Sparing effect of leptin on liver glycogen stores in rats during the fed-to-fasted transition

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Sparing effect of leptin on liver glycogen stores in rats during the fed-to-fasted transition. Am. J. Physiol. 277 (Endocrinol. Metab. 40): E544–E550, 1999.—The effect of moderate hyperleptinemia (~20 ng/ml) on liver and skeletal muscle glycogen metabolism was examined in Wistar rats. Animals were studied ~90 h after receiving recombinant adenoviruses encoding rat leptin (AdCMV-leptin) or β-galactosidase (AdCMV-βGal). Liver and skeletal muscle glycogen levels in the fed and fasted (18 h) states were similar in AdCMV-leptin- and AdCMV-βGal-treated rats. However, after delivery of a glucose bolus, liver glycogen levels were significantly greater in AdCMV-leptin compared with AdCMV-βGal rats (P < 0.05). To investigate the mechanism(s) of these differences, glycogen levels were measured immediately after the cessation of a 3- or 6-h glucose infusion or 3, 6, and 9 h after the cessation of a 6-h glucose infusion. Similar increases in liver and skeletal muscle glycogen occurred in hyperleptinemic and control rats in response to glucose infusions. However, 3 and 6 h after the cessation of a glucose infusion, liver glycogen levels were approximately twofold greater (P < 0.05) in AdCMV-leptin-treated compared with AdCMV-βGal-treated animals. Skeletal muscle glycogen levels were similar in AdCMV-leptin-treated and AdCMV-βGal-treated animals at the same time points. Glycogen phosphorylase, phosphodies- terase 3B, and glycogen synthase activities were unaltered by hyperleptinemia. We conclude that moderate increases in plasma leptin levels decrease liver glycogen degradation during the fed-to-fasted transition.

The liver and skeletal muscle play central roles in the regulation of glucose homeostasis. The liver acts as an organ of net glucose uptake and storage in the absorptive state and an organ of net glucose production during fasting, and skeletal muscle is the principal user and storage site of glucose, by mass, in the body. Glycogen metabolism plays a vital role in allowing liver and skeletal muscle to fulfill these critical functions, and it is therefore not surprising that it is regulated by a variety of hormonal, biochemical, and molecular mechanisms.

Leptin, the adipocyte-derived hormone that alters food intake, basal metabolic rate, thermogenesis, and lipid metabolism, has been implicated in the regulation of carbohydrate metabolism in skeletal muscle, liver, and adipose tissue (1, 4, 10, 13, 14, 16, 22, 27). The effects of leptin on glycogen metabolism in vivo, however, remain unclear because both inhibitory (1, 22) and stimulatory (13) actions of leptin have been reported. Both acute and chronic leptin-induced enhancements of the inhibitory effects of insulin on hepatic glucose production, caused by a suppression of hepatic glycogenolysis, have been noted (1, 22). In these studies, whole body glucose uptake and glycogen synthesis were unchanged by acute leptin treatment, whereas skeletal muscle glycogen synthesis was increased by chronic leptin treatment. These effects, however, were observed under hyperinsulinemic conditions, making it difficult to determine the effects of leptin under normal physiological conditions. Another study in partially fasted mice (13) demonstrated decreased liver glycogen levels (suggesting increased glycogenolysis) and increased skeletal muscle glucose uptake in animals receiving a 5-h intracerebroventricular or intravenous leptin infusion compared with animals receiving a saline infusion. Thus the aims of the current study were twofold. First, we sought further clarification of leptin effects on glycogen metabolism in vivo to reconcile the seemingly disparate previous findings noted above. Second, given that studies to date have been performed under hyperinsulinemic clamp conditions or in partially fasted animals, we sought to determine the effects of leptin on glycogen metabolism in the context of the two most relevant physiological situations, i.e., the absorptive state and the fed-to-fasted transition.

We have previously demonstrated that hyperleptinemia can be efficiently achieved in rats with a replication-defective recombinant adenovirus gene delivery system (3). In the present study, we have used this system to induce moderate, short-term increases in plasma leptin. Subsequently, we have investigated liver and skeletal muscle glycogen metabolism in hyperleptinemic rats and rats that have received a control saline infusion. Thus the aims of the current study were twofold. First, we sought further clarification of leptin effects on glycogen metabolism in vivo to reconcile the seemingly disparate previous findings noted above. Second, given that studies to date have been performed under hyperinsulinemic clamp conditions or in partially fasted animals, we sought to determine the effects of leptin on glycogen metabolism in the context of the two most relevant physiological situations, i.e., the absorptive state and the fed-to-fasted transition.

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MATERIALS AND METHODS

Animal maintenance and recombinant adenovirus administration. All procedures were carried out in accordance with
Animals were allowed to recover to their pre-surgery weights with a 3:1 mix of glycerol and heparin, and flame sealed.

The common carotid artery was catheterized (PE-50, Clay Adams, Charles, MO). Plasma triglycerides and free fatty acids were measured with kits (Sigma). Plasma glucose was measured with a HemoCue glucose analyzer (HemoCue AB, Angelholm, Sweden). Tissue glycogen was measured by an amyloglucosidase method that has been described previously (9). Glycogen phosphorylase activity was measured by an adaptation of the method of Gilboe et al. (8). Briefly, 150 mg of tissue were homogenized in 1 ml of a buffer containing, as final concentrations, 50 mM potassium fluoride, 10 mM EDTA, 6% glycerol, 2 mM sodium vanadate, 10 mM pyrophosphate, 1 µg/ml leupeptin, 10 µg/ml aprotinin, and 0.1 mM phenylmethylsulfonyl fluoride, with a Polytron homogenizer (Polytron, Lausanne, Switzerland). The sample was centrifuged for 10 min at 1,000 g at 4°C, and 20 µl of the supernatant were added to 40 µl of a mix containing, as final concentrations, 200 mM potassium fluoride, 100 mM [U-14C]glucose-1-phosphate (0.1 µCi/reaction), 1% glycogen, and 10 mM caffeine. Phosphorylase activity was measured over a period of 15 min in a 30°C water bath. The reaction was stopped by spotting 50 µl of the reaction onto Whatman Grade 3 paper (Whatman International, Maidstone, England) and immediate submersion in 66°C ice-cold ethanol. After two further washes in 66°C ethanol, filters were washed briefly in acetic acid, allowed to dry, and placed in scintillation vials containing 10 ml scintillate for radioactivity counting. Phosphodiesterase 3B (PDE3B) activity was measured with the method of Zhang et al. (29). Briefly, 20 µg of pulverized tissue were homogenized with a sonicator in 2 ml of a lysis buffer containing 50 mM NaF, 150 mM NaCl, 10 mM sodium phosphate, pH 7.2, 2 mM EDTA, 0.1% Triton X-100, 0.5% Lubrol, 3 µM benzamidine, 5 µg/ml leupeptin, 20 µg/ml pepstatin A, 300 µM Na3VO4, and 100 nM okadaic acid. PDE3B was immunoprecipitated from 200 µl of tissue extract overnight with a PDE3B polyclonal antibody and immediate submersion in 6°C ice-cold ethanol. The sample was centrifuged for 10 min at 1,000 g at 4°C, and 20 µl of the supernatant were added to 40 µl of a mix containing, as final concentrations, 200 mM potassium fluoride, 100 mM [U-14C]glucose-1-phosphate (0.1 µCi/reaction), 1% glycogen, and 10 mM caffeine. Phosphorylase activity was measured over a period of 15 min in a 30°C water bath. The reaction was stopped by spotting 50 µl of the reaction onto Whatman Grade 3 paper (Whatman International, Maidstone, England) and immediate submersion in 66°C ice-cold ethanol. After two further washes in 66°C ethanol, filters were washed briefly in acetic acid, allowed to dry, and placed in scintillation vials containing 10 ml scintillate for radioactivity counting. Phosphodiesterase 3B (PDE3B) activity was measured with the method of Zhang et al. (29). PDE activities in the PDE3B immunoprecipitates were measured with 1 µM cAMP as substrate. The results were normalized to PDE3B protein, as estimated by Western blot analysis, in the PDE3B immunoprecipitates.

Statistical analysis. Data are expressed as means ± SE. Statistical significance was determined by an unpaired Student’s t-test with the statistics module of Microsoft Excel, Version 5.0 (Microsoft, Seattle, WA). Statistical significance was assumed at P < 0.05.

RESULTS
Basal plasma parameters, body weight, and epididymal fat pad weight in AdCMV- leptin- and AdCMV-βGal-treated rats. Treatment of rats with AdCMV-leptin resulted in moderate increases in plasma leptin levels from 4.6 ± 0.9 to 20.0 ± 1.7 ng/ml (P < 0.05). These levels are substantially below those reported in a number of other in vivo and in vitro studies of leptin action (1, 13, 15, 17). A number of plasma parameters of glucose and lipid metabolism were monitored before initiation of experiments (Table 1). Plasma glucose, free fatty acids, triglycerides, and insulin, and glucagon were unaffected by moderate hyperleptinemia. Additionally, the short term of the hyperleptinemia (−70 h) did not result in significant differences in body weight or epididymal fat pad weights between AdCMV-leptin- and AdCMV-βGal-treated animals (Table 1), indicating that hyperleptinemic rats had adequate reserves of adipose tissue for energy requirements throughout the course of the studies.

Liver and skeletal muscle glycogen metabolism in AdCMV- leptin- and AdCMV-βGal-treated rats. We first determined if leptin alters steady-state glycogen stores...
Male Wistar rats received recombinant adenoviruses containing the leptin (AdCMV-leptin) or β-galactosidase (AdCMV-βGal) cDNA. Results are means ± SE. Approximately 86 h after viral delivery blood samples were obtained from ad libitum-fed AdCMV-leptin-treated and calorically matched AdCMV-βGal-treated rats and a number of plasma variables were measured. FFA, free fatty acids. Also during this period food intake and weight were monitored. Epididymal fat pads were obtained at time of death (6–15 h after initial blood samples). Epididymal fat pads were obtained at time of death (6–15 h after initial blood samples). *Significant difference (P < 0.001) between AdCMV-leptin- and AdCMV-βGal-treated animals at indicated time points.

Mechanism of leptin-induced alterations in liver glycogen metabolism. To determine the mechanism underlying differences in liver glycogen levels in AdCMV-leptin-treated and AdCMV-βGal-treated animals, studies were performed to address the effects of hyperleptinemia on glycogen accumulation and glycogen degradation. First, leptin effects on accumulation of liver and skeletal muscle glycogen during a glucose infusion were assessed in AdCMV-leptin- and AdCMV-βGal-treated animals subsequent to an 18-h glycogen-depleting fast. A 3- or 6-h glucose infusion resulted in similar glycogen levels in the liver (Fig. 2A) and the skeletal muscle (Fig. 2B) of hyperleptinemic and control animals, demonstrating that a moderate increase in leptin does not alter the capacity of the liver or skeletal muscle to replenish glycogen stores. Next, the effects of hyperleptinemia on the mobilization of liver and skeletal muscle glycogen during the fed-to-fasting transition was assessed. A 6-h glucose infusion administered to fed animals raised levels of hepatic glycogen to a similar degree in AdCMV-leptin- and AdCMV-βGal-treated rats (0 h time point, Fig. 3A). However, 3 h after the cessation of the glucose infusion, liver glycogen levels in AdCMV-leptin-treated animals were 198 ± 30 µg/mg protein compared with 94 ± 9 µg/mg protein in AdCMV-βGal-treated animals (Fig. 3A, P < 0.05). If one assumes that glycogen levels before beginning of the fast were similar to the levels in the 0-h groups, the difference in glycogen levels at the 3-h time point equates to a glycogenolytic rate of 0.96 µg·kg⁻¹·min⁻¹ in AdCMV-leptin-treated animals compared with 2.06 µg·kg⁻¹·min⁻¹ in AdCMV-βGal-treated animals during the 3 h of fasting. Six hours of fasting resulted in liver glycogen levels in AdCMV-leptin-treated animals that were 156 ± 21 µg/mg protein compared with 93 ± 22 µg/mg protein in AdCMV-βGal-treated animals (Fig. 3A, P < 0.05). Plasma insulin, glucagon, glucose, free fatty acids, and triglycerides were similar in both groups at both time points (Table 3). Increasing the length of the fast to 9 h resulted in similar liver glycogen levels in AdCMV-leptin-treated compared with AdCMV-βGal-treated animals (Fig. 3A). In skeletal muscle, glycogen levels were similar in AdCMV-leptin-
and AdCMV-βGal-treated animals at all times during the fast (Fig. 3B).

Glycogen phosphorylase, glycogen synthase, and PDE3B activity in AdCMV-leptin- and AdCMV-βGal-treated rats. The recent observations (1, 22) that leptin decreases hepatic glycogenolysis during hyperinsulinemia and that leptin increases PDE3B activity in primary hepatocytes (28) prompted us to test the hypothesis that the decreased rate of mobilization of hepatic glycogen during the fed-to-fasted transition is due to a decrease in glycogen phosphorylase activity mediated by increased PDE3B activity. In animals that had previously been shown to have significant differences in liver glycogen levels (Fig. 3A, 3-h time point), glycogen phosphorylase activity was 224.7 ± 36.0 mU/mg protein in AdCMV-leptin- and AdCMV-βGal-treated animals compared with 268.4 ± 29.8 mU/mg protein in AdCMV-βGal-treated animals, a nonsignificant difference (P > 0.10). PDE3B activity was similarly unchanged (61.9 ± 1.1 and 60.7 ± 0.9 pmol·min⁻¹·ml⁻¹ in AdCMV-leptin- and AdCMV-βGal-treated animals, respectively), as was active glycogen synthase (1.37 ± 0.32 and 1.86 ± 0.39 mU/mg protein in AdCMV-leptin- and AdCMV-βGal-treated animals, respectively). In a further set of experiments designed to address the effects of acute leptin administration on PDE3B activity, saline or recombinant leptin was infused into ad libitum-fed and 2-h-fasted Wistar rats and then liver PDE3B activity was assessed. Plasma leptin levels were ~35 ng/ml in the leptin-infused animals, compared with normal fed levels of ~4.5 ng/ml. In saline-infused rats, PDE3B activity was 59.7 ± 2.7 pmol·min⁻¹·ml⁻¹ (ad libitum fed) and 69.5 ± 1.5 pmol·min⁻¹·ml⁻¹ (2 h fasted), whereas in leptin-infused animals PDE3B activity was 56.3 ± 2.9 pmol·min⁻¹·ml⁻¹ (ad libitum fed) and 70.3 ± 7.8 pmol·min⁻¹·ml⁻¹ (2 h fasted). Differences between groups were not significant. Thus acute leptin administration had no effect on liver PDE3B activity under these conditions.

Fig. 2. Male Wistar rats received AdCMV-leptin or AdCMV-βGal. Approximately 72 h later, all animals undertook an 18-h fast. One group of animals was killed at end of fast (0-h group). All other animals received either a 3- or a 6-h glucose infusion (3- and 6-h groups) and were then killed. At each time point, livers and skeletal muscle were isolated and glycogen content was measured (A: liver; B: muscle). Results are means ± SE; n = 5 animals for each time point and each condition.

Fig. 3. Male Wistar rats received AdCMV-leptin or AdCMV-βGal. Approximately 86 h later all animals received a 6-h glucose infusion. One group of animals was killed immediately after cessation of glucose infusion (0-h group). Further groups of animals were killed 3, 6, or 9 h after cessation of glucose infusion. At each time point, livers and skeletal muscle were isolated and glycogen content was measured (A: liver; B: muscle). Results are means ± SE; n = 4 for both AdCMV-leptin and AdCMV-βGal for 0- and 6-h time points; n = 7 (AdCMV-leptin) and 9 (AdCMV-βGal) for 3-h time point; n = 3 for both AdCMV-leptin and AdCMV-βGal for 9-h time point. *Significant difference (P < 0.05) between the AdCMV-leptin- and the AdCMV-βGal-treated animals at indicated time points.
DISCUSSION

Leptin has multiple metabolic effects that include reductions in food intake, increased basal metabolic rate, and acute and chronic alterations in lipid and carbohydrate metabolism. Although a number of studies have addressed leptin effects on lipid metabolism, leptin effects on carbohydrate metabolism are less well understood. Previous studies have suggested that leptin alters glucose metabolism in a number of tissues (1, 4, 10, 13, 14, 16, 22, 27). However, the effects of leptin on hepatic glycogen metabolism in vivo remain unclear, because both stimulatory and inhibitory effects have been reported (1, 4, 22). Additionally, the effects of leptin during the two physiologically relevant situations, the absorptive phase and the fed-to-fasting transition, have not been investigated. The current study addressed these issues with glucose infusions to approximate the absorptive phase and fasting after a glucose infusion to mimic the fed-to-fasted transition. The major finding of the present study, that hyperleptinemia slows liver glycogen mobilization during the fed-to-fasted transition, lends further support to an important role for leptin in the regulation of carbohydrate metabolism, substrate mobilization, and energy partitioning.

The decreased rate of liver glycogen mobilization in hyperleptinemic rats compared with controls during a fast suggests that the use of glucose as an energy substrate may be decreased in leptin-overexpressing animals. Previous studies have demonstrated leptin-induced alterations in lipid metabolism. Thus depletion of fat mass occurs with chronic hyperleptinemia (3), the respiratory quotient decreases when leptin is administered to the ob/ob mouse (18), fatty acid oxidation increases when isolated soleus muscles are incubated with leptin (17), and the expression of enzymes of fatty acid oxidation are increased in islets incubated with leptin (24, 30). These data suggest that leptin may partition energy expenditure toward the use of fatty acids. Given the interdependent relationship between glucose and fatty acid metabolism (19), in which increases in fatty acid oxidation are offset by decreases in glucose oxidation, it is plausible to suggest that the slower mobilization of liver glycogen stores observed in the current study may be a consequence of leptin-induced increases in fatty acid oxidation at the periphery. However, further studies are required to address this possibility.

In addition to the possibility that leptin-induced increases in fatty acid oxidation may decrease the use of glucose as an energy source in the initial period of a fast, more direct actions of leptin on hepatic glycogen metabolism may also be considered. Recent in vivo studies (1, 22) and studies in perfused rat liver (4) have suggested that leptin alters intrahepatic glucose fluxes. Two studies in hyperinsulinemic rodents (1, 22) have shown that both acute and chronic leptin administration increase gluconeogenesis and decrease glycogenolysis, while Cohen et al. (4) have demonstrated increased incorporation of [13C]pyruvate into glycogen in leptin-perfused livers. These data suggest that leptin has direct effects on both glycogen synthesis and glycogenolysis, possibly mediated via leptin receptors present in the liver or via leptin action at the hypothalamus. In the current study, we observed no effects of leptin on liver glycogen accumulation during a glucose infusion or on the activation state of glycogen synthase during a subsequent fast. One possible explanation for these data is that the current study was performed at physiological insulin levels, whereas the perfused liver study was performed in the absence of insulin. At the molecular level, PDE3B activity, which has been implicated in insulin inhibition of glycogenolysis, is increased, and cAMP levels are decreased in primary hepatocytes (28) incubated with leptin. Taken together with observations of leptin-induced decreases in liver glycogenolysis, these data suggest that leptin may inhibit glycogen phosphorylase activity via PDE3B-mediated cAMP reductions. In the current study, we investigated this possibility but found no differences in phosphorylase a or PDE3B activity in AdCMV-leptin vs. AdCMV-βGal-treated animals. Furthermore, an independent set of experiments that acutely administered recombinant leptin had no effect on PDE3B activity in the absorptive phase or under glycogenolytic conditions. However, it remains possible that changes in activity of these proteins are involved in leptin-induced alterations in hepatic glycogen metabolism but that they occur at time points not investigated in the current study.

Although liver glycogen metabolism was substantially altered by hyperleptinemia, there were no effects of leptin on skeletal muscle glycogen metabolism. Thus muscle glycogen in fed and 18-h-fasted rats was similar in hyperleptinemic and control animals. Additionally, leptin did not alter the capacity of skeletal muscle to accumulate glycogen during a glucose infusion or mobilize glycogen during a fast. These results are consistent with previous in vivo (22) and in vitro (7, 17, 20, 31)
observations that have reported no effects of leptin administration on skeletal muscle glucose metabolism. It is interesting to speculate on the physiological significance of the present findings and those of others regarding the potential role of leptin in the regulation of energy partitioning. Plasma leptin levels are higher in the fed vs. the fasted state in the rat (R. Buettner, C. Rhodes, C. Newgard, and R. M. O’Doherty, unpublished observations) and human (2). Additionally, a diurnal rhythm of plasma leptin has been observed (23, 25, 26), while chronic increases in leptin are associated with increased adiposity in the rat (R. Buettner, C. Rhodes, C. Newgard, and R. M. O’Doherty, unpublished observations), mouse (6), and humans (5, 15, 21). Although roles for these alterations in leptin levels have been proposed, it is unknown what effects these changes have on energy partitioning. On the basis of the current study and others (12, 17), we suggest that increases in leptin will increase lipid oxidation while lowering glucose oxidation. Furthermore, differences between individuals in the magnitude of fasted-fed, diurnal, and more chronic alterations in leptin levels and/or leptin sensitivity could result in patterns of energy expenditure that favor fat oxidation over glucose oxidation and hence influence the propensity to accumulate adipose tissue. Further studies that mimic physiological alterations in endogenous leptin levels are required to more fully understand the potential role of altered leptin levels in substrate mobilization and energy partitioning.

In conclusion, the current study demonstrates that leptin spares liver glycogen stores during the fed-to-fasted transition, lending further support to the hypothesis that leptin plays a fundamental role in substrate mobilization and energy partitioning. The mechanism appears to be a decrease in the rate of glycogen breakdown. However, the molecular mechanisms underlying the leptin effect on hepatic glycogen metabolism remain to be determined.

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REFERENCES


