Short Term Effects of Leptin on Hepatic Gluconeogenesis and in Vivo Insulin Action*

Luciano Rossetti, Duna Massillon, Nir Barzilai, Patricia Vugun, Wei Chen, Meredith Hawkins, Jie Wu, and Jali Wang

From the Diabetes Research and Training Center and Division of Endocrinology, Albert Einstein College of Medicine, Bronx, New York 10461

Long term administration of leptin decreases caloric intake and fat mass and improves glucose tolerance. Here we examine whether leptin acutely regulates peripheral and hepatic insulin action. Recombinant mouse leptin (0.3 mg/kg h, Leptin +) or vehicle (Leptin −) were administered for 6 h to 4-month-old rats (n = 20), and insulin (3 milliunits/kg min) clamp studies were performed. During physiologic hyperinsulinemia (plasma insulin −65 microunits/ml), the rates of whole body glucose uptake, glycolysis, and glycogen synthesis and the rates of 2-deoxyglucose uptake in individual tissues were similar in Leptin − and Leptin +. Post-absorptive hepatic glucose production (HGP) was similar in the two groups. However, leptin enhanced insulin’s inhibition of HGP (4.1 ± 0.7 and 6.2 ± 0.7 mg/kg min; p < 0.05). The decreased HGP in the Leptin + group was due to a marked suppression of hepatic glycogenolysis (0.7 ± 0.1 versus 4.1 ± 0.6 mg/kg min, in Leptin + versus Leptin −, respectively; p < 0.001), whereas the % contribution of gluconeogenesis to HGP was markedly increased (82 ± 3% versus 36 ± 4% in Leptin + and Leptin −, respectively; p < 0.001). At the end of the 6-h leptin infusion, the hepatic abundance of glucokinase mRNA was decreased, whereas that of phosphoenolpyruvate carboxykinase mRNA was increased compared with Leptin −. We conclude that an acute increase in plasma leptin 1) enhances insulin’s ability to inhibit HGP, 2) does not affect peripheral insulin action, and 3) induces a redistribution of intrahepatic glucose fluxes and changes in the gene expression of hepatic enzymes that closely resemble those of fasting.

The recent discovery of the ob gene (1) and preliminary analysis of the properties of its product, leptin (2–7), have shed new light on the regulation of energy homeostasis (8). Since the most common alteration in energy balance, obesity, is tightly associated with insulin resistance, it has been proposed that leptin may play a role in carbohydrate metabolism and insulin action (8–11). Indeed, recent work in cultured adipose cells (12, 13) and hepatocytes (9) has suggested that leptin may regulate the gene expression of a key gluconeogenic enzyme, phosphoenolpyruvate carboxykinase (9), and may thus regulate the intrahepatic distribution of glucose fluxes.

To delineate whether leptin acutely regulates hepatic and peripheral insulin action in vivo, we examined the effects of systemic infusion of recombinant leptin on whole body glucose disposal, hepatic glucose fluxes, and glucokinase and PEPCK mRNA in the presence of euglycemic hyperinsulinemic clamp studies in conscious rats.

EXPERIMENTAL PROCEDURES

Experimental Animals—Two groups of male Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, MA) were studied. Group 1 consisted of 8 rats receiving an infusion of vehicle for 6 h (Leptin −) and group 2 consisted of 12 rats receiving an infusion of recombinant mouse leptin (Amgen, Inc., Thousand Oaks, CA; >95% pure by SDS-polyacrylamide gel electrophoresis) at the rate of 5 µg/kg min (Leptin +). Rats were housed in individual cages and subjected to a standard light (6 a.m. to 6 p.m.)/dark (6 p.m. to 6 a.m.) cycle. One week before the in vivo study, rats were anesthetized with an intraperitoneal injection of pentobarbital (50 mg/kg body weight), and indwelling catheters were inserted in the right internal jugular vein and in the left carotid artery. The venous catheter was extended to the level of the right atrium and the arterial catheter was advanced to the level of the aortic arch (23–26). Studies were performed in awake, unfasted, and chronically catheterized rats (18, 19).

Body Composition—Fat-free mass and fat mass were calculated from the whole body volume of distribution of water, estimated by tritiated water bolus injection in each experimental rat (20). Briefly, boluses of 20 µCi of [3H]2O (NEN Life Science Products) were injected intra-arterially on the morning of the study (9 a.m.). Steady state for [3H]2O-specific activity in rats is generally achieved within 45 min; 5 samples were collected between 1 and 2 h after injection. The distribution space of water was obtained by dividing the total radioactivity injected by the steady-state specific activity of plasma water which was assumed to be 93% of the total plasma volume. Fat-free mass was calculated from the whole body water distribution space divided by 0.73.

* This work was supported by an unrestricted research grant from Amgen, Inc., by National Institutes of Health Grants DK 45024 and DK 48321, by the Juvenile Diabetes Foundation, and by the Core Laboratories of the AECOM Diabetes Research and Training Center Grant DK 20541. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed: Albert Einstein College of Medicine, 1300 Morris Park Ave., Bronx, NY 10461. Tel.: 718-430-4118/2415; Fax: 718-430-8557; E-mail: rossetti@aecom.yu.edu.

The abbreviations used are: PEPCK, phosphoenolpyruvate carboxykinase; FFA, free fatty acids; GK, glucokinase; HGP, hepatic glucose production; MOPS, 4-morpholinepropanesulfonic acid.

This paper is available online at http://www.jbc.org
Euglycemic Hyperinsulinemic Clamp Studies—Studies were performed in unrestrained rats using the insulin clamp technique (18, 19, 21), in combination with high pressure liquid chromatography purified [3-3H]glucose, [U-14C]2-deoxyglucose, and [U-14C]lactate infusions, as described previously (21, 22). Food was removed for ~5 h before the in vivo studies lasted 300 min and included a 120-min equilibration period for assessment of the space of distribution of water, a 120-min basal period for assessment of the post-absorptive rates of glucose turnover, and a 120-min hyperinsulinemic clamp period. At the beginning of the basal period and 120 min before starting the glucose/insulin infusions, a primed continuous infusion of high pressure liquid chromatography purified [3H]glucose (NEN Life Science Products; 200 μCi bolus, 0.2 μCi/min) was initiated and maintained throughout the remaining 4 h of the study. A bolus of [U-14C]2-deoxyglucose (20 μCi) was injected 30 min before the end of the study. [U-14C]Lactate (5 μCi bolus/0.25 μCi/min) was infused during the last 10 min of the study.

The protocol followed during the insulin clamp study was similar to that described previously (18, 19, 21, 22). Briefly, a primed continuous infusion of regular insulin (3 millimolars/kilogram/min) was administered, and a variable infusion of a 25% glucose solution was started at time 0 and periodically adjusted to clamp the plasma glucose concentration at ~7 mEq/L. To control for possible effects of leptin on the endocrine pancreas (23), somatostatin (1.5 μg/kg/min) was also infused to inhibit endogenous insulin secretion in both groups. Plasma samples for determination of [3H]glucose and [3H]water-specific activities were obtained at 10, 20, 30, 40, 60 min during the basal and clamp periods. Steady-state conditions for the plasma glucose concentration and specific activity were achieved within 90 min in both the basal and clamp periods of the studies (Fig. 1). Plasma samples for determination of plasma insulin and FFA concentrations were obtained at 30-min intervals during the study. Plasma samples for determination of plasma [U-14C]2-deoxyglucose-specific activity were obtained at 90, 91, 93, 95, 98, 100, 105, 110, and 120 min during the clamp studies. The total volume of blood withdrawn was ~3.0 ml/study; to prevent volume depletion and anemia, a solution (1:1 v/v) of 4.0 ml of fresh blood (obtained by heart puncture from litters of the test animals) and heparinized saline (10 units/ml) was infused at a rate of 20 μl/min. Furthermore, following larger samples, red blood cells were resuspended in saline and immediately returned through the sampling catheter. All determinations were also performed on portal vein blood obtained at the end of the experiment.

At the end of the in vivo studies, rats were anesthetized (pentobarbital 60 mg/kg body weight, intravenously); the abdomen was quickly opened; portal vein blood was obtained, and rectus abdominal and perirenal hindlimb muscle and liver were freeze-clamped in situ with tongs pre-cooled in liquid nitrogen. The time from the injection of the anesthetic until freeze-clamping of the tissues was less than 45 s. All tissue samples were stored at ~80 °C for subsequent analysis. The study protocol was reviewed and approved by the Institutional Animal Care and Use Committee of the Albert Einstein College of Medicine.

Analytical Procedures—Plasma glucose was measured by the glucose oxidase method (Glucose Analyzer II, Beckman Instruments, Palo Alto, CA). Plasma insulin, glucagon, and leptin (rat Leptin radioimmunoassay kit, Linco Research Inc., St. Charles, MO) concentrations were measured by radioimmunoassay. The plasma concentration of free fatty acids was determined by an enzymatic method with an automated kit according to the manufacturer’s specifications (Waco Pure Chemical Industries, Osaka, Japan). Plasma [3H]glucose radioactivity was measured in duplicates in the supernatants of Ba(OH)2 and ZnSO4 precipitates (Somogyi procedure) of plasma samples (20 μl) after evaporation to dryness to eliminate tritiated water. The rates of glycolysis were estimated as described previously (19). Briefly, plasma-tritiated water-specific activity was determined by liquid scintillation counting of the protein-free supernatant (Somogyi filtrate) before and after evaporation to dryness. Because tritium on C-3 of glucose is lost to water during glycolysis, it can be assumed that plasma tritium is present either in tritiated water or [3-3H]glucose. To measure plasma [U-14C]2-deoxyglucose, samples were deproteinized as described above, and an aliquot of the supernatant was counted in a double channel beta-counter after addition of 500 μl of water and 5 ml of liquid scintillation mixture. To measure plasma [U-14C]2-deoxyglucose-specific activity, an aliquot of the supernatant was weighed and dissolved in 0.5 ml of 1 M NaOH kept in a shaking water bath at 60 °C for 1 h. Following neutralization with 0.5 ml of 1 M HCl, 2 aliquots were taken. One was deproteinized with Ba(OH)2 and ZnSO4 and the other with 6% HClO4. The HClO4 supernatant contains both phosphorylated and unphosphorylated 2-deoxyglucose, whereas the Ba(OH)2 and ZnSO4 supernatant contains only the unphosphorylated form. The difference in dpm between the two supernatants measures the muscle content of 2-deoxyglucose-phosphate (24). Uridine-diphosphoglucose (UDP-Glc), uridine-diphosphogalactose (UDP-galactose), and phosphoenolpyruvate (P-enolpyruvate) concentrations and specific activities in the liver were obtained through two sequential chromatographic separations, as previously reported (21, 22, 25, 26). Hepatic glucose-6-phosphate (G6P) and phosphoenolpyruvate carboxykinase (PEPCK) mRNA abundance was assessed by Northern blot analysis (27–29). Total RNA was isolated on freeze-clamped liver tissues according to the RNA-STAT kit (Tel-TEST “B” Inc. Friendswood, TX). The isolated RNA was assessed for purity by the 260/280 ratio absorbency. Twenty μg of total RNA were electrophoresed on a 1.2% formaldehyde denatured agarose gel in 1 × MOPS running buffer. The RNA was visualized with ethidium bromide and transferred to a hybird-N membrane (Amer- sham Corp.). The GK and PEPCK cDNA probes were labeled with 32P using the Megaprime labeling system kit (Amerham Corp.). Prehybridization and hybridization were carried out using the rapid hybridization buffer (Amerham Corp.). The filters were then exposed to Fuji x-ray films for ~48 h at ~80 °C with intensifying screens. Quantification of GK and PEPCK mRNA was done by scanning densitometry, normalized for ribosomal RNA signal to correct for loading irregularities.

Terminology (21)—The term total glucose output is intended as total in vivo flux through Glc-6-Pase. The term hepatic glucose production (HGP) is intended as the net rates of Glc-6-P dephosphorylation to glucose. Finally, glucose cycling is defined as the input of extracellular glucose into the Glc-6-P pool followed by exit of plasma-derived Glc-6-P back into the extracellular pool.

Calculations—Under steady-state conditions for plasma glucose concentrations, the rate of glucose appearance (Ra) is calculated as the ratio of the rate of infusion of [3H]glucose (dpm/min) and the steady-state plasma [3H]glucose-specific activity (dpm/mg). When exogenous glucose was given, the rate of endogenous glucose production was calculated as the difference between Ra and the infusion rate of glucose. The rate of 2-deoxyglucose uptake was calculated as described by Kraegen et al. (24). The percent of the hepatic glucose 6-phosphate pool directly derived from plasma glucose was calculated as the ratio of [14C]UDP-Glc and plasma [3H]glucose-specific activities. The percent of the hepatic glucose 6-phosphate pool derived from P-enolpyruvate gluconeogenesis was calculated as the ratio of the specific activities of [14C]UDP-Glc and 2 × [3H]enolpyruvate following in vivo labeling with [U-14C]lactate (21, 22). Comparisons between groups were made with Student’s t test for unpaired samples, and all values are presented as the mean ± S.E. of at least three basal measurements.

RESULTS AND DISCUSSION

The discovery by positional cloning of the murine ob gene (1) and of its product, a 16-kDa protein named leptin (15), has dramatically altered our understanding of energy metabolism (8). However, although it is now well established that circulating leptin is produced by adipose cells (1–3, 6, 15, 30) and exerts pronounced metabolic effects through specific hypothalamic receptors (7, 14, 31–34), there is minimal information on putative metabolic roles of leptin in carbohydrate or lipid homeostasis. Studies of the interactions between leptin and insulin action have been limited to isolated cell systems in culture (9, 12). Although the nutritional state and insulin levels per se can...
regulate the gene expression of ob in adipose cells and leptin levels in plasma (6, 8, 10, 11), it is presently unknown whether leptin plays any role in the regulation of in vivo insulin action and hepatic glucose fluxes.

**General Characteristics of the Experimental Animals (Table I)—** To examine the effect of the acute administration of leptin on whole body, skeletal muscle, and liver insulin action, 12 rats were infused with 5 mg/kg min leptin for 6 h and were compared with 8 control rats receiving a vehicle infusion. There were no differences in the mean body weights, average food intake, and fat-free mass between the two groups of rats. Similarly, following a 5-h fast (post-absorptive state), the plasma insulin, glucose, and FFA concentrations were similar in the rats assigned to the two experimental groups.

**Euglycemic Hyperinsulinemic Clamp Studies (Table II and Fig. 1)—** To assess the metabolic effects of insulin in vivo, a physiologic increase in the plasma insulin concentrations was generated for 120 min, and the plasma glucose concentrations were maintained at the basal levels (~7 mM) by a variable glucose infusion. Thus, conscious rats were compared in the presence of similar steady-state hyperinsulinemia and normoglycemia in the presence or absence of exogenous leptin (Table II). Steady-state conditions for plasma glucose concentration and specific activity were achieved during the last 30 min of the basal period and of the clamp study.

### Table II

| Group       | Leptin – | Leptin + |
|-------------|----------|----------|
| Glucose (mM) | 7.7 ± 0.2 | 7.4 ± 0.2 |
| Insulin (microunits/ml) | 25 ± 1 | 64 ± 2a |
| FFA (meq/liter) | 0.97 ± 0.09 | 0.75 ± 0.05a |
| GIR (mg/kg/min) | 0 | 12.9 ± 0.7 |
| Glucose (mM) | 7.7 ± 0.4 | 7.4 ± 0.2 |
| Insulin (microunits/ml) | 23 ± 2 | 65 ± 2a |
| FFA (meq/liter) | 1.19 ± 0.07a | 0.74 ± 0.07a |
| GIR (mg/kg/min) | 0 | 13.1 ± 0.9 |

*a p < 0.05 versus basal.

*b p < 0.05 versus Leptin –.

![FIG. 1. Time course of the plasma glucose concentrations (A), of the specific activities of plasma tritiated glucose (B), and of the plasma free fatty acid (FFA) concentrations (C) during the basal period and during the euglycemic hyperinsulinemic clamp studies in Leptin – and Leptin + rats. Steady-state conditions for plasma glucose concentration and specific activity were achieved during the last 30 min of the basal period and of the clamp study.](image)

![FIG. 2. Effect of hyperleptinemia on the rates of glucose disappearance (A) and on the rates of glycolysis and glycogen synthesis (B) during the hyperinsulinemic clamp studies. A, Rd was measured by tracer dilution technique during the basal period (Basal) and during the euglycemic hyperinsulinemic clamp studies (Insulin) in Leptin – and Leptin + rats. B, rates of glycogen synthesis and glycolysis during the euglycemic hyperinsulinemic clamp studies in Leptin – and Leptin + rats. Hyperleptinemia did not alter the actions of insulin on whole body glucose uptake (Rd), glycogen synthesis, and glycolysis.](image)
FIG. 3. Rates of 2-deoxyglucose uptake in striated muscle and adipose tissue of Leptin − and Leptin + rats at the end of the euglycemic hyperinsulinemic clamp studies. [U-14C]2-Deoxyglucose was given as a intra-arterial bolus injection 30 min before the end of the insulin clamp studies, and plasma samples for determination of plasma [U-14C]2-deoxyglucose-specific activity were obtained at 90, 91, 93, 95, 98, 100, 105, 110, 115, and 120 min during the clamp studies. At the completion of the in vivo studies hindlimb (H. Muscle) and rectus abdominis (R. Muscle), epididymal (E. Fat), perinephric (P. Fat), and mesenteric (M. Fat) fat, and heart were rapidly sampled, and the accumulation of labeled deoxyglucose-phosphate was quantitated. The calculated rates of deoxyglucose uptake in individual tissues confirmed that hyperleptinemia did not alter the in vivo action of insulin on glucose disposal.

Effect of Leptin on Insulin-mediated Glucose Disposal and on the Partitioning of Glucose Fluxes (Fig. 2)—The effect of a similar increase in the circulating insulin concentrations on the rates of tissue glucose uptake (Rd), glycolysis, and glycogen synthesis are displayed in Fig. 2. All measurements were performed during the final 30 min of the clamp study, a time when steady-state conditions were achieved for plasma glucose and insulin concentrations, glucose-specific activity, and rates of glucose infusion. The rates of whole body glucose disappearance (18.6 ± 1.0 and 17.7 ± 0.7 mg/kg/min in Leptin − and Leptin +, respectively; Fig. 2A) were similar in the two groups. Thus, leptin did not alter the whole body glucose disposal in response to physiologic increments in the plasma insulin concentration. We next examined whether leptin modifies the partitioning of glucose fluxes into the two major pathways of glucose disposal, glycogen synthesis and glycolysis. As shown in Fig. 2B the rates of glycogen synthesis and glycolysis during the hyperinsulinemic clamp studies were also similar in Leptin + compared with Leptin − rats. Thus, short term leptin infusion did not modulate the whole body fluxes of glucose through glycogen synthesis and glycolysis. Since hyperinsulinemia markedly stimulates the uptake of glucose in striated muscle and adipose tissue, the rates of glucose uptake were next assessed in muscle and fat sites sampled at the completion of the in vivo studies.

Effect of Leptin on 2-Deoxyglucose Uptake (Fig. 3)—The rate of skeletal muscle glucose uptake during the last 30 min of the hyperinsulinemic clamp studies was measured using 14C-labeled 2-deoxyglucose. The rates of 2-deoxyglucose transport in hindlimb and rectus muscle, heart, epididymal, perinephric, and mesenteric fat were similar in Leptin + and Leptin −. Taken together with the above results on the whole body glucose fluxes, these results indicate that, under the conditions of hyperinsulinemic clamp studies, short term leptin administration does not influence insulin action on glucose disposal.

Effect of Leptin on Hepatic Glucose Fluxes (Table III and Fig. 4 and 5)—Fig. 4 depicts the rate of glucose production (HGP) during the basal period and its suppression by hyperinsulinemia. It should be pointed out that it is highly unlikely that renal glucose production contributed to overall glucose production in our experimental setting. In fact, animals were fasted for just 5–6 h, and we had previously shown that marked decreases in renal mass has no effect on tracer-determined HGP in conscious rats (35). Basal HGP was similar in the Leptin − and Leptin + rats. However, the inhibition of HGP during the hyperinsulinemic clamp studies was moderately increased in the Leptin + compared with the Leptin − rats. In fact, the percent decrease in HGP from their basal values was 68 ± 6 and 52 ± 6% in Leptin + and Leptin −, respectively. Furthermore, the residual HGP during the clamp studies was also 34% lower in Leptin + than in Leptin −. We next examined whether leptin altered the relative contributions of plasma glucose, gluconeogenesis, and glycogenolysis to the hepatic glucose 6-phosphate pool. Leptin exerted its most striking metabolic effect on the intrahepatic partitioning of glucose fluxes. In fact, a marked increase in the contribution of gluconeogenesis to HGP was consistently demonstrated in the rats receiving exogenous leptin with P-enolpyruvate gluconeogenesis almost entirely accounting for HGP. Table III displays the [3H]UDP-glucose and [3H]UDP-galactose and the [3H]glucose-specific activities that are used to calculate the contribution of plasma glucose (Direct in Table III) to the hepatic glucose 6-phosphate pool. The UDP-galactose-specific activities confirmed the values obtained with
UDP-glucose, suggesting rapid and complete isotopic equilibration between the two intracellular pools. The ratio of the specific activities of 3H-labeled hepatic UDP-glucose/galactose and portal vein plasma glucose provided an estimate of the contribution of the direct pathway. As shown in Table III, the contributions of the direct pathway to the hepatic UDP-hexose pool measured at the end of the clamp studies were similar in the Leptin1 compared with the Leptin2 rats.

Table III also displays the [14C]UDP-glucose and [14C]UDP-galactose and the [14C]P-enolpyruvate-specific activities that are used to calculate the contribution of P-enolpyruvate gluconeogenesis (Indirect in Table III) to the hepatic glucose 6-phosphate pool. The indirect pathway accounted for ~25% of the hepatic UDP-glucose pool in the vehicle-infused group. This contribution was more than doubled in the rats receiving leptin for 6 h. These data allowed us to estimate the in vivo fluxes through glucose-6-phosphatase and the rates of glucose cycling, gluconeogenesis, and glycogenolysis in Leptin1 and Leptin + rats. As shown in Fig. 4B the decreased HGP in Leptin + was paralleled by similar decreases in the rates of glucose cycling and total glucose output compared with the Leptin – rats. The glucose carbons in HGP can be derived from gluconeogenesis and/or glycogenolysis. Under the hyperinsulinemic conditions of these studies glycolysis accounts for the majority of HGP (~55%) as shown in the control group (Leptin – in Fig. 5). However, following leptin infusion gluconeogenesis was markedly increased and accounted for 82% of HGP. Similar results were also obtained in rats studied with the same experimental protocol but with a leptin infusion of 0.5 μg/kg/min (plasma leptin concentration ~ 46 ± 9 ng/ml). In this additional group, gluconeogenesis accounted for an average of 77 ± 6% of HGP. Thus, short term administration of the product of the ob gene, leptin, leads to marked alterations in the intrahepatic distribution of hepatic glucose fluxes with stimulation of gluconeogenesis and inhibition of glycogenolysis.

Effect of Leptin on the Hepatic Gene Expression of GK and PEPCK (Fig. 6)—The relative abundance of GK and PEPCK mRNAs was determined by Northern blot analysis of total RNA obtained from liver samples at the completion of the in vivo studies. 20 μg of RNA were used in each lane, and equal loading was confirmed by analysis of ethidium-stained ribosomal RNA bands. The figure depicts pooled samples (n = 3/each) from the two experimental groups. Leptin administration resulted in a significant decrease in GK mRNA and increase in PEPCK mRNA.
Leptin and Hepatic Glucose Fluxes

(Example is shown in Fig. 6) shows that the rats receiving leptin for 6 h manifested a ~3-fold increase in PEPCK mRNA concentrations compared with vehicle-infused rats. Most importantly, this marked stimulation of PEPCK gene expression by leptin was observed in the presence of equal and physiologically elevated plasma insulin concentrations. Thus, leptin appeared to antagonize the effect of insulin on PEPCK gene expression in vivo. The latter effect of leptin resembles the effects of the hormone on PEPCK mRNA levels previously reported in an hepatoma cell line (9). Similarly, the effect of leptin on the hepatic abundance of GK mRNA was opposite to that of insulin. In fact, rats receiving leptin displayed a ~50% decrease in GK mRNA concentrations compared with vehicle-injected rats.

These results indicate that short term administration of exogenous leptin antagonizes the action of insulin on the gene expression of GK and PEPCK in the liver. It may be postulated that this represents the molecular mechanism by which leptin regulates the intrahepatic partitioning of glucose fluxes with marked increase in gluconeogenesis and decrease in glycolysis. However, it remains to be delineated whether these metabolic effects of leptin participate in the regulation of hepatic glucose metabolism under physiologic conditions and whether they are mediated via peripheral and/or central receptors. Recent evidence suggests that leptin may play a major metabolic role in the regulation of energy stores and in the choice of fuels to be utilized under various nutritional states (8, 12, 36). Since the rate of glycolysis gluconeogenesis is a major determinant of the hepatic concentration of malonyl-CoA and has been shown to be rate-limiting for the regulation of FFA re-esterification to triglyceride in the liver (36), our results suggest that leptin limits the hepatic formation of triglycerides by favoring β-oxidation of FFA in the mitochondria. These observations are consistent with the reported effects of leptin on adipose cells (12, 13) and pancreatic beta cells (36). Future studies will need to identify further the biochemical mechanism(s) by which leptin switches the hepatic energy source from predominantly carbohydrates to lipids. Since malonyl-CoA is a key metabolic regulator of the partitioning of substrate oxidation in the liver, a lowering effect of leptin on the hepatic concentration of malonyl-CoA relieving the inhibition on carnitine-palmitoyltransferase 1 (CPT-1) might be considered (37, 38). The latter effect could be mediated by either or both increased gluconeogenesis and decreased intrahepatic glycolysis as observed in the present study and/or inhibition of acetyl-CoA carboxylase by leptin (13).

Acknowledgments—We thank Dr. Michael McCaleb (Amgen, Inc., Thousand Oak, CA) for providing leptin and for helpful discussions and Rong Liu and Meizhu Hu for their excellent technical assistance.

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