Recombinant Human Leptin Treatment Does Not Improve Insulin Action in Obese Subjects With Type 2 Diabetes

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OBJECTIVE—Leptin therapy improves insulin sensitivity in people with leptin deficiency, but it is not known whether it improves insulin action in people who are not leptin deficient. The purpose of the current study was to determine whether leptin treatment has weight loss–independent effects on insulin action in obese subjects with type 2 diabetes.

RESEARCH DESIGN AND METHODS—We conducted a randomized, placebo-controlled trial in obese subjects (BMI: 35.4 ± 0.6 kg/m^2; mean ± SE) with newly diagnosed type 2 diabetes. Subjects were randomized to treatment with placebo (saline), low-dose (30 mg/day), or high-dose (80 mg/day) recombinant methionyl human (r-Met hu) leptin for 14 days. Multiorgan insulin sensitivity before and after treatment was evaluated by using the hyperinsulinemic-euglycemic clamp procedure in conjunction with stable isotopically labeled tracer infusions to measure glucose, glycerol, and fatty acid kinetics.

RESULTS—Low-dose and high-dose leptin treatment resulted in a threefold ($P < 0.01$) and 150-fold ($P < 0.001$) increase in basal plasma leptin concentrations, respectively. However, neither low-dose nor high-dose therapy had an effect on insulin-mediated suppression of glucose, glycerol, or palmitate rates of appearance into plasma compared with placebo. In addition, leptin treatment did not increase insulin-mediated stimulation of glucose disposal compared with placebo (14.3 ± 3.1, 18.4 ± 3.6, 16.7 ± 2.4 vs. 17.5 ± 2.5, 20.7 ± 3.0, 19.1 ± 3.3 μmol/kg body wt/min before vs. after treatment in the placebo, low-dose, and high-dose leptin groups, respectively).

CONCLUSIONS—r-Met hu leptin does not have weight loss–independent, clinically important effects on insulin sensitivity in obese people with type 2 diabetes. ***Diabetes*** **60:1474–1477, 2011**

Data from studies conducted in animal models indicate that leptin has beneficial effects on insulin action on glucose metabolism (1–3). Leptin also has profound metabolic effects in people. Leptin deficiency is associated with increased body weight and insulin resistance (4), and leptin replacement therapy improves insulin sensitivity in people with congenital leptin deficiency and leptin deficiency as a result of lipodystrophy or HIV-induced lipoatrophy (5–8). In contrast, obesity is commonly associated with insulin resistance despite high plasma leptin concentrations (9–11). Moreover, obesity is associated with resistance to many of the metabolic effects of leptin (12), which has led to the notion that resistance to leptin is involved in the pathogenesis of obesity-related insulin resistance. We hypothesized that increasing plasma leptin concentrations by exogenous leptin administration can improve insulin sensitivity in obese, insulin-resistant subjects. Accordingly, we conducted a randomized, placebo-controlled trial (NCT01207934) to evaluate the effect of low-dose and high-dose leptin treatment on insulin action on glucose production, glucose uptake, and lipolysis (by using a two-stage euglycemic-hyperinsulinemic clamp in conjunction with stable isotopically labeled tracer infusions) in obese subjects with newly diagnosed type 2 diabetes.

RESEARCH DESIGN AND METHODS

Subjects. Eighteen sedentary obese subjects with newly diagnosed type 2 diabetes who were not being treated with diabetes medications were randomized to one of three treatment groups: *1*) placebo (saline; four men and two women; age: 60 ± 3 years; BMI: 36 ± 1 kg/m^2; HbA_{1c}: 7.8 ± 0.3%; fasting plasma glucose concentration: 8.0 ± 0.4 mmol/L), *2*) low-dose (30 mg/day) recombinant methionyl human (r-Met hu) leptin (two men and four women; age: 53 ± 7 years; BMI: 35 ± 1 kg/m^2; HbA_{1c}: 8.2 ± 0.4%; fasting plasma glucose concentration: 8.5 ± 0.6 mmol/L), or *3*) high-dose (80 mg/day) r-Met hu leptin (three men and three women; age: 54 ± 4 years; BMI: 36 ± 1 kg/m^2; HbA_{1c}: 7.8 ± 0.3%; fasting plasma glucose concentration: 9.5 ± 0.5 mmol/L). None of the subjects smoked tobacco, were pregnant or lactating, had significant organ system dysfunction, or took medications known to affect metabolism. All subjects provided written, informed consent before participating in the study, which was approved by the Human Studies Committee of Washington University School of Medicine.

Experimental protocol

Body composition analyses and hyperinsulinemic-euglycemic clamp procedure. Subjects were admitted to the Clinical Research Unit (CRU) in the afternoon before the clamp procedure. Total body mass, fat mass, and fat-free mass were determined by using dual-energy X-ray absorptiometry (Hologic QDR 4500, Waltham, MA).

At 0600 h the next morning, after an overnight fast, one catheter was inserted into a forearm vein for infusion and a second catheter was inserted into a hand vein, which was heated to 55°C by using a thermostatically controlled box, to obtain arterialized blood samples. At 0700 h, a primed, continuous infusion of [6,6-$^2H_2$]glucose was started and maintained until the end of the study, 10.5 h later. At 0900 h, a continuous infusion of [1-^{13}C]palmitate and a primed, continuous infusion of [1,1,2,3,3-$^2H_5$]glycerol were started and maintained for 5 h (until the end of stage 1 of the clamp). At 1030 h, a two-stage (3.5 h each) euglycemic-hyperinsulinemic clamp was started. During stage 1 (3.5 to 7.0 h), insulin was infused at a rate of 20 mU/m^2 body surface area (BSA)/min (initiated by 80 mU/m^2 BSA/min for 5 min and then 40 mU/m^2 BSA/min for 5 min). During stage 2 (7.0 to 10.5 h), insulin was infused at a rate of 50 mU/m^2 BSA/min (initiated by 200 mU/m^2 BSA/min for 5 min and then 100 mU/m^2 BSA/min for 5 min). These two insulin infusion rates were chosen to evaluate liver and adipose tissue insulin sensitivity (low-dose insulin infusion to submaximally suppress hepatic glucose production and lipolysis of adipose tissue triglycerides) and skeletal muscle insulin sensitivity (high-dose insulin infusion to adequately stimulate muscle glucose uptake). Euglycemia was maintained at a blood glucose concentration of ~5.5 mmol/L by variable rate infusion of 20% dextrose enriched to 2.5% with [6,6-$^2H_2$]glucose. The infusion rates of [6,6-$^2H_2$]glucose, [1-^{13}C]palmitate, and [1,1,2,3,3-$^2H_5$]glycerol were reduced by 50% during stage 1, and the infusion rate of [6,6-$^2H_2$]glucose was reduced to 25% during stage 2 to account for changes in endogenous glucose production and lipolysis.

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Blood samples to determine plasma leptin and insulin concentrations and substrate (glucose, palmitate, and glycerol) tracer-to-tracee ratios (TTRs) were obtained before beginning the tracer infusion and every 10 min during the final 30 min of the basal period and stages 1 and 2 of the clamp.

Plasma insulin and leptin concentrations were measured by using radioimmunoassay (Linco Research, St. Charles, MO). Plasma glucose, palmitate, and glycerol TTRs were determined by using gas chromatography-mass spectroscopy.

Substrate rate of appearance (R_a) in plasma was calculated by dividing the substrate tracer infusion rate by the average plasma substrate TTR. Endogenous glucose production rate during the clamp procedure was calculated by subtracting the exogenous glucose infusion rate from glucose R_a; glucose R_d was calculated as the sum of endogenous glucose R_a and the rate of infused glucose. Hepatic insulin sensitivity was assessed by using the hepatic insulin sensitivity index, calculated as the product of basal endogenous glucose Ra and plasma insulin concentration (13).

Intervention. After completion of the clamp procedure, subjects were randomized to treatment with subcutaneous low-dose r-Met hu leptin (15-mg bid), high-dose r-Met hu leptin (40-mg bid), or placebo (saline, 2-mL bid) for 14 days, given by a research nurse during twice-daily home visits. The low dose was intended to provide a modest (~threefold) increase in plasma leptin concentrations, whereas the high dose was intended to provide a pharmacological exposure (~200-fold above baseline). Subjects were instructed to maintain their usual dietary and physical activity habits and were weighed daily by the research nurse. An increase in dietary intake was encouraged in any subject who demonstrated a trend toward a decrease in body weight. On day 14 of treatment, subjects were readmitted to the CRU, where the body composition analyses and the clamp procedure were repeated. The final doses of treatment were given in the evening before the study.

Statistical analyses and sample size considerations. ANOVA with repeated measures was used to compare between (placebo vs. low-dose and high-dose leptin) and within (before vs. after treatment) group differences. Tukey post hoc procedure was used if a significant main effect was found. A P value of ≤ 0.05 was considered statistically significant. Based on our own data evaluating the reproducibility of the effect of insulin on glucose and fatty acid kinetics during a euglycemic-hyperinsulinemic clamp in obese, insulin-resistant subjects (14), we estimated that six subjects in each group would allow us to detect a 25, 30, and 35% between-group difference in insulin-mediated effects of glucose R_a, palmitate R_a, and glucose R_d, respectively, at the 0.05 α-level and a β-value of 0.20 (i.e., 80% power).

RESULTS

Body composition and plasma leptin. Baseline body weight and body composition were similar between groups, and body weight and body composition did not change in any group after 2 weeks of treatment (Table 1). Plasma leptin concentrations were not different between groups before intervention. Plasma leptin remained unchanged in the placebo group and increased by ~threefold and ~150-fold, respectively, in the low-dose and high-dose leptin treatment groups (Table 1).

Substrate kinetics and insulin sensitivity. There were no differences between groups in basal plasma insulin concentrations, the hepatic insulin sensitivity index, and basal substrate kinetics before and after placebo or leptin treatment (Table 2). Plasma insulin concentrations and substrate kinetics during the clamp procedure were also not different between groups before and after placebo or either dose of leptin treatment (Table 2). Plasma insulin concentration increased from ~17 mU/L during basal conditions to ~35 mU/L during stage 1 and to ~85 mU/L during stage 2 both before and after 14 days of therapy. Insulin-mediated suppression of endogenous glucose R_a (~60 and ~80% decrease during stages 1 and 2, respectively) and insulin-mediated increase in glucose R_d (~75% increase during stage 2) was not different between groups before and after placebo or either dose of leptin treatment. Palmitate and glycerol R_a decreased by ~50 and ~35%, respectively, during stage 1 of the clamp procedure and were not different between groups before and after placebo or either dose of leptin treatment.

DISCUSSION

Leptin is an important regulator of insulin action; both leptin deficiency and leptin resistance are associated with insulin-resistant glucose metabolism (4,12). Furthermore, leptin replacement improves insulin sensitivity in subjects with leptin deficiency (5–8). Part of the beneficial effect of leptin replacement therapy could be a result of reductions in body weight in both leptin-deficient rodents and rodents with high-fat diet–induced obesity (3,11,15). However, leptin therapy increases insulin sensitivity in the absence of a significant decrease in body weight or causes a greater reduction in blood glucose concentration after leptin-induced weight loss than after weight loss induced by pair-feeding alone in wild-type and leptin-deficient rodent models (1,2,16) and improves insulin sensitivity in leptin-deficient subjects, even in the absence of significant changes in body weight (7,8,17). In contrast, our data demonstrate that increasing leptin availability above normal plasma concentrations by treatment with r-Met hu leptin does not have weight-loss–independent, clinically important effects on insulin sensitivity in obese people with type 2 diabetes. These results are consistent with data from previous leptin weight loss trials, which found that 8–12 weeks of leptin therapy and a low-calorie diet did not cause a greater change in plasma glucose or insulin concentrations than placebo therapy and a low-calorie diet (18,19).

The reason(s) for the discrepancy in results from our study and those conducted in leptin-deficient subjects and rodent obesity models is unclear. We studied only subjects

TABLE 1
Body composition and plasma leptin concentrations before and after placebo and leptin treatment

	Placebo		Leptin			
			Low dose (30 mg/day)		High dose (80 mg/day)	
	Before	After	Before	After	Before	After
BMI (kg/m^2)	36 ± 1	36 ± 1	35 ± 1	35 ± 1	36 ± 1	36 ± 1
Total body mass (kg)	108 ± 4	108 ± 4	93 ± 6	92 ± 6	106 ± 5	105 ± 5
Fat-free mass (kg)	64 ± 4	64 ± 4	58 ± 6	57 ± 6	64 ± 5	64 ± 5
Fat mass (kg)	44 ± 3	44 ± 3	35 ± 3	36 ± 3	42 ± 4	42 ± 4
Fat mass (% total body)	41 ± 3	41 ± 3	38 ± 3	39 ± 3	40 ± 4	40 ± 4
Plasma leptin (μg/L)	27 ± 7	25 ± 5	24 ± 8	76 ± 19*	35 ± 10	5,024 ± 500†

Values are means ± SE. *Value significantly different from corresponding value before, $P \leq 0.05$. †Value significantly different from corresponding value before, $P < 0.001$.

TABLE 2
Plasma insulin concentrations and metabolic kinetics during basal conditions and during the hyperinsulinemic-euglycemic clamp before and after placebo and leptin treatment

	Placebo		Leptin			
			Low dose (30 mg/day)		High dose (80 mg/day)	
	Before	After	Before	After	Before	After
Insulin (mU/L)						
Basal	19.9 ± 3.5	17.9 ± 2.9	15.1 ± 1.6	15.0 ± 0.9	17.6 ± 3.3	17.3 ± 4.2
Stage 1	37.6 ± 3.3	41.4 ± 2.0	35.9 ± 4.5	34.5 ± 2.8	33.8 ± 3.1	34.5 ± 3.2
Stage 2	91.7 ± 3.7	92.9 ± 4.0	86.1 ± 14.2	77.3 ± 5.3	81.8 ± 4.4	79.7 ± 6.5
Hepatic insulin sensitivity index	301 ± 41	270 ± 37	259 ± 21	260 ± 18	305 ± 61	298 ± 82
Endogenous glucose R_a (μmol/kg body wt/min)						
Basal	9.3 ± 0.4	9.1 ± 0.3	10.8 ± 0.6	10.6 ± 0.7	10.2 ± 0.5	9.9 ± 0.4
Stage 1	3.8 ± 0.9	2.8 ± 0.8	5.1 ± 0.8	4.6 ± 0.7	5.1 ± 0.6	4.4 ± 0.5
Stage 2	1.5 ± 0.6	1.3 ± 0.4	2.8 ± 0.5	2.5 ± 0.4	2.4 ± 0.9	2.2 ± 0.6
Glucose R_d (μmol/kg body wt/min)						
Stage 1	8.5 ± 1.3	9.6 ± 0.7	10.1 ± 1.5	12.2 ± 0.9	7.9 ± 1.2	8.6 ± 1.6
Stage 2	14.3 ± 3.1	17.5 ± 2.5	18.4 ± 3.6	20.7 ± 3.0	16.7 ± 2.4	19.1 ± 3.3
Palmitate R_a (μmol/kg body wt/min)						
Basal	1.3 ± 0.1	1.2 ± 0.1	1.5 ± 0.1	1.4 ± 0.2	1.3 ± 0.1	1.4 ± 0.1
Stage 1	0.8 ± 0.1	0.6 ± 0.1	0.7 ± 0.2	0.7 ± 0.2	0.8 ± 0.1	0.7 ± 0.1
Glycerol R_a (μmol/kg body wt/min)						
Basal	2.8 ± 0.2	2.6 ± 0.2	3.2 ± 0.3	2.9 ± 0.4	2.6 ± 0.3	2.8 ± 0.3
Stage 1	1.9 ± 0.2	1.6 ± 0.2	2.0 ± 0.3	1.9 ± 0.3	2.1 ± 0.3	1.8 ± 0.3

Values are means ± SE. Basal, stage 1, and stage 2 refer to the hyperinsulinemic-euglycemic clamp as described in the text. No group × time (before vs. after) interactions are shown.

with newly diagnosed, and presumably more reversible, type 2 diabetes and used sensitive measures to evaluate insulin sensitivity in vivo to increase our ability to detect an effect of leptin therapy on insulin action. Furthermore, we gave low and high doses of r-Met hu leptin to our subjects to ensure adequate plasma leptin concentrations were achieved and to evaluate potential dose-dependent effects. It is unlikely that the 2-week intervention in our study was not long enough to affect insulin action, because leptin administration alters glucose metabolism within several hours in rodents (11) and the beneficial metabolic effects of leptin replacement therapy in subjects with leptin deficiency occur within 1 week of treatment (20). In addition, current treatment strategies in obese insulin-resistant subjects, such as weight loss and pharmacotherapy, demonstrate improved insulin sensitivity can occur within days (13,21,22). Therefore, our data demonstrate that leptin treatment has different metabolic effects in subjects with leptin deficiency (who have almost no body fat) than in obese subjects (who have high plasma leptin concentrations and large amounts of body fat). However, we cannot exclude the possibility that leptin treatment would affect insulin action in overweight or obese subjects who have a lower BMI than our cohort.

In addition, our results demonstrate that leptin does not worsen insulin sensitivity, which has been suggested because of data obtained from studies conducted in obese people and isolated adipocytes. At any given BMI, increased plasma leptin concentration is associated with greater insulin resistance (9,11), and withdrawal of chronic leptin therapy in leptin-deficient subjects improves insulin sensitivity (23). Furthermore, large doses of leptin decrease insulin signaling and metabolic actions of insulin in isolated rat adipocytes (24,25). In our study, however, even remarkably high plasma leptin concentrations did not cause insulin resistance.

Although our study was conducted in a small number of subjects, it is unlikely we missed an important therapeutic effect of leptin as a result of inadequate statistical power, because there was not even a trend in leptin-induced changes in substrate kinetics with either dose of leptin therapy compared with placebo. In contrast, current treatment strategies for insulin resistance, such as weight loss and pharmacotherapy, improve insulin-mediated glucose uptake by ~25% or more (13,26,27).

In summary, we found that short-term treatment with either low-dose or high-dose r-Met hu leptin did not improve liver, skeletal muscle, or adipose tissue insulin sensitivity in weight stable, obese subjects with type 2 diabetes. The absence of a therapeutic effect of leptin in our study, within the context of the observed beneficial effects of leptin replacement therapy in subjects with leptin deficiency (4,7,8,12), suggests that a small amount of leptin is important for normal insulin action, but increasing leptin availability above normal plasma concentrations does not have weight loss–independent effects on insulin action.

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B.M. and J.F.H. were involved in conducting the infusion studies, processing the study samples, collecting data, performing the final data analyses, and writing the manuscript. A.M.D. and M.A.M. were involved in designing the study and writing the manuscript. B.W.P. was involved in sample processing and analyses. S.K. was involved in designing and conducting the infusion studies, processing

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