Leptin Acts Peripherally to Limit Meal-Induced Increases in Plasma Insulin Concentrations in Mice: A Brief Communication

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Leptin inhibits food intake and lowers plasma insulin concentrations. This study was designed to determine whether leptin acts independent of food-intake regulation to affect meal-induced increases in plasma insulin concentrations. Leptin-deficient, Lep^{ob}/Lep^{ob} mice were administered 1 μg leptin intracerebroventricularly (ICV) or intraperitoneally. Food intake and plasma insulin concentrations of mice administered leptin ICV before a meal were lower, as expected, than were intakes and plasma insulin concentrations of mice administered vehicle ICV. However when food intake was controlled, meal-induced increases in plasma insulin were unaffected by ICV administration of leptin. Intraperitoneal administration of 1 μg leptin before a meal lowered meal-induced increases in plasma insulin concentrations without influencing the size of the meal. We conclude that plasma leptin concentrations can affect meal-induced insulin secretion independent of the central nervous system actions of leptin associated with food-intake regulation. Exp Biol Med 229:1033–1037, 2004

Key words: leptin; plasma insulin; food intake; Lep^{ob}/Lep^{ob} mice

Leptin, a polypeptide hormone secreted predominately by adipocytes, crosses the blood–brain barrier to bind and activate the long form of the leptin receptor, the primary receptor isoform coupled to signal transduction pathways, within the hypothalamus (1). This action of leptin within the hypothalamus plays a pivotal role in regulation of body-weight homeostasis. The long form of the leptin receptor is also present in pancreatic islets and other organs, indicating that leptin has distinct actions on peripheral organs as well (2–5).

Leptin-deficient, Lep^{ob}/Lep^{ob} mice, as well as mice that do not express the long form of the leptin receptor, develop hyperinsulinemia neonatally, before they begin to overeat (6, 7). This indicates that leptin might directly target the pancreas to help restrain insulin secretion. Alternatively, leptin may function *via* a hypothalamic–pancreatic, autonomic nervous system pathway to regulate insulin secretion. In support of direct leptin–pancreatic islet actions, pancreatic beta-cells express the long form of the leptin receptor, and in some reports, but not in all, leptin has been shown to inhibit insulin secretion from isolated pancreatic islets and various insulinoma cells [reviewed in (8)]. It is, however, difficult, based on these *in vitro* studies, to predict the extent to which direct actions of leptin on pancreatic islets might contribute to the regulation of insulin secretion in an animal.

Chronic administration of leptin to Lep^{ob}/Lep^{ob} mice lowers their plasma insulin concentration (9–12), but this treatment also lowers food intake, body weight, and fat content and consequently improves peripheral insulin sensitivity. It is, thus, not possible to attribute the lowered plasma insulin concentrations in these mice to direct effects of leptin on their pancreatic islets *per se*, as the other metabolic consequences of leptin action, including a lowered food intake, would indirectly lead to lowered plasma insulin. Kulkarni et al. (3) administered leptin intraperitoneally to Lep^{ob}/Lep^{ob} mice 30 mins after a 1 hr meal. They observed a marked lowering in plasma insulin concentration, and an elevation in plasma glucose, within 10 min after administration of leptin. Plasma insulin concentrations were also lower in Lep^{ob}/Lep^{ob} mice administered

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leptin intraperitoneally (IP) for 8 days than in control Lep^{ob}/Lep^{ob} mice pair-fed to the leptin-treated mice (13). These reports support a role for leptin in regulation of insulin secretion independent of leptin-induced changes in food intake. It is not, however, possible to conclude from these studies whether the administered leptin acted peripherally or was first translocated to the hypothalamus to indirectly influence insulin secretion *via* regulation of the autonomic nervous system.

This study was undertaken to determine whether leptin acts peripherally to influence meal-induced increases in plasma insulin concentrations in mice. This objective was accomplished by comparisons of plasma insulin concentrations of leptin-deficient, Lep^{ob}/Lep^{ob} mice after injection of leptin either IP or intracerebroventricularly (ICV). If leptin acts centrally to influence insulin secretion, the ICV-administered leptin should be more effective than the same amount of leptin administered IP. Conversely, if leptin acts peripherally to lower plasma insulin, the IP-administered leptin should be most effective. A second objective of this study was to evaluate the relationship between the timing of leptin administration and the consumption of a meal on plasma insulin. This objective was prompted by our earlier study with isolated pancreatic islets, in which leptin administration before initiation of acetylcholine-induced insulin secretion attenuated the insulin secretion response, but leptin administration after initiation of acetylcholine-induced insulin secretion failed to lower the rate of insulin secretion (14). Mice, thus, were treated with leptin before initiation of a meal, or after a meal.

Materials and Methods

Animals. Lep^{ob}/Lep^{ob} female mice were obtained from our breeding colony (C57BL/6J-$Lep^{ob/+}$). The Guide for Care and Use of Laboratory Animals (National Research Council, 1985) and local institutional guidelines were followed for the care and treatment of the mice. Mice were weaned at 3 weeks of age and fed a nonpurified diet (Teklad Rodent Diet 8640; Harlan, Bartonville, IL). They were group housed at 25°C in solid-bottom cages with wood shavings for bedding. Lights were on from 0700 to 1900 hrs. Lep^{ob}/Lep^{ob} female mice at 14–16 weeks of age and weighing approximately 50 g were individually housed approximately 1 week before use.

Experimental Design. Mice were food deprived for 4, 16, or 24 hrs, as indicated in the table legends, before food was provided at ~1000 hrs. A 4-hr period of food deprivation is the minimum, in our experience, that ensures that mice will reliably consume food within a short time after it is presented. A 24-hr period of food deprivation was used in one trial to maximize intake during a 1-hr period. The 16-hr period of food deprivation was used to ensure substantial intake of a predetermined amount (i.e., 0.25 g) of food. With this approach of restricting food intake, saline-injected and leptin-injected mice consumed similar amounts of food. Preweighed pellets of the nonpurified diet were placed in a shallow container on the floor of a clean cage with minimal wood shavings for bedding cage, so mice had easy access to the food and the remaining food was easily recoverable. Mice were injected IP or ICV with saline or 1 μg murine leptin at the time indicated in the tables. Mice were decapitated to collect blood at the times indicated in the table legends, and plasma was obtained and frozen for subsequent analyses.

Procedures and Analyses. Mice were injected IP with 100 μl saline or 100 μl saline containing 1 μg leptin. Other mice were lightly anesthetized with ether before ICV injection (15) into the lateral ventricle with 2 μl saline or 2 μl saline containing 1 μg leptin. Plasma insulin was measured as described earlier (16). After the animals were sacrificed, the brain was sectioned to determine by visualization of the needle tract whether the lateral ventricle had been entered. Plasma glucose and leptin were measured with a glucose oxidase–peroxidase kit (Sigma Chemical Co., St. Louis, MO) and a mouse leptin assay kit (Linco Research Inc., St. Charles, MO), respectively. Student's *t*-tests were used to compare control versus leptin-treated mice. Differences were considered statistically significant at $P < 0.05$.

Results

ICV, but not IP, administration of 1 μg leptin lowered food intake of Lep^{ob}/Lep^{ob} mice within 1–3 hrs of administration (Table 1), consistent with earlier observations (15). Twenty-four hrs after ICV administration of leptin, mice had consumed only ~50% as much food as control mice, and they also had lower plasma insulin concentrations than control mice (Table 1). The lowered plasma insulin may be secondary to the lowered food intake of these mice. IP administration of 1 μg leptin failed to influence any of the parameters measured during the 24-hr postinjection period (Table 1). This dose of leptin was thus used in all subsequent trials to evaluate the effects of IP-administered leptin on plasma insulin, independent of effects on food intake.

To determine whether the route of leptin administration would affect plasma insulin independent of differences in food intake, and following a meal, mice were food deprived for 4 hrs and then fed for 1 hr before IP or ICV administration of leptin. Mice consumed 0.42 ± 0.04 g during this 1-hr period. Postmeal administration of leptin by either route failed to influence plasma insulin or glucose concentrations 30 mins later (Table 2). In a second trial, mice were food deprived for a longer period (i.e., 24 hrs) to further increase the amount of food subsequently consumed, and they were then fed for 1 hr. They consumed 0.73 ± 0.04 g. These mice were then injected IP with saline or 1 μg leptin and were sacrificed 30 mins later. Consistent with the data in Table 2, the plasma insulin concentrations of these control and postmeal, leptin-treated, Lep^{ob}/Lep^{ob} mice were similar (14 ± 1 versus 16 ± 5 ng/ml, respectively). Plasma

Table 1. Food Intake, Plasma Insulin, and Plasma Glucose Concentrations of Lep^{ob}/Lep^{ob} Mice After Intraperitoneal or Intracerebroventricular Administration of Leptin Before Access to Food[a]

	Intraperitoneal		Intracerebroventricular	
Parameter	Saline (18)	Leptin (20)	Saline (18)	Leptin (15)
Food intake				
g/1 hr	0.28 ± 0.06	0.28 ± 0.04	0.30 ± 0.03	0.20 ± 0.03*
g/2 hrs	0.38 ± 0.04	0.38 ± 0.07	0.35 ± 0.03	0.24 ± 0.04*
g/3 hrs	0.50 ± 0.08	0.50 ± 0.06	0.47 ± 0.04	0.28 ± 0.04*
g/24 hrs	5.70 ± 0.26	5.56 ± 0.34	5.20 ± 0.30	2.70 ± 0.50*
Plasma insulin (ng/ml)	43 ± 10	39 ± 10	26 ± 4	7 ± 4*
Plasma glucose (mg/dl)	279 ± 50	235 ± 30	270 ± 38	230 ± 49

[a] Values are means ± SE; numbers of animals are indicated in parantheses. Mice were food-deprived for 4 hrs, injected with saline or saline containing 1 μg leptin, and fed. Cumulative food intake was measured. Blood was obtained 24 hrs after injection.
* Significantly different ($P < 0.05$) than the saline-treated group at the same time point.

leptin concentrations of the Lep^{ob}/Lep^{ob} mice administered leptin IP averaged 9.6 ± 4.2 ng/ml 30 mins after leptin treatment.

Next, Lep^{ob}/Lep^{ob} mice were administered leptin just before meal onset to determine whether pretreatment with leptin would influence subsequent meal-induced increases in plasma insulin. Mice were food deprived for 16 hrs, administered leptin, and then fed for 30 mins. Before the meal, plasma insulin concentrations averaged 4 ± 1 ng/ml, and plasma glucose concentrations averaged 186 ± 21 mg/dl (n = 14). Plasma insulin increased fourfold in mice administered saline and fed *ad libitum* (Table 3). Administration of 1 μg leptin IP did not significantly influence food intake, although it was about 30% lower than in saline-treated mice. Plasma insulin concentration, but not plasma glucose, was, however, 50% lower in *ad libitum*–fed mice administered leptin IP than in control mice (Table 3), indicating that leptin acts peripherally. Leptin was not administered ICV before *ad libitum* presentation of food to the mice because ICV-administered leptin would significantly lower food intake and confound interpretation of any observed changes in plasma insulin.

To directly test effects of the route of leptin administration on meal-induced plasma insulin concentrations independent of differences in food intake, mice were fed a restricted amount of food (i.e., 0.25 g). This approach limited food intake of the saline-administered mice to closely approximate that of leptin-treated mice (Table 3). Under these conditions, mice administered 1 μg leptin IP had 50% lower plasma insulin concentrations than control mice, without exhibiting differences in plasma glucose concentrations (Table 3). Administration of leptin ICV failed to influence plasma insulin or glucose concentrations (Table 3).

Table 2. Effects of Leptin Administration After a 1-hr Meal on Plasma Insulin and Glucose Concentrations[a]

Treatment	Insulin (ng/ml)	Glucose (mg/dl)
Intraperitoneal		
Saline	18 ± 2	319 ± 31
Leptin	16 ± 3	271 ± 41
Intracerebroventricular		
Saline	25 ± 5	274 ± 8
Leptin	17 ± 6	242 ± 40

[a] Values are means ± SE for 6 Lep^{ob}/Lep^{ob} mice per group. Mice were food-deprived for 4 hrs and then fed for 1 hr. Mice consumed 0.42 ± 0.04 g. Following this 1-hr meal, mice were administered intraperitoneally or intracerebroventricularly saline or saline containing 1 μg leptin without further access to food, and sacrificed 30 mins later to obtain blood.

Discussion

Insulin and leptin play critical roles in regulation of metabolism, food intake, and body fatness. The focus of this study addressed the acute effects of leptin on plasma insulin concentrations in mice. The major finding was that leptin administered peripherally was able to limit meal-induced increases in plasma insulin concentration independent of the central actions of leptin associated with lowered food intake. The leptin-induced suppression of meal-induced increases in plasma insulin did not cause a concomitant lowering of plasma glucose. The fact that these adult obese mice are insulin resistant and that meal size, and consequently glucose intake, was not a variable may have contributed to the lowered plasma insulin without a concomitantly lowered postprandial plasma glucose in this acute study.

Our conclusion that leptin acts peripherally to limit meal-induced increases in plasma insulin is based on responses of mice administered 1 μg leptin ICV versus IP. This dose of leptin is well-established to limit food intake of mice within 1 hr, and for up to 24 hrs, when administered ICV (15). Plasma insulin concentrations are also lowered in these mice; however, it is difficult to determine whether the lowered plasma insulin is secondary to a lowered food intake *per se*, or to a more direct action of leptin on the autonomic nervous system regulation of insulin secretion (Table 1). When food intake of saline-injected Lep^{ob}/Leb^{ob} mice was restricted to the amount consumed by Lep^{ob}/Leb^{ob} mice administered leptin ICV, plasma insulin concentrations of the two groups were similar (Table 3). This indicates that

Table 3. Effects of Leptin Administration Before a 30-min Meal on Food Intake, Plasma Insulin, and Plasma Glucose Concentrations[a]

		Food intake (g/30 min)		Insulin (ng/ml)		Glucose (mg/dl)	
Feeding Schedule	Injection Method	Saline	Leptin	Saline	Leptin	Saline	Leptin
Ad libitum	IP	0.35±0.04 (6)	0.24±0.07 (6)	16±4	8±3*	257±37	263±21
Restricted	IP	0.18±0.02 (13)	0.19±0.02 (17)	8±2	4±1*	203±30	204±28
Restricted	ICV	0.23±0.02 (9)	0.22±0.01 (12)	7±2	7±2	222±31	234±26

[a] Values are means ± SEM; numbers of Lep^{ob}/Lep^{ob} are indicated in parentheses. Mice were food-deprived for 16 hrs and injected intraperitoneally (IP) or intracerebroventricularly (ICV) with saline or saline containing 1 μg leptin. Food was presented either *ad libitum* or restricted to 0.25 g/mouse immediately after the saline ± leptin injection. Mice were sacrificed 30 mins later to obtain blood.
* Significantly different ($P < 0.05$) than the saline-treated group.

the acute effect of centrally-administered leptin on plasma insulin occurs *via* control of food intake *per se*.

IP administration of 1 μg leptin to Lep^{ob}/Leb^{ob} mice was sufficient to acutely elevate plasma leptin to a high physiological concentration (i.e., ~10 ng leptin/ml plasma 30 minutes postinjection; see results) but was insufficient to acutely inhibit food intake. Under these conditions, leptin still limited meal-induced increases in plasma insulin (Table 3). As discussed below, these results are consistent with the hypothesis that leptin has direct physiological effects on pancreatic islets to control meal-induced insulin secretion, but the possibility that peripherally administered leptin might activate afferent nerve terminals to transmit signals to the central nervous system and then to the pancreas cannot be totally excluded. Because food intake was not affected by this peripheral dose of leptin, any activation of nerve terminals would have presumably been selective to the pancreas.

Administration of leptin after consumption of the meal did not reduce the already elevated plasma insulin concentrations (Table 2). This observation is consistent with our earlier report that leptin coadministered with acetylcholine reduced the ability of acetylcholine to potentiate glucose-induced insulin secretion from pancreatic islets of Lep^{ob}/Leb^{ob} mice, but that leptin was ineffective in lowering insulin secretion when added to pancreatic islets after they were first exposed to acetylcholine (14). We speculate that leptin might acutely regulate a signal transduction pathway involved in the insulin secretion cascade pathway, but once gastrointestinal hormones and neurotransmitters associated with meal onset acutely stimulate insulin secretion, leptin may be less effective in acutely reversing this process. Our results and this speculation are not, however, totally consistent with the report of Kulkarni et al. (3), who trained mice for a week to eat a 1-hr meal daily. Leptin administered IP 30 mins after a meal effectively and rapidly lowered plasma insulin concentrations in these mice (3), in contrast to the ineffectiveness of leptin when administered postmeal to our acutely food-deprived, refed mice. It remains to be determined whether the training process *per se* contributed to the ability of leptin to acutely suppress plasma insulin concentrations when administered postmeal. For example, mice restricted to only a 1-hr meal daily likely would be in negative energy balance. Further studies are needed to fully define the effectiveness of leptin administration to regulate plasma insulin concentrations relative to the timing of the meal.

In earlier studies we used isolated pancreatic islets from leptin-deficient, Lep^{ob}/Leb^{ob} mice to examine the possible role for leptin in the control of insulin secretion (14). Lep^{ob}/Leb^{ob} mice have elevated plasma insulin concentrations by 2 weeks of age, but their pancreatic islets still secreted insulin normally in response to glucose at this age (17, 18). This indicates that factors other than sensitivity of the islets to glucose *per se* must contribute to the hyperinsulinemia in young Lep^{ob}/Leb^{ob} mice.

Consumption of a meal stimulates release of gastrointestinal hormones and neurotransmitters that markedly potentiate nutrient-induced insulin secretion (19). Islets from young Lep^{ob}/Leb^{ob} mice respond normally to potentiation of glucose-induced insulin secretion with GLP-1, an activator of the protein kinase A signal transduction system, but they hypersecrete insulin in response to acetylcholine or cholecystokinin, activators of the phospholipase C-protein kinase C (PLC-PKC) signal transduction pathway (17, 18, 20). Hyperactivity of this PLC-PKC pathway within pancreatic islets of young Lep^{ob}/Leb^{ob} mice thus appears to be a major contributor to their initial hyperinsulinemia. Further studies showed that leptin addition to pancreatic islets from these mice specifically inhibited this PLC-PKC-induced hypersecretion of insulin (14, 20). Leptin is known to activate phosphatidylinositol 3-kinase, which inhibits PLC-PKC-induced insulin secretion (21). We have proposed that this leptin-regulated pathway in pancreatic islets participates in the control of insulin secretion by regulating the extent to which nutrient-induced insulin secretion is potentiated by the PLC-PKC signal transduction pathway (20).

Leptin exerts multiple actions that contribute to the regulation of plasma insulin. Our focus was on the role of leptin in acutely regulating plasma insulin in association with a meal. Clearly, the ability of leptin to acutely inhibit food intake *via* central nervous system mechanisms constrains insulin secretion, but even with food intake controlled, leptin-deficient mice still have higher plasma

insulin concentrations than leptin-sufficient mice. This food intake-independent role for leptin in the control of plasma insulin appears to be mediated in part of least by peripheral actions of leptin. Leptin may act within the pancreas to participate in the regulation of phosphatidylinositol 3-kinase activity within beta-cells (20, 21). This action may in turn determine the extent to which release of gastrointestinal hormones and neurotransmitters in association with a meal stimulates insulin secretion. On the basis of this hypothesis, either leptin deficiency as occurs in Lep^{ob}/Lep^{ob} mice or leptin resistance as occurs in many obese people would lead to an exaggerated meal-induced increase in plasma insulin secondary to the diminished ability of phosphatidylinositol 3-kinase activity to constrain PLC-PKC-induced potentiation of insulin secretion. Under normal physiological conditions, plasma leptin concentrations are likely sufficient to activate the leptin-regulated pathways within the pancreas that function to limit meal-induced increases in plasma insulin.

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