Induced Adiposity and Adipocyte Hypertrophy in Mice Lacking the AMP-Activated Protein Kinase-α2 Subunit

Josep A. Villena,1 Benoit Viollet,2 Fabrizzio Andreelli,2 Axel Kahn,2 Sophie Vaulont,2 and Hei Sook Sul1

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 AMPKα2 knockout (KO) mice to a high-fat diet to examine the effect of AMPK on adipose tissue formation. Compared with the wild type, AMPKα2 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/EBPα), and adipocyte fatty acid-binding protein (aP2), or total cell number. Unlike impaired glucose homeostasis observed on normal diet feeding, when fed a high-fat diet AMPKα2 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 AMPKα2 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 AMPKα2 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 (α1, α2, β1, β2, γ1, γ2, and γ3), encoded by different genes, are known. As for the catalytic α-subunit, the α1 isohomolog is widely distributed, whereas the α2 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-aminomidazole-4-carboxamide ribonucleoside (AICAR), inhibits their differentiation into adipocytes. Since energy balance

From the 1Department of Nutritional Sciences and Toxicology, University of California, Berkeley, California, and the 2Department of Genetic, Development, and Molecular Pathology, Institute Cochin, Paris, France.

Address correspondence and reprint requests to Hei Sook Sul, Department of Nutritional Sciences and Toxicology, University of California, Berkeley, CA 94720. E-mail: hsul@nature.berkeley.edu.

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J.A.V. and B.V. contributed equally to this work.

ACC, acetyl CoA carboxylase; AICAR, 5-aminimidazole-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 α2 subunit, regulates adipose tissue development. We report that AMPKα2 knockout (KO) mice exhibit higher body weight, with a specific increase in adipose tissue mass. However, expression of genes that control adipogenesis, including C/EBPα 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 AMPKα2 does not regulate adipocyte differentiation in vivo. We show that the augmentation in adipose tissue mass in AMPKα2 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, AMPKα2 KO mice on a high-fat diet show glucose tolerance and insulin sensitivity similar to those of wild-type mice.

RESEARCH DESIGN AND METHODS

AMPKα2 KO mice on a mixed 129/Sv and C57BL/6 background were generated as described (17). After weaning, wild-type and homozygous AMPKα2 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 AMPKα2 KO male mice was fixed overnight in 10% formalin, dehydrated, and embedded in paraffin for subsequent sectioning. Sections (8 μm) 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 μg 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/EBPα, PPARγ, 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′- TTCGCTTGCTGGCAT-3′; DGAT1 reverse, 5′- AGATCGGCCCCACATCCA-3′; GPAT forward, 5′- ATGGACAGGCTCGTTTGA-3′; and GPAT reverse, 5′- CCTACCCACCAACAGAG-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 with a commercially available triglyceride reagent (Sigma, St. Louis, MO).

Results

AMPKα2 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 AMPKα2 subunit on adipose tissue mass and development. At weaning, there was no difference in body weight between wild-type and AMPKα2 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, AMPKα2 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, with a glucometer (AccuChek; Roche Diagnostics, Indianapolis, IN). Serum triglycerides were analyzed as described.

FIG. 1. Body weight and food intake in AMPKα2 KO mice. A: AMPKα2 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 AMPKα2 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 ± SE.
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 AMPKα2 KO mice was not due to a differential energy input because no difference in food intake between wild-type and AMPKα2 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 AMPKα2 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 AMPKα2 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 α2 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 AMPKα2 KO mice was due to an increase in adipose tissue mass.

**Increased adipocyte size without changes in cell number or adipogenesis in AMPKα2 KO mice.**

Increased adipocyte size in adipose tissue mass can be the result of an increase in adipocyte 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 adipose depots of the two experimental groups were found (Fig. 3A), indicating that the increase in the mass of the various fat pads of AMPKα2 KO mice was not due to an increase in cell number. In contrast, microscopic analysis of gonadal adipose tissue from wild-type and AMPKα2 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 AMPKα2 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 AMPKα2 KO mice was not reflected in the change in the expression of adipocyte marker genes such as C/EBPα, PPARγ, 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 AMPKα2 subunit (Table 1). Interestingly, however, we found a considerable reduction, up to 50%, in the expression of ADSF/resistin in adipose tissue of the AMPKα2 KO mice.
mice. Overall, these results demonstrate that the increase in adipose tissue mass observed in AMPKα2 KO mice is not due to changes in cell number or adipocyte differentiation but due to adipocyte hypertrophy.

**AMPKα2 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 AMPKα2 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 AMPKα2 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, AMPKα2 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 AMPKα2 KO mice when compared with wild-type mice (Fig. 6B). This is in contrast to the results observed when AMPKα2 KO mice were fed a normal diet. Unlike wild-type mice, AMPKα2 KO 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 AMPKα2 knockout mice on normal diet disappeared when these mice were fed a high-fat diet. These results suggest that the lack of AMPKα2 subunit and the concomitant increase in lipid storage in adipose tissue in AMPKα2 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 AMPKα2 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 AMPKα2 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 AMPKα2 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 AMPKα2 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 AMPKα2 ablation and the subsequent reduction in AMPK activity. However, no difference in whole-body oxidative metabolism monitored by indirect calorimetry has been observed in AMPKα2 KO mice (17). Thus, the accretion of triglycerides in adipose tissue seems to have resulted from an enhanced anabolic...
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/EBPα and PPARγ (16), two master genes that control adipocyte differentiation (24). It is worth noting that a fraction of AMPKα2 subunit has been found in the nucleus of the cell (25) and that the coactivator p300, a known modulator of PPARγ (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 AMPKα2 KO mice, however, no changes in the expression of adipocyte transcription factors, PPARγ, C/EBPα, or the mature adipocyte markers, including aFABP/aP2, were observed. This indicates that the increase in adipose tissue mass observed in AMPKα2 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 AMPKα2 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 AMPKα2 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 AMPKα2 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 AMPKα2 KO mice seems unlikely because we did not find any difference in heparin-stimulated lipoprotein lipase activity in AMPKα2 KO and wild-type mice (data not shown). A dysregulated lipolysis could also be considered a possible cause for the aug-

**FIG. 5.** Adipocyte markers expression is not altered in AMPKα2 KO mice. *A* Total RNA from inguinal and gonadal fat pads of three wild-type mice and three AMPKα2 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 ± SE. Statistical significance is indicated: *P* < 0.05.

**TABLE 1**

Serum parameters  

<table>
<thead>
<tr>
<th></th>
<th>Fasted</th>
<th>AMPKα2 KO</th>
<th>Fed</th>
<th>AMPKα2 KO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>101.7 ± 6.6</td>
<td>102.6 ± 18.0</td>
<td>157.6 ± 9.8</td>
<td>155.3 ± 6.3</td>
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<tr>
<td>Triglycerides (mg/ml)</td>
<td>0.24 ± 0.03</td>
<td>0.24 ± 0.03</td>
<td>0.36 ± 0.02</td>
<td>0.45 ± 0.05</td>
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<tr>
<td>FFA (mEq/l)</td>
<td>0.78 ± 0.05</td>
<td>1.09 ± 0.17</td>
<td>0.37 ± 0.04</td>
<td>0.54 ± 0.05*</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>2.51 ± 0.40</td>
<td>2.42 ± 0.46</td>
<td>3.72 ± 0.06</td>
<td>3.10 ± 0.36</td>
</tr>
<tr>
<td>Adiponectin (mg/ml)</td>
<td>21.5 ± 0.15</td>
<td>21.2 ± 0.26</td>
<td>20.5 ± 0.16</td>
<td>17.6 ± 0.17</td>
</tr>
<tr>
<td>Insulin (ng/ml)</td>
<td>1.01 ± 0.07</td>
<td>0.70 ± 0.06*</td>
<td>1.67 ± 0.20</td>
<td>1.34 ± 0.08</td>
</tr>
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Data are means ± SE. *P* < 0.05.
mented adipose mass in AMPKα2 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 AMPKα2 KO mice, one would expect an inhibition of lipolysis. However, we did not observe any difference in cytosolic HSL activity in adipose tissue of AMPKα2 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 AMPKα2 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 AMPKα2 KO mice.

In the previous characterization of AMPKα2 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 AMPKα2 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 AMPKα2 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, AMPKα2 KO mice showed glucose disposal rates similar to those of wild-type mice. These results can be interpreted as a resistance of AMPKα2 KO mice to further develop high-fat diet–induced glucose intolerance. AMPKα2 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 AMPKα2 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 AMPKα2 KO mice as compared with wild-type mice when fed a high-fat diet, whereas AMPKα2 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 AMPKα2 KO mice on a high-fat diet because other parameters known to influence glucose homeostasis are not substantially different in AMPKα2 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 AMPKα2 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 AMPKα2 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 AMPKα2 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 AMPKα2 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 adipocyte markers, ADSF/resistin expression is significantly lower in AMPKα2 KO mice on a high-fat diet compared with wild-type mice. The contribution of lower ADSF-resistin expression to the glucose metabolism in AMPKα2 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 AMPKα2 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 AMPKα2 KO mice on a normal diet.

In conclusion, our study demonstrates that ablation of AMPKα2 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 enlarged fat mass and enhanced lipid accumulation in adipocytes but not in other tissues. The increase in adipocyte 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 α1 and α2 AMPK subunits may provide valuable information to help us understand the role of AMPK in adipocytes and its impact on whole-body metabolism.


