Leptin Enhances Glycogen Storage in Hepatocytes by Inhibition of Phosphorylase and Exerts an Additive Effect With Insulin

Susan Aiston and Loranne Agius

The effects of the adipocyte-derived hormone leptin on glucose metabolism in hepatocytes were investigated. Incubation of hepatocytes from Wistar rats with leptin for 16 h caused a dose-dependent increase in incorporation of [^{14}C]glucose into glycogen, with a maximal effect at 30 nmol/l leptin. This effect of leptin was observed over a range of glucose concentrations (10–25 mmol/l) and was associated with stimulation of net glycogen deposition. It was not counteracted by mercaptopicolinate, an inhibitor of phosphoenolpyruvate carboxykinase, indicating that it is not due to increased gluconeogenic flux. Leptin also enhanced the short-term stimulation of glycogen synthesis by insulin. These effects of leptin were associated with inhibition of phosphorylase *a*, which occurred after 4 h of exposure to leptin. Culture with leptin for 16 h did not affect the activities of glucose-6-phosphatase or glucokinase or the activation state of glycogen synthase. Leptin did not affect glycolysis determined from the detritiation of [3-^{3}H]glucose. The inhibitory effects of leptin on phosphorylase *a* were counteracted by short-term incubation with glucagon but were additive with the inhibitory effects of insulin and also with the inhibition caused by resorcinol (25 µmol/l), which inhibits phosphorylase kinase by a mechanism analogous to the antidiabetic drug proglycosyn. These results show that leptin has additive effects with insulin in inhibiting phosphorylase and stimulating glycogen storage in hepatocytes, indicating that these hormones act by separate but convergent mechanisms. It is concluded that the primary action of leptin in hepatocytes is to enhance glycogen storage. This may be an important compensatory mechanism for the inhibition of insulin secretion. *Diabetes* 48:15–20, 1999

Leptin, the protein encoded by the *Ob* gene, is a hormone that is produced by adipocytes and acts through distinct receptors in target organs to control food intake and energy metabolism (1,2). Rodents that are homozygous for defects in either leptin production (*ob/ob* mouse) or leptin receptor function (Zucker *fa/fa* rats and *db/db* mice) develop early-onset massive obesity that is associated with hyperphagia, hyperinsulinemia, hypertriglyceridemia, and other metabolic abnormalities (1,2). Treatment of the *ob/ob* mouse with leptin reverses the metabolic abnormalities in part by suppression of appetite but also by additional mechanisms (3).

Various effects of leptin on target organs have recently been identified. These include suppression of glucose-induced insulin secretion in pancreatic islets (4,5); increased partitioning of long-chain fatty acids toward oxidation as opposed to esterification in pancreatic islets (6) and soleus muscle of normal rats (7); inhibition of glucose incorporation into glycogen in soleus muscle of *ob/ob* mice (8); and stimulation of lipolysis (9) and counteraction of the metabolic actions of insulin in adipocytes (10). In hepatoma cell lines, leptin has been reported to counteract insulin action (11) or exert cytokine-like effects (12), whereas in other cell lines, insulin-like effects have been reported (13).

A recent in vivo study (14) showed that leptin infusion for 6 h did not affect the peripheral actions of insulin but enhanced the ability of insulin to suppress hepatic glucose production. This was associated with an increased proportional contribution of gluconeogenesis to hepatic glucose production. It was suggested that an increased gluconeogenic flux may underlie the effects of leptin in the liver in vivo. It was unclear whether these in vivo effects of leptin were due to direct effects of leptin on hepatocytes or to secondary endocrine changes. There have as yet been no in vitro studies on the effects of leptin on differentiated hepatocytes. The aim of this study was to characterize the direct effects of leptin on glucose metabolism in hepatocytes.

RESEARCH DESIGN AND METHODS

Materials. Recombinant human leptin was from Peninsula Laboratories, Belmont, CA. Radiochemicals ([U-^{14}C]glucose, [2-^{3}H]glucose, [3-^{3}H]glucose, and UDP[1-^{3}H]glucose) were from Du Pont-NEN, Boston, MA. Glucose dehydrogenase (*Bacillus megaterium*) was from Calbiochem, Nottingham, U.K. Other enzymes and cofactors and insulin (porcine) were from Sigma, St. Louis, MO.

Hepatocyte isolation and culture. Hepatocytes were isolated by collagenase perfusion of the liver of male Wistar rats (body wt 210–280 g) fed ad libitum (15). Hepatocytes were suspended in minimum essential medium (MEM) containing 7% neonatal calf serum and inoculated either in 24-well plates (for metabolic studies) or in 12-well or 6-well plates (for enzyme activity determination) (15). After cell attachment (~4 h), the medium was replaced with fresh serum-free MEM containing 5 mmol/l glucose, 10 nmol/l dexamethasone, and other hormones as indicated. Cells were cultured in this medium for 16 h. In experiments describing the long-term effects of leptin or insulin, these were added to the medium after cell attachment, and the metabolic studies (3-h incubations) or enzyme activity determinations were performed after the 16-h culture. In experiments describing short-term effects of leptin (2–6 h), cells were cultured for 16 h in the absence of hormones and then incubated with the hormones for the time intervals indicated.

From the Human Diabetes and Metabolism Research Center, the Medical School, University of Newcastle upon Tyne, Newcastle upon Tyne, U.K.

Address correspondence and reprint requests to Loranne Agius, DPhil, Human Diabetes and Metabolism Research Center, Level 4, Leech Bldg., The Medical School, University of Newcastle upon Tyne, Newcastle upon Tyne NE2 4HH, U.K. E-mail: loranne.agius@ncl.ac.uk.

Received for publication 22 June 1998 and accepted in revised form 16 September 1998.

G-6-P, glucose-6-phosphate; IRS, insulin receptor substrate; MEM, minimum essential medium.

Determination of glycogen synthesis and glycolysis. After culture with the hormones indicated for 16 h, hepatocytes were incubated for 3 h in fresh MEM containing the glucose concentration indicated and either [U-^{14}C]glucose, [2-^{3}H]glucose, or [3-^{3}H]glucose (2 µCi/ml) without or with 10 nmol/l insulin as indicated. On termination of the incubations, the medium was collected for determination of $^{3}H_2O$ (16), and the hepatocyte monolayers were washed with 150 mmol/l NaCl and extracted in 0.1 mmol/l NaOH. Glycogen synthesis was determined from the incorporation of ^{14}C-label into glycogen by ethanol precipitation (15), and glycogen deposition was determined enzymically from the increase in cellular glycogen during 3 h (15). Protein was determined by a Lowry method, and glucose-6-phosphate (G-6-P) was determined as previously described (16).

Enzyme activities. Glucose-6-phosphatase was determined spectrometrically using glucose dehydrogenase (17) in hepatocytes that were cultured without or with leptin for 16 h. Glucokinase (free and bound activity) was determined in hepatocytes that were cultured for 16 h without or with leptin (500 ng/ml) and then incubated for 30 min in medium supplemented with varying glucose (5–35 mmol/l) concentrations. Free and bound glucokinase activity was determined as previously described (18). For determination of phosphorylase *a* and glycogen synthase, the hepatocyte monolayers were incubated for 30 min in MEM containing 15 mmol/l glucose and then extracted in buffer containing 10 mmol/l Tris, 150 mmol/l KF, 15 mmol/l EDTA, 600 mmol/l sucrose, 1 mmol/l phenylmethylsulfonyl fluoride, 1 mmol/l benzamidine, 50 mmol/l mercaptoethanol, and 5 µg/ml leupeptin, pH 7.5. Extracts were sonicated and centrifuged for 10 min at 13,000*g*. Phosphorylase *a* was determined on the supernatants spectrometrically in the presence of caffeine to inhibit phosphorylase *b* (19). The assay contained 50 mmol/l imidazole, 50 mmol/l NaF, 2 mmol/l $MgCl_2$, 0.5 mmol/l EDTA, 1 mg/ml oyster glycogen, 1 mmol/l caffeine, 1 mmol/l NADP, 10 µmol/l glucose-1,6-bisphosphate, 2.5 U/ml phosphoglucomutase, and 2 U/ml G-6-P dehydrogenase, pH 7.5. The reaction was started with 10 mmol/l phosphate. Glycogen synthase was assayed radiochemically from the incorporation of UDP[1-^{3}H]glucose into glycogen in the absence (active) and presence of 10 mmol/l G-6-P (total activity) as previously described (20). Protein was determined by the Bio-Rad (Richmond, CA) protein assay reagent using bovine serum albumin as standard. Enzyme activities are expressed in milliunits per milligram of cell protein, where one milliunit is the amount of enzyme catalyzing the metabolism of one nanomole of substrate per minute. The fractional activity of glycogen synthase represents the active form as a percentage of total activity.

Statistics. Results are presented as means ± SE for the number of cell cultures indicated. Statistical analysis was by the paired Student's *t* test.

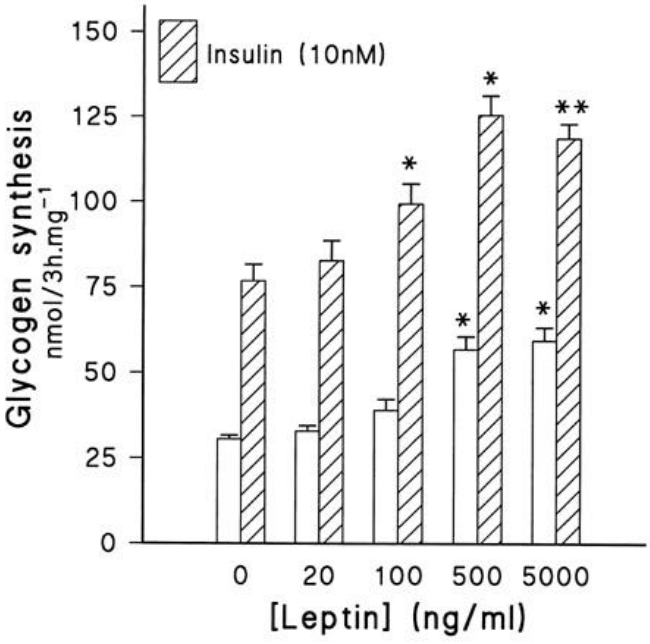

FIG. 1. Effects of leptin concentration on glycogen synthesis and its short-term stimulation by insulin. Hepatocytes were incubated for 16 h in MEM containing 5 mmol/l glucose and the concentrations of leptin indicated. They were then incubated for 3 h in fresh medium containing 15 mmol/l [U-^{14}C]glucose in either the absence (□) or presence (▨) of 10 nmol/l insulin. Glycogen synthesis is expressed as nanomoles of [^{14}C]glucose incorporated into glycogen per 3 h per milligram of cell protein. Values are means ± SE for three cell preparations. *P < 0.05; **P < 0.01 relative to corresponding controls without leptin. All values with insulin are greater (P < 0.05) than corresponding controls.

RESULTS

Effects of leptin and insulin on glycogen synthesis. Glycogen synthesis was determined in hepatocytes that were precultured for 16 h in medium containing 5 mmol/l glucose and then incubated for 3 h in medium with an elevated glucose concentration (10–25 mmol/l). Insulin (10 nmol/l) present during the 3-h incubation increased glycogen synthesis, determined from the incorporation of [U-^{14}C]glucose into glycogen at 15 mmol/l glucose, by more than twofold (Fig. 1). Leptin (20 ng/ml to 5 µg/ml) had no effect on glycogen synthesis when added during the 3-h incubation with 15 mmol/l glucose in either the absence or presence of insulin (results not shown). However, when leptin was present during the 16-h preculture, it caused a dose-dependent increase in glycogen synthesis when the cells were subsequently incubated (3 h) both in the absence of insulin (Fig. 1; twofold increase at 500 ng/ml or 30 nmol/l) and the presence of insulin (Fig. 1). The incremental increase in glycogen synthesis by insulin was 46 nmol · 3 h^{-1} · mg^{-1} in cells cultured without leptin and 68 nmol · 3 h^{-1} · mg^{-1} in cells cultured with 500 ng/ml leptin (Fig. 1). Leptin infusion in vivo for 6 h has been shown to increase hepatic PEPCK mRNA levels (14). To investigate whether the effects of leptin on glycogen synthesis are due to increased flux through PEPCK with increased cycling of 3-carbon precursors via the gluconeogenic pathway, we used mercaptopicolinate (1 mmol/l), an inhibitor of PEPCK, which inhibits conversion of 3-carbon precursors into glycogen in hepatocytes (21). Leptin stimulated the incorporation of glucose into glycogen in the presence of mercaptopicolinate (Fig. 2*A*), indicating that the stimulation of glycogen synthesis is not due to increased flux through PEPCK. Figure 2*B* shows the stimulatory effects of leptin (500 ng/ml, 16 h) on glycogen synthesis, determined over a range of glucose concentrations (10–25 mmol/l). The percentage stimulation by leptin was greater at 10 mmol/l compared with 25 mmol/l glucose (107 ± 20 vs. 41 ± 10%, P < 0.053), whereas the incremental increase was greater at 25 mmol/l glucose (41 ± 8 vs. 11 ± 3 nmol · 3 h^{-1} · mg^{-1}, P < 0.015). Glycogen deposition determined enzymically (Fig. 2*C*) showed changes similar to the incorporation of [^{14}C]glucose, indicating that leptin stimulates net glycogen storage rather than cycling of label between synthesis and degradation.

Lack of effect of leptin on glucose-6-phosphatase, glucokinase, and glycolysis. Culture of hepatocytes with leptin (20 ng/ml to 5 µg/ml) for 16 h had no effect on total cell protein. Leptin (500 ng/ml) did not affect the total activities of either glucose-6-phosphatase (control 39 ± 6 vs. leptin 42 ± 6 mU/mg protein, n = 6) or glucokinase (control 22 ± 2 vs. leptin 22 ± 2 mU/mg protein, n = 5). Leptin had no effect on the translocation of glucokinase determined after incubation of the hepatocytes for 30 min with varying glucose concentrations (5 mmol/l: 24 ± 2 vs. 26 ± 3; 10 mmol/l: 31 ± 4 vs. 32 ± 3; 15 mmol/l: 39 ± 3 vs. 40 ± 5; 25 mmol/l: 47 ± 5 vs. 48 ± 5; 35 mmol/l: 55 ± 5 vs. 53 ± 4 free glucokinase percent total activity, n = 5). Leptin (500 ng/ml, 16 h) had no effect on the G-6-P content that was determined after a 30-

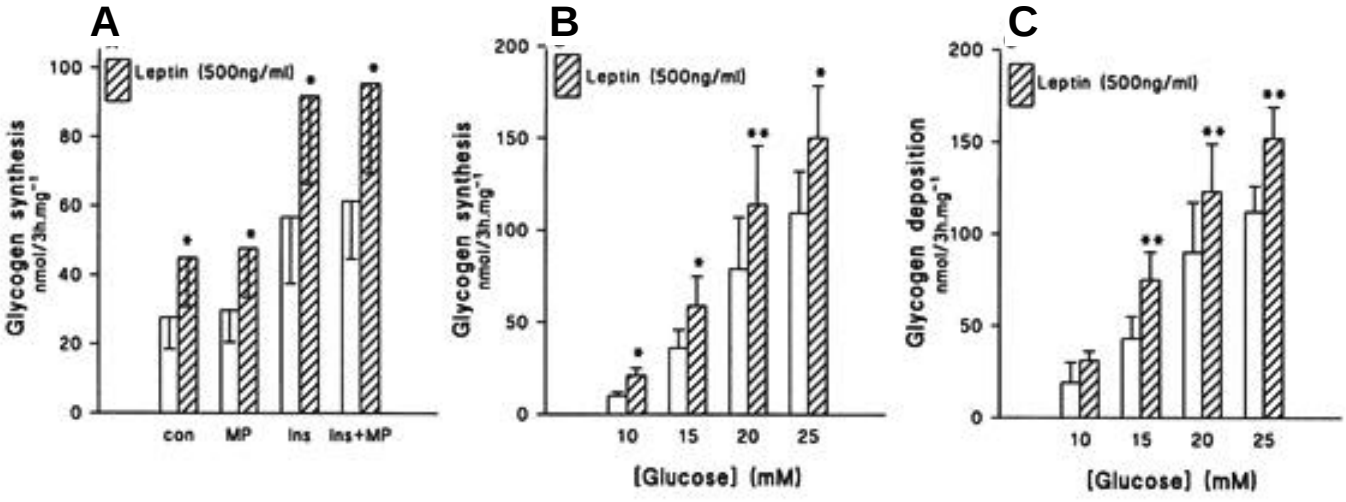

FIG. 2. Effects of leptin on glycogen synthesis and deposition at different glucose concentrations. Hepatocytes were incubated for 16 h in MEM containing 5 mmol/l glucose in either the absence (□) or presence (▨) of 500 ng/ml leptin. They were then incubated for 3 h in fresh medium containing [U-^{14}C]glucose and the additions indicated. *A*: Incubations contained 15 mmol/l glucose, 5 mmol/l pyruvate, and either 1 mmol/l mercaptopicolinate (MP) or 10 nmol/l insulin (ins). *B* and *C*: Incubations were at the glucose concentrations indicated. *A* and *B*: Glycogen synthesis expressed as nanomoles of [^{14}C]glucose incorporated into glycogen per 3 h per milligram of cell protein. *C*: Glycogen accumulation determined enzymically expressed as nanomoles of glucosyl units formed per 3 h per milligram cell protein. Values are means ± SE for four cell preparations. *P < 0.05; **P < 0.01.

min incubation with 25 mmol/l glucose (control 1.11 ± 0.06 vs. leptin 1.07 ± 0.10 nmol/mg protein, n = 5). Leptin had no effect on the rate of glucose phosphorylation, determined from the detritiation of [2-^{3}H]glucose, or the rate of glycolysis, determined from the detritiation of [3-^{3}H]glucose, in the same experiments as those reported in Figs. 1 and 2 (results not shown).

Effects of leptin and insulin on glycogen synthase. After incubation with hormones for 16 h, the total activity of glycogen synthase was increased by 10 nmol/l insulin (P < 0.001) but not by 500 ng/ml leptin (control 3.0 ± 0.3; leptin 3.1 ± 0.3; insulin 3.9 ± 0.3; insulin plus leptin 4.0 ± 0.3 mU/mg protein, n = 9), and the activation state of glycogen synthase (active enzyme percent total activity) was increased by insulin (P < 0.001) but not by leptin (control 17.1 ± 2.6; leptin 19.4 ± 3.2; insulin 29.2 ± 3.8; insulin plus leptin 30.1 ± 3.9 percent active, n = 9). Glycogen synthesis determined after a 16-h incubation with hormones was increased by 83% by leptin alone, by 165% by insulin alone, and by 253% by insulin plus leptin (control 26.3 ± 3.9; leptin 48.1 ± 9.1; insulin 69.6 ± 17.9; leptin plus insulin 92.9 ± 20.6 nmol · 3 h^{-1} · mg^{-1}, P < 0.03 relative to insulin alone, n = 5). This suggests that the increase in glycogen synthesis by leptin (500 ng/ml, 16 h) in either the absence or presence of insulin (10 nmol/l, 16 h) cannot be explained by an increase in the activation state of glycogen synthase.

Effects of leptin and insulin on glycogen phosphorylase activity. Hepatocytes cultured with either leptin (500 ng/ml) or insulin (10 nmol/l) for 16 h had significantly lower activities of phosphorylase *a* (P < 0.03) than respective controls cultured without hormones, and the combined effects of leptin and insulin were significantly greater (P < 0.03) than with either hormone alone (control 14.8 ± 1.5; leptin 12.7 ± 1.5; insulin 9.7 ± 1.5; leptin plus insulin 8.0 ± 1.6 mU/mg protein, n = 7). Figure 3 shows the time course of the effects of leptin on phosphorylase *a* expressed as the percentage inhibition relative to controls incubated for the same time interval. Significant inhibition by leptin was observed after 4 h but not after 2 h. The inhibition by leptin was smaller than the inhibition by 10 nmol/l insulin. However, the combined inhibition

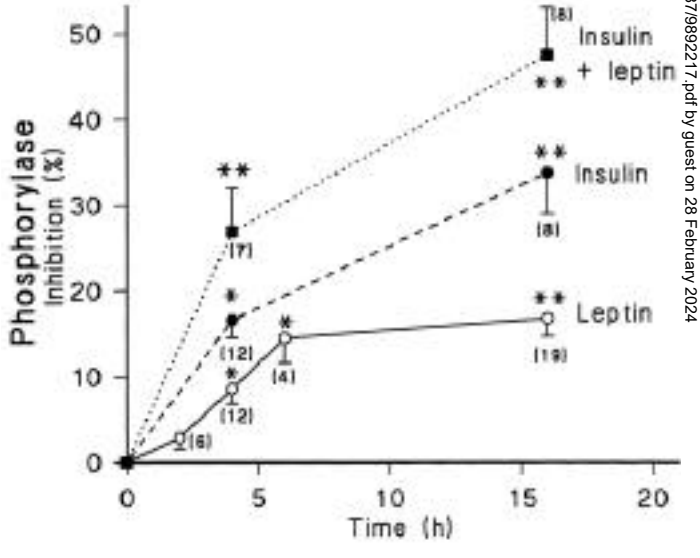

FIG. 3. Inhibition of phosphorylase *a* by leptin and insulin. Hepatocytes were incubated without or with 500 ng/ml leptin and/or 10 nmol/l insulin for the time intervals indicated, and phosphorylase *a* activity was determined. The inhibition of phosphorylase activity by leptin (○), insulin (●), or insulin plus leptin (■) is expressed as the percentage inhibition relative to controls incubated without hormones for the same time interval. Statistical analysis refers to the phosphorylase activity in the presence of hormones compared with the respective controls. *P < 0.05; **P < 0.001. Values are means ± SE for the number of cell preparations indicated in parentheses.

by insulin and leptin was greater than by either hormone alone at both 4 and 16 h ($P < 0.03$).

Interactions with short-term glucagon. When hepatocytes that had been cultured without or with leptin for 16 h were incubated with 100 nmol/l glucagon during the last 30 min of the incubation to activate phosphorylase *a*, there was no significant difference in activity between cells cultured without or with leptin, indicating that glucagon totally overrides the inhibitory effects of leptin (control 15.0 ± 5.6; leptin 12.4 ± 4.8 ($P < 0.01$); control plus 100 nmol/l glucagon 25.6 ± 9.0; leptin plus 100 nmol/l glucagon 25.0 ± 8.5 mU/mg cell protein, $n = 6$).

Interactions with resorcinol. Previous studies have shown that the mechanism of action of the antidiabetic drug proglycosyn, which counteracts the hyperglycemia of insulin-resistant obese diabetic mice, is at least in part due to inactivation of phosphorylase *a* by inhibition of phosphorylase kinase (22). This effect is mimicked by resorcinol (22). We examined whether resorcinol (added during the last 30 min) and leptin (500 ng/ml, 16 h) have additive effects on phosphorylase activity. Resorcinol caused maximum inhibition of phosphorylase (40%) at 25 µmol/l. At this resorcinol concentration, the effects of leptin (16 h) and resorcinol (30 min) on phosphorylase *a* were additive (control 18.7 ± 0.5; leptin 16.6 ± 0.8; control plus resorcinol 11.0 ± 0.7; leptin plus resorcinol 7.4 ± 0.4 mU/mg cell protein, $n = 3$, $P < 0.03$ relative to resorcinol alone or leptin alone).

DISCUSSION

The metabolic effects of leptin on differentiated hepatocytes in vitro have not been reported previously. The present study demonstrates that in hepatocytes in short-term primary culture (<24 h), leptin does not counteract the short-term effects of insulin on glucose metabolism, but it stimulates glycogen storage and exerts an additive effect with insulin on glucose incorporation into glycogen. The stimulatory effect of leptin on glycogen deposition and glucose incorporation into glycogen differs from the effects of insulin both in relation to the time course of hormone action and in the regulatory mechanisms involved. Insulin increases glycogen synthesis when present during a 3-h incubation with elevated glucose concentration (short-term exposure to hormone) and when the hepatocytes are precultured with insulin for 16 h. Leptin, in contrast, does not increase glycogen synthesis when present only during a 3-h incubation. However, glycogen deposition and glucose incorporation into glycogen are increased when hepatocytes are precultured with leptin for 16 h and then incubated for 3 h in the absence of leptin. Whereas the stimulation of glycogen synthesis by a 16-h culture with insulin is associated with inhibition of phosphorylase as well as with a marked increase in the activation state of glycogen synthase and an increase in total synthase activity, the effect of leptin is associated only with inhibition of phosphorylase.

It is generally assumed that the incorporation of [^{14}C]glucose into glycogen is a measure of the rate of glycogen synthesis, whereas glycogen deposition determined analytically reflects the difference between rates of synthesis and degradation. In most metabolic conditions associated with net glycogen deposition in hepatocytes, for example, the responses to elevated glucose concentration or insulin (21), there is a good correlation between the rate of [^{14}C]glucose incorporation and the rate of glycogen deposition determined analytically (21). It has been inferred from these observations that glycogen degradation is negligible under these conditions. The observations that most metabolic conditions that stimulate glycogen deposition and [^{14}C]glucose incorporation into glycogen in hepatocytes also activate glycogen synthase (23) appeared to support the assumption that [^{14}C]glucose incorporation into glycogen is a measure of the rate of glycogen synthesis. However, the present finding that leptin increases glycogen deposition and [^{14}C]glucose incorporation into glycogen without activation of glycogen synthase or translocation of glucokinase suggests that the incorporation of [^{14}C]glucose into glycogen (like glycogen deposition) may be a measure of the difference between rates of glycogen synthesis and degradation. The inactivation of phosphorylase by leptin suggests that in hyperglycemic conditions, the increase in glycogen deposition by leptin is due to decreased glycogen degradation. Because acute exposure to glucagon counteracts the inhibitory effects of leptin on phosphorylase *a*, the most likely explanation for the effect of leptin is inactivation of phosphorylase by dephosphorylation. The lack of effect of leptin on glucokinase and glucose-6-phosphatase and the failure of mercaptopicolinate to suppress the effects of leptin on the incorporation of [^{14}C]glucose into glycogen further suggest that inactivation of phosphorylase is the predominant mechanism of action of leptin on glycogen metabolism. It is noteworthy that while the inhibitory effect of insulin on phosphorylase is approximately twofold greater than the effect of leptin, the time courses of action of insulin and leptin on phosphorylase were not markedly different. Thus, the difference between the time courses of action of leptin and insulin on glycogen storage (short-term stimulation by insulin but not by leptin) can be best explained by the additional mechanisms that are involved in insulin action, for example, short-term activation of glycogen synthase (23) and stimulation of glucokinase translocation (24) that are not shared by leptin.

The finding that the increase in glycogen deposition and incorporation of [^{14}C]glucose into glycogen by leptin are associated with inactivation of phosphorylase is significant on four accounts. First, as discussed above, it suggests that changes in [^{14}C]glucose incorporation into glycogen may arise from differences in glycogen degradation rather than synthesis. Second, it may explain why the stimulation of glycogen deposition by insulin is greater than the activation of glycogen synthase (23), since inactivation of phosphorylase by insulin may be a significant contributing factor to the effect of insulin. Third, it shows that inactivation of phosphorylase can occur in the absence of sequential activation of synthase. Phosphorylase *a* is a potent inhibitor of glycogen synthase phosphatase (25), and in several metabolic conditions, inactivation of phosphorylase precedes activation of synthase by synthase phosphatase. The activation of synthase by synthase phosphatase has been explained by the decrease in the concentration of phosphorylase *a*, its inhibitor, when this is dephosphorylated to phosphorylase *b*. Although the present results do not exclude the possibility that inactivation of phosphorylase may potentiate activation of synthase in response to a rise in G-6-P (26), they clearly show that inactivation of phosphorylase without an increase in G-6-

P occurs in the absence of activation of synthase. Fourth, the similarity in the time courses of the effects of insulin and leptin on phosphorylase raises the issue of whether insulin may activate synthase and inactivate phosphorylase by distinct or separate signaling pathways, and the effects of insulin and leptin on phosphorylase inactivation may share similar mechanisms. A recent report suggests that leptin administration in vivo causes an activation of phosphatidylinositol 3-kinase similar to that of insulin when activity is determined in immunoprecipitates of the p85 regulatory subunit of this kinase, but it causes a smaller activation than insulin when activity is determined in immunoprecipitates of either insulin receptor substrate (IRS)-1 or IRS-2 (27). This supports the suggestion that inactivation of phosphorylase by insulin and leptin might involve similar mechanisms. The additive effects of leptin and insulin on phosphorylase inhibition provide an explanation for the findings of Rossetti et al. (14) that leptin infusion in vivo (6 h) suppressed hepatic glucose production during a hyperinsulinemic clamp. However, an in vivo study on wild-type mice showed that leptin infusion at a concentration of 33 ng/ml decreased hepatic glycogen (28). This effect is probably mediated at the level of the central nervous system, since similar metabolic effects were elicited by a lower dose of leptin administered by the intracerebroventricular route. Higher concentrations of leptin were effective in the present study (100 or 500 ng/ml in the absence or presence of insulin, respectively) at increasing glycogen storage. Whether use of human leptin may account for the apparent low sensitivity of rat hepatocytes is unclear. It is noteworthy, however, that a recent study demonstrated that the stomach epithelium secretes large quantities of leptin into the blood after a meal (29). Thus, the liver might be exposed to a higher concentration of leptin in the absorptive state than the periphery. This supports a physiological role for the present findings of direct effects of leptin on glycogen metabolism in hepatocytes.

A key question concerns the possible mechanism(s) by which leptin inactivates phosphorylase. Phosphorylase is activated by phosphorylation on a single residue (Ser-14) catalyzed by phosphorylase kinase, which is activated in response to a rise in cAMP and/or Ca^{2+}. Dephosphorylation (inactivation) of phosphorylase or phosphorylase kinase is mediated by protein phosphatases type 1 and type 2A. Thus, inactivation of phosphorylase could result from a decrease in cell cAMP or Ca^{2+} or from activation of protein phosphatases. The additive effects of resorcinol, an inhibitor of phosphorylase kinase (22), and leptin on phosphorylase inhibition suggest that the effects of leptin may be due in part to activation of protein phosphatases. Leptin inhibits insulin secretion in pancreatic islets (4,5). It has been suggested that the primary function of leptin may be to dampen insulin release during fasting rather than during feeding (5). This is consistent with the observation that circulating leptin levels show a circadian rhythm that peaks in the early morning (30). We hypothesize that the primary function of the inhibition of hepatic phosphorylase by leptin is a compensatory mechanism for the inhibition of insulin secretion. Thus, the combined effect of leptin on the pancreatic islets and the liver is to lower circulating insulin levels with a consequent suppression of glucose utilization by the periphery while conserving hepatic glycogen stores. The inhibition of phosphorylase by leptin supports, rather than opposes (31), the thrifty genotype hypothesis by conserving glucose in the late absorptive state.

ACKNOWLEDGMENTS

We thank the Medical Research Council, the British Diabetic Association, and the Juvenile Diabetes Foundation for support.

REFERENCES

1. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM: Positional cloning of the mouse obese gene and its human homologue. *Nature (Lond)* 372:425–432, 1994
2. Flier JS: Leptin expression and action: new experimental paradigms. *Proc Natl Acad Sci U S A* 94:4242–4245, 1997
3. Levin N, Nelson C, Gurney A, Vandlen R, De Sauvage F: Decreased food intake does not completely account for adiposity reduction after ob protein infusion. *Proc Natl Acad Sci U S A* 93:1726–1730, 1996
4. Emilsson V, Liu Y-L, Cawthorne MA, Morton NM, Davenport M: Expression of the functional leptin receptor mRNA in pancreatic islets and direct inhibitory action of leptin on insulin secretion. *Diabetes* 46:313–316, 1997
5. Kieffer TJ, Heller RS, Leech CA, Holz GG, Habener JF: Leptin suppression of insulin secretion by the activation of ATP-sensitive K^+ channels in pancreatic β-cells. *Diabetes* 46:1087–1093, 1997
6. Shimabukoro M, Koyama K, Chen G, Wang MY, Trieu F, Lee Y, Newgard CB, Unger RH: Direct antidiabetic effect of leptin through triglyceride depletion of tissues. *Proc Natl Acad Sci U S A* 94:4637–4641, 1997
7. Muoio DM, Dohn GL, Fiedoorek FT, Tapscott EB, Coleman RA: Leptin directly alters lipid partitioning in skeletal muscle. *Diabetes* 46:1360–1363, 1997
8. Liu YL, Emilsson V, Cawthorne MA: Leptin inhibits glycogen synthesis in the isolated soleus muscle of obese (*ob*/*ob*) mice. *FEBS Lett* 411:351–355, 1997
9. Fruhbeck G, Aguado M, Martinez JA: In vitro lipolytic effect of leptin on mouse adipocytes: evidence for a possible autocrine/paracrine role of leptin. *Biochem Biophys Res Commun* 240:590–594, 1997
10. Muller G, Ertl J, Gerl M, Preibisch G: Leptin impairs metabolic actions of insulin in isolated rat adipocytes. *J Biol Chem* 272:10585–10593, 1997
11. Cohen B, Novick D, Rubinstein M: Modulation of insulin activities by leptin. *Science* 274:1185–1188, 1996
12. Wang Y, Kuropatwinski KK, White DW, Hawley TS, Hawley RG, Tartaglia LA, Baumann H: Leptin receptor action in hepatic cells. *J Biol Chem* 272:16216–16223, 1997
13. Berti L, Kellerer M, Capp E, Haring HU: Leptin stimulates glucose transport and glycogen synthesis in C_2C_{12} myotubes: evidence for a PI-3-kinase–mediated effect. *Diabetologia* 40:606–609, 1997
14. Rossetti, L, Massillon D, Barzilai N, Vuguin, P, Chen W, Hawkins M, Wu JW, Wang J: Short-term effects of leptin on hepatic gluconeogenesis and in vivo insulin action. *J Biol Chem* 272:27758–27763, 1997
15. Agius L, Peak M, Alberti KGMM: Regulation of glycogen synthesis from glucose and gluconeogenic precursors in periportal and perivenous rat hepatocytes. *Biochem J* 266:91–102, 1990
16. Agius L: Involvement of glucokinase translocation in the mechanism by which resorcinol inhibits glycolysis in hepatocytes. *Biochem J* 325:667–673, 1997
17. Alegre M, Ciudad CJ, Fillat C, Guinovart JJ: Determination of glucose-6-phosphatase activity using the glucose dehydrogenase coupled reaction. *Anal Biochem* 173:185–189, 1988
18. Agius L, Peak M, Newgard CB, Gomez-Foix AM, Guinovart JJ: Evidence of glucose-induced translocation of glucokinase in the control of hepatic glycogen synthesis. *J Biol Chem* 271:30479–30486, 1996
19. Stalmans W, Hers H-G: The stimulation of liver phosphorylase *b* by AMP, fluoride, and sulphate: a technical note on the specific determination of the *a* and *b* forms of liver glycogen phosphorylase. *Eur J Biochem* 54:341–350, 1975
20. Thomas JA, Schlender KK, Larner J: A rapid filter paper assay for UDP glucose-glycogen glucosyltransferase, including an improved biosynthesis of UDP-^{14}C-glucose. *Anal Biochem* 25:486–499, 1968
21. Agius L, Tosh D, Peak M: The contribution of pyruvate cycling to loss of [6-^{3}H]glucose during conversion of glucose to glycogen in hepatocytes: effects of insulin, glucose, and acinar origin of hepatocytes. *Biochem J* 289:255–262, 1993
22. Van Schaftingen E: Involvement of phosphorylase kinase inhibition in the effect of resorcinol and proglycosyn on glycogen metabolism in the liver. *Eur J Biochem* 234:301–307, 1995
23. Peak M, Rochford JJ, Borthwick AJ, Yeaman SJ, Agius L: Signalling pathways involved in the stimulation of glycogen synthesis by insulin in rat hepatocytes.

Diabetologia 41:16–25, 1998
24. Agius L, Peak M: Intracellular binding of glucokinase in hepatocytes and translocation by glucose, fructose, and insulin. *Biochem J* 296:785–796, 1993
25. Stalmans W, Bollen M, Toth B, Gergley P: Short-term hormonal control of protein phosphatases involved in hepatic glycogen metabolism. *Adv Enzyme Regul* 30:305–327, 1990
26. Villar-Palasi C, Guinovart JJ: The role of glucose-6-phosphate in the control of glycogen synthase. *FASEB J* 11:544–558, 1997
27. Kim YB, Uotani S, Flier JS, Kahn BB: In vivo administration of leptin rapidly activates phosphoinositide 3-kinase (PI 3-kinase) in insulin-sensitive tissues (Abstract). *Diabetes* 47:A317, 1998
28. Kamohara S, Burcelin R, Halaas JL, Friedman JM, Charron MJ: Acute stimulation of glucose metabolism in mice by leptin treatment. *Nature* 389:374–377, 1997
29. Bado A, Levasseur S, Attoub S, Kermorgant S, Laigneau JP, Bortoluzzi MN, Moizo L, Lehy T, Guerre-Millo M, Le Marchand Brustel Y, Lewin MJM: The stomach is a source of leptin. *Nature* 394:790–703, 1998
30. Sinha MK, Ohannesian JP, Heiman M, Kriauciunas A, Stephens TW, Magonsin S, Marco C, Caro JF: Nocturnal rise of leptin in lean, obese, and non-insulin-dependent diabetes mellitus subjects. *J Clin Invest* 97:1344–1347, 1996
31. Flier JS: What's in a name? In search of leptin's physiological role. *J Clin Endocrinol Metab* 83:1407–1413, 1998

Author Queries (please see Q in margin and underlined text)