

Increased Fatty Acid Re-esterification by PEPCK Overexpression in Adipose Tissue Leads to Obesity Without Insulin Resistance

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Adipose tissue glyceroneogenesis generates glycerol 3-phosphate, which could be used for fatty acid esterification during starvation. To determine whether increased glyceroneogenesis leads to increased fat mass and to explore the role of obesity in the development of insulin resistance, we overexpressed PEPCK, a regulatory enzyme of glyceroneogenesis in adipose tissue. Transgenic mice showed a chronic increase in PEPCK activity, which led to increased glyceroneogenesis, re-esterification of free fatty acids (FFAs), increased adipocyte size and fat mass, and higher body weight. In spite of increased fat mass, transgenic mice showed decreased circulating FFAs and normal leptin levels. Moreover, glucose tolerance and whole-body insulin sensitivity were preserved. Skeletal muscle basal and insulin-stimulated glucose uptake and glycogen content were not affected, suggesting that skeletal muscle insulin sensitivity is normal in transgenic obese mice. Our results indicate the key role of PEPCK in the control of FFA re-esterification in adipose tissue and, thus, the contribution of glyceroneogenesis to fat accumulation. Moreover, they suggest that higher fat mass without increased circulating FFAs does not lead to insulin resistance or type 2 diabetes in these mice. *Diabetes* 51: 624–630, 2002

In the Western world, there has been a marked increase in obesity, which is characterized by an alteration of the control of fat deposition and is frequently associated with type 2 diabetes, hypertension, and dyslipidemia. Moreover, obesity is often associated with increased circulating free fatty acid (FFA) levels (1).

Fatty acids released into 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 re-esterification.

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2-DG, 2-[1-³H]deoxy-D-glucose; aP2, adipocyte lipid-binding protein gene; BAT, brown adipose tissue; FFA, free fatty acid; TNF- α , tumor necrosis factor- α ; WAT, white adipose tissue.

FFAs are esterified with glycerol 3-phosphate. In adipose tissue, glucose is considered to be the main precursor of glycerol 3-phosphate. When the supply of glucose is limited, as in starvation or a low-carbohydrate diet, glyceroneogenesis occurs in adipose tissue from pyruvate and amino acids (2–4). In humans, hepatic glyceroneogenesis from pyruvate may be a major contributor to triglyceride synthesis during starvation and may be important for the regulation of VLDL triglyceride production (5). A regulatory step of this pathway is the conversion of oxaloacetate to phosphoenolpyruvate catalyzed by PEPCK (2). Changes in PEPCK activity occur through modifications of *PEPCK* gene expression, which is hormonally regulated (6,7). β -Adrenergic agonists acting through cyclic AMP stimulate *PEPCK* gene transcription, whereas insulin and glucocorticoids are inhibitory (8,9). However, the physiological role of PEPCK and glyceroneogenesis is not fully understood.

The aim of this study was to examine whether increased PEPCK activity in adipose tissue leads to higher glyceroneogenesis and, if so, whether this would increase lipid re-esterification and fat mass. To this end, transgenic mice overexpressing *PEPCK* specifically in adipose tissue were generated by using a chimeric gene in which *PEPCK* gene transcription was under the control of the constitutive and adipose-specific adipocyte lipid-binding protein gene (*aP2*) promoter (10,11).

RESEARCH DESIGN AND METHODS

Generation of transgenic mice expressing the *aP2-PEPCK* chimeric gene. A 6-kb *ApaI-ApaI* fragment containing the entire coding sequence and the polyadenylation signal of the rat *PEPCK* gene (10) was introduced downstream of the *aP2* promoter gene at a *SmaI* site in the *pAP-2* plasmid (11). This plasmid was designated *pKS/aP2-PCK1*. A 11.4-kb *NotI-SalI* fragment, containing the entire *aP2-PEPCK* chimeric gene, was microinjected into fertilized eggs. The general procedures for microinjection of the chimeric gene were as described elsewhere (12). At 3 weeks of age, the animals were tested for the presence of the transgene by Southern blot, prepared with 10 μ g tail DNA that was digested with *EcoRI*. Blots were hybridized with a 3.85-kb *SphI-SphI* probe containing a *PEPCK* gene fragment radiolabeled with [α -³²P]dCTP (3,000 Ci/mmol; Amersham) by random oligoprimering (Roche Diagnostics, Mannheim, Germany). 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 9:00 A.M.). When stated, mice were starved for 24 h. Animals were killed and samples were taken between 9:00 and 10:00 A.M. In the experiments described below, male mice, aged 4 and 6 months, were used. All the experimental procedures were approved by the Ethical Committee of the Catalan Government.

RNA analysis. Total RNA was obtained from white adipose-tissue (WAT), brown adipose tissue (BAT), liver, and kidney by using TriPure Isolation Reagent (Roche Diagnostics). RNA samples were electrophoresed on a 1%

agarose gel containing 2.2 mol/l formaldehyde. Northern blots were hybridized to a ^{32}P -labeled *PEPCK* cDNA probe. The expression of both the chimeric and the endogenous genes led to a 2.8-kb mRNA transcript.

Enzymatic activity. Cytosolic PEPCK activity was measured in adipose tissue and liver extracts by the method of Chang and Lane (13). WAT and liver samples were homogenized, and 100,000g supernatants corresponding to the cytosolic fraction were incubated in the presence of [^{14}C]-bicarbonate. Radioactivity incorporated into malate was taken to be a direct reflection of PEPCK activity. Protein content was assayed in the homogenates using the Bio-Rad protein assay (Bio-Rad, München, Germany). Glucokinase activity was determined in liver cytosolic extracts from fed mice (14). Glucokinase activity was calculated as the difference between the glucose phosphorylation capacity at 100 and 0.5 mmol/l glucose.

Histological analysis. Epididymal WAT and interscapular BAT were fixed for 12–24 h in formalin, embedded in paraffin, and sectioned. Sections were stained with hematoxylin/eosin.

Body fat content. Mouse carcasses were analyzed following the method of Salmon and Flatt (15). Carcasses were digested in alcoholic KOH at 60°C for 48 h. Aliquots of the digestion were adjusted to a final concentration of 0.5 mol/l MgCl_2 , placed on ice for 10 min, and then centrifuged for 10 min. Triglyceride concentrations were measured in supernatants.

Hormones and metabolites determination. Serum insulin levels were measured by radioimmunoassay (CIS; Biotinternational, Gif-Sur-Yvette, France). Leptin concentration was determined in 5 μl serum using an enzyme-linked immunosorbent assay kit (Crystal Chemical, Chicago, IL) following the manufacturer's instructions. Serum triglycerides were determined enzymatically using GPO-PAP (Roche Diagnostics). FFAs were measured by the acyl-CoA synthase and acyl-CoA oxidase method (Wako Chemicals, Neuss, Germany) in serum, adipose tissue extracts, or incubation medium. Lactate was measured in serum by the lactate dehydrogenase method (Roche Diagnostics). Glucose was measured in blood with a Glucometer Elite analyzer (Bayer, Tarrytown, NY). Glycerol concentration was determined enzymatically (Roche Diagnostics) in serum, adipose tissue extracts, or incubation medium. Glycogen was determined in skeletal muscle and liver from fed mice as previously described (16).

Fatty acid synthesis and glyceroneogenesis. Epididymal fat pads were incubated at 37°C in 3 ml Krebs-Ringer bicarbonate HEPES buffer, pH 7.4, containing 3% BSA (fatty acid-free) and 5 mmol/l pyruvate (0.3 μCi pyruvate-2- ^{14}C). After 2 h of incubation, lipids were extracted from the tissue with chloroform-methanol (2:1), and fatty acids and glyceride-glycerol were isolated as previously described (17).

FFA re-esterification. The method used for the measurement of FFA re-esterification by adipose tissue in vitro was described by Vaughan (18). Epididymal fat pads were incubated for 2 h at 37°C in 3 ml Krebs-Ringer bicarbonate HEPES buffer, pH 7.4, containing 3% BSA (fatty acid-free) and 5 mmol/l pyruvate. FFAs and glycerol were analyzed in both the tissue and the medium before and after the incubation, as previously described.

In vivo glucose utilization index. An intravenous flash injection of 1 μCi of the nonmetabolizable glucose analog 2-[1- ^3H]deoxy-D-glucose (2-DG; Amersham Pharmacia Biotech, Piscataway, NJ) was administered to fed mice. When stated, 0.75 IU/kg insulin (Humulin Regular; Eli Lilly, Indianapolis, IN) was given intraperitoneally to the mice at time zero. The specific blood 2-DG clearance was determined by the Somogyi procedure (19), with 25 μl blood samples (tail vein) obtained 1, 10, 20, and 30 min after injection. Skeletal muscle (gastrocnemius and quadriceps) and WAT (epididymal) samples were removed 30 min after injection. The glucose utilization index was determined by measuring the accumulation of radiolabeled compounds (20). The amount of 2-DG-6 phosphate per milligram of protein was divided by the integral of the concentration ratio of 2-DG to unlabeled glucose measured. Because values were not corrected by a "discrimination constant" for 2-DG in glucose metabolic pathways, the results are expressed as the index of glucose utilization, in picomoles per milligram of protein per minute.

Statistical analysis. All values are expressed as means \pm SE. Statistical analysis was carried out using the Student-Newman-Keuls test. Differences were considered significant at $P < 0.05$.

RESULTS

Adipose-specific overexpression of PEPCK in transgenic mice. The 5.4 kb of the fat-specific regulatory region of the *aP2* gene was used to direct the expression of the *PEPCK* gene to adipose tissue. Four independent lines (Tg1–Tg4) of transgenic mice bearing the *aP2-PEPCK* chimeric gene (Fig. 1A) were obtained. *PEPCK* mRNA expression and activity were analyzed in several tissues.

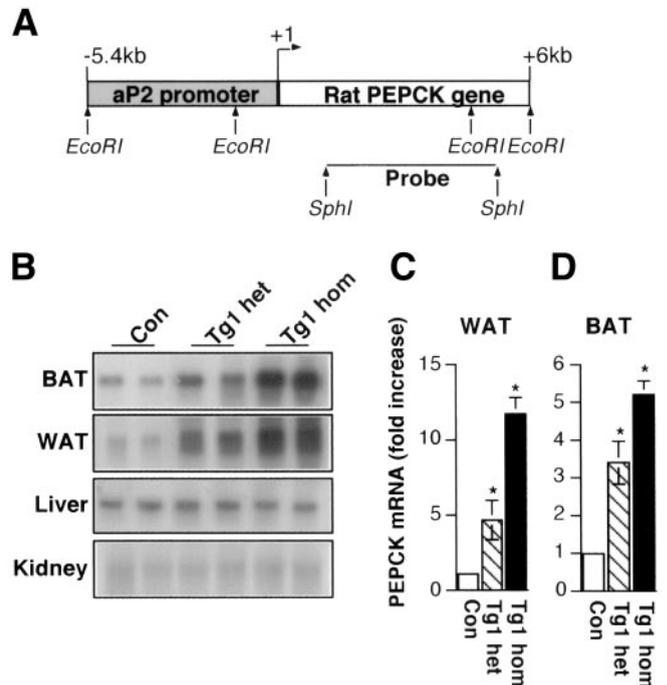


FIG. 1. Adipose-specific expression of the *PEPCK* gene in transgenic mice. **A:** Schematic representation of the *aP2-PEPCK* chimeric gene. A 11.4-kb fragment containing the entire *aP2-PEPCK* chimeric gene was microinjected into fertilized eggs. The transgene was detected by Southern blot using the indicated probe. **B:** Expression of the transgene in WAT and BAT. Representative Northern blots of RNA from epididymal WAT, interscapular BAT, liver, and kidney of starved control (Con) and heterozygous (Tg1 het) or homozygous (Tg1 hom) line 1 transgenic mice, hybridized with a *PEPCK* probe, are shown. **C** and **D:** *PEPCK* mRNA was quantified in WAT and BAT, and the results represent the means \pm SE of six mice.

Both control and transgenic mice showed a 2.8-kb mRNA transcript that hybridized with a *PEPCK* cDNA probe, resulting from the expression of both the endogenous *PEPCK* gene and the transgene. A twofold increase in *PEPCK* mRNA was observed in adipose tissue of Tg2–Tg4 (data not shown). However, a higher level of *PEPCK* mRNA was detected in WAT from heterozygous and homozygous Tg1 mice (5- and 11-fold increase) (Fig. 1B and C). In addition, in BAT, Tg1 heterozygous and homozygous mice showed three- and fivefold increases of *PEPCK* mRNA levels, respectively, compared with controls (Fig. 1B and D). *PEPCK* mRNA levels were unchanged in the liver and kidney of transgenic mice (Fig. 1B). When *PEPCK* activity was measured in WAT cytosolic extracts, about a twofold increase was observed in fed heterozygous transgenic animals from lines Tg2–Tg4 (Fig. 2A). This increase in *PEPCK* activity was higher in fed heterozygous Tg1 mice (3.5-fold) (Fig. 2A). Moreover, starved heterozygous and homozygous Tg1 mice showed 4- and 13-fold increases in adipose *PEPCK* activity, respectively, over controls (Fig. 2B). As expected, no changes were observed in hepatic *PEPCK* activity (Fig. 2B). In the study described below, heterozygous and homozygous Tg1 mice and non-transgenic littermates were used.

Glyceroneogenesis and FFA re-esterification were studied in vitro in WAT from transgenic mice. ^{14}C -pyruvate conversion into glycerol was increased (2- to 2.5-fold) in transgenic compared with control mice (Fig. 2C), indicating an increase in glyceroneogenesis. In contrast, de novo

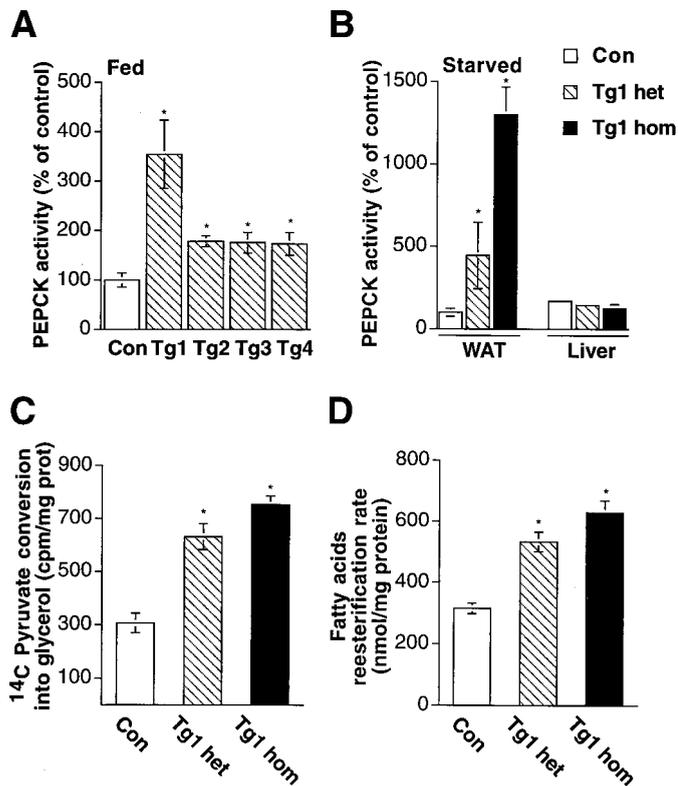


FIG. 2. PEPCK activity, glyceroneogenesis, and fatty acid re-esterification rate in control and transgenic mice. **A:** PEPCK activity was measured in epididymal adipose cytosolic extracts obtained from fed control and transgenic mice. Four transgenic lines (Tg1–Tg 4) were analyzed. Results are presented as percentage of PEPCK activity in control mice (3.9 mU/mg protein). Data represent the mean \pm SE of at least six animals for each group. **B:** PEPCK activity in adipose tissue and liver from overnight-starved control (Con) and heterozygous (Tg1 het) or homozygous (Tg1 hom) line 1 transgenic mice. Results are presented as percentage of PEPCK activity in WAT of control mice (5.8 mU/mg protein). Glyceroneogenesis (**C**) and fatty acid (**D**) re-esterification rate were measured in epididymal fat pad from starved animals as indicated in RESEARCH DESIGN AND METHODS. Data represent the means \pm SE of at least six animals for each group. * $P < 0.05$.

fatty acid synthesis was not significantly affected (control 32.4 ± 10 vs. heterozygous Tg1 25.0 ± 3.5 cpm/mg protein). Furthermore, the FFA re-esterification rate was higher (about twofold) in WAT from transgenic mice than in controls (Fig. 2D). These results indicate that in *aP2-PEPCK* transgenic mice, higher PEPCK activity in WAT led to an increase in glyceroneogenesis and FFA re-esterification.

Although lactate is a substrate of glyceroneogenesis, circulating lactate levels were similar in control and transgenic mice (control 5.29 ± 0.19 vs. homozygous Tg1 5.33 ± 0.17 mmol/l, $n = 8$).

PEPCK overexpression led to adipocyte hypertrophy. Body weight, epididymal fat pad weight, and adipose cell size were measured in 6-month-old mice. Although a small increase in body weight was observed in heterozygous Tg1 mice compared with controls, the body weight of homozygous transgenic mice increased markedly (~33%) (Fig. 3A). Gonadal fat pad weight increased significantly in both heterozygous (~180%) and homozygous (~340%) mice compared with controls (Fig. 3B). Moreover, body fat content increased ~125% in heterozygous and 200% in homozygous transgenic mice (control 5.2 ± 0.1 , heterozy-

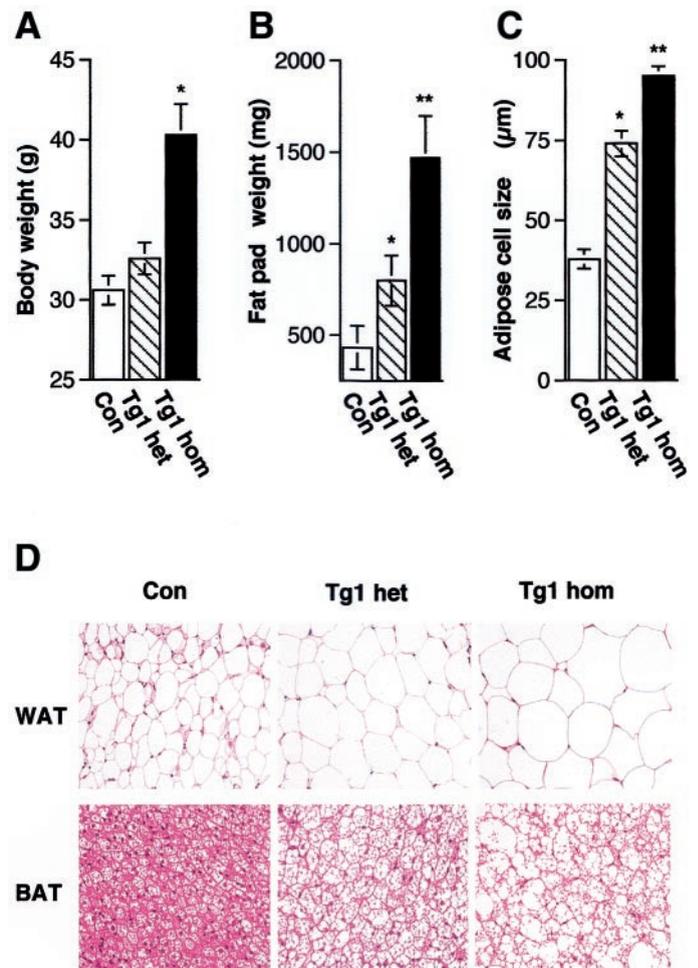


FIG. 3. Body weight (**A**), epididymal fat pad weight (**B**), and adipose cell diameter (**C**) of 6-month-old control (Con) and heterozygous (Tg1 het) or homozygous (Tg1 hom) transgenic mice were determined as indicated in RESEARCH DESIGN AND METHODS. Results are means \pm SE of eight animals for each group. * $P < 0.01$. **D:** Representative sections of epididymal WAT (top panel) and interscapular brown fat (bottom panel) from nontransgenic and heterozygous or homozygous transgenic mice stained with hematoxylin and eosin ($\times 400$).

gous Tg1 6.5 ± 0.1 , and homozygous Tg1 $11.3 \pm 0.1\%$ body fat, $n = 8$), indicating that the body weight gain was probably due to higher lipid deposition. Although transgenic mice had increased fat mass, food intake was similar to that of control mice (3.3 ± 0.1 , 3.2 ± 0.1 , and 3.4 ± 0.1 g/day for control, heterozygous, and homozygous transgenic mice, respectively). Histological analysis of fat tissue from 6-month-old mice showed gonadal white adipocytes with a typical large unilocular lipid droplet, whereas interscapular brown adipocytes contained multilocular lipid droplets (Fig. 3D). In transgenic mice, white adipocytes were much larger than controls, and lipid deposition was also higher in interscapular BAT (Fig. 3D). WAT hypertrophy was quantified by measuring adipocyte diameter. Heterozygous transgenic white adipocytes had a larger (about twofold) mean diameter than control adipocytes (Fig. 3C). This increase was greater (about threefold) in homozygous transgenic mice (Fig. 3C) and was parallel to the fat-pad weight increase (Fig. 3B), indicating that the obesity was probably due to hypertrophy of adipose tissue.

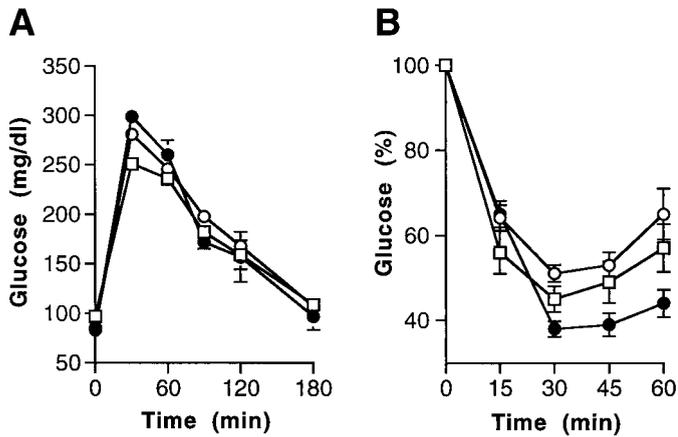


FIG. 4. Intraperitoneal glucose and insulin tolerance tests. **A:** Intraperitoneal glucose tolerance test. Awake overnight starved control (Con, \square) and heterozygous (Tg1 het, \circ) and/or homozygous (Tg1 hom, \bullet) transgenic mice (4 months old) were given an intraperitoneal injection of glucose (1 mg/g of body wt). Blood samples were taken at the times indicated from the tail vein of the same animal. Glucose was measured as indicated in RESEARCH DESIGN AND METHODS. Results are means \pm SE of six animals for each group. **B:** Intraperitoneal insulin tolerance test. Insulin (0.75 IU per kg of body wt, Humulin Regular) was injected intraperitoneally into awake, fed control and transgenic mice. Blood samples were taken from the tail vein of the same animals at the time indicated, and glucose levels were determined as indicated in RESEARCH DESIGN AND METHODS. Results were calculated as percentage of blood glucose values at time zero for each mouse and are means \pm SE of six animals for each group.

Obese aP2-PEPCK mice are not insulin resistant.

Four- to 6-month-old fed and starved heterozygous and homozygous transgenic mice had similar blood glucose levels to nontransgenic mice (fed: control 141 ± 6 vs. heterozygous Tg1 157 ± 10 and homozygous Tg1 139 ± 6 mg/dl; starved: control 87 ± 4 vs. heterozygous Tg1 84 ± 5 and homozygous Tg1 90 ± 11 mg/dl, $n = 8$). Similarly, they showed normal fed and fasted insulin levels (fed: control 27.4 ± 3.7 vs. heterozygous Tg1 27.3 ± 2 and homozygous Tg1 25.9 ± 5 μ U/ml; starved: control 14.6 ± 2 vs. heterozygous Tg1 12.6 ± 2 and homozygous Tg1 14.3 ± 1 μ U/ml). An intraperitoneal glucose tolerance test was also performed in overnight-starved 4-month-old mice. In control as well as homozygous and heterozygous Tg1 mice, glycemia reached similar maximum levels 30 min after glucose injection and then returned to the basal value after 180 min (Fig. 4A). This indicated that transgenic obese mice had normal glucose tolerance. In addition, whole-body insulin sensitivity was measured with an insulin tolerance test. Fed control and transgenic mice showed a similar blood glucose concentration decrease 30 min after insulin injection (Fig. 4B). However, homozygous Tg1 mice presented a tendency to lower glucose levels at 45 and 60 min, indicating that the hypoglycemic response to insulin was higher in transgenic mice (Fig. 4B).

Moreover, in transgenic mice, a significant increase in the basal [14 C]2-deoxyglucose uptake index was detected in WAT (control 280 ± 59 vs. homozygous Tg1 524 ± 67 $\text{pmol} \cdot \text{mg}^{-1} \text{protein} \cdot \text{min}^{-1}$, $n = 4$). This indicated that adipose tissue was not insulin resistant in transgenic mice and may explain the tendency toward higher whole-body insulin sensitivity in homozygous transgenic mice.

In vivo insulin-stimulated glucose uptake into skeletal muscle of transgenic mice was also measured. Skeletal

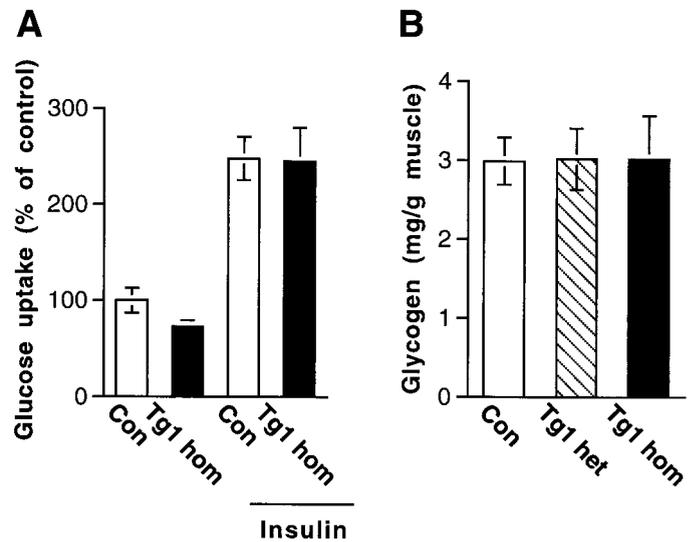


FIG. 5. Skeletal muscle glucose utilization index and glycogen content. **A:** Glucose utilization index was measured in skeletal muscle (quadriceps and gastrocnemius) of fed control and homozygous transgenic mice treated and nontreated (basal) with insulin intraperitoneally as indicated in RESEARCH DESIGN AND METHODS. Results are presented as percentage of basal glucose utilization in muscle of control mice ($20 \text{ pmol} \cdot \text{mg protein}^{-1} \cdot \text{min}^{-1}$). Results are means \pm SE of four animals for each group. **B:** Glycogen concentration in skeletal muscle was measured in perchloric extracts as described in RESEARCH DESIGN AND METHODS. Results are means \pm SE of at least six animals for each group.

muscle basal [14 C]2-deoxyglucose uptake was similar in homozygous transgenic and control mice (Fig. 5A). When insulin was injected, glucose uptake was stimulated about 2.5-fold in control mice. This increase was similar in homozygous transgenic mice (Fig. 5A). In addition, skeletal muscle glycogen content (Fig. 5B) and *Glut-4* gene expression (data not shown) were not altered in transgenic mice.

Furthermore, neither glucokinase activity (control 32 ± 1 , heterozygous Tg1 31 ± 2 , and homozygous Tg1 33 ± 4 mU/mg protein, $n = 8$) nor glycogen content (control 30 ± 3 , heterozygous Tg1 33 ± 3 , and homozygous Tg1 34 ± 4 mg/g liver, $n = 8$) were affected in liver from transgenic obese mice. In addition, serum triglyceride levels were unchanged between transgenic and control animals in fed (control 137 ± 24 , heterozygous Tg1 138 ± 23 , and homozygous Tg1 140 ± 18 mg/dl, $n = 8$) and starved conditions (control 99 ± 14 , heterozygous Tg1 103 ± 12 , and homozygous Tg1 116 ± 6 mg/dl, $n = 8$). Moreover, histological analysis did not show hepatic steatosis (data not shown).

Thus, this suggests that PEPCK overexpression led to altered adipose tissue metabolism and obesity without affecting skeletal muscle or liver metabolism. Moreover, these results indicate that, although they were obese, transgenic mice did not develop insulin resistance.

Obese transgenic mice did not present higher circulating FFA and leptin levels. In fed animals, most of the circulating FFAs came from food, since adipose tissue lipolysis is inhibited by insulin (1). Thus, fed control and transgenic mice showed similar FFA concentrations (Fig. 6A). Starved control mice had serum FFA levels fourfold higher than fed animals due to increased adipose tissue lipolysis. In contrast, both starved heterozygous and ho-

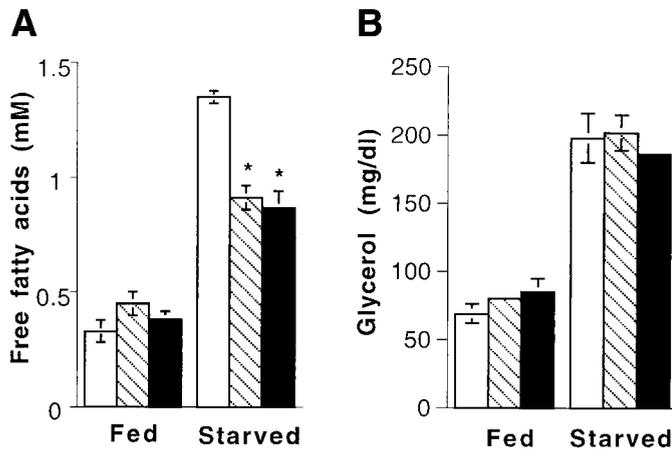


FIG. 6. Circulating FFA and glycerol levels in *aP2-PEPCK* transgenic mice. FFA (A) and glycerol (B) levels were measured in serum from fed or starved six-month-old control (Con, □) and heterozygous (Tg1 het, ▨) or homozygous (Tg1 hom, ■) transgenic animals as indicated in RESEARCH DESIGN AND METHODS. Data represent the means \pm SE of at least eight animals for each group. * $P < 0.05$.

mozygous transgenic animals only showed a twofold increase (Fig. 6A). This indicates that FFA release from adipose tissue was markedly reduced in these mice. However, in starved conditions, similar serum glycerol levels were observed between control and heterozygous and homozygous transgenic mice (Fig. 6B), indicating that the lipolysis rate was the same in both groups. FFA release from adipose tissue is usually increased in obesity (1). However, our results indicate that circulating FFA levels were even lower in obese transgenic animals than in nonobese control mice, probably as a result of the increased rate of intra-adipose tissue re-esterification.

Because adipose tissue production of leptin and tumor necrosis factor- α (TNF- α) are increased during obesity and can affect glucose homeostasis (21,22), serum leptin levels and TNF- α mRNA expression were measured. In WAT from obese transgenic mice, TNF- α mRNA expression was similar to that of controls (data not shown). Moreover, in 6-month-old fed mice, circulating leptin levels were unchanged (control 5.4 ± 0.4 , heterozygous Tg1 5.4 ± 0.6 , and homozygous Tg1 5.4 ± 0.4 ng/ml, $n = 8$). These results suggest that higher fat mass without increased circulating FFA levels did not alter leptin or TNF- α expression.

DISCUSSION

Here, we demonstrate that a primary metabolic alteration in adipose tissue, i.e., *PEPCK* overexpression, increased glyceroneogenesis, FFA re-esterification, and lipid deposition. This indicates direct involvement of *PEPCK* in the synthesis of glycerol 3-phosphate and shows the key role of glyceroneogenesis in FFA re-esterification and subsequent fat accumulation. Furthermore, increased fat accumulation in BAT was also observed, indicating that *PEPCK* is a key enzyme in this tissue. Our results are consistent with previous reports suggesting that *PEPCK* provides glycerol 3-phosphate during starvation, which maintains triglyceride synthesis by FFA re-esterification (23,24). This may be considered as a negative-feedback control on adipose tissue FFA output, which avoids the toxic effects of excess circulating FFAs (25,26). Our results suggest that

an increase in glycerol 3-phosphate concentration is sufficient to increase triglyceride synthesis. This suggests that the enzymatic steps of triglyceride synthesis are not limiting and that regulation of glycerol 3-phosphate supply contributes to the control of triglyceride synthesis. Furthermore, it raises the possibility that dysregulation of glyceroneogenesis might influence lipid deposition and therefore contribute to obesity. However, the role of increased *PEPCK* activity in fat tissue is unknown in human obesity.

Overexpression of the glucose transporter GLUT4 in adipocytes of transgenic mice also results in increased glycerol-glyceride and triglyceride synthesis due to enhanced glucose uptake (27,28). These mice are mildly obese and present adipocyte hyperplasia without hypertrophy (27). In these fed transgenic mice, increased lipogenesis may be balanced by higher rates of lipolysis favored by the lower serum insulin levels and thus would not lead to an increase in adipose cell size (28). In contrast, in homozygous *aP2-PEPCK* transgenic mice, the increased fat mass was associated with higher white adipocyte diameter, demonstrating that the obesity was, at least in part, due to adipose hypertrophy. Consistent with these results, adipose tissue activation of peroxisome proliferator-activated receptor γ by thiazolidinediones, a class of antidiabetic drugs, upregulates GLUT4 and *PEPCK* gene transcription and increases fat accumulation by hypertrophy and hyperplasia (29–31). Most forms of obesity result from adipose cell hypertrophy, although more severe forms also show adipose cell hyperplasia (32). Several spontaneous or genetically engineered animal models of hypertrophic obesity, such as *ob/ob* mice (33), yellow mice with the *agouti* gene mutation (34), or mice lacking the serotonin receptor (35) result from alterations in energy intake or expenditure and are associated with insulin resistance. In contrast, *aP2-PEPCK* transgenic mice had similar food intake to controls. Moreover, these animals were normoglycemic and normoinsulinemic and did not show alterations in glucose or insulin tolerance tests. Skeletal muscle basal and insulin-stimulated glucose uptake and glycogen content were also unaffected, indicating that skeletal muscle of *aP2-PEPCK* transgenic mice was insulin sensitive. Similar hepatic glucokinase activity and glycogen content in transgenic obese and control mice and normal serum triglyceride levels suggested that triglyceride and glucose liver metabolism was not affected. Thus, obesity in homozygous *aP2-PEPCK* transgenic mice was due to a primary alteration in fat deposition because glucose homeostasis, whole-body and skeletal muscle insulin sensitivity, or liver glucose and triglyceride metabolism were not altered. In contrast, *PEPCK* overexpression in the liver leads to increased chronic hepatic glucose production and then to increased insulin secretion, hyperinsulinemia, hyperglycemia, and insulin resistance (12,36,37). Because of the *aP2* promoter adipose specificity (11), *PEPCK* was not overexpressed in the liver of *aP2-PEPCK* transgenic mice.

Obesity is generally associated with insulin resistance, thus favoring the development of diabetes. How enlarged adipose tissue mass affects insulin resistance is unclear, although several mechanisms have been proposed (38). In humans, there is a positive correlation between the

amount of visceral fat and the degree of insulin resistance (1). The increased rate of lipolysis in this fat depot and the resulting increased plasma FFA level are believed to be responsible for the development of insulin resistance (1). High-circulating FFAs may have deleterious effects on insulin uptake by the liver (39) and increase insulin secretion by the pancreatic β -cell (26), therefore leading to hyperinsulinemia. Moreover, FFAs increase gluconeogenesis in liver and inhibit insulin-stimulated glucose utilization in skeletal muscle (40,41). Therefore, in obese *aP2-PEPCK* mice, the absence of insulin resistance may result from the low levels of circulating FFAs due to enhanced re-esterification. Similarly, mice lacking *TNF- α* develop high-fat, diet-induced obesity without hyperinsulinemia and are more sensitive to insulin than obese control animals (42). The *TNF- α ^{-/-}* animal has lower levels of circulating FFAs, which may protect against diet-induced insulin resistance (42).

Furthermore, adipose tissue deficiency in lipodystrophy is associated with elevated circulating lipids and insulin resistance (43). A fatless mouse model, obtained when adipogenesis was disrupted by the expression of a dominant-negative protein inhibiting the function of B-ZIP transcription factor, develops severe insulin resistance (44). These mice show lipid deposition in the liver and high levels of circulating FFAs in a fed state (44). Fat transplantation into this model can restore insulin sensitivity, probably by restoring the capacity of adipose tissue to store lipids (45). Our results together with these findings suggest that increasing fat storage may protect against insulin resistance.

Insulin sensitivity in wild-type mice and rats may also be improved by leptin treatment (21). This hormone, involved in the control of food intake, also enhances whole-body glucose utilization and reduces β -cell insulin secretion (21). Moreover, leptin lowers triglyceride content in non-adipocytes by increasing fatty acid oxidation and decreasing lipogenesis, suggesting that leptin may be involved in counterbalancing fat accumulation and the development of insulin resistance (46). However, in *aP2-PEPCK* transgenic mice, leptin levels were not increased. Therefore, the low circulating FFA levels and the absence of insulin resistance in obese *aP2-PEPCK* transgenic mice were probably not the result of the leptin action. Leptin has also been described as a sensor for adipose tissue triglyceride stores (21). Circulating leptin levels are strongly correlated with body fat mass, and rodent models of obesity generally show hyperleptinemia (21). The fact that no differences were observed in circulating leptin levels between obese *aP2-PEPCK* transgenic mice and controls suggests that an increase in WAT triglyceride content is not sufficient to trigger an increase in leptin production. Furthermore, it has been shown that increased circulating FFAs stimulate leptin secretion (47,48). Thus, the absence of higher levels of circulating FFAs in obese *aP2-PEPCK* transgenic mice was consistent with the lack of increased circulating leptin. In addition, obese *aP2-PEPCK* transgenic mice did not show increased *TNF- α* levels. Although *TNF- α* was reported to impair insulin signaling and may play a role in insulin resistance in obesity, not all studies have found it to be increased in obesity (49,50).

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