild-type mice fed a normal diet (data not shown). However, when fed a high-fat diet, AMPK α 2 KO mice could clear glucose from circulation at rates similar to those of wild-type mice

(Fig. 6A). Neither did we observe differences in the insulin sensitivity of AMPK α 2 KO mice when compared with wild-type mice (Fig. 6B). This is in contrast to the results observed when AMPK α 2 KO mice were fed a normal diet. Unlike wild-type mice, AMPK α 2 mice on normal diet were glucose intolerant and insulin resistant (17). This indicates that the differences in glucose tolerance and insulin sensitivity observed between wild-type and AMPK α 2 knockout mice on normal diet disappeared when these mice were fed a high-fat diet. These results suggest that the lack of AMPK α 2 subunit and the concomitant increase in lipid storage in adipose tissue in AMPK α 2 KO mice probably prevented the further impairment of glucose homeostasis normally occurring from eating a high-fat diet. We also determined the triglyceride content in liver and muscle of AMPK α 2 KO mice. Lipid accumulation in tissues other than adipose tissue, such as muscle and liver, is considered to be a cause of the development of insulin resistance associated with obesity. We found no differences in triglyceride content in liver or muscle between the two experimental groups (Fig. 4). In concordance, we only observed a slight increase in circulating levels of FFAs and triglycerides (Table 1) that did not seem to be sufficient to promote triglyceride accumulation in other organs and to produce further impairment of glucose homeostasis in AMPK α 2 KO mice on a high-fat diet.

DISCUSSION

AMPK plays a central role in the modulation of the energy metabolism. It has been proposed (19,20) that a deficiency in AMPK activity could be a factor contributing to the development of obesity. The higher body weight and the increased fat mass exhibited by AMPK α 2 KO mice in this study are, indeed, consistent with such a hypothesis. The effect we have observed on adipose tissue mass by the ablation of AMPK α 2 subunit is in agreement with various studies linking AMPK activity and alterations in fat mass. Sustained AMPK activation in obese Zucker rats by long-term administration of AICAR diminished the mass of epididymal and retroperitoneal fat pads up to 30–40%, although no difference in total body weight was observed in these studies (21–23). The authors speculated that the adipose mass reduction observed in these studies was due to a decrease in lipogenesis and an increase in the whole-body oxidative metabolism by AICAR activation of AMPK. AMPK activation in adipose tissue has been shown to inhibit lipogenesis by phosphorylation and inhibition of ACC. In muscle it has been shown that the reduction in malonyl-CoA, which acts as an inhibitor of the carnitine-palmitoyl transferase, resulting from the ACC inactivation, releases carnitine-palmitoyl transferase inhibition and increases fatty acid influx into the mitochondria to be oxidized. Therefore, the increase in adipose tissue mass and fat cell size observed in our study could be due to an enhanced lipogenesis and changes in oxidative metabolism as a consequence of AMPK α 2 ablation and the subsequent reduction in AMPK activity. However, no difference in whole-body oxidative metabolism monitored by indirect calorimetry has been observed in AMPK α 2 KO mice (17). Thus, the accretion of triglycerides in adipose tissue seems to have resulted from an enhanced anabolic

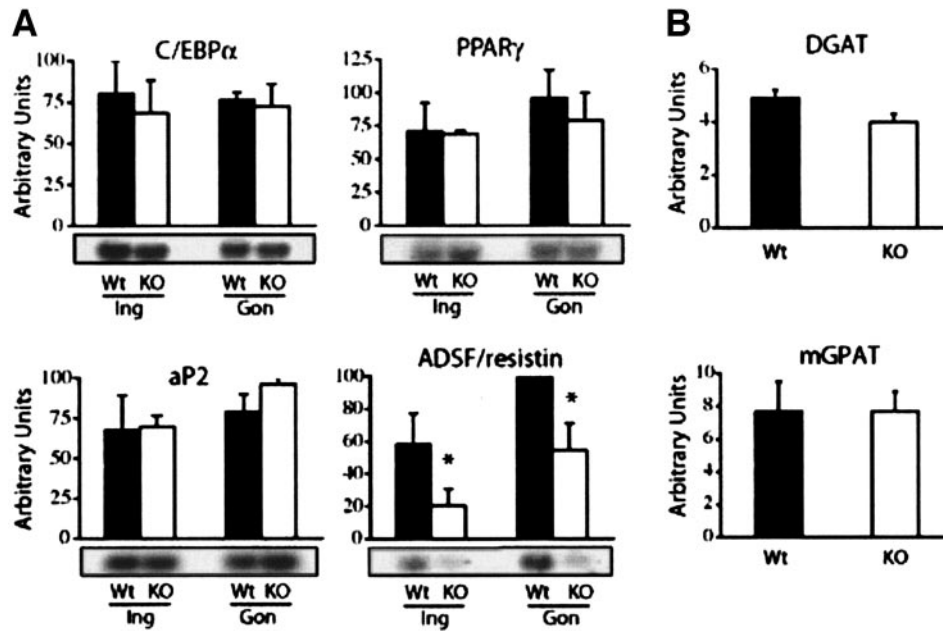


FIG. 5. Adipocyte markers expression is not altered in AMPK α 2 KO mice. *A*: Total RNA from inguinal and gonadal fat pads of three wild-type mice and three AMPK α 2 KO mice was probed with cDNAs probes for different adipocyte markers. *B*: DGAT and GPAT mRNA were quantified by real-time PCR. Data are expressed in arbitrary units corrected by β -actin mRNA level in each sample. Results are means \pm SE. Statistical significance is indicated: * $P < 0.05$.

metabolism in fat tissue rather than a defective oxidative capacity of muscle and liver.

The molecular mechanisms underlying the effect of AMPK in adipose tissue mass are poorly understood. Increase in adipose tissue mass may arise from an increase in the number of adipose cells as a consequence of an enhanced adipogenesis, an increase in triglyceride storage in the preexisting adipocytes, or a combination of both processes. It has been reported that AICAR treatment of 3T3-L1 cells, a well-characterized model for in vitro adipocyte differentiation, causes inhibition of 3T3-L1 preadipocyte conversion into adipocytes by preventing the expression of C/EBP α and PPAR γ (16), two master genes that control adipocyte differentiation (24). It is worth noting that a fraction of AMPK α 2 subunit has been found in the nucleus of the cell (25) and that the coactivator p300, a known modulator of PPAR γ (26), has recently been reported to be phosphorylated by AMPK, reducing its affinity for nuclear receptors (27). These findings support the hypothesis that AMPK may directly regulate gene transcription. In our AMPK α 2 KO mice, however, no changes in the expression of adipocyte transcription factors, PPAR γ , C/EBP α , or the mature adipocyte markers,

including aFABP/aP2, were observed. This indicates that the increase in adipose tissue mass observed in AMPK α 2 KO mice is not due to an enhancement of preadipocyte differentiation into adipocytes. It is possible that the observed effect in 3T3-L1 preadipocytes may not be due to a specific activation of AMPK by AICAR but by its effect on other AMP-sensitive molecules or processes (28,29).

The increase in adipose tissue mass observed in AMPK α 2 KO mice is, however, accompanied by a cellular hypertrophy without changes in the number of cells in the fat depots. Therefore, we conclude that the effect of AMPK α 2 null on adipose tissue mass is more likely due to an increased triglyceride accumulation in the preexisting adipocytes rather than an increase in cell number or differentiation. The metabolic cause of the enhanced triglyceride accumulation in adipose tissue of AMPK α 2 KO mice could be by either an increase in triglyceride synthesis or a decrease in lipolysis. A preferential fatty acid uptake by adipose tissue of AMPK α 2 KO mice seems unlikely because we did not find any difference in heparin-stimulated lipoprotein lipase activity in AMPK α 2 KO and wild-type mice (data not shown). A dysregulated lipolysis could also be considered a possible cause for the aug-

TABLE 1
Serum parameters

	Fasted		Fed	
	Wild type	AMPK α 2 KO	Wild type	AMPK α 2 KO
Glucose (mg/dl)	101.7 \pm 6.6	102.6 \pm 18.0	157.6 \pm 9.8	155.3 \pm 6.3
Triglycerides (mg/ml)	0.24 \pm 0.03	0.24 \pm 0.03	0.36 \pm 0.02	0.45 \pm 0.05
FFA (mEq/l)	0.78 \pm 0.05	1.09 \pm 0.17	0.37 \pm 0.04	0.54 \pm 0.05*
Leptin (ng/ml)	2.51 \pm 0.40	2.42 \pm 0.46	3.72 \pm 0.06	3.10 \pm 0.36
Adiponectin (mg/ml)	21.5 \pm 0.15	21.2 \pm 0.26	20.5 \pm 0.16	17.6 \pm 0.17
Insulin (ng/ml)	1.01 \pm 0.07	0.70 \pm 0.06*	1.67 \pm 0.20	1.34 \pm 0.08

Data are means \pm SE. * $P < 0.05$.

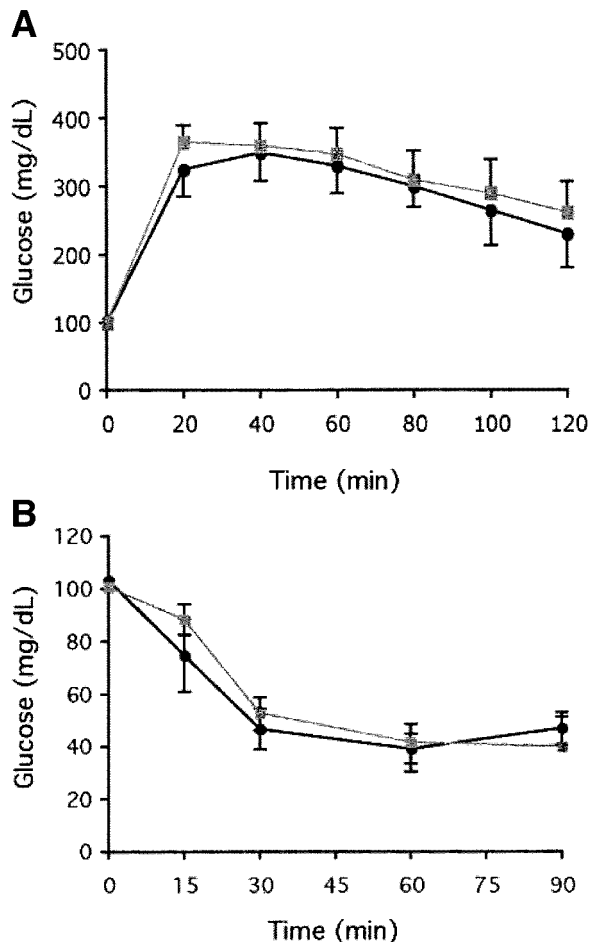


FIG. 6. AMPK α 2 KO and wild-type mice exhibited similar glucose tolerance and insulin sensitivity when fed a high-fat diet. **A:** Glucose tolerance test. Overnight-fasted mice were given an intraperitoneal injection of glucose (2 mg/g body wt). Blood samples were collected from the tail at the indicated time points and analyzed for glucose concentration. **B:** Insulin tolerance test. Insulin (0.5 units/kg body wt) was intraperitoneally injected after fasting for 5 h, and glucose levels were monitored at the indicated time points (■, wild type; □, AMPK α 2). Results are the means \pm SE from five to six animals in each group.

mented adipose mass in AMPK α 2 KO mice. It has been reported (30) that hormone-sensitive lipase (HSL) can be phosphorylated at S565 by AMPK, and this may facilitate its translocation to the lipid droplets (31), activating its lipolytic capacity (15). Therefore, in AMPK α 2 KO mice, one would expect an inhibition of lipolysis. However, we did not observe any difference in cytosolic HSL activity in adipose tissue of AMPK α 2 KO mice and wild-type mice (data not shown). We cannot rule out, however, the possibility of a defective HSL translocation into lipid droplets in AMPK α 2 KO mice, preventing the interaction of HSL with its substrates and thereby causing an increase in lipid storage in adipose tissue. In this regard, expression of dominant-negative AMPK has been reported (31) to inhibit lipolysis in 3T3-L1 adipocytes. As for triglyceride synthesis, we did not observe any differences in mRNA levels for the mitochondrial GPAT and DGAT, two enzymes involved in the esterification of fatty acids to triglycerides. However, although direct phosphorylation of mitochondrial GPAT has yet to be demonstrated, mitochondrial GPAT has been suggested to be a target for

AMPK (32). Further studies addressing in vivo changes in fatty acid and fat synthesis as well as lipolysis in AMPK knockout mice are needed to understand the exact nature of the enhanced triglyceride accumulation in adipocytes of AMPK α 2 KO mice.

In the previous characterization of AMPK α 2 KO mice (17), no differences in body weight or fat mass was observed when mice were fed a normal diet. The only striking characteristic of these mice was a severe insulin resistance. In the present work, a long-term high-fat feeding was conducted on AMPK α 2 KO mice. High-energy intake is known to be a factor that contributes to the development of obesity and insulin resistance (11). Consequently, under these conditions, we expected that the impairment in glucose tolerance and insulin sensitivity observed in AMPK α 2 KO mice on a normal diet would be potentiated by high-fat feeding. To our surprise, when fed a high-fat diet, despite the increased adiposity, AMPK α 2 KO mice showed glucose disposal rates similar to those of wild-type mice. These results can be interpreted as a resistance of AMPK α 2 KO mice to further develop high-fat diet-induced glucose intolerance. AMPK α 2 KO mice model is not the only model in which obesity or increased fat mass is dissociated from impaired glucose tolerance. The aP2-null mice develop obesity without insulin resistance when fed a high-fat diet (33). Obesity is also dissociated from impaired glucose disposal in mice that overexpress DGAT1 in adipose tissue (34). Overexpression of PEPCK in white adipose tissue also leads to an augmentation of adipose mass but does not cause insulin resistance (35). These models, as well as our AMPK α 2 KO mice, provide strong evidence that increased adiposity per se does not necessarily contribute to insulin resistance.

The main question that remains is why there was no difference in glucose disposal in AMPK α 2 KO mice as compared with wild-type mice when fed a high-fat diet, whereas AMPK α 2 KO mice exhibit insulin resistance and glucose intolerance when fed a normal diet. Regardless, the observed phenotype must be associated with the increased fat mass in AMPK α 2 KO mice on a high-fat diet because other parameters known to influence glucose homeostasis are not substantially different in AMPK α 2 KO mice fed either a normal or high-fat diet (17). High concentrations of plasma FFAs are believed to be a cause for the development of peripheral insulin resistance. An increased availability and uptake of FFAs by liver or muscle can lead to the accumulation of triglycerides or intermediate metabolites, such as long-chain fatty acyl-CoA or diacylglycerol, which ultimately could be responsible for the insulin resistance (36,37). The fact that serum FFA concentration is only slightly elevated in AMPK α 2 KO as compared with wild-type mice and that these mice have a similar triglyceride content in liver and muscle, either on high-fat or normal diets, rule out the lipid accumulation in these tissues as a major determinant of their glucose homeostasis.

In addition to its function as a lipid storage organ, adipose tissue plays a key role in the regulation of energy metabolism as an endocrine organ by the secretion of a wide variety of hormones that also regulate glucose homeostasis. Despite the increase in adipose cell size, the lack of AMPK α 2 subunit did not alter the secretion of

leptin and adiponectin, adipokines known to stimulate glucose utilization and fatty acid oxidation by peripheral tissues. This is probably due to the fact that the degree of adipocyte differentiation is not affected by the lack of the AMPK α 2 subunit. Moreover, the lack of differences in the circulating levels of leptin and adiponectin support this observation, as it has been reported (38–41) in different rodent models that altered adipocyte differentiation is frequently associated with changes in the levels of these adipokines. Interestingly, the effects of leptin and adiponectin on oxidative metabolism in muscle have recently been described (7,42) to be mediated in part through the activation of AMPK. However, the reduction in AMPK activity as a result of AMPK α 2 subunit ablation does not seem to have any compensatory effects in the expression of these adipokines, ruling out a feedback regulation of leptin and adiponectin levels by AMPK. It is interesting to note that, contrary to other adipokines or mature adipocytes markers, ADSF/resistin expression is significantly lower in AMPK α 2 KO mice on a high-fat diet compared with wild-type mice. The contribution of lower ADSF/resistin to the glucose metabolism in AMPK α 2 KO mice is not clear. However, because ADSF/resistin has been associated with the development of diabetes (43), the lower ADSF/resistin levels observed in AMPK α 2 KO mice as compared with wild-type mice on a high-fat diet could have, in part, prevented the further impairment in glucose tolerance and insulin resistance anticipated from increased adiposity and high-fat feeding as compared with those observed in AMPK α 2 KO mice on a normal diet.

In conclusion, our study demonstrates that ablation of AMPK α 2 subunit leads to the development of obesity when animals are fed a high-fat diet, as a result of an enhanced lipid accumulation in adipocytes but not in other tissues. The increase in adipose mass is due to an enlargement of the preexisting adipocytes and does not affect cell number or differentiation. Further studies are needed to unravel the mechanisms by which AMPK modulates adipose tissue function and peripheral glucose disposal. To this end, the generation of a conditional KO in adipose tissue for both α 1 and α 2 AMPK subunits may provide valuable information to help us understand the role of AMPK in adipocytes and its impact on whole-body metabolism.

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REFERENCES

- Hardie DG, Carling D, Carlson M: The AMP-activated/SNF1 protein kinase subfamily: metabolic sensors of the eukaryotic cell? *Annu Rev Biochem* 67:821–855, 1998
- Winder WW, Hardie DG: AMP-activated protein kinase, a metabolic master switch: possible roles in type 2 diabetes. *Am J Physiol* 277:E1–E10, 1999
- Stapleton D, Mitchelhill KI, Gao G, Widmer J, Michell BJ, Teh T, House CM, Fernandez CS, Cox T, Witters LA, Kemp BE: Mammalian AMP-activated protein kinase subfamily. *J Biol Chem* 271:611–614, 1996
- Verhoeven AJ, Woods A, Brennan CH, Hawley SA, Hardie DG, Scott J, Beri RK, Carling D: The AMP-activated protein kinase gene is highly expressed in rat skeletal muscle: alternative splicing and tissue distribution of the mRNA. *Eur J Biochem* 228:236–243, 1995
- Woods A, Johnstone SR, Dickerson K, Leiper FC, Fryer LG, Neumann D, Schlattner U, Wallimann T, Carlson M, Carling D: LKB1 is the upstream kinase in the AMP-activated protein kinase cascade. *Curr Biol* 13:2004–2008, 2003
- Ponticos M, Lu QL, Morgan JE, Hardie DG, Partridge TA, Carling D: Dual regulation of the AMP-activated protein kinase provides a novel mechanism for the control of creatine kinase in skeletal muscle. *EMBO J* 17:1688–1699, 1998
- Minokoshi Y, Kim YB, Peroni OD, Fryer LG, Muller C, Carling D, Kahn BB: Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. *Nature* 415:339–343, 2002
- Kishi K, Yuasa T, Minami A, Yamada M, Hagi A, Hayashi H, Kemp BE, Witters LA, Ebina Y: AMP-activated protein kinase is activated by the stimulations of G(q)-coupled receptors. *Biochem Biophys Res Commun* 276:16–22, 2000
- Friedman JM, Halaas JL: Leptin and the regulation of body weight in mammals. *Nature* 395:763–770, 1998
- Fruebis J, Tsao TS, Javorschi S, Ebbets-Reed D, Erickson MR, Yen FT, Bihain BE, Lodish HF: Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. *Proc Natl Acad Sci U S A* 98:2005–2010, 2001
- Hill JO, Peters JC: Environmental contributions to the obesity epidemic. *Science* 280:1371–1374, 1998
- Comuzzie AG, Allison DB: The search for human obesity genes. *Science* 280:1374–1377, 1998
- Sullivan JE, Brocklehurst KJ, Marley AE, Carey F, Carling D, Beri RK: Inhibition of lipolysis and lipogenesis in isolated rat adipocytes with AICAR: a cell-permeable activator of AMP-activated protein kinase. *FEBS Lett* 353:33–36, 1994
- Corton JM, Gillespie JG, Hawley SA, Hardie DG: 5-aminoimidazole-4-carboxamide ribonucleoside: a specific method for activating AMP-activated protein kinase in intact cells? *Eur J Biochem* 229:558–565, 1995
- Yin W, Mu J, Birnbaum MJ: Role of AMP-activated protein kinase in cyclic AMP-dependent lipolysis in 3T3-L1 adipocytes. *J Biol Chem* 278:43074–43080, 2003
- Habinowski SA, Witters LA: The effects of AICAR on adipocyte differentiation of 3T3-L1 cells. *Biochem Biophys Res Commun* 286:852–856, 2001
- Viollet B, Andreelli F, Jorgensen SB, Perrin C, Goloen A, Flamez D, Mu J, Lenzner C, Baud O, Bennoun M, Gomas E, Nicolas G, Wojtaszewski JF, Kahn A, Carling D, Schuit FC, Birnbaum MJ, Richter EA, Burcelin R, Vaulont S: The AMP-activated protein kinase α 2 catalytic subunit controls whole-body insulin sensitivity. *J Clin Invest* 111:91–98, 2003
- Folch J, Lees M, Sloane Stanley GH: A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* 226:497–509, 1957
- Ruderman NB, Saha AK, Kraegen EW: Minireview: malonyl CoA, AMP-activated protein kinase, and adiposity. *Endocrinology* 144:5166–5171, 2003
- Winder WW: Energy-sensing and signaling by AMP-activated protein kinase in skeletal muscle. *J Appl Physiol* 91:1017–1028, 2001
- Winder WW, Holmes BF, Rubink DS, Jensen EB, Chen M, Holloszy JO: Activation of AMP-activated protein kinase increases mitochondrial enzymes in skeletal muscle. *J Appl Physiol* 88:2219–2226, 2000
- Saha AK, Kurowski TG, Kaushik VK, Dean D, Tomas E, Ye J, Kraegen EW, Ruderman N: Pharmacological activation of AMP-activated protein kinase: a target for the treatment of obesity (Abstract). *Diabetes* 51 (Suppl. 2):A254, 2002
- Buhl ES, Jessen N, Pold R, Ledet T, Flyvbjerg A, Pedersen SB, Pedersen O, Schmitz O, Lund S: Long-term AICAR administration reduces metabolic disturbances and lowers blood pressure in rats displaying features of the insulin resistance syndrome. *Diabetes* 51:2199–2206, 2002
- Rosen ED, Spiegelman BM: Molecular regulation of adipogenesis. *Annu Rev Cell Dev Biol* 16:145–171, 2000
- Salt I, Celler JW, Hawley SA, Prescott A, Woods A, Carling D, Hardie DG: AMP-activated protein kinase: greater AMP dependence, and preferential nuclear localization, of complexes containing the α 2 isoform. *Biochem J* 334:177–187, 1998
- Gelman L, Zhou G, Fajas L, Raspe E, Fruchart JC, Auwerx J: p300 interacts with the N- and C-terminal part of PPAR γ 2 in a ligand-independent and -dependent manner, respectively. *J Biol Chem* 274:7681–7688, 1999
- Yang W, Hong YH, Shen XQ, Frankowski C, Camp HS, Leff T: Regulation of

- transcription by AMP-activated protein kinase: phosphorylation of p300 blocks its interaction with nuclear receptors. *J Biol Chem* 276:38341–38344, 2001
28. Vincent MF, Erion MD, Gruber HE, Van den Berghe G: Hypoglycaemic effect of AICARiboside in mice. *Diabetologia* 39:1148–1155, 1996
 29. Vincent MF, Marangos PJ, Gruber HE, Van den Berghe G: Inhibition by AICA riboside of gluconeogenesis in isolated rat hepatocytes. *Diabetes* 40:1259–1266, 1991
 30. Garton AJ, Campbell DG, Carling D, Hardie DG, Colbran RJ, Yeaman SJ: Phosphorylation of bovine hormone-sensitive lipase by the AMP-activated protein kinase: a possible antilipolytic mechanism. *Eur J Biochem* 179: 249–254, 1989
 31. Su CL, Sztalryd C, Contreras JA, Holm C, Kimmel AR, Londos C: Mutational analysis of the hormone-sensitive lipase translocation reaction in adipocytes. *J Biol Chem* 278:43615–43619, 2003
 32. Muoio DM, Seefeld K, Witters LA, Coleman RA: AMP-activated kinase reciprocally regulates triacylglycerol synthesis and fatty acid oxidation in liver and muscle: evidence that sn-glycerol-3-phosphate acyltransferase is a novel target. *Biochem J* 338:783–791, 1999
 33. Hotamisligil GS, Johnson RS, Distel RJ, Ellis R, Papaioannou VE, Spiegelman BM: Uncoupling of obesity from insulin resistance through a targeted mutation in aP2, the adipocyte fatty acid binding protein. *Science* 274:1377–1379, 1996
 34. Chen HC, Stone SJ, Zhou P, Buhman KK, Farese RV Jr: Dissociation of obesity and impaired glucose disposal in mice overexpressing acyl coenzyme A:diacylglycerol acyltransferase 1 in white adipose tissue. *Diabetes* 51:3189–3195, 2002
 35. Franckhauser S, Munoz S, Pujol A, Casellas A, Riu E, Otaegui P, Su B, Bosch F: Increased fatty acid re-esterification by PEPCCK overexpression in adipose tissue leads to obesity without insulin resistance. *Diabetes* 51:624–630, 2002
 36. McGarry JD: Banting Lecture 2001: dysregulation of fatty acid metabolism in the etiology of type 2 diabetes. *Diabetes* 51:7–18, 2002
 37. Yu C, Chen Y, Cline GW, Zhang D, Zong H, Wang Y, Bergeron R, Kim JK, Cushman SW, Cooney GJ, Atcheson B, White MF, Kraegen EW, Shulman GI: Mechanism by which fatty acids inhibit insulin activation of insulin receptor substrate-1 (IRS-1)-associated phosphatidylinositol 3-kinase activity in muscle. *J Biol Chem* 277:50230–50236, 2002
 38. Lin J, Arnold HB, Della-Fera MA, Azain MJ, Hartzell DL, Baile CA: Myostatin knockout in mice increases myogenesis and decreases adipogenesis. *Biochem Biophys Res Commun* 291:701–706, 2002
 39. Lee K, Villena JA, Moon YS, Kim KH, Lee S, Kang C, Sul HS: Inhibition of adipogenesis and development of glucose intolerance by soluble preadipocyte factor-1 (Pref-1). *J Clin Invest* 111:453–461, 2003
 40. Jones ME, Thorburn AW, Britt KL, Hewitt KN, Wreford NG, Proietto J, Oz OK, Leury BJ, Robertson KM, Yao S, Simpson ER: Aromatase-deficient (ArKO) mice have a phenotype of increased adiposity. *Proc Natl Acad Sci U S A* 97:12735–12740, 2000
 41. He W, Barak Y, Hevener A, Olson P, Liao D, Le J, Nelson M, Ong E, Olefsky JM, Evans RM: Adipose-specific peroxisome proliferator-activated receptor (γ) knockout causes insulin resistance in fat and liver but not in muscle. *Proc Natl Acad Sci U S A* 100:15712–15717, 2003
 42. Yamauchi T, Kamon J, Minokoshi Y, Ito Y, Waki H, Uchida S, Yamashita S, Noda M, Kita S, Ueki K, Eto K, Akanuma Y, Froguel P, Foufelle F, Ferre P, Carling D, Kimura S, Nagai R, Kahn BB, Kadowaki T: Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nat Med* 8:1288–1295, 2002
 43. Stepan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, Patel HR, Ahima RS, Lazar MA: The hormone resistin links obesity to diabetes. *Nature* 409:307–312, 2001