Overfeeding Rapidly Induces Leptin and Insulin Resistance

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In common forms of obesity, hyperphagia, hyperinsulinemia, and hyperleptinemia coexist. Here, we demonstrate rapid induction of insulin and leptin resistance by short-term overfeeding. After 3 and 7 days on the assigned diet regimen, rats were tested for their biological responses to acute elevations in plasma insulin and leptin concentrations. Severe resistance to the metabolic effects of both leptin and insulin ensued after just 3 days of overfeeding. During the insulin clamp studies, glucose production was decreased by ~70% in control rats and 28–53% in overfed rats. Similarly, leptin infusion doubled the contribution of gluconeogenesis to glucose output in control rats but failed to modify gluconeogenesis in overfed animals. These findings demonstrate a paradoxical and rapid collapse of the leptin system in response to nutrient excess. This partial failure is tightly coupled with the onset of insulin resistance. *Diabetes* 50:2786–2791, 2001

Hyperphagia and elevated levels of both insulin and leptin are common features of obesity (1–8). This is paradoxical because leptin is a potent inhibitor of feeding (9–15) and is expected to decrease insulin levels via improved insulin action (16–21) and inhibition of insulin secretion (22). To reconcile these findings, it has been proposed that obesity is associated with resistance to the biological effects of both insulin and leptin (1–8). However, although it is generally assumed that leptin resistance contributes to hyperphagia (1,4,6,7), it is also possible that hyperphagia may induce leptin resistance and other metabolic sequelae of obesity. This rapid adaptation to increased energy availability may be designed to curtail the leptin system in order to facilitate storage of nutrients into lipid stores (1,3,23–25). This may be accomplished by restraining leptin biosynthesis (23–25) and/or by inducing leptin resistance (1,3–6,24,26). These mechanisms would be particularly well developed in individuals or animals predisposed to weight gain and diabetes (1,11,24,25,27). Consistent with the "thrifty genotype" hypothesis, this sequence of events would be tightly coupled to the onset of insulin resistance (1,11,24,28).

In addition to its anorectic actions, leptin is also a potent modulator of biochemical pathways and metabolic fluxes (17–19,29–32). In particular, we have shown that acute administration of leptin to postabsorptive rats caused a marked redistribution of intrahepatic glucose fluxes, with a marked increase in the relative contribution of gluconeogenesis and a parallel decrease in the contribution of glycogenolysis to hepatic glucose fluxes (19,32). These acute metabolic effects of leptin can be utilized to evaluate leptin sensitivity as a measurable biological response to an acute challenge with the hormone. Recent evidence in rodents (25) and humans (27) indicate that inadequate early increase in leptin secretion and biosynthesis in response to overeating may also play a role in the development of obesity and glucose intolerance. We have suggested that in an obesity-prone strain of rats, this may be partly due to the operation of a negative feedback loop between circulating leptin and its own biosynthesis (leptin autoregulation) (23).

To test whether voluntary overfeeding leads to rapid onset of leptin and insulin resistance in a rodent strain, which is susceptible to age- and diet-dependent weight gain (33,34), we assessed its impact on the metabolic actions of insulin and leptin.

RESEARCH DESIGN AND METHODS

One week before the in vivo study, catheters were inserted in the left carotid artery and the right internal jugular vein of male Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, MA), as previously described (19,32,35,36). 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. Rats received either normal chow (cat. no. 5001; Purina Mills Ltd.), with 59% calories provided by carbohydrates, 20% by protein, and 21% by fat or a highly palatable diet (cat. no. 9389; Purina Mills Ltd.), with 22% of calories provided by protein, 33% by fat, and 45% by carbohydrates. Starch was the main carbohydrate source in both diets, whereas sucrose accounted for ~3.5%. One group on the latter diet was allowed to eat ad libitum, and these rats rapidly increased their caloric intake by almost twofold. This was due to the increased energy content of the diet (37% vs. standard chow) and to increased food intake (47% vs. standard chow). Two control groups (control and pair-fed) received either normal chow or the high-fat diet at ~80% of the preintervention caloric intake. This modest decrease in food consumption is commonly observed after surgical implantation of the indwelling catheters. Rats were studied 3 and 7 days after implementation of the above diet regimens. Overall, we studied six groups of animals: *1*) 3-day control; *2*) 3-day overfed (ad libitum); *3*) 3-day pair-fed; *4*) 7-day overfed (ad libitum); *5*) 7-day control; and *6*) 7-day pair-fed. Food was removed for 5 h before all infusion protocols.

Additional groups of 3-day control and ad libitum rats were used to test whether short-term overfeeding alters leptin's inhibitory action on food intake. These latter groups did not undergo catheterization. All rats were placed in metabolic cages throughout the feeding experiments to monitor daily food intake. On the fourth day of the assigned diet regimen, food was withdrawn between 8:00 A.M. and 6:00 P.M. At 2 h before initiation of the dark

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HPLC, high-performance liquid chromatography; PEP, phosphoenolpyruvate.

TABLE 1
Effect of overfeeding (ad libitum) on body weight and metabolic parameters

	Control		Pair-fed		Ad libitum	
	3 Days	7 Days	3 Days	7 Days	3 Days	7 Days
n	6	6	8	12	8	10
Body weight (g)	298 ± 8.0	301 ± 7.0	304 ± 6.0	289 ± 6.0	332 ± 14	322 ± 6.0*
Δ Body weight (g)	−6.0 ± 4.0	−8.0 ± 4.0	−13 ± 4.0	−7.0 ± 3.0	25 ± 5.0*	26 ± 4.0*
Calorie intake (kcal/d)	56 ± 4.0	57 ± 5.0	51 ± 2.0	51 ± 2.0	112 ± 8.0*	105 ± 7.0*
Plasma glucose (mmol/l)	6.9 ± 0.2	6.7 ± 0.3	6.8 ± 0.2	6.9 ± 0.1	8.1 ± 0.1*	7.1 ± 0.2
Plasma FFA (mmol/l)	0.9 ± 0.1	0.8 ± 0.2	1.0 ± 0.2	0.9 ± 0.1	0.9 ± 0.2	1.0 ± 0.2
Plasma insulin (ng/ml)	1.0 ± 0.2	0.9 ± 0.2	0.9 ± 0.2	0.8 ± 0.3	1.7 ± 0.4*	1.9 ± 0.3*
Plasma leptin (ng/ml)	0.8 ± 0.3	0.7 ± 0.3	1.2 ± 0.2	0.9 ± 0.1	2.8 ± 0.5*	1.8 ± 0.3*
Basal GP ($mg \cdot kg^{-1} \cdot min^{-1}$)	11.1 ± 0.2	10.2 ± 0.3	8.7 ± 0.3	10.0 ± 0.4	14.1 ± 0.4*	12.1 ± 0.7

Biochemical parameters are the means ± SE of three basal measurements from each rat. Caloric intake is the mean ± SE of the cumulative intake during the 3 or 7 days preceeding the in vivo study. Food was withdrawn 5 h before plasma sampling. FFA, free fatty acid; GP, rate of endogenous glucose production. *$P < 0.01$ vs. control and pair-fed.

cycle, 3-day control animals were randomized to receive a subcutaneous injection of either vehicle or leptin (2 mg/kg body wt), whereas 3-day ad libitum rats were randomized to receive subcutaneous vehicle, leptin 2 mg/kg body wt, or leptin 5 mg/kg body wt. After injection, all rats were returned to the metabolic cages for monitoring of food intake.

The infusion protocols were designed to examine the effect of physiological increases in circulating insulin and leptin on carbohydrate metabolism. Rats received a 6-h intra-arterial infusion of either vehicle or recombinant mouse leptin (gift of Dr. M. McCaleb, Amgen, Thousand Oaks, CA) that was >95% pure by SDS-PAGE. Leptin was infused at the rate of 500 $ng \cdot kg^{-1} \cdot min^{-1}$.

The studies lasted 360 min and included three 120-min periods (equilibration, basal, and hyperinsulinemic clamp). The protocol followed during the insulin clamp study was similar to that previously described (19,23,32,37). Briefly, a primed-continuous infusion of regular insulin (3 $mU \cdot kg^{-1} \cdot min^{-1}$) was administered, and a variable infusion of a 25% glucose solution was started at time zero and periodically adjusted to clamp the plasma glucose concentration at ~7 mmol/l. To control for possible effects of leptin on the endocrine pancreas, somatostatin (1.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$) was also infused to inhibit endogenous insulin secretion in all groups. Plasma samples for determination of plasma insulin and leptin concentrations were obtained at 30-min intervals during the study. To assess glucose kinetics, we administered a primed-continuous infusion of high-performance liquid chromatography (HPLC)-purified [3-^{3}H]glucose (40 μCi bolus for 0.4 μCi/min) (New England Nuclear, Boston, MA) for the duration of the study. Samples for the determination of ^{3}H-glucose specific activity were obtained throughout infusions at 10-min intervals. At 10 min before the end of the in vivo studies, [U-^{14}C]lactate (20 μCi bolus for 1.0 μCi/min) (New England Nuclear, Boston, MA) was administered to determine the contribution of gluconeogenesis to the hepatic glucose-6-phosphate pool. At the end of the in vivo studies, rats were anesthetized (pentobarbital 60 mg/kg body wt i.v.), and tissue samples were freeze-clamped in situ with aluminum tongs precooled in liquid nitrogen. All tissue samples were stored at −80°C for subsequent analysis. The hepatic ^{14}C-phosphoenolpyruvate (PEP) and ^{3}H/^{14}C-UDP-glucose specific activities were measured by HPLC, and the rates of PEP-gluconeogenesis were calculated. Gluconeogenesis was estimated from the specific activities of ^{14}C-labeled hepatic UDP-glucose (assumed to reflect the specific activity of hepatic glucose-6-phosphate) and hepatic PEP after the infusion of [U-^{14}C]lactate and [3-^{3}H]glucose with the formula: GNG = TGO × ^{14}C-UDP-glucose SA/^{14}C-PEP SA × 2, where GNG is gluconeogenesis, SA is specific activity, and TGO is total glucose output. Statistical analysis was performed using unpaired Student's *t* test or analysis of variance.

RESULTS

To generate a model of voluntary hyperphagia, we fed a palatable diet to Sprague-Dawley rats and monitored their food intake and their adaptation to the increased caloric content of the diet. Two control groups were pair-fed at equal daily caloric consumption with either normal chow or the same palatable diet. After 3 days of overfeeding, plasma insulin and leptin concentrations were markedly elevated. However, animals fed ad libitum failed to adapt to the enhanced caloric content of the diet and markedly increased their daily energy intake (Table 1). Furthermore, despite hyperinsulinemia, rates of postabsorptive glucose production and plasma glucose levels were also transiently increased in ad libitum–fed rats.

Voluntary overfeeding rapidly induces insulin resistance. We examined whether short-term hyperphagia led to changes in the in vivo actions of insulin. Insulin action on carbohydrate metabolism was assessed in conscious rats using a combination of the insulin clamp and tracer dilution techniques (19,23,35,36). Somatostatin was also infused to prevent potential effects of leptin on endogenous insulin secretion (37). During the clamp studies, plasma glucose (~7 mmol/l) and insulin (~400 pmol/l) concentrations were similar in all groups. In the presence of physiologic hyperinsulinemia, overfeeding markedly decreased (by ~55% at 3 days and by ~40% at 7 days) the rate of glucose infusion required to maintain plasma glucose concentrations at basal levels (Fig. 1*A*). Thus, alterations in calorie intake result in rapid and dramatic changes in insulin's ability to promote glucose disposal. The two major effects of insulin on carbohydrate metabolism are to stimulate the uptake of glucose into peripheral tissues (mostly in skeletal muscle) and inhibit the production of glucose by the liver. The rate of glucose uptake was decreased after 7 days of overfeeding (17.6 ± 0.7 vs. 22.3 ± 0.6 $mg \cdot kg^{-1} \cdot min^{-1}$; $P < 0.01$ vs. control) (Fig. 1*B*). At this time, the skeletal muscle concentration of the end product of the hexosamine pathway, UDP-*N*-acetylglucosamine, was markedly increased in ad libitum compared with pair-fed and control rats (34.9 ± 3.1 vs. 20.8 ± 2.7 and 21.5 ± 1.7 nmol/g, respectively; $P < 0.01$) Similarly, voluntary overfeeding markedly impaired the action of insulin on glucose production. The rates of glucose production during the insulin clamp studies were approximately two- to fourfold higher in ad libitum rats than in pair-fed and control rats at both 3 and 7 days (Fig. 1*C*). The effect of insulin on GP can also be expressed as percent inhibition from basal levels (Fig. 1*D*). Overfeeding markedly diminished insulin's inhibition of glucose production. Thus, voluntary hyperphagia impaired insulin action on glucose production within just 3 days, whereas peripheral insulin action was decreased after 7 days.

Voluntary overfeeding blunts the acute metabolic effects of leptin. We next examined whether short-term hyperphagia led to changes in the in vivo effects of leptin

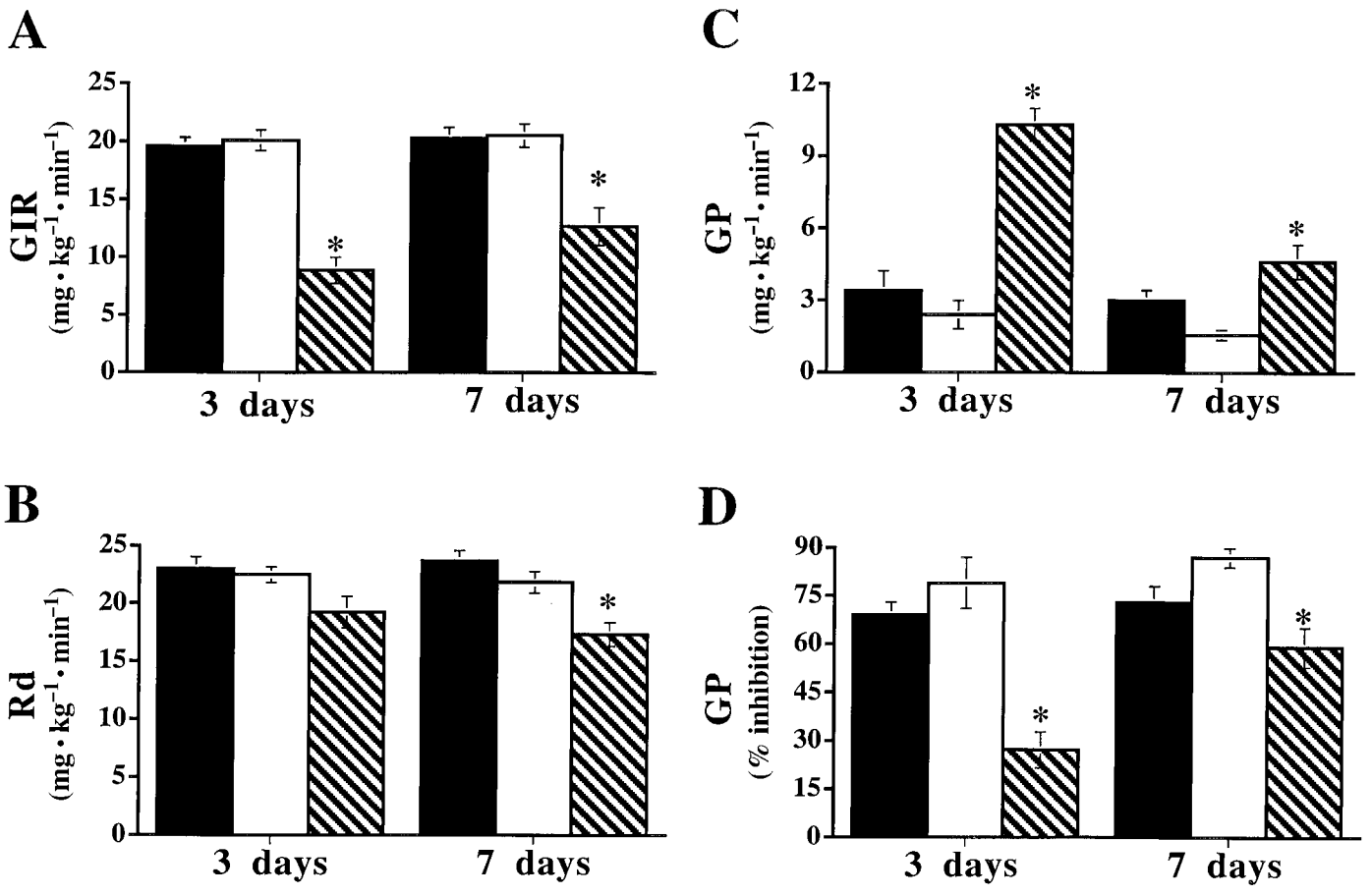

FIG. 1. Insulin action on peripheral glucose uptake and production during voluntary overfeeding. Physiological increases in plasma insulin concentrations were achieved by infusing insulin at the rate of 3 mU · kg^{-1} · min^{-1}. Steady-state plasma insulin concentrations were ~62 μU/ml in all groups. *A*: During the insulin clamp studies, the rate of glucose infusion was markedly decreased by 3 and 7 days of overfeeding (ad libitum, ▨) compared with controls (■) and pair-feeding (□). *B*: Insulin action on glucose uptake (Rd) was significantly decreased by 7 days of overfeeding (ad libitum). *C*: During the insulin clamp studies, the rate of glucose production (GP) was markedly increased in 3- and 7-day overfed rats (ad libitum) compared with both control groups (control and pair-fed). *D*: Inhibition of GP in response to physiological hyperinsulinemia was markedly decreased by 3 and 7 days of overfeeding. Values represent means ± SE. *$P < 0.01$ vs. control and pair-fed.

on hepatic glucose fluxes (19,32). To assess the acute metabolic response to leptin, we generated moderate increases in plasma leptin levels in conscious rats via systemic infusions of recombinant mouse leptin. Leptin infusions raised plasma leptin levels by ~23 ng/ml in all groups, whereas circulating leptin remained at basal levels during vehicle infusions. The contribution of gluconeogenesis to hepatic glucose fluxes was estimated by measuring the specific activities of liver UDP-glucose and PEP (Table 2) after the infusion of a labeled gluconeogenic precursor (lactate) (19,32,38). In pair-fed and control rats, acute elevations in circulating leptin levels resulted in a marked increase in the incorporation of labeled lactate into UDP-glucose (Table 2). This increase translated into a doubling of the contribution of gluconeogenesis to hepatic glucose fluxes (Fig. 2*A* and *B*). By contrast, a similar increase in plasma leptin levels failed to modify the contribution of gluconeogenesis in overfed rats (Fig. 2*C*). This lack of response to leptin was demonstrated after just 3 days of overfeeding.

Voluntary overfeeding diminishes the acute inhibitory effects of leptin on food intake. Of note, the action of leptin on feeding behavior also appeared to be rapidly impaired during voluntary overfeeding. In fact, despite significant elevations in circulating leptin and insulin concentrations, overfed rats failed to decrease their food intake in response to the increased caloric content of the diet. To provide more direct evidence that short-term overfeeding impairs leptin action on feeding behavior, we next tested the feeding responses to a single subcutaneous injection of leptin in 3-day control and ad libitum rats (Table 3). The subcutaneous injection of leptin at a dose of 2 mg/kg body wt decreased food intake by ~30% in chow-fed rats ($P < 0.01$). However, injection of the same dose in rats overfed for 3 days failed to alter food intake compared with subcutaneous vehicle injection. To further characterize the feeding response in this model, leptin was also injected at a 2.5-fold higher dose (5 mg/kg body wt) in 3-day overfed rats. At this higher dose, leptin decreased food intake by ~11% (P = NS).

DISCUSSION

Obesity and type 2 diabetes have been defined as "civilization syndromes" to emphasize the important synergism between environmental and genetic factors in their pathophysiology (27,28,39,40). An important tenet of this concept is that the activation of biological responses to increased availability of nutrients (adipostat) is muted in susceptible individuals. This altered response would con-

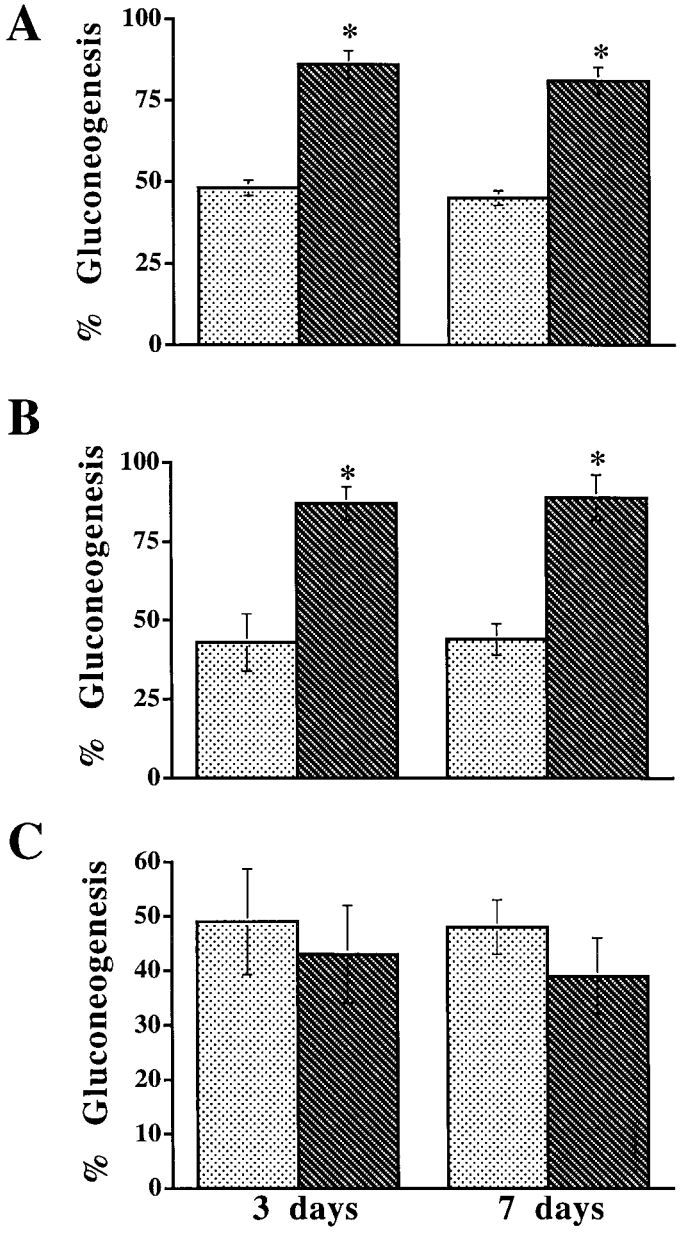

FIG. 2. Leptin action on hepatic gluconeogenesis during voluntary overfeeding. Plasma leptin concentrations were increased in Lep+ groups by infusing recombinant leptin at the rate of 500 ng · kg^{-1} · min^{-1}. During the leptin infusions (Lep+), steady-state plasma leptin concentrations averaged ~25 ng/ml in all groups. *A* and *B*: In control and pair-fed rats, physiological increases in plasma leptin levels resulted in a doubling of the contribution of gluconeogenesis to total glucose output. This effect was similar after 3 and 7 days. *C*: In overfed rats (ad libitum), physiological increases in plasma leptin levels failed to modify the contribution of gluconeogenesis to total glucose output. ▨, Lep−; ▧, Lep+. Values represent means ± SE. *$P < 0.01$ vs. control and pair-fed.

tribute to the onset of weight gain and insulin resistance, and it is likely to involve a partial failure of the leptin system. In line with this conceptual framework, Coleman (10,41) demonstrated that decreased expression (heterozygous mutants) of leptin or leptin receptor genes was associated with a survival advantage during food deprivation. Can a similar downregulation of the leptin system be acquired as an adaptation to overfeeding? Here, we report rapid induction of leptin and insulin resistance after voluntary overfeeding in an obesity-prone rat strain. The rapid time course of this adaptation is particularly important because it suggests that hyperphagia per se might lead to leptin and insulin resistance in susceptible individuals.

TABLE 2
Effect of leptin infusion on the indirect pathway of hepatic UDP-glucose formation

	[^{14}C]PEP (dpm/nmol)	[^{14}C]UDP-glucose (dpm/nmol)	Indirect pathway (%)
3-Day control			
Vehicle	12.2 ± 2.5	11.8 ± 1.5	48 ± 5
Leptin	13.2 ± 1.9	21.8 ± 3.1	83 ± 5*
3-Day pair-fed			
Vehicle	14.3 ± 3.1	11.9 ± 1.8	43 ± 9
Leptin	11.9 ± 2.0	21.0 ± 3.6	87 ± 6*
3-Day ad libitum			
Vehicle	11.4 ± 1.8	11.3 ± 1.2	49 ± 10
Leptin	14.1 ± 4.2	10.4 ± 3.4	43 ± 9
7-Day control			
Vehicle	14.0 ± 2.1	11.9 ± 1.8	44 ± 6
Leptin	15.1 ± 2.5	23.8 ± 3.1	79 ± 4*
7-Day pair-fed			
Vehicle	19.7 ± 2.5	18.8 ± 1.1	44 ± 5
Leptin	20.3 ± 2.2	35.6 ± 4.9	89 ± 7*
7-Day ad libitum			
Vehicle	10.9 ± 1.4	10.4 ± 0.7	48 ± 5
Leptin	14.6 ± 2.1	11.8 ± 2.4	39 ± 6

Data are means ± SE. Specific activities of UDP-glucose and PEP were used to calculate the contribution of PEP-gluconeogenesis (indirect pathway) to the hepatic UDP-glucose pool after [U-^{14}C]lactate infusion in rats during vehicle or leptin administration. The indirect pathway is the percent of the hepatic UDP-glucose pool derived from PEP-gluconeogenesis, calculated as the ratio of the specific activities of [^{14}C]UDP-glucose and 2 × [^{14}C]PEP. *$P < 0.01$ vs. vehicle.

Overfeeding rapidly induced hepatic and then peripheral insulin resistance. The first manifestations of impaired insulin action were the transient increase in postabsorptive glucose production and plasma glucose levels and the marked resistance to the inhibitory action of insulin on glucose production. Peripheral insulin resistance was only shown after 7 days of overfeeding. It is

TABLE 3
Effect of leptin on the food intake

	Preinjection (kcal/d)	Postinjection (kcal/d)	Decrease (%)
3-Day control			
Vehicle	72.4 ± 7.8	69.2 ± 6.6	4 ± 2
Leptin	71.4 ± 5.9	50.5 ± 3.4*	29 ± 3*
3-Day ad libitum			
Vehicle	109.2 ± 4.5	103.9 ± 3.8	5 ± 1
Leptin	107.3 ± 9.6	105.3 ± 4.3	2 ± 1
High leptin	98.1 ± 8.7	87.1 ± 6.7	11 ± 5

Data are means ± SE. The effect of a single subcutaneous injection of leptin or vehicle was tested in rats fed either a high-fat diet or normal chow for 3 days. The subcutaneous injection of leptin 2 mg/kg body wt decreased food intake by ~30% in chow-fed rats (3-day control). However, injection of the same dose in rats overfed for 3 days (3-day ad libitum) failed to alter food intake compared with subcutaneous vehicle injection. To further characterize the feeding response in this model, leptin was also injected at a 2.5-fold higher dose (high leptin, 5 mg/kg body wt) in 3-day overfed rats. Leptin modestly decreased food intake by ~11%. *$P < 0.01$ vs. vehicle.

possible that the early increase in glucose production during hyperphagia led to peripheral insulin resistance via a sustained increase in skeletal muscle glucose metabolism (36). Because increased flux in the hexosamine pathway has been proposed to mediate this secondary form of insulin resistance (42–45), we measured the concentration of the end product of this pathway in skeletal muscle of overfed, control, and pair-fed rats. Indeed, UDP-*N*-acetyl-glucosamine levels were markedly increased (by 68%) after 7 days but not after 3 days of overfeeding. Thus, one can hypothesize that the early increase in hepatic glucose output was designed to redirect carbohydrate flux from the liver to skeletal muscle and adipose tissue. This generated increased flux of glucose in muscle and adipose cells, which in turn caused peripheral insulin resistance (42–45) and increased leptin biosynthesis (35,46,47). It should be noted that the altered macronutrient composition in the pair-fed group did not result in significant changes in metabolic parameters compared with rats fed normal chow. Furthermore, it is unlikely that the modest caloric restriction imposed on the two control groups had a significant impact on insulin sensitivity (21). Conversely, the increase in body weight induced by overfeeding was largely accounted for by increased fat mass. This may in turn play a role in the onset of insulin resistance. Regardless of the underlying biochemical cause, hepatic insulin resistance was an early consequence of voluntary hyperphagia, and its onset coincided with the induction of leptin resistance.

Leptin resistance is rapidly acquired during voluntary overfeeding. Circulating leptin levels promptly increased in response to increased calorie intake. However, leptin failed to restrain feeding behavior and prevent weight gain. This may be an early indication of impaired leptin action on feeding behavior and/or leptin's inability to counteract the high palatability of the high-fat diet. Furthermore, the ability of exogenous leptin to restrain food intake was also blunted in overfed rats (Table 3). To provide further evidence of leptin resistance, we assessed the changes in hepatic glucose fluxes in response to an acute increase in plasma leptin concentrations (19,32). After 3 days of overfeeding, the action of leptin on hepatic gluconeogenesis was blunted. It is notable that hepatic insulin resistance and leptin resistance can be demonstrated after just 3 days of voluntary hyperphagia. The simultaneous appearance of both leptin and insulin resistance may indicate that these two processes are causally related. Recent evidence suggests that leptin can exert beneficial effects on insulin action (16–19,21). Thus, it may be hypothesized that leptin resistance led to hepatic insulin resistance. Alternatively, hyperphagia might have resulted in leptin and insulin resistance through altered function of a common hypothalamic network (17). Finally, it is also possible that hypothalamic insulin resistance is the primary consequence of overfeeding, and this in turn caused leptin resistance. In this regard, there is recent evidence suggesting a role of phosphoinositide-3 kinase downstream of both leptin and insulin signaling (48).

Overall, this is an experimental demonstration of one of the central tenets of the "thrifty genotype" hypothesis. Increased availability of nutrients (palatable food) in a susceptible population (obesity-prone rat strain) results in the rapid paralysis of the adipostat (leptin system) and impaired insulin action on carbohydrate metabolism. We suggest that the rapid onset of leptin resistance recapitulates the partial failure of the leptin system in Coleman's genetic models of obesity (41) and may provide a similar survival advantage by attenuating the response of the leptin/insulin system to overfeeding. This is consistent with the "thrifty genotype" concept, where once beneficial effects of currently deleterious genes may play a role in the development of susceptibility to obesity and type 2 diabetes (39).

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