

Adipose Overexpression of Phosphoenolpyruvate Carboxykinase Leads to High Susceptibility to Diet-Induced Insulin Resistance and Obesity

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Obesity and insulin resistance are associated with increased serum free fatty acids (FFAs). Thus, a reduction in circulating FFAs may increase insulin sensitivity. This could be achieved by increasing FFA reesterification in adipose tissue. Transgenic mice with increased adipose tissue glyceroneogenesis, caused by overexpression of phosphoenolpyruvate carboxykinase (PEPCK), show increased FFA reesterification and develop obesity but are insulin sensitive. Here, we examined whether these transgenic mice were protected from diet-induced insulin resistance. Surprisingly, when fed a high-fat diet for a short period (6 weeks), transgenic mice developed severe obesity and were more hyperinsulinemic, glucose intolerant, and insulin resistant than controls. The high triglyceride accumulation prevented white adipose tissue from buffering the flux of lipids in circulation and led to increased serum triglyceride levels and fat deposition in liver. Furthermore, circulating leptin and FFA concentrations increased to similar levels in transgenic and control mice, while adiponectin levels decreased in transgenic mice compared with controls. In addition, transgenic mice showed fat accumulation in brown adipose tissue, which decreased uncoupling protein-1 expression, suggesting that these mice had impaired diet-induced thermogenesis. These results indicate that increased PEPCK expression in the presence of high-fat feeding may have deleterious effects and lead to severe insulin resistance and type 2 diabetes. *Diabetes* 55:273–280, 2006

Obesity is a major health problem in Western societies. This alteration of the metabolic and endocrine functions of adipose tissue is frequently associated with insulin resistance and type 2 diabetes. How enlarged adipose tissue mass leads to liver and muscle insulin resistance and to pancreas insulin hypersecretion is unclear. Adipose tissue secretes several proteins named adipocytokines that may influence glucose

metabolism and insulin sensitivity and link increased adiposity and insulin resistance (1,2). However, the excess of free fatty acids (FFAs) released by adipose tissue in obesity may also be responsible for the development of insulin resistance (3–7). Increases in plasma FFA levels diminish the extraction of insulin by the liver and enhance hepatic gluconeogenesis (4,7). In muscle, the increased rate of fat oxidation impairs insulin-mediated glucose disposal by inhibiting glucose oxidation and glycogen synthesis (3). Moreover, insulin resistance is matched by stimulation of β -cell proliferation and insulin secretion (6).

Fatty acids released to the bloodstream result from the difference between hydrolysis of triglycerides in adipocytes during lipolysis and reutilization of the FFAs by fat cells through a futile cycle termed reesterification (8,9). Adipose tissue buffers lipid fluxes by suppressing the release of FFAs and increasing triglyceride clearance (10). However, in obesity, the impairment of this buffering action may contribute to triglyceride accumulation in liver, skeletal muscle, and pancreatic β -cells, which in turn may lead to insulin resistance (10).

The thiazolidinediones (TZDs), specific activators of peroxisome proliferator-activated receptor (PPAR)- γ , improve insulin sensitivity in type 2 diabetic patients (11). TZDs have a direct antidiabetic effect on glucose metabolism in skeletal muscle and liver (12). In addition, TZDs increase insulin sensitivity by increasing lipid storage capacity of adipose tissue and reducing circulating FFA and triglyceride levels (13). TZDs decrease fatty acid release from adipose tissue by increasing FFA reesterification via the induction of both phosphoenolpyruvate carboxykinase (PEPCK), a regulatory enzyme of glyceroneogenesis, and glycerol kinase (14,15).

The increase in glyceroneogenesis in transgenic mice overexpressing PEPCK in adipose tissue leads to increased FFA reesterification; higher adipocyte size, fat mass, and body weight; and decreased circulating FFAs (16). Moreover, despite obesity, glucose tolerance and whole-body insulin sensitivity are preserved (16). This suggests that obesity without increased circulating FFAs does not lead to insulin resistance or type 2 diabetes. Thus, we examined these transgenic mice to determine whether increased FFA reesterification counteracts the insulin resistance induced by a high-fat diet. After 6 weeks on this diet, transgenic mice gained more body weight and displayed more pronounced glucose intolerance and insulin resistance than control mice. Thus, paradoxically, PEPCK overexpression causes insulin sensitivity on a normal diet but insulin resistance on a high-fat diet.

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BAT, brown adipose tissue; FFA, free fatty acid; PEPCK, phosphoenolpyruvate carboxykinase; PGC, PPAR- γ coactivator; PPAR, peroxisome proliferator-activated receptor; TZD, thiazolidinedione; UCP, uncoupling protein; WAT, white adipose tissue.

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RESEARCH DESIGN AND METHODS

Treatment of mice. Transgenic mice expressing the PEPCK gene in white adipose tissue (WAT) and brown adipose tissue (BAT) under the control of the $\alpha P2$ promoter in a C57Bl6XSJL mixed genetic background were used (16). We used littermates as controls. Mice were fed ad libitum with a standard diet (Panlab, Barcelona, Spain) and maintained under a light-dark cycle of 12 h (lights on at 8:00 A.M.). Control and homozygous transgenic male mice aged 3 months were kept in individual cages and fed a high-fat diet (TD 88137; Teklad, Madison, WI) or a standard diet for up to 6 weeks. Where stated, mice were fasted for 16 h. Animals were anesthetized and killed between 9:00 and 11:00 A.M. Blood and tissue samples were immediately frozen. Energy expenditure was measured using an indirect open-circuit calorimeter (Oxylet; Panlab, Cornell, Spain), which allowed us to monitor oxygen consumption (V_{O_2}), carbon dioxide production (V_{CO_2}), food intake, and locomotor activity in four metabolic chambers simultaneously. Mice were acclimated to the metabolic chambers for 24 h, and data were collected every 15 min for 3 min in each cage. Data for energy expenditure were taken from the dark photoperiod and adjusted for body weight. All experimental procedures involving mice were approved by the Ethics and Experimental Animal Committee of the Universitat Autònoma de Barcelona.

RNA analysis. Total RNA was obtained from WAT and BAT by using Qiazol Isolation Reagent (Qiagen, Hilden, Germany). RNAs were separated by electrophoresis on a 1% agarose gel containing 2.2 mol/l formaldehyde. Northern blots were hybridized to ^{32}P -labeled *PEPCK*, *PGC-1 α* , *PPAR- γ* , *18S rRNA*, and *UCP-1* cDNA probes (17–20).

Protein detection by Western blot. Western blot analysis was performed by standard procedures from total cellular homogenates of WAT and BAT (21). Tissues were homogenized in a buffer containing 20 mmol/l HEPES pH 7.9, 25% (vol/vol) glycerol, 420 mmol/l NaCl, 10 mmol/l KCl, 0.5 mmol/l dithiothreitol, 1.5 mmol/l $MgCl_2$, 0.2 mmol/l EDTA, 0.5 mmol/l phenylmethylsulfonyl fluoride, 20 μ mol/l leupeptin, 20 μ mol/l pepstatin, 20 μ mol/l aprotinin, 50 mmol/l NaF, and 2 mmol/l Na vanadate. Samples were centrifuged (15,000g), and an aliquot of the supernatant was assayed for protein concentration by the Bradford method as described by the manufacturer (Bio-Rad protein assay; Bio-Rad, Hercules, CA). Twenty-five micrograms of protein was analyzed by 10% SDS-PAGE and transferred to nitrocellulose membranes. Proteins were detected using rabbit polyclonal anti-PPAR- γ coactivator (PGC)-1 (Chemicon International, Temecula, CA) antibody diluted 1:2,000, rabbit polyclonal anti-uncoupling protein (UCP)-1 (Abcam, Cambridge, U.K.) antibody diluted 1:1,000, and rabbit polyclonal anti-pyruvate carboxylase (PCK)-1 (Abgent, San Diego, CA) diluted 1:500.

Enzyme, metabolite, and hormone assays. Tissue triglyceride content was determined by extracting total lipids from liver and BAT samples with chloroform-methanol (2:1 vol/vol) as described previously (22), separating the chloroform and methanol-water phases. Triglycerides were then quantified spectrophotometrically using an enzymatic assay kit (GPO-PAP; Roche Diagnostics, Basel, Switzerland). Glucose was measured enzymatically (Glucoquant; Roche Diagnostics) in serum. Glucose was also determined in 5 μ l blood by using a Glucometer Elite analyzer (Bayer, Leverkusen, Germany). Serum triglycerides were determined enzymatically (GPO-PAP). FFAs were measured in serum by the acyl-CoA synthase and acyl-CoA oxidase methods (Wako Chemicals, Neuss, Germany). Serum insulin levels were measured by radioimmunoassay (CIS Biointernational, Gif-Sur-Yvette, France). Leptin concentration was determined in 5 μ l serum using an enzyme-linked immunosorbent assay kit (Crystal Chemical, Chicago, IL) following the manufacturer's instructions. Serum adiponectin levels were measured by radioimmunoassay (Linco, St. Charles, MO).

Glucose and insulin tolerance tests. For glucose tolerance tests, awake control and transgenic mice fasted overnight (16 h) with free access to water and were given an intraperitoneal injection of glucose (1 g/kg body wt). Blood samples were obtained from the tail vein before the glucose injection and at the indicated time points after the glucose load, and the glucose concentration was measured. For insulin tolerance tests, insulin (0.75 IU/kg body wt; Humulin Regular; Eli Lilly, Indianapolis, IN) was injected intraperitoneally into awake fed control and transgenic mice. Glucose concentration was determined in blood samples obtained from the tail vein at the indicated time points after the insulin injection.

Histological analysis. The epididymal fat pad, interscapular BAT, and liver from control and transgenic mice were fixed for 12–24 h in formalin, embedded in paraffin, and sectioned. Sections were stained with hematoxylin/eosin. For adipocyte size quantification, sections were viewed with a Nikon Eclipse E800 microscope (Nikon, Tokyo, Japan) at 10 \times magnification. Images were obtained with a video camera connected to a color monitor and to an image analyzer (analySIS 3.0; Soft Imaging System, Lakewood, CO). Surface areas of adipocytes were measured using the analySIS software. The mean

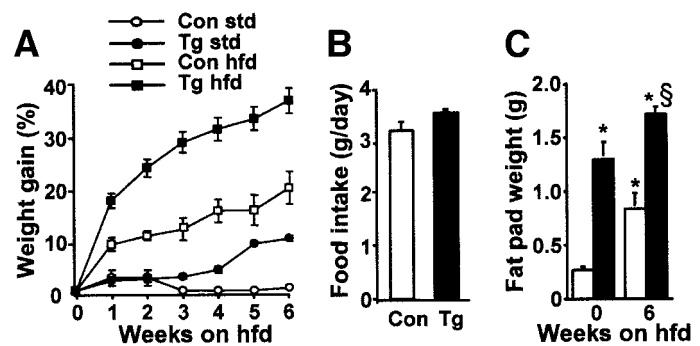


FIG. 1. Fat mass gain of high-fat diet-fed mice. **A:** Changes in body weight of control (Con std) and transgenic (Tg std) mice fed a standard diet and control (Con hfd) and transgenic (Tg hfd) mice fed a high-fat diet for 6 weeks. Data are means \pm SEM of at least 12 mice for each group. **B:** Food consumption during the high-fat diet. Data are means \pm SEM of 10 mice for each group. **C:** Epididymal fat pad weight from control and transgenic mice before and after a high-fat diet. * $P < 0.01$ vs. standard diet-fed control mice; $\$P < 0.01$ vs. high fat-fed control mice.

surface area and the frequency distribution were calculated from >500 cells for each mouse.

Statistical analysis. Enzyme activities, serum parameters, and metabolite concentrations are expressed as means \pm SEM. The significance of differences between data were analyzed using the Student-Newmann-Keuls test. Differences were considered significant at $P < 0.05$.

RESULTS

Transgenic mice overexpressing PEPCK developed severe obesity after being fed a high-fat diet. Three-month-old male mice were fed a high-fat diet for 6 weeks. During this period, control mice gained $\sim 20\%$ of their initial body weight, whereas transgenic mice gained $\sim 40\%$ (Fig. 1A), although food intake was similar in both groups (Fig. 1B). Epididymal fat pad weight was greatly increased in transgenic compared with control mice fed a standard diet (Fig. 1C). When fed a high-fat diet, fat pad weight increased in both groups and was higher in transgenic mice (Fig. 1C). Histological analysis of fat tissue showed larger white adipocytes in transgenic mice than in control mice (Fig. 2A). Furthermore, after a high-fat diet, adipocyte size increased in both groups, especially in the transgenic mice (Fig. 2A). WAT hypertrophy was quantified by measuring mean surface adipocyte area. Transgenic white adipocytes had larger (~ 2.5 -fold) mean surface area than control adipocytes in mice fed a standard diet (controls fed standard diet $408 \pm 20 \mu m^2$ vs. transgenics fed standard diet $948 \pm 52 \mu m^2$). After a high-fat diet, adipocyte surface area was increased about 2.5-fold in control mice and ~ 3.2 -fold in transgenic mice compared with controls on standard diet (controls fed high-fat diet $1,006 \pm 66 \mu m^2$ vs. transgenics fed high-fat diet $1,301 \pm 182 \mu m^2$). High fat-fed transgenic mice also showed a higher number of small cells than controls, as well as the presence of very large adipocytes (Fig. 2B). PEPCK mRNA expression was also analyzed in WAT. A sixfold increase in PEPCK gene expression was observed in WAT from transgenic mice fed a standard diet (Fig. 2C). A similar increase was observed when these transgenic mice were fed a high-fat diet (Fig. 2C). Moreover, higher protein levels of PEPCK were measured in WAT and BAT of transgenic mice (Fig. 2D). This indicates that PEPCK overexpression in adipose tissue was not altered by the high-fat diet.

PEPCK overexpression in adipose tissue aggravates hyperinsulinemia and insulin resistance induced by a

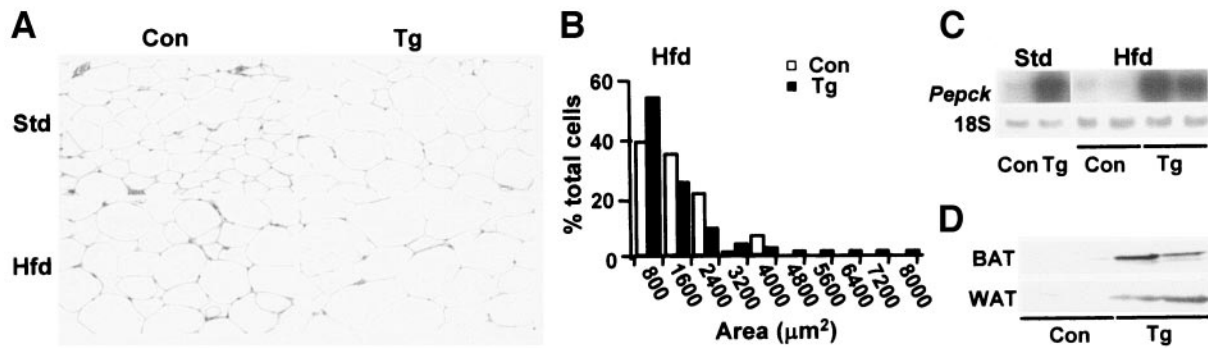


FIG. 2. Histological analysis of adipose tissue and PEPCK overexpression in adipose tissue from high-fat diet-fed transgenic mice. *A*: Representative sections stained with hematoxylin/eosin of epididymal WAT from control (Con) and transgenic (Tg) mice before and after a high-fat diet. *B*: Frequency distribution of adipocyte cell surface area from control and transgenic mice fed a high-fat diet. *C*: Expression of PEPCK in WAT. Representative Northern blots of RNA from epididymal WAT from control and transgenic mice fed either a standard (Std) or a high-fat (Hfd) diet hybridized with a PEPCK or a 18S probe are shown. *D*: Protein levels of PEPCK were detected by Western blot in WAT and BAT from control and transgenic mice fed a high-fat diet. Representative Western blots are shown.

high-fat diet. When transgenic mice were fed a standard diet, circulating glucose levels were unchanged in either fed or fasted conditions (Fig. 3A). After 6 weeks on a high-fat diet, blood glucose concentrations increased in both conditions in both control and transgenic mice (Fig. 3A). Transgenic mice fed a standard diet showed similar insulin levels to controls (Fig. 3B). However, after feeding a high-fat diet, control mice were mildly hyperinsulinemic (~1.5-fold increase), whereas transgenic mice showed a strong increase (~14-fold) in insulinemia (Fig. 3B). After 6 weeks on a high-fat diet, transgenic mice had higher blood glucose levels and did not recover basal glucose at 180 min, indicating that they had become more glucose intolerant than controls (Fig. 3C). Whole-body insulin sensitivity of transgenic mice was also measured. In fat-fed transgenic mice, the hypoglycemic effect of insulin was abolished, while the insulin response of fat-fed control mice was slightly lower than that of control mice fed a standard diet (Fig. 3D). This indicated that transgenic mice had developed higher insulin resistance than controls when fed a high-fat diet.

Transgenic mice presented lipid accumulation in liver and increased triglyceridemia. Mice fed a high-fat diet for 6 weeks showed higher hepatic fat deposition than mice fed a standard diet (Fig. 4A). Although control mice showed mild lipid accumulation, transgenic mice developed high liver steatosis. Consistent with this morphological alteration, hepatic triglyceride content was increased about sevenfold in transgenic mice fed a high-fat diet compared with control mice fed a standard diet and twofold compared with controls fed a high-fat diet (Fig. 4B). Transgenic mice fed a standard diet had unchanged serum triglyceride concentrations compared with controls (Fig. 4C). However, after 6 weeks on a high-fat diet, transgenic mice presented hypertriglyceridemia, whereas circulating triglycerides in control mice remained unaltered (Fig. 4C). These results indicate that increased reesterification in adipose tissue associated with a high-fat diet leads to lipid deposition in liver and increased circulating triglyceride levels, which may also have contributed to the development of insulin resistance in transgenic mice.

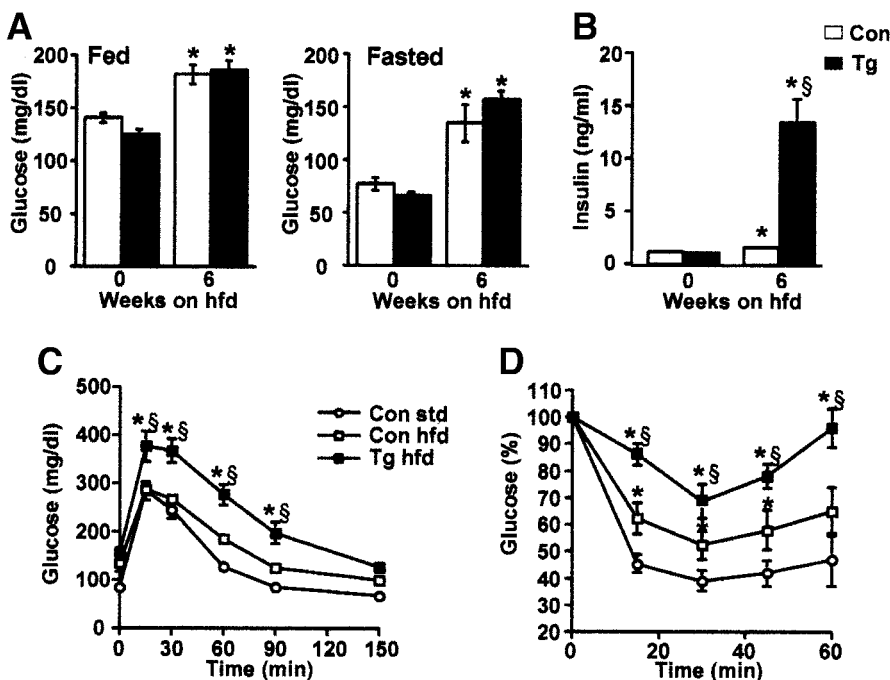


FIG. 3. Circulating glucose and insulin levels and intraperitoneal glucose and insulin tolerance tests. *A*: Glucose was measured in control and transgenic mice in fed and fasted conditions before and after a high-fat diet (hfd). Data are means \pm SEM of 12 animals for each group. *B*: Insulin was measured in control and transgenic mice before and after a high-fat diet in fed conditions. Data are means \pm SEM of 12 animals for each group. *C*: Awake overnight-fasted control mice fed a standard diet (Con std) and control (Con hfd) and transgenic (Tg hfd) mice after 6 weeks on a high-fat diet were given an intraperitoneal injection of glucose (1 mg/kg body wt). Blood samples were taken from the tail vein of the same animal at the times indicated. Results are means \pm SEM of six animals for each group. *D*: Insulin was injected intraperitoneally into awake control mice fed a standard diet (Con std) and control (Con hfd) and transgenic (Tg hfd) mice fed a high-fat diet. Blood samples were taken from the tail vein of the same animals at the time indicated, and glucose levels were determined. Results are means \pm SEM of six animals for each group. * $P \leq 0.01$ vs. standard diet-fed control mice; § $P \leq 0.05$ vs. high fat-fed control mice.

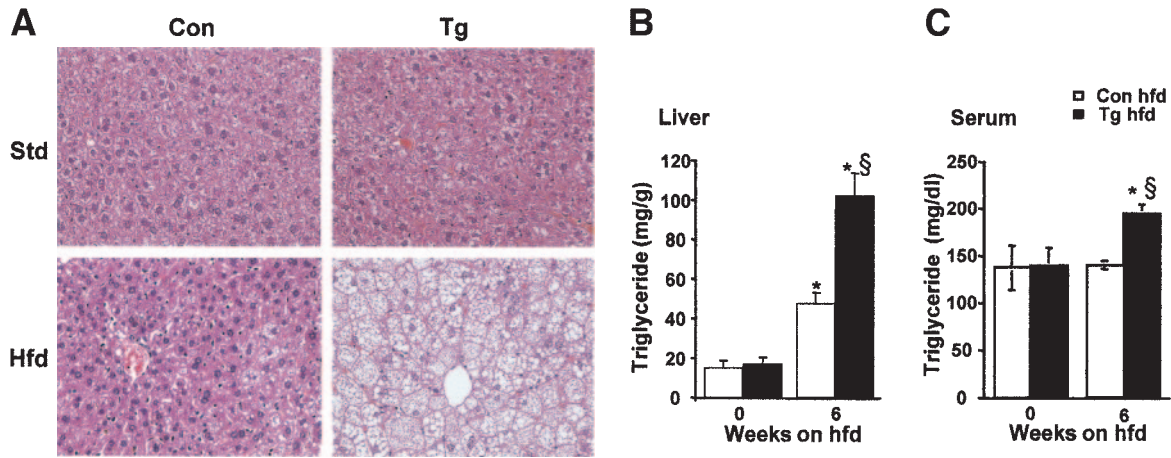


FIG. 4. Lipid accumulation in the liver of transgenic mice. **A:** Representative sections stained with hematoxylin/eosin of liver from control (Con) and transgenic (Tg) mice fed either a standard (Std) or a high-fat (Hfd) diet for 6 weeks. **B:** Triglyceride content in the liver of control and transgenic mice before and after 6 weeks on a high-fat diet. **C:** Triglyceride levels in serum from control and transgenic mice before and after 6 weeks on a high-fat diet. Data are means \pm SEM of six mice for each group. * $P \leq 0.01$ vs. control mice fed a standard diet; § $P \leq 0.01$ vs. high fat-fed control mice.

Circulating FFA levels were similar in high fat-fed transgenic and control mice. In fed animals, most of the circulating FFAs came from food because adipose tissue lipolysis is inhibited by insulin. Fat-fed control and transgenic mice showed a similar circulating FFA increase to mice fed a standard diet, probably due to the high-fat diet and to adipose tissue insulin resistance (Fig. 5A). In fasted control mice, FFA release was increased (approximately fourfold), as compared with fed control mice, due to increased lipolysis. In contrast, fasted transgenic mice on a standard diet only showed a twofold increase in FFA levels, resulting from the higher reesterification rate. However, after 6 weeks on a high-fat diet, fasted transgenic mice showed a similar increase in circulating FFAs to controls (Fig. 5A). Thus, although transgenic mice were more obese than control mice, they did not show higher circulating FFA levels. This suggested that the higher insulin resistance observed in transgenic mice did not result from increased circulating FFA levels.

Adiponectin levels were decreased in transgenic mice. Interleukin (IL)-6, tumor necrosis factor (TNF)- α , leptin, and adiponectin levels have been described to be altered during obesity and may contribute to insulin resistance. The concentration of these hormones was also determined in serum from transgenic and control mice

before and after a 6-week high-fat diet. Circulating IL-6 and TNF- α concentration were not detected (data not shown), indicating that in our transgenic mice, insulin resistance was probably not the result of alterations of these hormones. Leptin levels were the same in both groups feeding on a standard diet and were increased to a similar extent in both groups by a high-fat diet (Fig. 5B). In contrast, adiponectin levels under standard diet were higher in transgenic mice (Fig. 5C). Under high-fat diet, adiponectin levels increased ($\sim 300\%$) in control mice, while they were only slightly increased ($\sim 30\%$) in transgenic mice and were 30% lower than in high-fat fed controls (Fig. 5C). This lower adiponectin concentration, together with the increased leptinemia, may also have contributed to the development of insulin resistance in high fat-fed transgenic mice.

Transgenic mice showed alterations in BAT. Brown adipocytes from transgenic mice accumulated more lipid than control mice when both groups were fed a standard diet (Fig. 6A). Moreover, a high-fat diet increased fat deposition in both groups, resulting from increased FFA reesterification (16). However, the multilocular lipid droplet size was higher in transgenic mice, and in some adipocytes, lipid deposition appeared unilocular (Fig. 6A). Lipid content of BAT was increased ~ 16 -fold in transgenic

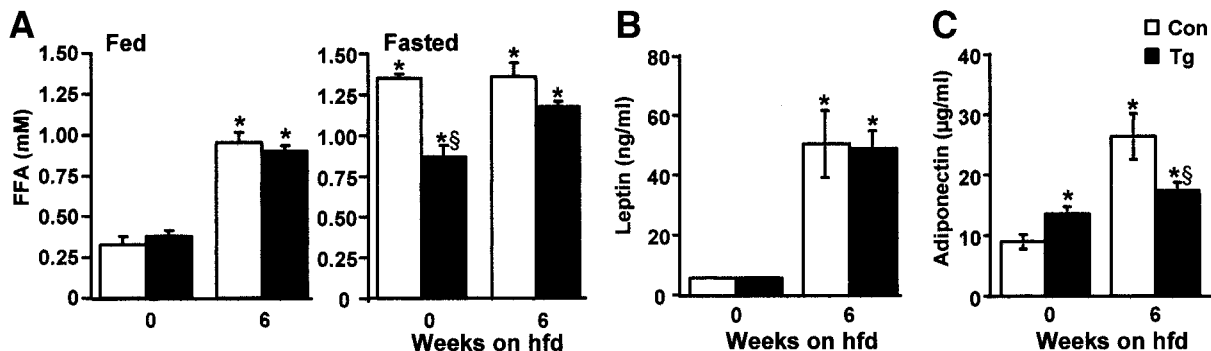


FIG. 5. Serum FFA, leptin, and adiponectin levels. **A:** FFA levels were measured in serum from control (Con) and transgenic mice (Tg) in fed and fasted conditions before and after a high-fat diet (hfd). Results are means \pm SEM of 12 animals for each group. * $P \leq 0.01$ vs. standard diet-fed control mice; § $P \leq 0.01$ vs. standard diet-fasted control mice. **B:** Leptin (A) and adiponectin (B) levels in serum from control and transgenic mice before and after a high-fat diet. Data are means \pm SEM of 12 mice for each group. * $P \leq 0.01$ vs. standard diet-fed control mice; § $P \leq 0.01$ vs. high fat-fed control mice.

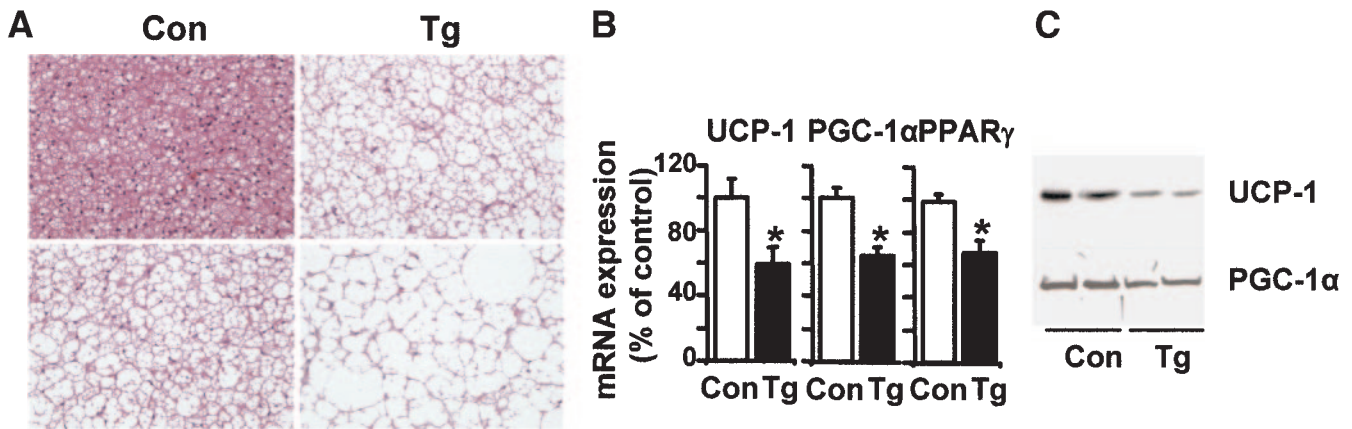


FIG. 6. BAT alterations in transgenic mice. **A:** Representative sections stained with hematoxylin/eosin of interscapular BAT from control (Con) and transgenic (Tg) mice fed either a standard (Std) or a high-fat (Hfd) diet. **B:** Expression of key genes in BAT. Northern blots of RNA from interscapular BAT from control and transgenic mice fed a high-fat diet were hybridized with UCP-1, PPAR- γ , PGC-1 α , and 18S cDNA probes. The signals obtained were quantified and normalized for loading inequalities with 18S and expressed as a percentage of control mice fed a high-fat diet (100%). Data are means \pm SEM of at least eight mice for each group. * $P \leq 0.05$ vs. high fat-fed control mice. **C:** Protein levels of UCP-1 and PGC-1 α were detected by Western blot in BAT from control and transgenic mice fed a high-fat diet. Representative Western blots are shown.

mice compared with control mice fed a high-fat diet (controls fed high-fat diet 0.225 ± 0.024 mg triglyceride/mg protein vs. transgenics fed high-fat diet 3.637 ± 1.770 mg triglyceride/mg protein; $n = 8$). The expression of key genes in energy expenditure was next analyzed in these mice. UCP-1 mRNA levels decreased 40% in transgenic mice (Fig. 6B). Moreover, protein levels of UCP-1 were strongly decreased in BAT from transgenic mice (Fig. 6C). To further assess the molecular mechanisms leading to downregulation of UCP-1, the expression of two positive regulators of UCP-1, PGC-1 α and PPAR- γ , was determined. Transgenic mice showed a decrease of PGC-1 α mRNA ($\sim 30\%$) and protein, which may be responsible for decreased UCP-1 (Fig. 6B and C). Moreover, Northern blot analysis showed a decrease in PPAR- γ mRNA ($\sim 40\%$) in transgenic BAT (Fig. 6B). These results suggest that the fat deposition in BAT may have led to decreased UCP-1 and impaired diet-induced thermogenesis, as well as contributed to the development of insulin resistance in transgenic mice.

Energy expenditure but not physical activity is decreased in transgenic mice. Energy expenditure was measured by indirect calorimetry in mice fed either a standard or a high-fat diet (dark cycle). Whereas food intake was similar in transgenic and control mice during the experiment, energy expenditure was lower in transgenic mice than in controls fed either a standard or a high-fat diet (Fig. 7A). Moreover, high fat-fed control and transgenic mice showed lower energy expenditure than

control mice fed a standard diet (Fig. 7A). However, no difference in physical activity was observed between groups (Fig. 7B). This indicates that the decreased energy expenditure was not due to lower activity. It also suggests that increased body fat gain was due to decreased metabolic rate and thermogenesis.

DISCUSSION

These results show that transgenic mice overexpressing PEPCK in adipose tissue gain twice as much body weight as control mice when fed a high-fat diet. However, food intake was similar in transgenic and control mice, which was consistent with similar leptin levels in both groups. Transgenic mice also accumulated more fat in WAT and BAT. These mice presented strong hyperinsulinemia and were more insulin resistant and glucose intolerant than control mice fed a high-fat diet. Moreover, they displayed higher levels of circulating triglycerides associated with a higher degree of liver steatosis. This steatosis may be responsible for the alterations in glucose homeostasis observed in transgenic mice. The lipid accumulation in the liver inhibits glucose metabolism, thus contributing to hyperglycemia, and decreases hepatic insulin extraction contributing to hyperinsulinemia (23,24). Excess triglyceride deposition in liver, referred to as nonalcoholic fatty liver disease, is a common disorder predominantly found in individuals with type 2 diabetes, obesity, and other components of the metabolic syndrome (23). In humans,

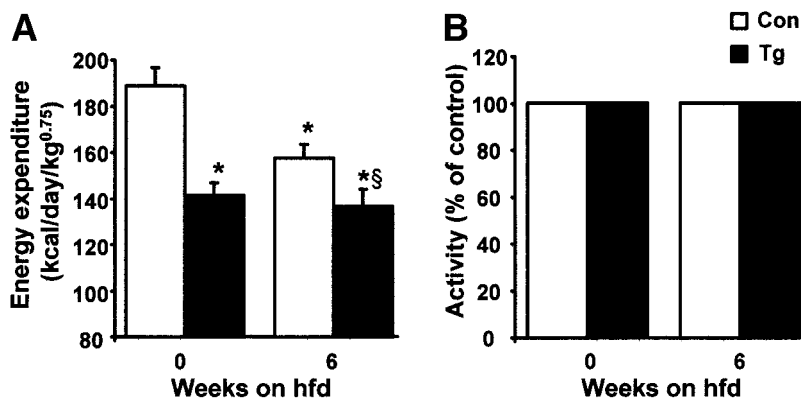


FIG. 7. Energy expenditure and physical activity. **A:** Energy expenditure was calculated from oxygen consumption and CO₂ production, measured by indirect calorimetry during the dark photoperiod, as indicated in RESEARCH DESIGN AND METHODS. **B:** Spontaneous locomotor activity was recorded during a 24-h period. Data were collected from control (Con) and transgenic (Tg) mice before and after a high-fat diet (hfd) and are reported as means \pm SEM of four mice for each group. * $P \leq 0.05$ vs. standard diet-fed control mice; § $P \leq 0.05$ vs. high fat-fed control mice.

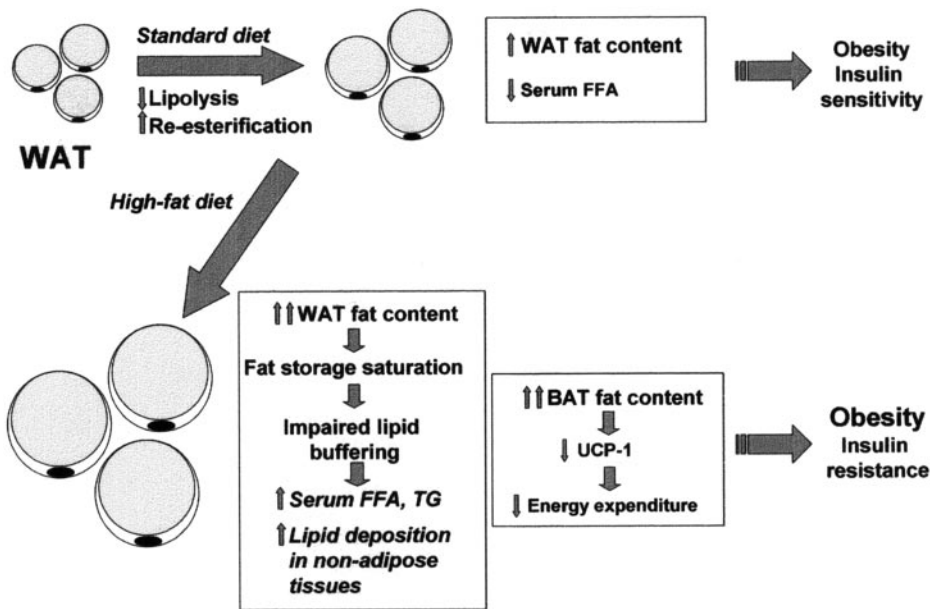


FIG. 8. High-fat feeding in the presence of increased PEPCK expression impairs adipose tissue lipid buffering and leads to obesity and insulin resistance. TG, triglycerides.

fat accumulation in the liver is associated with hepatic insulin resistance, fasting hyperinsulinemia, and hypertriglyceridemia (25). This suggests that insulin resistance is a consequence of lipid deposition in the liver, thus contributing to hyperglycemia and impaired glucose tolerance. Consistent with this, the increase of the glycolytic flux by long-term glucokinase overexpression in the liver of transgenic mice leads to increased hepatic triglyceride content and circulating lipid levels, which cause insulin resistance (22). Hepatic steatosis, elevated circulating lipids, and insulin resistance are also associated with adipose tissue deficiency in lipodystrophy (26,27). Insulin sensitivity can be restored in a mouse lipodystrophic model by fat transplantation, probably by recovering the capacity of adipose tissue to buffer lipids and reducing hepatic fat (28). Thus, probably because of the lack of nonadipose tissue fat deposition, transgenic mice fed a standard diet were obese without insulin resistance (16). In contrast, more severe obesity induced by a high-fat diet led to a saturation of the storage capacity of adipose tissue, which led to fat deposition in the liver and then to insulin resistance, glucose intolerance, and hyperinsulinemia. Therefore, this suggests a threshold in the increase of adipose tissue mass, which is probably crucial for the appearance of fat deposition in nonadipose tissues.

In addition, the development of insulin resistance in obesity may be caused by the excess of FFAs released by adipose tissue (3–7). As expected, circulating FFA levels were decreased in fasted transgenic mice when fed a standard diet because of the increased reesterification rate in adipose tissue (16). However, in transgenic mice fed a high-fat diet, FFA levels increased to similar levels to controls. This increase in FFA levels induced by high-fat diet in transgenic mice compared with standard diet-fed transgenic mice may be the result of either increased adiposity or increased insulin resistance in adipose tissue of these mice. However, the absence of higher circulating FFA levels in transgenic compared with high fat-fed controls suggests that PEPCK overexpression in adipose tissue leads to fatty liver and insulin resistance through another mechanism. Adipose tissue also secretes factors that may affect insulin sensitivity and link obesity with type 2 diabetes (1,2). For instance, adiponectin increases

insulin sensitivity and is lower in obese patients (29–32). However, in the present study, 6 weeks of high-fat feeding increased adiponectin in controls. Similarly, previous studies demonstrated that adiponectin is upregulated in rats fed a high-fat diet for a short period (33,34). Thus, this increase may be a response to diet-induced insulin resistance and obesity. Furthermore, adiponectin levels in transgenic mice fed a standard diet were higher than in control mice, which may account for the absence of insulin resistance, despite the obesity of these mice when fed a standard diet. This is also consistent with a suggestion that adiponectin decrease is more closely related to insulin resistance than obesity (35). However, when fed a high-fat diet, circulating adiponectin levels were reduced in transgenic compared with control mice, and this decrease may contribute to the development of insulin resistance in transgenic mice. This is consistent with reports that adiponectin levels lower in later stages of obesity (30). Moreover, adiponectin treatment decreases liver fat content in obese mice (31). This suggests that decreased expression of adiponectin in high fat-fed transgenic mice may be partly responsible for their higher hepatic steatosis, thus contributing to insulin resistance and glucose intolerance.

Our results indicate that PEPCK is involved in adipose tissue triglyceride storage and may play a crucial role in lipid storage regulation. PEPCK activity is controlled at transcriptional level, and PPAR- γ is one of the transcription factors involved in PEPCK promoter activation (36–38). Consistent with this, a deletion of a critical PPAR- γ binding site in the PEPCK gene promoter ablated PEPCK expression in WAT and led to lipodystrophy (39). Activation of PPAR- γ by TZD improves glucose tolerance in diabetic patients and diabetic animal models (11–13). However, these drugs, probably by increasing FFA reesterification through PEPCK and glycerol kinase activation (14,15), induce weight gain. TZD treatment leads to a redistribution of adipose tissue from visceral to subcutaneous depots (40–43). This shift may improve insulin sensitivity. However, our results indicate that an increase in FFA reesterification associated with a high-fat diet may have deleterious effects

The PEPCK overexpression also led to higher fat accu-

mulation in BAT of transgenic mice (16). Transgenic brown adipocytes showed increased lipid droplet size, and some cells had a white adipocyte appearance. Fat accumulation in BAT may impair thermogenesis through alteration of UCP-1. UCP-1 is a mitochondrial inner-membrane protein that uncouples proton entry from ATP synthesis leading to heat production (44,45). This protein is the main factor responsible for thermogenesis in BAT (46). While the expression of UCP-1 was not altered in standard-fed transgenic mice (data not shown), the high lipid deposition in BAT was accompanied by a downregulation of UCP-1 expression in fat-fed transgenic mice. Its expression is controlled by PPAR- γ and PGC-1 α (18,47). However, a recent report demonstrates that PPAR- γ 2 is not required for BAT development or UCP-1 gene expression (48). Thus, the decreased UCP-1 mRNA levels observed in high fat-fed transgenic mice may have been due to the reduced expression of PGC-1 α gene. Moreover, this UCP-1 reduction may have decreased energy expenditure and increased diet-induced obesity. Consistent with this, mice lacking all three subtypes of β adrenergic receptors (β 1, β 2, and β 3) showed decreased UCP-1 expression, which led to impaired thermogenesis and increased susceptibility to diet-induced insulin obesity (49). Moreover, these mice presented increased lipid deposition in BAT (49). Similarly, chronic-intense PPAR- γ activation by TZD treatment increased the size of lipid vacuoles and suppressed UCP-1 mRNA and protein expression in BAT (50). These results suggest that the decreased energy expenditure and the higher susceptibility to diet-induced obesity and insulin resistance of our transgenic mice are due in part to BAT alterations.

In summary, our results indicate that PEPCK overexpression in adipose tissue and the consequent increased reesterification in transgenic mice under a standard diet lead to obesity without higher circulating FFAs or insulin resistance (Fig. 8). Paradoxically, under a high-fat diet, these mice were insulin resistant. High-fat feeding in the presence of PEPCK overexpression leads to triglyceride accumulation in WAT and BAT and to fat storage saturation. This impairs the role of WAT in buffering the flux of lipids in circulation, leading to fat deposition in liver, hypertriglyceridemia, and insulin resistance (Fig. 8). Furthermore, fat accumulation in BAT probably reduced diet-induced thermogenesis. Thus, all these results suggest that the regulation of the lipid storage capacity of adipose tissue is crucial to the maintenance of insulin sensitivity.

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