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PRELIMINARY REPORT

Absence of Short-Term Effects of Glucagon-Like Peptide-1 and of Hyperglycemia on Plasma Leptin Levels in Man

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In rodents, leptin and the incretin glucagon-like peptide-1 (7-36) amide (GLP-1) affect feeding at least in part via interaction with hypothalamic neuropeptide Y (NPY), suggesting that cross talk may exist between GLP-1 and the *ob* gene product. Besides insulin, acute hyperglycemia has recently been shown to induce *ob* gene expression. To address the question of whether leptin plasma levels in humans are affected by GLP-1 infusion and/or hyperglycemia, eight healthy volunteers were studied during euglycemia and hyperglycemic clamping with or without GLP-1 administration while insulin levels were kept constant by somatostatin infusion. Under all conditions, leptin plasma levels remained unchanged, demonstrating that in humans leptin plasma concentrations are affected neither by short-term peripheral GLP-1 infusion nor by hyperglycemia, which suggests that postprandial GLP-1 release and hyperglycemia do not modulate secretion of the *ob* gene product.

LEPTIN, the *ob* gene product, is produced by adipocytes and is found in the serum of normal mice and rats and in humans. In mice, it has been shown to reduce body weight by increasing energy expenditure and decreasing food intake,[1] most likely via central inhibition of the expression of hypothalamic neuropeptide Y (NPY),[2] a known appetite stimulant.

The glucose-dependent, insulinotropic incretin glucagon-like peptide-1 (7-36) amide (GLP-1) is secreted postprandially by intestinal L-cells[3] and plays a crucial role in glucose homeostasis.[4] Despite the fact that mice with a null mutation in the GLP-1 receptor do not develop a pathological feeding behavior (probably due to genetic redundancy), GLP-1 has recently been found to act as a potent satiety factor when administered intracerebroventricularly to fasted mice[4] and to rats.[5] In rats, the GLP-1 receptor agonist, exendin-4, decreased food intake,[6] whereas the GLP-1 antagonist, exendin (9-39), showed synergistic effects on NPY-induced feeding,[5] suggesting that GLP-1 may interact with NPY and with leptin. Interestingly, both leptin[7] and rat and human GLP-1 receptors[6,8,9] are found in the hypothalamus. However, the potential interaction of GLP-1 and leptin is not necessarily at the central level. GLP-1 could also augment leptin plasma levels indirectly via induction of pancreatic secretion of insulin, a known stimulus for leptin.[10,11] Even though the presence of specific GLP-1 receptors on adipocytes is controversial,[8,12] GLP-1 has been shown to have peripheral extrapancreatic effects, including adipose tissue.[13,14] It is therefore tempting to speculate that the postprandial increase of circulating GLP-1 levels would stimulate adipose tissue to secrete more leptin and thereby confer a feedback loop regulating satiety or energy balance. Despite the fact that peripheral GLP-1 administration did not affect feeding behavior in rats,[5,6] systemic GLP-1 significantly reduced body weight gain in a dose-dependent manner,[6] which would be consistent with the increased energy expenditure caused by leptin.

In lean mice, intraperitoneal glucose injection led within minutes to increased expression of the *ob* gene, which correlated with plasma glucose but not with plasma insulin concentrations,[10] suggesting that hyperglycemia by itself might have a stimulatory effect on leptin.

Leptin plasma levels have been shown to correlate with adipose mass and to be stimulated by insulin, whereas the effects of GLP-1 and of hyperglycemia have not been examined in man. The aims of the present study were to determine (1) whether systemic GLP-1 infusion increases leptin plasma concentrations in humans and (2) whether acute hyperglycemia is able to augment leptin plasma levels in man. Therefore, leptin plasma concentrations were measured in eight lean volunteers receiving GLP-1 once and saline once during hyperglycemic

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clamping while endogenous insulin secretion was blocked by somatostatin infusion (pancreatic clamping) to eliminate the confounding effects of hyperinsulinemia due to GLP-1 or glucose and to determine the insulin-independent effects of peripheral GLP-1 and glucose. In control experiments, leptin plasma levels were assessed while somatostatin was not infused, allowing GLP-1 and hyperglycemia to induce endogenous insulin secretion.

SUBJECTS AND METHODS

Eight healthy male volunteers aged 23 to 29 years and with a body mass index of 20 to 24 kg/m^2 provided written informed consent to participate. The study protocol was approved by the Human Ethics Committee, Department of Medicine, University of Basel, Switzerland. Every volunteer was studied twice after an overnight fast, once with GLP-1 and once with saline in randomized order. For infusions, a teflon cannula was inserted into an antecubital vein, and for blood withdrawal, a butterfly needle was inserted into a dorsal hand vein. After a 30-minute euglycemic baseline period, pancreatic clamping was started as described previously.[15] Thirty minutes later, an infusion of synthetic GLP-1 (1.2 pmol · kg^{-1} · min^{-1}, sterile and pyrogen-free, net peptide content 91.68%, peptide purity > 97%; Saxon Biochemicals, Hannover, Germany)[16] or placebo (NaCl 0.9% wt/vol) was started and continued for 180 minutes.[15] During the infusion period, hyperglycemia of 8 mmol/L was achieved and maintained by a glucose infusion (20% wt/vol) that was frequently adjusted depending on the actual plasma glucose level as measured by a Yellow Springs Glucose Analyzer (model 23 AM; Yellow Springs, OH). Plasma GLP-1 concentration was measured by a new radioimmunoassay specific for the amidated carboxyl terminus of GLP-1.[17] Plasma concentrations of insulin and leptin were measured by radioimmunoassays (INSIK-5 (P2796); Sorin Biomedica, Saluggia, Italy, and Linco Research, St Charles, MO, respectively). For statistical analyses, paired *t* tests and ANOVA with

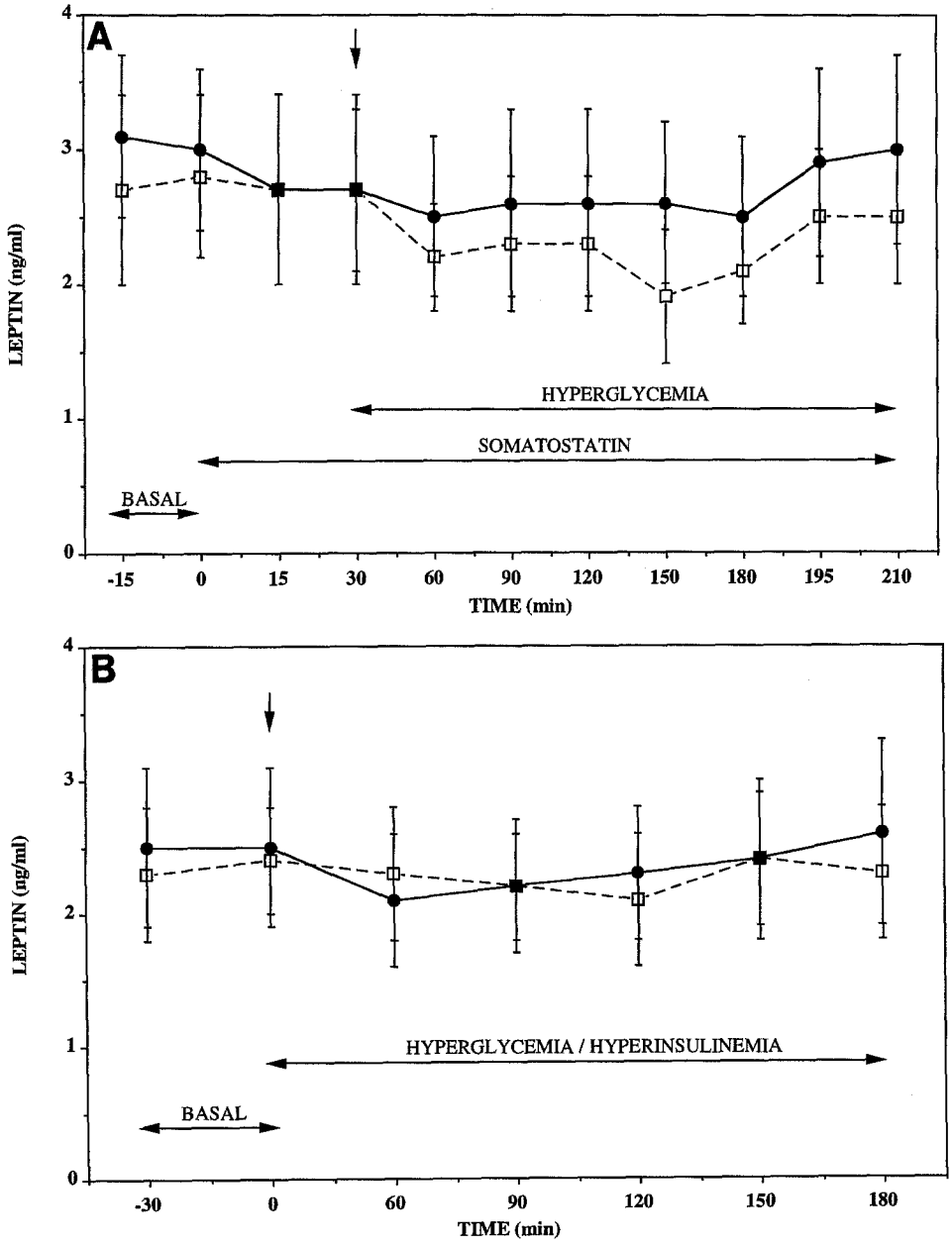

Fig 1. Leptin plasma concentrations during the euglycemic basal period and the 3-hour hyperglycemic infusion period without (saline, □) or with (●) GLP-1 infusion (A) with and (B) without somatostatin infusion. Results are the mean ± SEM; n = 8 subjects per protocol.

repeated measures were performed using STATVIEW software (Macintosh; Apple Computer, Switzerland).

RESULTS AND DISCUSSION

Leptin plasma concentrations did not change during systemic pharmacological GLP-1 infusion compared with saline (Fig 1A). Insulin plasma levels were kept constant throughout the experiments with GLP-1 and saline (94 ± 12 and 84 ± 7 pmol/L, respectively), and glucose plasma levels were elevated from basal 5.1 ± 0.1 to 8.0 ± 0.1 mmol/L during hyperglycemic clamping in both protocols. However, plasma concentrations of leptin were also not affected by hyperglycemia compared with the euglycemic basal period (Fig 1A). These data suggest that (1) acute peripheral GLP-1 infusion is not able to alter plasma leptin concentrations in humans and (2) isolated acute hyperglycemia does not affect plasma leptin levels in healthy man. Even in experiments without pancreatic clamping that were accompanied by a marked stimulation of endogenous insulin secretion due to GLP-1 and/or hyperglycemia (plasma insulin, 1,486 ± 145 and 185 ± 12 pmol/L, respectively),[15] no changes in plasma leptin levels could be observed during 3 hours (Fig 1B). These findings are in agreement with other studies in humans showing no effect of exogenous insulin administration for up to 48 hours.[11] In addition, these latter experiments with unblocked endogenous insulin secretion provide a control for the biological potency of synthetic GLP-1 administration. The dose of 1.2 pmol · kg^{-1} · min^{-1} has been shown[16] to increase plasma levels to pharmacological concentrations of 120 pmol/L and to increase insulin to similar levels as observed in the present experiments. Even though plasma GLP-1 concentrations increased only from a basal value of 12 ± 2 to 40 ± 8 pmol/L during GLP-1 infusion,[15] the discrepancy in measured GLP-1 levels seems to be due to the different radioimmunoassays used rather than to differences in circulating levels of active GLP-1.

Thus, in contrast to observations in rodents, acute hyperglycemia with and without hyperinsulinemia does not increase plasma leptin levels in humans. Three hours of peripheral GLP-1 infusion in man also has no effect on plasma leptin concentrations, whereas it remains to be determined whether long-term, or, as suggested by the rat model, central GLP-1 administration is effective. However, the present findings suggest that postprandial elevation of neither GLP-1 nor plasma glucose affects leptin plasma levels in man.

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