Acute and Chronic Effect of Insulin on Leptin Production in Humans

Studies In Vivo and In Vitro

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This study was undertaken to investigate the changes in obesity (*OB*) gene expression and production of leptin in response to insulin in vitro and in vivo under euglycemic and hyperglycemic conditions in humans. Three protocols were used: *1*) euglycemic clamp with insulin infusion rates at 40, 120, 300, and 1,200 mU · m^{-2} · min^{-1} carried out for up to 5 h performed in 16 normal lean individuals, 30 obese individuals, and 31 patients with NIDDM; *2*) 64- to 72-h hyperglycemic (glucose 12.6 mmol/l) clamp performed on 5 lean individuals; *3*) long-term (96-h) primary culture of isolated abdominal adipocytes in the presence and absence of 100 nmol/l insulin. Short-term hyperinsulinemia in the range of 80 to >10,000 μU/ml had no effect on circulating levels of leptin. During the prolonged hyperglycemic clamp, a rise in leptin was observed during the last 24 h of the study ($P < 0.001$). In the presence of insulin in vitro, *OB* gene expression increased at 72 h ($P < 0.01$), followed by an increase in leptin released to the medium ($P < 0.001$). In summary, insulin does not stimulate leptin production acutely; however, a long-term effect of insulin on leptin production could be demonstrated both in vivo and in vitro. These data suggest that insulin regulates *OB* gene expression and leptin production indirectly, probably through its trophic effect on adipocytes. *Diabetes* 45:699–701, 1996

The obesity (*ob*) gene and its protein product, leptin, have recently been discovered in mice (1,2). Mutations in the *ob* gene are the cause of increased food intake, reduced energy expenditure, and obesity in the *ob/ob* mouse (2). We reported the complete coding sequence of the human obesity (*OB*) gene and found that no such mutations were present in human obesity (3). Furthermore, we developed a radioimmunoassay for human leptin and found that its circulating levels in a cohort of obese patients are several-fold higher than in lean individuals (4). Most importantly, observations suggesting a direct relationship between leptin and insulin were found in these patients (4). Specifically, changes in insulin levels paralleled those in leptin during weight loss (4), fasting, and overfeeding (J.W. Kolaczynski, J.F. Caro, unpublished observations). However, the relationship between insulin and leptin appeared to be complex. There was no postprandial rise in serum leptin (5), and leptin increased rather than decreased during the night when insulin was at nadir (5). Both in vivo and in vitro studies in rodents have also yielded conflicting results. Acute administration of insulin (hours) in vivo or in vitro increased *ob* mRNA in rodents (6). Prolonged administration of insulin (days) had a similar effect (7). In contrast, a direct effect of insulin on *ob* mRNA could not be demonstrated in isolated rat adipocytes (8) or fully differentiated 3T3-L1 adipocytes (9). It should be recognized, however, that only *ob* mRNA, not leptin, was investigated in the previous studies (6–9). We report here the acute and prolonged effect of insulin on leptin production studied in vivo and in vitro in humans.

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FBG, fasting blood glucose; FI, fasting insulin.

RESEARCH DESIGN AND METHODS

Three different protocols were used: *1*) Euglycemic clamp studies involved 77 subjects (69 men, 8 women), of whom 16 were healthy lean individuals (BMI 23.8 ± 0.6 kg/m^2, age 38.7 ± 1.8 years, fasting blood glucose [FBG] 95.3 ± 1.5 mg/dl, fasting insulin [FI] 5.7 ± 0.8 μU/ml), 30 were normal obese individuals (BMI 33.1 ± 0.6 kg/m^2, age 43.1 ± 1.8 years, FBG 96.0 ± 2.1 mg/dl, FI 11.7 ± 1.6 μU/ml), and 31 were obese NIDDM patients (BMI 32.8 ± 0.6 kg/m^2, age 52.9 ± 1.5 years, FBG 201.8 ± 11.7 mg/dl, FI 18.1 ± 2.2 μU/ml). The healthy and obese group was free of clinically or biochemically apparent disease and did not take any medications known to affect glucose tolerance and/or insulin secretion. The participants underwent clamp studies (10) with primed constant insulin infusions at 40, 60, 120, 300, and 1,200 mU/m^2 body surface area, carried out for 120–300 min. *2*) Prolonged hyperglycemic clamp studies involved five healthy volunteers (four men, one woman, age 34.0 ± 2.0 years, BMI 24.9 ± 0.9 kg/m^2) who were infused with 20% dextrose in water beginning at 0800 after overnight fast. The rate of dextrose infusion was adjusted to maintain hyperglycemia at 12.6 mmol/l, with the intention of continuing the infusion for 64–72 h. *3*) For in vitro studies, subcutaneous abdominal adipose tissue samples (10–30 g) were obtained from individuals undergoing elective surgery or needle biopsy as previously reported (11). The tissue was immediately transported to the laboratory in saline and digested with collagenase, and the isolated adipocytes were then cultured for up to 96 h as described previously (11). The viability of the cultured cells was assessed by basal and insulin-simulated transport assay (11). No significant cell breakdown was noted as assessed by measurement of initial and final lipocrit of the cultured adipocytes. *OB* mRNA was measured in adipocytes by reverse transcription–polymerase chain reaction as previously reported (3). Leptin was measured in culture media and the serum by radioimmunoassay as reported previously (4). The results are presented as individual

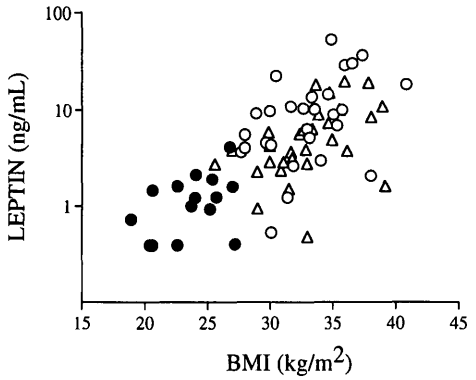

FIG. 1. Basal serum leptin concentration as a function of BMI in 77 normal lean (●) and obese (○) subjects and NIDDM patients (△).

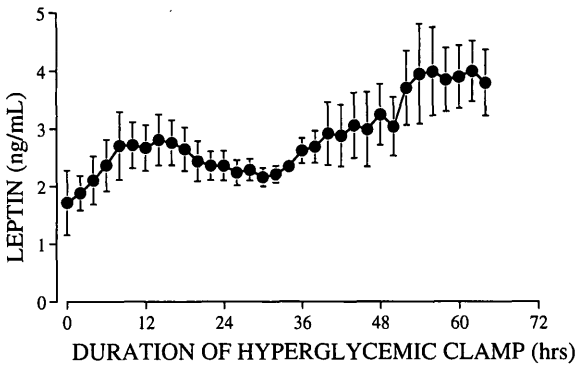

FIG. 3. Response of leptin to hyperinsulinemia under hyperglycemic clamp conditions carried out for up to 72 h.

values, means ± SE. The statistical analysis was performed with analysis of variance and Student's *t* test where applicable.

RESULTS

Figure 1 illustrates the direct relationship between basal leptin and BMI in the study patients ($r = 0.681$, $P < 0.001$); a similar relationship was previously reported in a larger cohort (4). Figure 2 summarizes responses of serum leptin levels to different doses of insulin infusions over the range from 40 to 1,200 mU · m^{-2} · min^{-1} (steady-state insulinemia from 73.9 ± 5.4 to 12,007.0 ± 841.8 μU/ml) maintained for up to 300 min. There was neither a distinction in the leptin responses among lean, obese, and NIDDM patients (NS) nor an insulin dose-response effect at any of the sampling times (NS). In few individuals, both downward and upward regulation of serum leptin concentration was noted, suggestive of independent leptin oscillations. To examine this phenomenon, three euglycemic clamp studies (insulin infusion rate 300 mU · m^{-2} · min^{-1}) were carried out for 4 h with frequent (every 5–10 min) determination of leptin. Oscillations in the hormone concentration with over a twofold difference between nadir and maximal values could be observed throughout the course of the experiment, but without any consistent insulin-induced increase (data not shown). The long-term in vivo effect of insulin is shown in Fig. 3. During the last 24-h period of the hyperglycemic clamp, the mean serum leptin concentration was considerably higher than that in the first or second 24-h period (2.5 ± 0.1 and 2.49 ± 0.1 vs. 3.73 ± 0.1 ng/ml, $P < 0.001$). There was no difference in mean serum insulin concentrations in each 24-h period (172.0 ± 19.1 vs. 169.0 ± 15.0 vs. 137.3 ± 9.2 μU/ml, respectively).

As shown in Fig. 4, exposure to 100 nmol/l insulin in vitro for up to 72 h had no effect on leptin release into the medium. However, a twofold increase ($P < 0.005$) in leptin release to the medium was observed by 96th hour of the culture. This increase in leptin release was preceded by a 200% insulin-induced increase ($P < 0.01$) in *OB* gene expression, which returned to baseline 24 h later.

DISCUSSION

The present study documents that insulin infusions creating hyperinsulinemia in the range of 75 to >10,000 μU/ml under euglycemic conditions for up to 5 h did not produce noticeable changes in the circulating leptin levels. There was no difference in the response of insulin-sensitive (lean) or insulin-resistant (obese) individuals or patients with NIDDM. On the other hand, a rise in serum leptin concentrations during the final 24 h of the 72-h hyperglycemic clamp was observed. Likewise, in in vitro experiments, the presence of 100 nmol/l insulin resulted in an increase in mRNA at 72 h, followed by a sharp increase in leptin release to the medium. These data thus show that some (7–9) but not all (6) findings in rodents can be observed in humans both in vivo and in vitro. Several explanations can be offered to reconcile the differences. First, the stimulatory effect of insulin on leptin production in humans is more inert. Second, the incorpora-

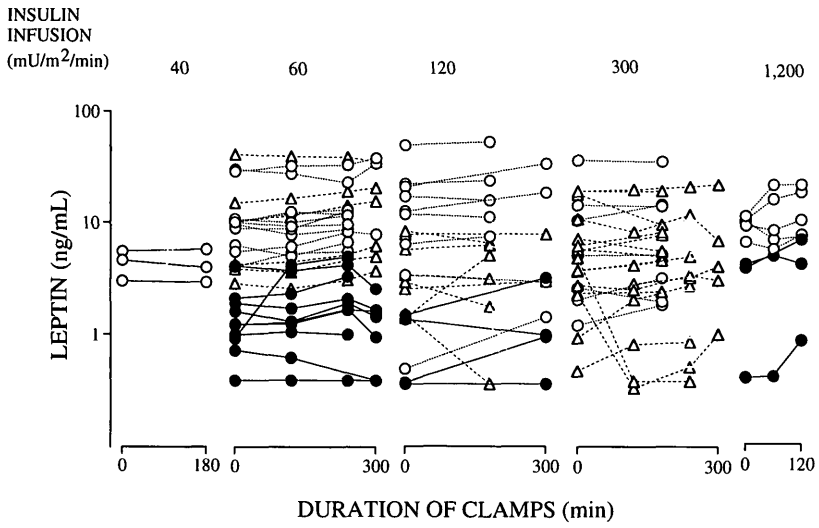

FIG. 2. Responses of leptin to short-term hyperinsulinemia under clamp conditions in lean (●) and obese (○) subjects and NIDDM patients (△).

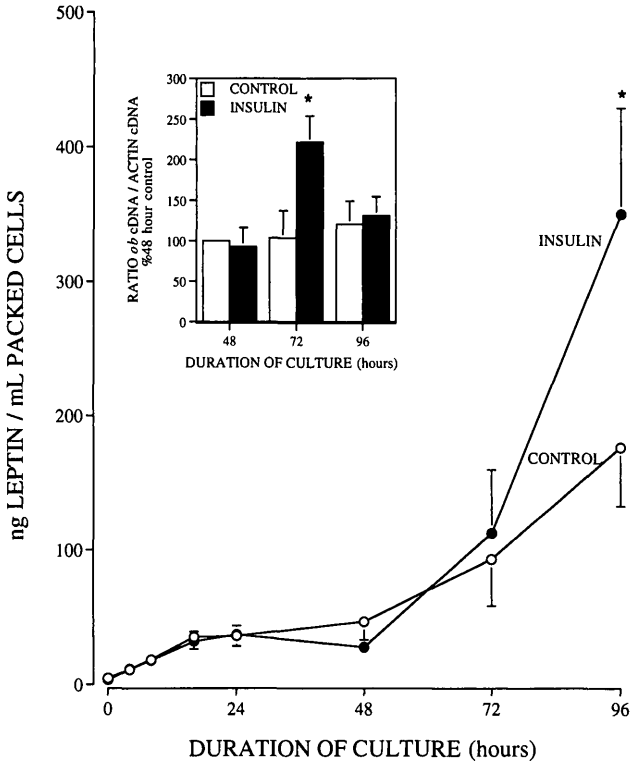

FIG. 4. Effect of hyperinsulinemia (100 nmol/l) on *OB* gene expression (inset; means ± SE, $n = 4$) and leptin release to the medium (means ± SE, $n = 3$ for 0- to 16-h and $n = 6$ for 24- to 96-h time points) in isolated human adipocytes in primary culture.

tion of glucose into adipose tissue triglycerides is a much more active process in rodents than in humans (12). Third, a difference between adipose tissue depots in leptin response to insulin may exist in rodents and humans (13). However, since in humans insulin action on leptin production was a long-term effect, the data do not clearly support a direct effect of insulin on *OB* gene expression. Rather, the results favor a concept that the observed effects are the consequence of the trophic effect of insulin on the adipocytes. In this context, it is important to note that both a 72-h hyperglycemic clamp and primary culture of adipocytes in the presence of insulin are de facto experimental models of massive overfeeding.

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