A moderate increase in carnitine palmitoyltransferase 1a activity is sufficient to substantially reduce hepatic triglyceride levels

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NONALCOHOLIC FATTY LIVER DISEASE (NAFLD) represents a spectrum of liver abnormalities with an estimated prevalence between 14 and 24% in the general population (4). Furthermore, NAFLD is present in the majority of patients with metabolic syndrome and type 2 diabetes mellitus and is strongly associated with insulin resistance (38) characterized by the inability of insulin to control hepatic gluconeogenesis (28). Although most patients with NAFLD have asymptomatic fatty liver, the condition predisposes toward the development of steatohepatitis, cirrhosis, and hepatocellular carcinoma. Furthermore, hepatic lipid accumulation is associated with a more atherogenic lipid profile (25), including hypertriglyceridemia, a higher plasma concentration of large very low density lipoproteins (VLDL) and small low-density lipoproteins (LDL), and with lower levels of high-density lipoproteins (HDL) and large LDL particles.

According to the “two-hit” hypothesis of fatty liver development (15), the first step involves the accumulation of triglycerides (TG) within hepatocytes. Since cellular fatty acid uptake is largely concentration dependent (8), the increased plasma levels of free fatty acids often seen in patients with metabolic syndrome or type 2 DM would be predicted to drive fatty acid entry into hepatocytes. In addition, increased availability of plasma glucose provides excessive substrate for hepatic de novo lipogenesis, with the first committed intermediate in this pathway, malonyl-CoA, simultaneously limiting β-oxidation via inhibition of the mitochondrial enzyme carnitine palmitoyltransferase 1a (CPT-1a) (31). Net accumulation of hepatocyte lipid will depend on the balance of fatty acid oxidation, de novo lipogenesis/esterification, and TG secretion, mainly in the form of VLDL particles. Although the exact mechanism of TG accumulation is not known (4), it may result from decreased oxidation, increased lipogenesis, or both. Recent studies have pointed to possible defects in both of these pathways in liver steatosis (12, 13, 16, 35).

On the basis of these data, we hypothesized that an intervention that stimulates fatty acid oxidation should result in reduced hepatic fat burden. In the current study, we tested this hypothesis by increasing the expression of CPT-1a, which is a critical rate-determining regulator of β-oxidation in hepatocytes (30). We then evaluated the effects of this intervention in vitro and in vivo on several critical pathways of hepatic lipid metabolism. The data demonstrate that increased expression of CPT-1a is associated with an elevated rate of hepatocyte β-oxidation, a decreased accumulation of TG, and decreased TG secretion in vitro. Consistent with these data, increased expression of CPT-1a in vivo stimulates fatty acid oxidation and substantially reduces the hepatic TG load on either a standard diet or high-fat diet. The data represent proof in principle that a pharmacological agent that stimulates hepatic fatty acid oxidation (perhaps acting on CPT-1a) could provide a novel approach to treatment of NAFLD.

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EXPERIMENTAL PROCEDURES

Materials. Dulbecco’s modified Eagle’s medium (DMEM) and fetal bovine serum (FBS) were obtained from Mediatech (Herndon, VA). [9, 10-3H]palmitate, [1, 2-14C]acetate, and [1-14C]water were obtained from NEN Life Science Products (Boston, MA). [1-14C]palmitic acid, 1-[methyl-13C]glutamate, d-[U-14C]glucose, and ECL Western blotting detection system were obtained from Amersham Biosciences (Piscataway, NJ). Collagen type I and collagenase type IA were obtained from Sigma-Aldrich (St. Louis, MO). Insoluble and Nembrutal were obtained from Abbott Laboratories (North Chicago, IL). SDS-PAGE (5%) gels were obtained from Bio-Rad Laboratories (Hercules, CA). Rabbit polyclonal antibodies against rat apolipoprotein B (apoB) were a kind gift from Dr. Roger A. Davis (San Diego State University, San Diego, CA). Anti-rabbit antibodies were obtained from Cell Signaling Technology (Beverly, MA). Bicinchoninic acid (BCA) protein assay kit was obtained from Pierce (Rockford, IL). TG determination kit was obtained from Roche Diagnostics (Mannheim, Germany), and the β-hydroxybutyrate kit was obtained from Stanbio Laboratory (Boerne, TX). All animals were purchased from Taconic Farms and fed either a standard diet (catalog no. 01351) or a high-fat (HF) diet (catalog no. 96001), purchased from Harlan Teklad (Madison, WI).

Recombinant adenoviruses. Adenoviruses (a kind gift from the late Dr. J. Denis McGarry, University of Texas Southwestern Medical Center, Dallas, TX) contained cDNAs encoding Escherichia coli β-galactosidase (Adv-β-gal), rat CPT-1a (Adv-CPT-1a), or rat carnitine palmitoyltransferase 2 (Adv-CPT-2), under the control of the cytomegalovirus promoter. Adenoviruses were amplified, purified, and stored as described previously (5).

Cell isolation, culture, and adenoviral transduction. Hepatocytes were isolated from male Sprague-Dawley rats (250–300 g) by a recirculating collagenase perfusion of the liver, as described previously (11), yielding 3–6 × 10^8 cells per liver (at 80–90% viability, as determined by trypan blue exclusion). Cells were seeded in six-well collagen-coated plates at 3 × 10^6 live cells per well in DMEM containing 10% (vol/vol) FBS, 100 U/ml penicillin G, and 100 μg/ml streptomycin and then cultured at 37°C in an atmosphere of 5% CO2. After 1 h, the medium was aspirated, the monolayer was washed with 2 ml of phosphate-buffered saline (PBS) to remove unattached cells, and serum-free medium was added (1 ml/well). Adenoviruses were introduced at 250 multiplicities of infection of viral suspension per well. After 1 h, the medium was aspirated, hepatocytes were washed with PBS, and 2 ml of fresh 10% FBS-DMEM containing antibiotics was added. Twenty-four hours following viral transduction, the culture medium was replaced and hepatocytes were incubated for an additional 18 h before initiation of the experiments described below.

Animal maintenance and adenovirus administration. All described procedures were approved by the University of Pittsburgh Institutional Animal Care and Use Committee. Adult male Sprague-Dawley rats weighing 250–300 g were housed under standardized conditions and provided free access to water and the standard diet, containing 65% carbohydrate, 15% fat, and 24% protein (per calories). Animals were accommodated for about 1 wk before receiving Adv-CPT-1a (n = 6), Adv-β-gal (n = 6), or Adv-CPT-2 (n = 6) by tail vein injection. The other group of male Sprague-Dawley rats (150–175 g) was started on the HF diet, containing 40% carbohydrate, 41% fat, and 19% protein (per calories) 6 wk before adenoviral injection. Animals were injected with Adv-CPT-1a (n = 7), Adv-β-gal (n = 8), or Adv-CPT-2 (n = 8). A single dose of 1 × 10^12 adenoviral particles, in a final volume of 500 μl of sterile saline, was administered using a 24-gauge catheter needle to animals anesthetized with isoflurane. Animals’ weight and food intake were recorded daily. Metabolic studies were performed 5 days after viral administration. Animals were fasted for 18 h before metabolic studies and then anesthetized with Nembutal, and about 3 ml of blood were obtained by cardiac puncture. Plasma was rapidly frozen in liquid nitrogen. The liver was removed, washed in ice-cold saline, and then divided into smaller pieces, which were either used fresh for CPT activity and fatty acid oxidation assay ex vivo or rapidly frozen in liquid nitrogen.

Incubation with palmitate. Palmitate was complexed with essentially fatty acid-free bovine serum albumin (BSA) to generate a stock solution of 25% (wt/vol) BSA and 7.5 mM palmitate in serum-free medium (34). The stock solution and a similar BSA stock prepared in the absence of fatty acid were diluted into 10% FBS-DMEM to give concentrations of 2.5% BSA and either 0.04 or 0.4 mM palmitate, concentrations that mimic physiological plasma levels of free fatty acids seen in postprandial and fasting states, respectively.

CPT assay. Activities of CPT-1 and CPT-2 were determined in the direction of acylcarnitine formation by using a radiochemical assay, as described previously (9). Briefly, hepatocyte monolayers were harvested in an ice-cold buffer containing 150 mM KCl and 5 mM Tris-HCl, pH 7.2, and broken with a glass homogenizer. The monolayer cell homogenate (with the mitochondria largely intact) was used directly for assay of CPT-1, located on the outer aspect of the organelle. In animal studies, 250 mM sucrose was added to the homogenization buffer, and liver fragments (~1 g) were homogenized for 5 s on ice, followed by serial centrifugation to prepare mitochondria, as described previously (32). For measurement of CPT-2 activity, a portion of the homogenate was made 1% (wt/vol) with the detergent octylglucoside, which inactivates CPT-1 and releases CPT-2 from the mitochondrial matrix in active form (42). Protein content was measured using the BCA method.

Palmitate oxidation. After 18-h incubation with unlabeled palmitate, the culture medium was replaced with fresh DMEM containing BSA and fatty acids as before, in the additional presence of [3H]palmitate (10 μCi/ml). The cells were incubated for a further 3 h in triplicate wells for each condition. The same medium was used for liver fragments, which were weighed (~200 mg/well) and incubated at 37°C for 2 h in duplicate wells for each animal. Tritiated water was determined in the collected medium by using a vapor-phase equilibration method, as described by Hughes et al. (23). The monolayers were washed twice with 2 ml of ice-cold PBS and collected in 1 ml of 1 N NaOH for determination of protein content.

Ketone body production. After 18 h in the presence of unlabeled palmitate, BSA-palmitate medium was replaced as fast as possible on fatty acid oxidation experiments, but with the addition of [1-14C]palmitate (0.4 μCi/ml). After 3 h, ketone bodies in the medium were determined according to Drynan et al. (19). Briefly, 100 μl of 20% perchloric acid were added to 1 ml of the culture medium, followed by centrifugation at 18,000 g for 10 min at room temperature. 14C-labeled acid-soluble products were determined in 400 μl of the supernatant. Cells were harvested for protein determination as described above.

Lipogenesis and fatty acid esterification. De novo lipogenesis and esterification of exogenous fatty acids were determined over two time periods. For 3-h experiments, cells were preincubated for 18 h with unlabeled palmitate before the medium was replaced with the identical one containing [14C]acetate (0.05 μCi/ml) or [14C]glucose (0.3 μCi/ml), to determine rates of de novo lipid synthesis (27), or [3H]palmitate (7.5 μCi/ml), to determine rates of fatty acid esterification for 3 h. Alternatively, hepatocytes were exposed to unlabeled palmitate and the same concentration of [3H]palmitate, [14C]acetate, or [14C]glucose throughout the initial 18-h incubation before harvest. Experiments were performed in triplicate wells for each condition. To determine incorporation of label into total lipids, the culture medium was removed and the monolayers were washed twice in 2 ml of ice-cold PBS before harvest in 1 ml of PBS and centrifugation at 18,000 g for 2 min at 4°C. The cell pellets were resuspended in 80 μl of PBS, and total lipids were extracted using the method of Bligh and Dyer (7). Briefly, 200 μl of methanol and 100 μl
of chloroform were added to each sample (with vigorous vortex mixing for 30 s after each addition) followed by centrifugation at 18,000 g for 5 min at room temperature. After collection of the supernatants, the pellets were redissolved in 0.5 ml of 1 N NaOH for determination of protein content. The supernatants were mixed with 100 μl of chloroform and 100 μl of water (with vortex mixing for 30 s after each step) and centrifuged at 18,000 g for 5 min at room temperature. The upper phase was discarded, and the lower, hydrophobic phase was dried by vacuum centrifugation for 20 min. Dried lipids were resuspended in 200 μl of chloroform for determination of radioactive content.

**TG assay.** Intracellular TG were extracted from the cell monolayers or from 30–50 mg of frozen liver tissue and quantified as described previously (34). For determination of secreted TG (in VLDL particles), apoB-containing lipoproteins were precipitated from the culture medium by using dextran sulfate, according to Waugh and Small (41). Briefly, 4 ml of the culture media were mixed gently with 200 μl of dextran sulfate (20 g/l) and 400 μl of 1.1 M magnesium sulfate, incubated at room temperature for 20 min, and centrifuged at 1,000 g for 10 min. The supernatants were removed, and the TG content of the pellets was determined as described for cells.

**Immunoblot analysis of apoB.** After 18-h incubation with unlabelled palmitate, apoB-containing lipoproteins were precipitated from 2 ml of culture medium using dextran sulfate, similarly to the procedure for secreted TG. The pellets were resuspended in 50 μl of SDS-PAGE sample buffer, heated at 90°C for 5 min, and resolved by 5% SDS-PAGE. Proteins were transferred onto polyvinylidene difluoride membranes (in the presence of 0.1% SDS) for immunoblotting by conventional means, using a polyclonal rabbit antibody directed against rat apoB (14). Signals were detected by chemiluminescence and exposure to X-ray film, and bands within the linear range were quantified using the NIH Image software.

**Plasma analyses.** Plasma measurements of glucose were performed using a glucose meter, insulin was measured using a radioimmunoassay kit, and TG, nonesterified fatty acids, and β-hydroxybutyrate were measured using appropriate colorimetric assay kits. To estimate insulin resistance, we calculated the homeostasis model assessment (HOMA) index as fasting plasma insulin (μIU/ml) × fasting plasma glucose (mM)/22.5.

**Statistical analysis.** Data from in vitro studies are means ± SE for at least three independent experiments and were analyzed using the SigmaStat program. Nonparametric analysis of variance (ANOVA) on ranks (Kruskal-Wallis method) was used for each data group, followed by individual pairwise comparisons using the Student-Newman-Keuls method. The difference among data was considered significant at P < 0.05. Results of animal experiments are means ± SE for at least six animals in each group. Data were analyzed using the SPSS program with one-way ANOVA followed by Tukey’s test for post hoc multiple comparisons. Statistical significance was assumed at P < 0.05.

**RESULTS**

**CPT-1a overexpression increases CPT-1a activity and rates of fatty acid oxidation and ketone body production in hepatocytes.** Initial experiments demonstrated that CPT activities in primary cultures of rat hepatocytes transduced with the control adenovirus Adv-β-gal were not different from those in non-transduced cells (not shown). Hence, for further experiments, all control cells received Adv-β-gal. Figure 1A shows the CPT-1a and -2 activities in the cells 24 h after transduction with Adv-β-gal, Adv-CPT-1a, and Adv-CPT-2. Adv-CPT-1a increased CPT-1a activity approximately three- to fourfold compared with cells expressing β-galactosidase or overexpressing CPT-2. Transduction with Adv-CPT-2 resulted in a fivefold increase in CPT-2 activity. For Adv-CPT-1a and Adv-CPT-2, maximal increases in corresponding enzyme activities were observed about 24 h after transduction and remained stable for a further 24 h (not shown). In addition, the presence of 0.04 or 0.4 mM palmitate in the context of 2.5% BSA, BSA alone, or 10% FBS in the culture medium did not significantly affect CPT-1a or CPT-2 activity (not shown).

For measurement of the oxidation rate of exogenous palmitate, cells were incubated with 0.4 or 0.04 mM palmitate for
18 h, with labeled substrate included for the final 3 h, during which period oxidation, as well as the other metabolic assays presented, were linear (data not shown). Overexpression of CPT-1a resulted in a significant increase (≈70%) in the rate of fatty acid oxidation compared with control cells (Fig. 1B). In proportional terms, the stimulatory effect of CPT-1a overexpression on β-oxidation was similar at the low or high levels of exogenous palmitate. However, the absolute rate of oxidation was driven by fatty acid concentration in the medium, regardless of the adenovirus used, a 10-fold increase in palmitate concentration resulting in a proportional increase in oxidation. Furthermore, overexpression of CPT-1a resulted in a 2.5-fold increase in ketone production relative to cells transduced with Adv-β-gal or Adv-CPT-2 (Fig. 1C). Similar to β-oxidation, ketogenesis was ~20-fold greater in the presence of 0.4 mM palmitate than in 0.04 mM substrate. Thus total oxidative usage of palmitate (β-oxidation plus ketone production) was increased ~70% in CPT-1a-overexpressing cells at both high and low palmitate concentrations.

CPT-1a overexpression does not affect de novo lipid synthesis and fatty acid esterification but does reduce the respective lipid pools in hepatocytes. [14C]acetate incorporation into intracellular lipids (primarily TG and cholesterol) assessed over a short period of time provides a measure of net de novo lipogenesis. When assessed over 3 h under the same conditions as those for measurement of oxidative pathways, no differences were observed between cells overexpressing β-galactosidase, CPT-1a, or CPT-2 (Fig. 2A). However, when cells are incubated with [14C]acetate for 18 h, the content of [14C]labeled lipids will be affected by the rate of synthesis as well as degradation of newly synthesized molecules, or in other words, their turnover. Under such a prolonged incubation, the elevated CPT-1a activity was associated with a 25% reduction in radio-labeled lipids (Fig. 2B). Since lipid synthesis is unchanged, decreased accumulation of lipids would suggest that CPT-1a overexpression results in an increase in the use of intracellular lipid pools for oxidative metabolism. This effect of CPT-1a overexpression was similar at either concentration of exogenous palmitate. Interestingly, the incorporation of [14C]acetate into lipids in all cell groups that did not reach statistical significance.

CPT-1a overexpression decreases cellular triglyceride content and release of VLDL TG but does not affect secretion of apoB by hepatocytes. Incubation with 0.4 mM palmitate for 18 h resulted in a twofold increase in intracellular TG in all treated cells compared with incubation in the presence of 0.04 mM fatty acid (Fig. 3A). However, at either palmitate concentration, TG content was reduced by 30–35% in hepatocytes overexpressing CPT-1a. Interestingly, TG content was also decreased significantly (15–20%) in cells with increased CPT-2 activity compared with β-galactosidase-expressing cells. As shown in Fig. 3B, Adv-CPT-1a-treated hepatocytes secreted 60% less VLDL TG than control cells, regardless of exogenous palmitate level. TG secretion was also decreased in CPT-2-overexpressing cells, albeit to a lesser degree (~30%). There was also a tendency toward increased TG secretion (20%) by Adv-β-gal-and Adv-CPT-2-transduced cells with the higher palmitate concentration. To assess the effect of CPT-1a overexpression on secretion of the large and small variants of apoB, VLDL particles were precipitated from the culture medium and apoB was quantified by immunoblotting (Fig. 4A). ApoB secretion was unchanged in the presence of elevated CPT-1a or CPT-2 activities at either 0.04 or 0.4 mM exogenous palmitate (Fig. 4B).

CPT-1a overexpression in rat liver increases CPT-1a activity and hepatic fatty acid oxidation capacity and decreases hepatic TG content. After performing in vitro experiments, we evaluated the effects of increased CPT-1a activity on liver lipid metabolism in the standard diet-fed rats using Adv-β-gal and Adv-CPT-2 as control. Five days after adenovirus delivery, hepatic CPT-1a activity was increased by ~45% (Fig. 5A) and the rate of fatty acid oxidation was increased by ~45% (Fig. 5B) in Adv-CPT-1a animals compared with age- and weight-matched Adv-CPT-2 and Adv-β-gal controls. Importantly, liver TG content was reduced by ~40% in Adv-CPT-1a vs. Adv-CPT-2 and Adv-β-gal animals (Fig. 5C), whereas plasma TG were unaffected by increased liver CPT-1a activity (Table 1).

There were no significant differences in fatty acid oxidation and liver TG content between the two control groups, despite significantly higher CPT 1 activity (32%) in Adv-CPT-2- vs. Adv-β-gal-overexpressing animals (Fig. 5, A–C). Furthermore, as shown in Table 1, fasting plasma levels of glucose, free fatty acids, TG, β-hydroxybutyrate, and the liver enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were similar among the three groups of animals. However, there was a trend toward decreased plasma insulin levels in Adv-CPT-1a animals, which did not reach a statistical significance and did not alter the HOMA index, a measure of insulin sensitivity. Finally, pre- and postinjection weights of animals were not significantly different among the three groups (Table 1).

CPT-1a overexpression in liver of obese rats increases CPT-1a activity and fatty acid oxidation capacity and decreases hepatic TG content. The same experimental protocol used in animals fed the standard diet was applied to obese Sprague-Dawley rats after 6 wk of a HF diet. At the time of adenovirus delivery, HF-fed animals were of similar age as standard diet-fed animals but were ~50% heavier (~440 vs.
290 g). Five days after injection, liver CPT-1a activity was increased by ~60% (Fig. 5A) and the rate of fatty acid oxidation was increased by ~45% (Fig. 5B) in obese Adv-CPT-1a animals compared with age- and weight-matched obese Adv-CPT-2 and Adv-β-gal animals. Importantly, liver TG content was reduced by ~70% in Adv-CPT-1a vs. control animals (Fig. 5C). Liver TG content was substantially higher in obese control rats compared with corresponding control animals fed the standard diet, fivefold higher in those injected with Adv-CPT-2, and 3.3-fold higher in animals treated with Adv-β-gal. However, CPT-1a-overexpressing animals on the HF diet were protected from steatosis. There were no differences in CPT activity, fatty acid oxidation, and liver TG content between two control groups of rats on the same diet. Muscle TG content was also similar between CPT-1a and control animals (data not shown). Finally, although CPT-1a activity in rats on the HF diet was comparable to that in corresponding groups of animals on the standard diet, the rate of fatty acid oxidation was increased twofold in rats on the HF diet (Fig. 5B).

As shown in Table 1, fasting plasma levels of glucose, free fatty acids, β-hydroxybutyrate, insulin, AST, and ALT were similar among all groups of animals fed the same diet, although the levels of free fatty acids and insulin were twofold higher in
obese rats compared with lean animals. Interestingly, obese animals injected with Adv-CPT-2 had the lowest plasma TG levels of all four groups (Table 1), but the difference was not statistically significant compared with the other two groups of obese rats. Furthermore, pre- and postinjection weights of animals (Table 1) and food intake (data not shown) did not differ significantly among the groups.

DISCUSSION

Although NAFLD is recognized as a major health problem, particularly prevalent in individuals suffering from the metabolic syndrome or type 2 DM, limited treatment options are currently available. Agonists of the nuclear receptor PPAR-γ, such as pioglitazone (6), or activators of PPAR-α, such as fenofibrate (39), have been shown to stimulate fatty acid oxidation and decrease liver TG content, raising hope that these compounds may be useful for the treatment of NAFLD. However, these drugs target a number of metabolic and inflammatory pathways, so it is unclear what specific mechanism contribute to their beneficial metabolic effects. We hypothesized that an agent that directly and specifically increases the rate of hepatic fatty acid oxidation would reduce hepatic fat burden and might present a potential novel therapeutic route. Since the mitochondrial outer membrane enzyme CPT-1 is known to be a primary intracellular regulator of β-oxidation, largely by virtue of its potent inhibition by malonyl-CoA, the first committed intermediate in the opposing pathway of de novo fatty acid synthesis, we chose to overexpress the liver isoform of CPT-1a in primary hepatocytes and liver of lean and obese rats. We predicted that this approach would result in a specific enhancement of the rate of fatty acid oxidation, leading to a lowered fat load on the hepatocyte. Indeed, we have previously shown that overexpression of CPT-1a in L6 myotubes increases β-oxidation in that cell type (34). Furthermore, it has been shown that overexpressed CPT-1a retains normal sensitivity to malonyl-CoA, with an I50 value comparable to that observed in intact mitochondria from fasted rats (10). The primary goal of our study was to determine whether a specific stimulation of fatty acid oxidation would be sufficient to alter intrahepatic triglyceride accumulation. We addressed these issues in three different models, namely, in primary hepatocytes and in vivo in the liver of lean and obese rodents. In all these models, increased CPT-1a activity substantially reduced intracellular levels of TG, and the apparent primary mechanism of this effect was an increase in fatty acid oxidation. Thus overexpression of CPT-1a in primary hepatocytes resulted in a three- to fourfold increase in CPT-1 activity that was sufficient to increase the rate of exogenous palmitate oxidation and

Fig. 3. Effect of CPT-1a overexpression on cellular accumulation and secretion of triglyceride (TG). Twenty-four hours after transduction, hepatocytes were exposed to exogenous palmitate at either 0.04 or 0.4 mM for 18 h, and cellular accumulation (A) and secretion (B) of TG into medium were measured as described in EXPERIMENTAL PROCEDURES. Values are means ± SE for 4 independent experiments. Secreted TGs were measured in the apolipoprotein B (apoB) fraction precipitated with dextran sulfate, and results are expressed as percentages of values in β-gal-expressing cells. *P < 0.05 relative to the corresponding control value in β-gal-expressing cells.

Fig. 4. Secretion of apoB by transduced hepatocytes. Cells were transduced with Adv-CPT-2 and treated 24 h later with either 0.04 or 0.4 mM palmitate. After an additional 18-h incubation, apoB-containing lipoproteins were precipitated using dextran sulfate and immunoblotted by conventional means using a polyclonal rabbit antibody directed against rat apoB. A: a typical immunoblot for rat apoB as detected by chemiluminescence. B: quantitative analysis of apoB bands using the NIH Image software, expressed as the relative arbitrary units of the corresponding β-gal control. Values are means ± SE for 3 independent experiments.
A hepatic mito CoA level by genetic deletion of the acetyl CoA carboxylase 2 gene (1, 2) or hepatic overexpression of MCD (3), deletion of stearoyl-CoA desaturase, which increases AMP kinase activity, leading to a decreased activity of acetyl-CoA carboxylase (18, 33), or stimulation of mitochondrial uncoupling by either 2,4-dinitrophenol (37) or adenovirus-mediated uncoupling protein-1 (UCP1) expression in liver (24). However, although these studies provided data consistent with ours, it was previously impossible to determine with precision how much of the effect could be ascribed directly to an increase in fatty acid oxidation, since those approaches would be predicted to target other metabolic functions as well. For example, AMP kinase activation and decreased malonyl-CoA content can reduce the rate of lipogenesis (1), and hepatic overexpression of UCP1 (24) or use of 2,4-dinitrophenol (37) is associated with reduced weight gain.

Plasma metabolic variables were unchanged in lean or obese animals overexpressing CPT-1a. Thus there were no significant differences in the plasma concentration of glucose, TG, free fatty acids, or β-hydroxybutyrate. There was a trend toward lower fasting insulin levels in Adv-CPT-1a-treated animals, but it did not reach statistical significance, and HOMAs were unaltered, suggesting that CPT-1a overexpression has no effects on insulin sensitivity. Some, but not all, of these data agree with those observed in rats overexpressing MCD (3). Specifically, elevated activity of MCD in obese animals was associated with a decrease in plasma insulin and free fatty acids and an increase in insulin sensitivity. This may be due to additional effects of reduced malonyl-CoA concentration, which may not be directly related to β-oxidation (1).

The lack of an effect of CPT-1a overexpression on plasma ketones is noteworthy, since we observed substantial effects on ketogenesis in primary hepatocytes. This difference is most likely due to the virtual absence of insulin in hepatocyte

ketone body production by ~70%. In lean or obese animals in vivo, a lesser level of CPT-1a overexpression also substantially elevated the fatty acid oxidation capacity. Together, these data confirm the high degree of control over mitochondrial fatty acid oxidation exerted by CPT-1a and are in agreement with previous studies where this enzyme was shown to regulate both β-oxidation and ketone body production in hepatocytes (19). This point is accentuated by the study of Dobbins et al. (17), who observed that treatment of rats with etomoxir, an irreversible inhibitor of CPT-1, led to a significant increase in hepatic TG content. Finally, our observation that a severalfold increase in CPT-1a activity leads to only 70% increase in β-oxidation is consistent with a study in which overexpression of CPT-1a in cultured pancreatic β-cells (36) resulted in a sixfold increase in enzyme activity and a twof- to threefold increase in oxidation, whereas hepatocytes transduced with adenovirus carrying cDNA for malonyl-CoA decarboxylase (MCD) increased the enzymatic activity sevenfold but increased fatty acid oxidation by about 80% compared with control cells (3). This suggests that the quantitative effect of CPT-1 stimulation on fatty acid oxidation is limited by one or more downstream steps, possibly at the level of the Krebs cycle, over which CPT-1a exerts very low control (19). Furthermore, the concentration of malonyl-CoA, which is under more complex control in the whole body, can limit CPT-1 function, possibly leading to more restricted activity in vivo compared with in vitro conditions as observed in our experiments. In addition to CPT-1, free fatty acids also seem to regulate hepatocyte fatty acid oxidation, since the rate of oxidation increases with increasing extracellular levels of fatty acids both in vitro and in vivo. This phenomenon appears to be independent of the degree of CPT-1a activity and could be the result of the mass action effect of increased fatty acid supply, since hepatocytes’ uptake of free fatty acids is only concentration dependent (8).

Although our study was specifically designed to affect directly only mitochondrial β-oxidation, the data are consistent with other studies in which indirect, less specific approaches were used to stimulate this pathway. These include lowering the hepatic malonyl-CoA level by genetic deletion of the acetyl-CoA carboxylase 2 gene (1, 2) or hepatic overexpression of MCD (3), deletion of stearoyl-CoA desaturase, which increases AMP kinase activity, leading to a decreased activity of acetyl-CoA carboxylase (18, 33), or stimulation of mitochondrial uncoupling by either 2,4-dinitrophenol (37) or adenovirus-mediated uncoupling protein-1 (UCP1) expression in liver (24). However, although these studies provided data consistent with ours, it was previously impossible to determine with precision how much of the effect could be ascribed directly to an increase in fatty acid oxidation, since those approaches would be predicted to target other metabolic functions as well. For example, AMP kinase activation and decreased malonyl-CoA content can reduce the rate of lipogenesis (1), and hepatic overexpression of UCP1 (24) or use of 2,4-dinitrophenol (37) is associated with reduced weight gain.

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carnitine palmitoyltransferase 1a (CPT-1a), and carnitine palmitoyltransferase 2 (CPT-2) to the day animals were killed (day 5). Data are means ± SE of at least 6 animals. HOMA, homeostasis model assessment; FFA, free fatty acids; TG, triglycerides; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

Changes in body weight were calculated from the day of injection (day 0) with adenovirus encoding β-galactosidase (β-gal), carnitine palmitoyltransferase 1a (CPT-1a), and carnitine palmitoyltransferase 2 (CPT-2) to the day animals were killed (day 5). Data are means ± SE of at least 6 animals. HOMA, homeostasis model assessment; FFA, free fatty acids; TG, triglycerides; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

Table 1. Metabolic variables in lean and obese rats 5 days after injection with adenovirus

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<th>Lean Animals</th>
<th>Obese Animals</th>
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<tr>
<td></td>
<td>β-gal</td>
<td>CPT-2</td>
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<tr>
<td>Weight change, g</td>
<td>2.5 ± 3.0</td>
<td>0.7 ± 6.2</td>
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<tr>
<td>Glucose, mg/dl</td>
<td>136 ± 11.4</td>
<td>117.7 ± 6.9</td>
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<tr>
<td>Insulin, ng/ml</td>
<td>0.75 ± 0.3</td>
<td>1.25 ± 0.4</td>
</tr>
<tr>
<td>HOMA</td>
<td>5.18 ± 2.33</td>
<td>10.15 ± 5.36</td>
</tr>
<tr>
<td>FFA, μM</td>
<td>134.0 ± 20</td>
<td>136.7 ± 46</td>
</tr>
<tr>
<td>TG, mg/dl</td>
<td>21.9 ± 9.0</td>
<td>14.0 ± 3.4</td>
</tr>
<tr>
<td>β-OH-butyrate, mg/dl</td>
<td>4.08 ± 1.19</td>
<td>3.28 ± 0.92</td>
</tr>
<tr>
<td>ALT, units/l</td>
<td>80 ± 23</td>
<td>81 ± 53</td>
</tr>
<tr>
<td>AST, units/l</td>
<td>176 ± 56</td>
<td>647 ± 396</td>
</tr>
</tbody>
</table>

Changes in body weight were calculated from the day of injection (day 0) with adenovirus encoding β-galactosidase (β-gal), carnitine palmitoyltransferase 1a (CPT-1a), and carnitine palmitoyltransferase 2 (CPT-2) to the day animals were killed (day 5). Data are means ± SE of at least 6 animals. HOMA, homeostasis model assessment; FFA, free fatty acids; TG, triglycerides; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

Table 1. Metabolic variables in lean and obese rats 5 days after injection with adenovirus

<table>
<thead>
<tr>
<th></th>
<th>Lean Animals</th>
<th>Obese Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β-gal</td>
<td>CPT-2</td>
</tr>
<tr>
<td>Weight change, g</td>
<td>2.5 ± 3.0</td>
<td>0.7 ± 6.2</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>136 ± 11.4</td>
<td>117.7 ± 6.9</td>
</tr>
<tr>
<td>Insulin, ng/ml</td>
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REFERENCES


