Leptin Does Not Directly Regulate the Pancreatic Hormones Amylin and Pancreatic Polypeptide

Interventional studies in humans

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OBJECTIVE — Leptin and the pancreatic hormones amylin and pancreatic polypeptide are being evaluated alone or in combination for the treatment of obesity, but their physiological regulation has not yet been fully elucidated. Thus, we examined whether amylin and pancreatic polypeptide are regulated by caloric intake and/or short- and long-term energy deprivation and whether any potential regulation is mediated by changes in leptin levels.

RESEARCH DESIGN AND METHODS — We measured circulating levels of amylin and pancreatic polypeptide after *1*) a 75-g glucose load in 28 healthy, normal-weight women, *2*) 72-h complete energy deficiency (severe hypoleptinemia) with administration of either placebo or replacement-dose recombinant methionyl human leptin (r-metHuLeptin) in normal-weight men ($n = 6$) and women ($n = 7$), and *3*) chronic mild energy deficiency (mild hypoleptinemia) in 7 women with hypothalamic amenorrhea before and after r-metHuLeptin administration for 3 months.

RESULTS — Amylin and pancreatic polypeptide levels increased 15 min after a 75-g glucose load and remained elevated at 60 and 120 min ($P < 0.0001$). Fasting for 72 h decreased leptin (to 21%) and amylin (to 67%) of baseline but not pancreatic polypeptide levels. Normalizing leptin levels with r-metHuLeptin did not alter the fasting-induced decrease in amylin and had no effect on pancreatic polypeptide levels. Neither amylin nor pancreatic polypeptide levels were different in leptin-deficient women with hypothalamic amenorrhea compared with weight-matched control subjects, and normalization of leptin levels with r-metHuLeptin treatment did not alter amylin or pancreatic polypeptide levels.

CONCLUSIONS — Circulating amylin levels increase after a glucose load and decrease in response to short-term complete fasting, but these changes are not mediated by leptin.

Diabetes Care **31:945–951, 2008**

The endocrine pancreas, in addition to producing insulin and glucagon, also secretes several other hormones important in energy homeostasis, including amylin and pancreatic polypeptide. Amylin, or islet amyloid polypeptide, is cosecreted with insulin from pancreatic β-cells and plays a complementary role to insulin in the regulation of glucose homeostasis (1). Pancreatic polypeptide is secreted from pancreatic F-cells and is also released in response to food intake (2). It has been proposed that both amylin and pancreatic polypeptide act as short-term satiety signals to decrease appetite and food intake, inhibit gastric emptying, and reduce gastric acid secretion (3,4).

Similar to long-term adiposity signals such as insulin and leptin, amylin and pancreatic polypeptide appear to play important roles in body weight regulation and energy homeostasis. In rats, amylin decreases food intake, body weight, and fat mass, whereas inhibition of amylin signaling has the opposite effect (5,6). Pramlintide, a Food and Drug Administration–approved synthetic amylin analog for the treatment of diabetes, induces weight loss in individuals with (7) and without diabetes (8). Transgenic mice overexpressing pancreatic polypeptide are leaner than controls (9), and chronic peripheral administration of pancreatic polypeptide to mice reduces body weight (10). Although observational studies of pancreatic polypeptide levels in humans are conflicting (11–14), intravenous infusion of pancreatic polypeptide in normal-weight subjects has been shown to reduce 24-h energy intake (15).

Because accumulating evidence suggests that amylin and pancreatic polypeptide may contribute to the regulation of body weight, understanding whether "cross-talk" exists between these molecules and other hormones important in energy homeostasis, such as leptin, has scientific importance and clinical relevance for the treatment of obesity. Of interest, leptin receptors have been identified on human pancreatic islet cells (16). In vitro, leptin suppresses insulin secretion from human islets (16) and reduces glucose-stimulated amylin secretion from mouse islets (17), suggesting the existence of an "adipoinsular axis" in which leptin can regulate the secretion of pancreatic hormones. In rats, amylin has a synergistic effect with leptin to induce weight loss (18), specifically causing greater loss of fat mass compared with pair-fed rats (19), and a recent clinical trial in humans involving administration

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Received for publication 21 December 2007 and accepted in revised form revised form 20 January 2008.

Published ahead of print at http://care.diabetesjournals.org on 5 February 2008. DOI: 10.2337/dc07-2433.

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Abbreviations: AUC, area under the curve; BIDMC, Beth Israel Deaconess Medical Center; GCRC, General Clinical Research Center; OGTT, oral glucose tolerance test; r-metHuLeptin, recombinant methionyl human leptin.

of amylin and leptin suggests a similar synergy (http://www.amylin.com). Even fewer data are available on the interaction between leptin and pancreatic polypeptide, but it has been shown that pancreatic polypeptide administration in leptin-deficient *ob*/*ob* mice decreases body weight (20).

Thus, we performed interventional studies in humans to evaluate whether circulating levels of amylin and pancreatic polypeptide are regulated by caloric intake and/or energy deprivation in a manner consistent with satiety signals and whether any potential regulation is mediated by leptin. We first measured the amylin and pancreatic polypeptide response to a 75-g oral glucose load in healthy, normal-weight individuals. We then evaluated whether complete energy deprivation alone (fasting-induced hypoleptinemic state) and/or fasting with administration of recombinant methionyl human leptin (r-metHuLeptin) to normalize the fasting-induced hypoleptinemia would alter amylin and/or pancreatic polypeptide levels in healthy, normal-weight subjects. Finally, we tested whether amylin and pancreatic polypeptide levels are different in women with hypothalamic amenorrhea, who have a mild chronic energy deficit resulting in relative leptin deficiency, compared with weight-matched control subjects, and whether r-met-HuLeptin administration for up to 3 months to normalize circulating leptin levels would alter circulating amylin and pancreatic polypeptide levels.

RESEARCH DESIGN AND METHODS

— The oral glucose tolerance test (OGTT) study was carried out at Harokopio University, Athens, Greece, in accordance with the Declaration of Helsinki and was approved by the university's ethics committee. Informed consent was obtained from participants. The short-term and chronic energy deficit study protocols were approved by the institutional review board of the Beth Israel Deaconess Medical Center (BIDMC). Clinical quality r-metHuLeptin was supplied by Amgen (Thousand Oaks, CA) and administered under an investigational new drug application submitted to the Food and Drug Administration by the investigators.

OGTT Study

Twenty-eight women from the area of Athens and Piraeus, Greece, were evaluated as part of a larger study to examine insulin sensitivity in offspring of patients with type 2 diabetes. Inclusion criteria included age of 20–45 years; nonsmoker; no history of hypertension, endocrine, or metabolic diseases; sedentary lifestyle; fasting glucose <126 mg/dl; BMI <27 kg/m^2; stable body weight ≥6 months before the study; not pregnant; and not taking medications (including birth control or hormone replacement therapy). Subjects abstained from alcohol or structured exercise for 24 h before the study. After a 12-h overnight fast and collection of a fasting blood sample, subjects ingested a solution containing 75 g anhydrous glucose, and blood samples were obtained at 15, 30, 60, 90, and 120 min for glucose, insulin, amylin (except in two subjects), and pancreatic polypeptide. Area under the curve (AUC) was calculated.

Short-term energy deprivation study

Eight men (mean ± SEM age 23.3 ± 1.2 years) and seven women (age 23.7 ± 1.5 years) with BMI <25 kg/m^2 were studied during three separate admissions in the BIDMC General Clinical Research Center (GCRC) as part of a larger study to evaluate the role of leptin in the neuroendocrine and immune response to fasting (21,22), baseline fed condition, 72-h fasting with administration of placebo, and 72-h fasting with administration of replacement dose r-metHuLeptin designed to normalize the fasting-induced decline in leptin levels. The same subjects participated in all three admissions except for two men for the r-metHuLeptin condition, and thus data for only six men are presented (age 23.5 ± 1.5 years). One woman did not complete the placebo condition. Each admission was separated by at least 7 weeks to permit recovery of hematocrit, leptin levels, and weight to baseline. Women had regular menstrual cycles and had not taken oral contraceptives for at least 6 months before the study. Subjects were admitted to the GCRC the night before day 1. During each study in the fed or fasting state, blood samples were obtained at 8 A.M. on day 1 (men and women) and at 8 A.M. on day 3 (men) or 4 (women) for measurement of leptin, amylin, pancreatic polypeptide, and insulin levels. During the baseline fed condition, subjects received a standardized isocaloric diet: 20% calories from breakfast (8 A.M.), 35% from lunch (2 P.M.), 35% from dinner (6 P.M.), and 10% from a snack (10 P.M.). During both fasting studies, subjects received only calorie-free liquids for 3 days and NaCl (500 mg), KCl (40 mEq), and a standard multivitamin with minerals daily. r-metHuLeptin was administered at a dose of 0.04 (men) or 0.08 (women) mg · kg^{-1} · day^{-1} on the first day and 0.1 (men) or 0.2 (women) mg · kg^{-1} · day^{-1} on the second and third days. The total daily r-metHuLeptin dose for each day was divided into four equal doses given every 6 h by subcutaneous injection. Placebo (a buffer solution) was administered according to the same schedule as r-metHuLeptin.

Chronic energy deprivation study

Seven normal-weight women (age 25.0 ± 2.2 years) with chronic energy deficit, hypothalamic amenorrhea for at least 6 months related to strenuous exercise or low weight, and relative leptin deficiency (baseline leptin level <4 ng/ml) who completed at least 2 months of treatment were evaluated as part of a larger study on the effects of r-metHuLeptin on neuroendocrine function (23). Subjects self-administered r-metHuLeptin (0.08 mg · kg^{-1} · day^{-1} for 2 months and then 0.2 mg · kg^{-1} · day^{-1} for the third month) subcutaneously twice daily with 40% of the daily dose at 8 A.M. and 60% at 8 P.M. to mimic the normal diurnal variation of leptin levels. Blood samples for measurement of leptin, amylin, and pancreatic polypeptide were obtained at an initial screening visit 1 month before the study and after 1, 3, 7, and 11 weeks of r-metHuLeptin treatment. Two subjects completed the study at 8 weeks because they achieved the primary outcome of ovulation; thus, these two subjects did not have data collected at 11 weeks and data for only five subjects was available at the 11th week. The six women from the short-term energy deficit study were used as control subjects.

Hormone measurements

All samples used were stored at −80 C. We evaluated whether freeze-thaw cycles would significantly degrade amylin and pancreatic polypeptide levels in test human plasma. There were no changes in amylin or pancreatic polypeptide with up to four freeze-thaw cycles (amylin: 14.6, 15.6, 15.5, and 16.5 pmol/l after one to four freeze-thaw cycles, respectively; pancreatic polypeptide: 101.7, 96.8, 101.3, and 104.8 pg/ml after one to four freeze-thaw cycles, respectively). Amylin and pancreatic polypeptide levels were measured by enzyme linked immunosorbent assay (Millipore, Billerica, MA) with sensitivities of 3.9 pg/ml (1 pmol/l) and 12.3

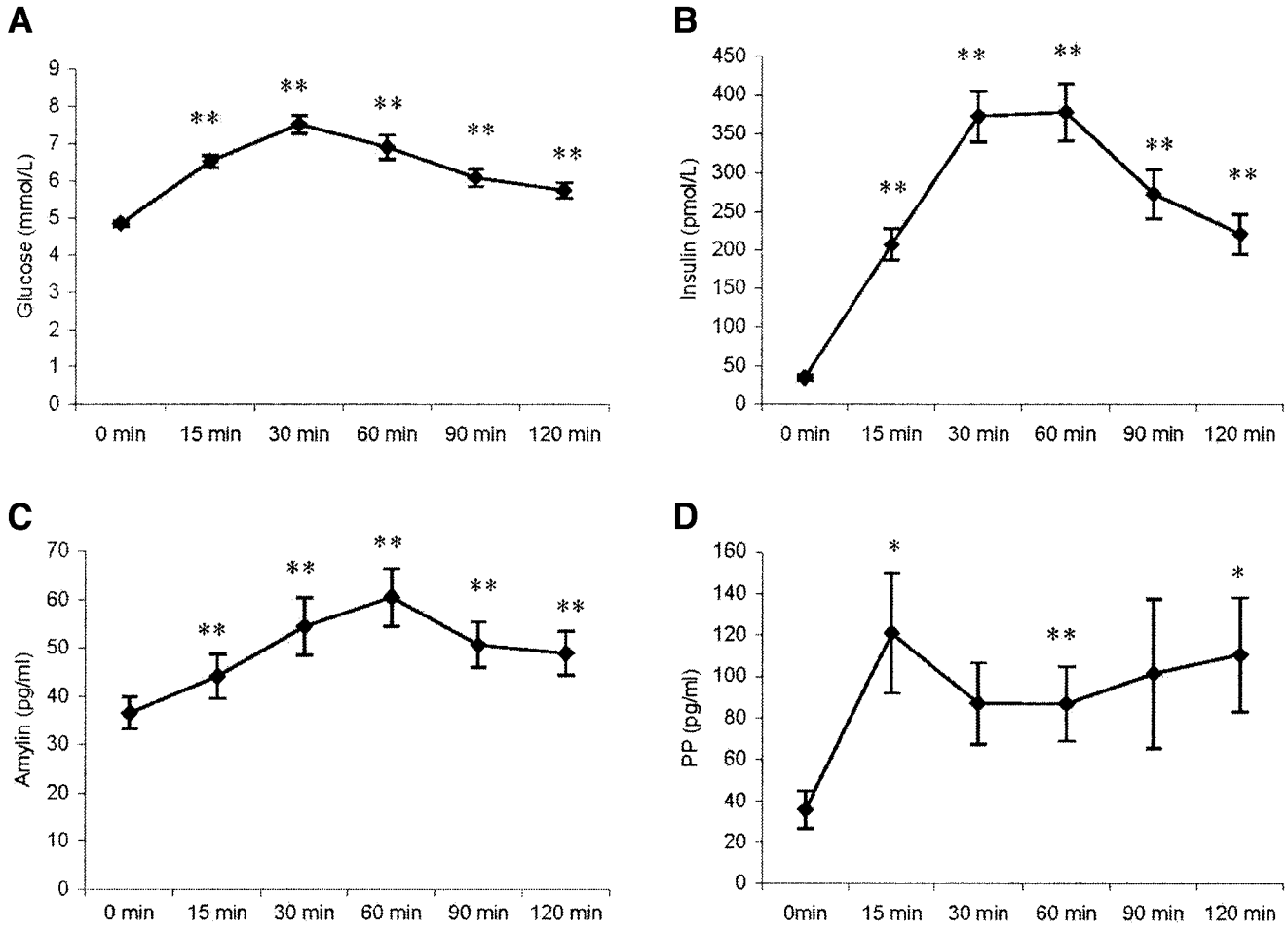

Figure 1—*Glucose* (A)*, insulin* (B)*, amylin* (C)*, and pancreatic polypeptide (PP)* (D) *levels after a 75-g oral glucose load* (n = *28 normal-weight women*). *P < *0.05*, **P < *0.01 vs. baseline, by repeated-measures ANOVA with post hoc tests.*

pg/ml, respectively. Leptin levels were measured by radioimmunoassay (Millipore) with a sensitivity of 0.5 ng/ml. Glucose was determined by the enzymatic calorimetric method (Roche Diagnostics [Mannheim, Germany] and Randox Laboratories [County Antrim, Ireland]). Insulin was measured by immunoradiometric assay using a radiolabeled mouse monoclonal anti-insulin and solid-phase guinea pig anti-insulin (supplied by the Scottish Antibody Production Unit). All samples were run in duplicate with quality controls, and inter- and intra-assay coefficients of variation were <10%.

Statistical analysis

Data are expressed as means ± SEM. Statistical analyses were performed by using SPSS 11.5 (SPSS, Chicago, IL). For the OGTT study, differences in hormone levels were analyzed across time points and between groups using repeated-measures ANOVA with Bonferroni adjustments in post hoc tests. AUCs of amylin and pancreatic polypeptide between relatives and control subjects and between lower and higher BMI groups was assessed using the Mann-Whitney *U* test. For the short-term energy deficit study, nonparametric Wilcoxon rank sum and paired *t* tests were used to assess changes in hormone levels for each condition, with similar results obtained except where noted. To determine whether changes in hormone levels varied between conditions, we compared mean final to initial day differences using one-way ANOVA and a Kruskal-Wallis test, with Wilcoxon rank-sum and pairwise *t* tests for post hoc analysis and Bonferroni-corrected *P* value = 0.017 to adjust for multiple comparisons. For the chronic energy deficit study, the Mann-Whitney *U* test was used to compare subjects with hypothalamic amenorrhea with control subjects, and changes in hormone levels were analyzed using a mixed-effects model repeated-measures ANOVA with Bonferroni adjustment for post hoc tests.

RESULTS

Amylin and pancreatic polypeptide levels increase in response to oral glucose load and remain elevated for at least 120 min

We first characterized the amylin and pancreatic polypeptide response to a 75-g glucose load in 28 healthy women (age 30.5 ± 1.1 years; BMI 22.4 ± 0.4 kg/m^2) (Fig. 1). Glucose levels increased significantly at 15 min, peaked to 1.5-fold of baseline at 30 min, declined after 60 min, but remained significantly higher than baseline at 120 min (overall $P < 0.0001$). Insulin levels peaked to 10-fold of baseline at 30 min and declined after 60 min but still remained higher than baseline after 120 min (overall $P < 0.0001$). Amylin levels increased significantly at 15 min, peaked to nearly 1.5-fold of baseline after 60 min, and then plateaued and remained higher than baseline at 120 min (overall $P < 0.0001$). Pancreatic polypeptide levels increased fourfold at 15 min ($P <$

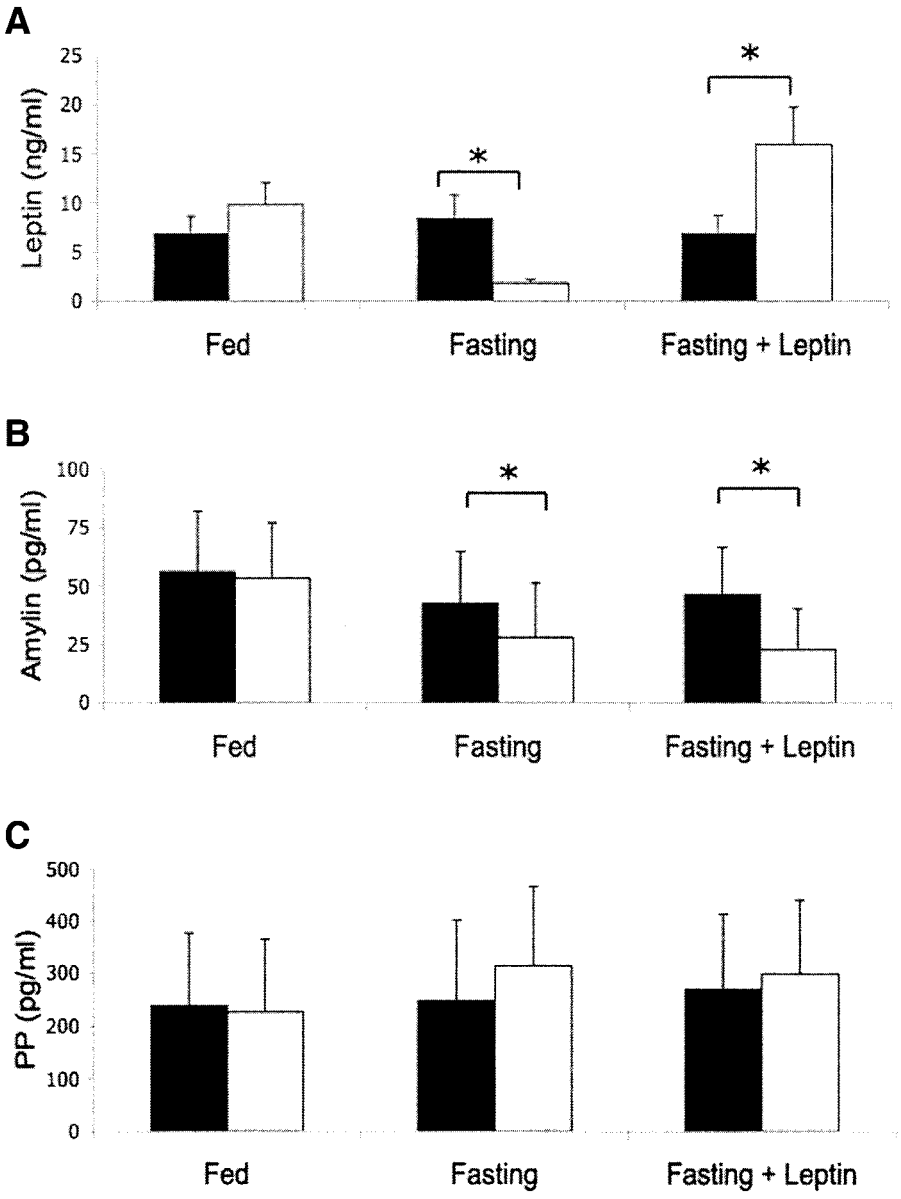

Figure 2—*Levels of leptin (*A*), amylin (*B*), and pancreatic polypeptide (PP) (*C*) at the beginning (day 1) and end (day 3 or 4) of a baseline fed condition, 72-h complete fasting with administration of placebo, and 72-h complete fasting with administration of r-metHuLeptin* (n = *6 normal-weight men and 7 normal-weight women*). ■, *day 1;* □, *final day.* *P < *0.0167.*

0.05), and levels remained significantly elevated above baseline at 60 and 120 min (overall $P = 0.02$).

Twelve subjects had at least one parent with type 2 diabetes. There was a tendency for those with a family history of diabetes to have a higher amylin AUC compared with those without a family history ($P = 0.04$ by Wilcoxon, $P = 0.12$ by *t* test), but pancreatic polypeptide AUC did not differ on the basis of family history ($P = 0.75$). When subjects were divided according to the median BMI value into lower and higher BMI subgroups (20.7 ± 0.3 vs. 24.1 ± 0.4 kg/m^2, $P = 0.001$), there was no significant difference in amylin and pancreatic polypeptide AUCs between subgroups (data not shown).

Short-term fasting significantly decreases amylin levels independently of leptin but has no effect on pancreatic polypeptide levels

We then evaluated the effects of fasting on amylin and pancreatic polypeptide levels and whether potential fasting-induced changes are mediated by leptin in normal-weight subjects (BMI 23.5 ± 0.4 kg/m^2 [men] and 21.7 ± 0.8 kg/m^2 [women]). During the baseline fed condition, amylin (56.0 ± 25.8 vs. 53.2 ± 23.8 pg/ml, $P = 0.29$) and pancreatic polypeptide (238.0 ± 138.7 vs. 226.0 ± 138.8 pg/ml, $P = 0.46$) levels remained stable (Fig. 2). There was no difference across the first day of all three conditions by ANOVA, indicating that parameters had returned to baseline between interventions.

After a 72-h complete fast, leptin levels decreased from 8.5 ± 2.3 ng/ml on the first day to 1.8 ± 0.4 ng/ml on the final day ($P = 0.002$). Similarly, amylin levels decreased from 42.4 ± 22.3 to 27.9 ± 23.2 pg/ml ($P = 0.002$). During fasting, administration of r-metHuLeptin normalized the fasting-induced decrease in leptin to levels higher than baseline but still within the physiologic range (7.0 ± 1.7 vs. 15.9 ± 3.8 ng/ml, $P = 0.002$). However, normalizing leptin levels did not alter the fasting-induced decrease in amylin levels (46.2 ± 20.3 vs. 22.6 ± 17.5 pg/ml, $P = 0.003$). Consistent with this finding, there was an overall difference in amylin levels across the three conditions ($P < 0.0001$ by ANOVA) because of differences between the fed condition and each fasting state, but not between fasting alone versus fasting with r-metHuLeptin administration ($P = 0.99$).

Because amylin and insulin are cosecreted and it has been suggested that changes in the amylin-to-insulin ratio may have physiologic relevance (24), we measured the amylin-to-insulin ratio but found no difference across the three conditions or between the first and final days of each condition (data not shown). Pancreatic polypeptide levels tended to increase with fasting (247.7 ± 153.4 vs. 314.0 ± 152.3 pg/ml, $P = 0.019$ by Wilcoxon, $P = 0.013$ by *t* test). During fasting with r-metHuLeptin replacement, there was no difference in pancreatic polypeptide levels (269.3 ± 143.5 vs. 299.0 ± 140.5 pg/ml, $P = 0.20$). Two subjects were excluded from the amylin analysis because their amylin levels were less than assay. One subject had amylin levels 5–7 times higher than those of the other subjects, accounting for the large SEM. Another subject had pancreatic polypeptide levels that were ~10 times greater than those of other subjects, again accounting for the large SEM. Results were similar when these subjects were excluded.

Chronic relative leptin deficiency and leptin replacement have no effect on amylin and pancreatic polypeptide levels

Finally, we evaluated whether amylin and pancreatic polypeptide levels were af-

fected by chronic leptin deficiency and leptin replacement in women with hypothalamic amenorrhea and relative leptin deficiency. Leptin levels were significantly lower in women with hypothalamic amenorrhea compared with weight-matched eumenorrheic control subjects (3.9 ± 0.8 vs. 11.4 ± 1.6 ng/ml, $P = 0.007$), despite similar BMI (20.6 ± 0.8 [hypothalamic amenorrhea] vs. 21.7 ± 0.8 [control subjects] kg/m^2, $P = 0.46$). Despite the difference in leptin levels, levels of amylin and pancreatic polypeptide in hypothalamic amenorrhea subjects were not different from control subjects (amylin, 45.2 ± 12.8 [hypothalamic amenorrhea] vs. 36.6 ± 23.0 pg/ml [control subjects], $P = 0.26$; pancreatic polypeptide, 249.6 ± 100.1 [hypothalamic amenorrhea] vs. 366.4 ± 255.2 pg/ml [control subjects], $P = 0.95$). Over 11 weeks of r-metHuLeptin treatment, leptin levels increased significantly to physiologic levels during the first 2 months (baseline, 3.9 ± 0.8 ng/ml; week 1, 8.9 ± 1.4 ng/ml; week 3, 10.0 ± 1.8 ng/ml; and week 7, 22.1 ± 7.3 ng/ml) and to mildly supraphysiologic levels during the third month at the higher dose (week 11, 39.1 ± 13.1 ng/ml). Despite the increase in leptin levels, there were no significant changes in amylin (baseline, 45.2 ± 12.8 pg/ml; week 1, 50.5 ± 17.2 pg/ml; week 3, 65.8 ± 21.7 pg/ml; week 7, 53.1 ± 13.2 pg/ml; and week 11, 43.5 ± 12.4 pg/ml; $P = 0.30$) or pancreatic polypeptide levels (baseline, 249.6 ± 100.1 pg/ml; week 1, 228.4 ± 105.8 pg/ml; week 3, 240.0 ± 74.1 pg/ml; week 7, 224.6 ± 70.7 pg/ml; and week 11, 159.3 ± 64.2; $P = 0.67$).

CONCLUSIONS — In these interventional studies in humans, we provide novel insights into the regulation of amylin and pancreatic polypeptide by caloric ingestion as well as acute and chronic states of energy deficit and show that changes in amylin and pancreatic polypeptide levels induced by energy deficit are not mediated by leptin. In lean individuals, amylin and pancreatic polypeptide levels increase in response to oral glucose intake and remain elevated for up to 2 h. Complete fasting for 72 h significantly decreases amylin levels, but this effect is not mediated by leptin. In contrast, pancreatic polypeptide levels are not significantly affected by fasting or leptin replacement. Finally, amylin and pancreatic polypeptide levels in women with hypothalamic amenorrhea (a model of chronic but milder energy deficit associated with hypoleptinemia) are not different from those of weight-matched control subjects with higher leptin levels nor altered by r-metHuLeptin for up to 3 months.

Because both amylin and pancreatic polypeptide may act as satiety signals regulating the body's immediate response to food intake, we first verified that acute caloric ingestion has an effect to increase these hormone levels in lean individuals. Prior studies have demonstrated that basal and glucose-stimulated amylin levels are higher in obese individuals (25,26). Although data are conflicting on whether patients with impaired glucose tolerance have decreased (27) or increased (25) amylin levels after a glucose load, patients with type 2 diabetes have a decreased amylin response to glucose (25), loss of the first-phase amylin response (28), and a decreased amylin-to-insulin ratio (26). Although earlier studies showed no difference in the amylin response to glucose between relatives of patients with type 2 diabetes and control subjects (29) and no correlation with markers of glucose metabolism (29,30), a more recent study showed that both amylin and insulin secretion are proportionally reduced in first-degree relatives of patients with type 2 diabetes after accounting for the effect of insulin sensitivity on β-cell function (31), suggesting that amylin may serve as a marker for β-cell function. In our study, subgroup analysis suggested a trend for amylin levels to be higher in offspring of patients with type 2 diabetes versus control subjects. However, subjects in our study were generally younger and leaner and comprised all women compared with the prior study in which the average age was ~40 years and average BMI was ~29 kg/m^2 (31). Further, larger studies are needed to clarify whether individuals with genetic risk factors for diabetes have alterations in amylin levels before obvious changes in insulin sensitivity.

By slowing gastric emptying, decreasing food intake, and suppressing glucagon secretion, amylin contributes to glucose regulation. In contrast with insulin and other medications for the treatment of diabetes (e.g., sulfonylureas or thiazolidinediones), amylin (pramlintide) improves appetite control and thus may promote weight loss in patients with type 2 (7) as well as type 1 diabetes (32). Because of this weight loss effect, there is considerable interest in the development of amylin for the treatment of obesity. A recent randomized, placebo-controlled study in non–insulin-treated obese subjects with and without type 2 diabetes demonstrated that amylin (at higher doses than that used for diabetes) induced greater weight loss compared with placebo (8).

More recently, administration of leptin in combination with amylin/pramlintide for 24 weeks in overweight and obese subjects resulted in greater weight loss (12.7%) compared with amylin/pramlintide alone (8.4%) (http://www.amylin.com). The synergistic effect of leptin and amylin/pramlintide to induce weight loss could occur through a central mechanism (see below); however, given the evidence for an adipoinsular axis and demonstration that leptin can regulate insulin (16) and amylin (17) secretion from pancreatic islets in vitro, it is reasonable to speculate whether this synergism is due, wholly or in part, to an effect of leptin to alter amylin levels. We thus conducted studies involving fasting and administration of r-metHuLeptin in healthy humans to evaluate this speculation. Consistent with the idea that amylin is cosecreted with insulin, lack of nutrient intake during short-term fasting for 3 days caused a significant decrease in amylin levels. However, normalizing leptin levels during fasting with r-metHuLeptin did not alter the fasting-associated decrease in amylin levels, indicating that the regulation of amylin is independent of leptin in the short-term. Because short-term regulation of hormones can differ from more long-term regulation, we also used a model of chronic energy deficit and longer duration of leptin replacement (up to 3 months) and found similar results with respect to lack of regulation of amylin by leptin. Taken together, these findings suggest that any potential synergistic effects of leptin and amylin on weight loss in obese individuals may occur centrally, either via restoration of leptin sensitivity with amylin and/or an increase in amylin sensitivity by leptin.

The area postrema of the hindbrain, which lacks a functional blood-brain barrier, is a critical site for the anorectic actions of amylin (5) (33), and leptin has been shown to regulate neuropeptide Y and proopiomelanocortin neurons in the arcuate nucleus that project to the lateral hypothalamic area (34), which are intimately interconnected with the AP. Other studies in rats have noted a synergistic effect of leptin and amylin to reduce food

intake (18) and suggest that amylin may restore leptin sensitivity in leptin-resistant animals (35). Administration of an amylin antagonist led to increased food intake in obese Zucker *fa/fa* rats with leptin receptor mutations but not in lean control subjects, suggesting that amylin may play some role as a lipostatic signal when leptin signaling systems are defective (36). Thus, it appears likely that amylin and leptin may act via different but closely interrelated and potentially synergistic pathways. Further studies are warranted to determine the exact mechanism(s) by which amylin and leptin may act synergistically and whether the effect of amylin and leptin to induce weight loss in humans can be sustained over a longer time frame.

The current evidence behind pancreatic polypeptide as a regulator of body weight remains unclear with observational cross-sectional studies in humans showing no difference in pancreatic polypeptide levels between lean and obese subjects (11,12) or lower pancreatic polypeptide levels in obese individuals (13,14). Patients with Prader-Willi syndrome have a blunted pancreatic polypeptide response to oral intake (37). Infusion of pancreatic polypeptide reduced food intake in patients with Prader-Willi syndrome (38) and reduced feeding at a buffet meal in normal-weight subjects with anorectic effects persisting for up to 24 h (15), suggesting a role for pancreatic polypeptide in the treatment of obesity. However, longitudinal prospective evaluation of Pima Indians over 5 years indicates that the role of pancreatic polypeptide in regulating energy balance may be complex, as higher fasting pancreatic polypeptide levels were associated with greater risk of weight gain, but higher postprandial pancreatic polypeptide levels were associated with decreased risk of weight gain (39). In our study, we found that that short-term fasting tended to increase pancreatic polypeptide levels, whereas pancreatic polypeptide levels were not significantly changed by fasting with r-metHuLeptin treatment. Although the fasting-induced change did not reach statistical significance after adjustment for multiple comparisons, the findings from the short-term fasting study suggest an effect of leptin to regulate pancreatic polypeptide. However, more chronic energy deficit and r-metHuLeptin replacement had no effect on pancreatic polypeptide levels. Thus, the role of pancreatic polypeptide in regulating energy homeostasis and body weight requires further evaluation, but our data do not support a role of pancreatic polypeptide as a major molecule implicated in energy homeostasis.

Strengths of our studies include the interventional administration of r-metHuLeptin in models of short-term and long-term energy deficit and the randomized, placebo-controlled design of the short-term study for examining the relationship between leptin, amylin, and pancreatic polypeptide. The relatively small sample size of these studies is one potential limitation. However, we were able to demonstrate statistically significant findings that a fasting-induced change in amylin is independent of leptin, whereas there may be an effect of normalizing leptin levels on pancreatic polypeptide levels during short-term fasting. On the basis of our data, ~80–100 subjects would be required to obtain 80–90% power to detect a statistically significant effect of leptin on pancreatic polypeptide.

In summary, we demonstrate novel findings using an interventional study design in healthy normal-weight humans that amylin levels are decreased during short-term complete fasting, but this effect is not mediated by leptin, and neither the amylin nor pancreatic polypeptide level is altered by chronic energy deficit or normalizing leptin levels for up to 3 months. Thus, any potential synergistic effect of amylin and leptin to mediate weight loss is not likely to be due to alterations of amylin levels by leptin but rather related to central mechanisms. These findings are consistent with our previous findings that leptin and gastrointestinal-secreted hormones (e.g., ghrelin [40] and peptide YY [41]) are independently regulated and support the existence of redundancy in the systems that regulate energy homeostasis.

Acknowledgments— This study was supported by National Institutes of Health (NIH) Grants MO1-RR01032 and R01-58785, a grant from Amgen, a discretionary grant from BIDMC (to C.S.M.), and NIH Grant K23 RR018860 (to J.L.C.).

We gratefully thank the GCRC nurses at BIDMC for assistance with collecting the samples for this research, the GCRC nutritionists for assistance with the isocaloric diet and fasting studies, and Jessica Fargnoli for assistance with the statistical analysis.

References

1. Young A, Denaro M: Roles of amylin in diabetes and in regulation of nutrient load. *Nutrition* 14:524–527, 1998
2. Adrian TE, Bloom SR, Bryant MG, Polak JM, Heitz PH, Barnes AJ: Distribution and release of human pancreatic polypeptide. *Gut* 17:940–944, 1976
3. Weyer C, Maggs DG, Young AA, Kolterman OG: Amylin replacement with pramlintide as an adjunct to insulin therapy in type 1 and type 2 diabetes mellitus: a physiological approach toward improved metabolic control. *Curr Pharm Des* 7:1353–1373, 2001
4. Huda MS, Wilding JP, Pinkney JH: Gut peptides and the regulation of appetite. *Obes Rev* 7:163–182, 2006
5. Lutz TA, Mollet A, Rushing PA, Riediger T, Scharrer E: The anorectic effect of a chronic peripheral infusion of amylin is abolished in area postrema/nucleus of the solitary tract (AP/NTS) lesioned rats. *Int J Obes Relat Metab Disord* 25:1005–1011, 2001
6. Rushing PA, Hagan MM, Seeley RJ, Lutz TA, D'Alessio DA, Air EL, Woods SC: Inhibition of central amylin signaling increases food intake and body adiposity in rats. *Endocrinology* 142:5035, 2001
7. Hollander P, Maggs DG, Ruggles JA, Fineman M, Shen L, Kolterman OG, Weyer C: Effect of pramlintide on weight in overweight and obese insulin-treated type 2 diabetes patients. *Obes Res* 12:661–668, 2004
8. Aronne L, Fujioka K, Aroda V, Chen K, Halseth A, Kesty NC, Burns C, Lush CW, Weyer C: Progressive reduction in body weight after treatment with the amylin analog pramlintide in obese subjects: a phase 2, randomized, placebo-controlled, dose-escalation study. *J Clin Endocrinol Metab* 92:2977–2983, 2007
9. Ueno N, Inui A, Iwamoto M, Kaga T, Asakawa A, Okita M, Fujimiya M, Nakajima Y, Ohmoto Y, Ohnaka M, Nakaya Y, Miyazaki JI, Kasuga M: Decreased food intake and body weight in pancreatic polypeptide-overexpressing mice. *Gastroenterology* 117:1427–1432, 1999
10. Malaisse-Lagae F, Carpentier JL, Patel YC, Malaisse WJ, Orci L: Pancreatic polypeptide: a possible role in the regulation of food intake in the mouse: hypothesis. *Experientia* 33:915–917, 1977
11. Pieramico O, Malfertheiner P, Nelson DK, Glasbrenner B, Ditschuneit H: Interdigestive cycling and post-prandial release of pancreatic polypeptide in severe obesity. *Int J Obes* 14:1005–1011, 1990
12. Wisen O, Bjorvell H, Cantor P, Johansson C, Theodorsson E: Plasma concentrations of regulatory peptides in obesity following modified sham feeding (MSF) and a liquid test meal. *Regul Pept* 39:43–54, 1992

13. Lassmann V, Vague P, Vialettes B, Simon MC: Low plasma levels of pancreatic polypeptide in obesity. *Diabetes* 29:428–430, 1980
14. Marco J, Zulueta MA, Correas I, Villanueva ML: Reduced pancreatic polypeptide secretion in obese subjects. *J Clin Endocrinol Metab* 50:744–747, 1980
15. Batterham RL, Le Roux CW, Cohen MA, Park AJ, Ellis SM, Patterson M, Frost GS, Ghatei MA, Bloom SR: Pancreatic polypeptide reduces appetite and food intake in humans. *J Clin Endocrinol Metab* 88:3989–3992, 2003
16. Seufert J, Kieffer TJ, Leech CA, Holz GG, Moritz W, Ricordi C, Habener JF: Leptin suppression of insulin secretion and gene expression in human pancreatic islets: implications for the development of adipogenic diabetes mellitus. *J Clin Endocrinol Metab* 84:670–676, 1999
17. Karlsson E, Stridsberg M, Sandler S: Leptin regulation of islet amyloid polypeptide secretion from mouse pancreatic islets. *Biochem Pharmacol* 56:1339–1346, 1998
18. Osto M, Wielinga PY, Alder B, Walser N, Lutz TA: Modulation of the satiating effect of amylin by central ghrelin, leptin and insulin. *Physiol Behav* 91:566–572, 2007
19. Roth JD, Hughes H, Kendall E, Baron AD, Anderson CM: Antiobesity effects of the β-cell hormone amylin in diet-induced obese rats: effects on food intake, body weight, composition, energy expenditure, and gene expression. *Endocrinology* 147: 5855–5864, 2006
20. Asakawa A, Inui A, Yuzuriha H, Ueno N, Katsuura G, Fujimiya M, Fujino MA, Niijima A, Meguid MM, Kasuga M: Characterization of the effects of pancreatic polypeptide in the regulation of energy balance.*Gastroenterology*124:1325–1336, 2003
21. Chan JL, Heist K, DePaoli AM, Veldhuis JD, Mantzoros CS: The role of falling leptin levels in the neuroendocrine and metabolic adaptation to short-term starvation in healthy men. *J Clin Invest* 111:1409–1421, 2003
22. Chan JL, Matarese G, Shetty GK, Raciti P, Kelesidis I, Aufiero D, De Rosa V, Perna F, Fontana S, Mantzoros CS: Differential regulation of metabolic, neuroendocrine, and immune function by leptin in humans. *Proc Natl Acad Sci USA* 103:8481–8486, 2006
23. Welt CK, Chan JL, Bullen J, Murphy R, Smith P, DePaoli AM, Karalis A, Mantzoros CS: Recombinant human leptin in women with hypothalamic amenorrhea. *N Engl J Med* 351:987–997, 2004
24. Blackard WG, Clore JN, Kellum JM: Amylin/insulin secretory ratios in morbidly obese man: inverse relationship with glucose disappearance rate. *J Clin Endocrinol Metab* 78:1257–1260, 1994
25. Enoki S, Mitsukawa T, Takemura J, Nakazato M, Aburaya J, Toshimori H, Matsukara S: Plasma islet amyloid polypeptide levels in obesity, impaired glucose tolerance and non-insulin-dependent diabetes mellitus. *Diabetes Res Clin Pract* 15:97–102, 1992
26. Ludvik B, Lell B, Hartter E, Schnack C, Prager R: Decrease of stimulated amylin release precedes impairment of insulin secretion in type II diabetes. *Diabetes* 40: 1615–1619, 1991
27. Kahn SE, Verchere CB, Andrikopoulos S, Asberry PJ, Leonetti DL, Wahl PW, Boyko EJ, Schwartz RS, Newell-Morris L, Fujimoto WY: Reduced amylin release is a characteristic of impaired glucose tolerance and type 2 diabetes in Japanese Americans. *Diabetes* 47:640–645, 1998
28. Makimattila S, Fineman MS, Yki-Jarvinen H: Deficiency of total and nonglycosylated amylin in plasma characterizes subjects with impaired glucose tolerance and type 2 diabetes. *J Clin Endocrinol Metab* 85:2822–2827, 2000
29. Nyholm B, Fineman MS, Koda JE, Schmitz O: Plasma amylin immunoreactivity and insulin resistance in insulin resistant relatives of patients with non-insulin-dependent diabetes mellitus. *Horm Metab Res* 30:206–212, 1998
30. Gulli G, Rossetti L, DeFronzo RA: Hyperamylinemia is associated with hyperinsulinemia in the glucose-tolerant, insulin-resistant offspring of two Mexican-American non-insulin-dependent diabetic parents. *Metabolism* 46:1157–1161, 1997
31. Knowles NG, Landchild MA, Fujimoto WY, Kahn SE: Insulin and amylin release are both diminished in first-degree relatives of subjects with type 2 diabetes. *Diabetes Care* 25:292–297, 2002
32. Ratner RE, Dickey R, Fineman M, Maggs DG, Shen L, Strobel SA, Weyer C, Kolterman OG: Amylin replacement with pramlintide as an adjunct to insulin therapy improves long-term glycaemic and weight control in type 1 diabetes mellitus: a 1-year, randomized controlled trial. *Diabet Med* 21:1204–1212, 2004
33. Lutz TA, Althaus J, Rossi R, Scharrer E: Anorectic effect of amylin is not transmitted by capsaicin-sensitive nerve fibers. *Am J Physiol* 274:R1777–R1782, 1998
34. Elias CF, Aschkenasi C, Lee C, Kelly J, Ahima RS, Bjorbaek C, Flier JS, Saper CB, Elmquist JK: Leptin differentially regulates NPY and POMC neurons projecting to the lateral hypothalamic area. *Neuron* 23:775–786, 1999
35. Roth J, Weyer C, Anderson C, Parkes D, Baron A: Leptin responsitivity restored in leptin-resistant diet-induced obese (DIO) rats: synergistic actions of amylin and leptin for reduction of body weight and fat (Abstract). In Proceedings of the 66th Scientific Sessions of the American Diabetes Association. Alexandria, VA, American Diabetes Association, 2006
36. Grabler V, Lutz TA: Chronic infusion of the amylin antagonist AC 187 increases feeding in Zucker *fa/fa* rats but not in lean controls. *Physiol Behav* 81:481–488, 2004
37. Zipf WB, O'Dorisio TM, Cataland S, Sotos J: Blunted pancreatic polypeptide responses in children with obesity of Prader-Willi syndrome. *J Clin Endocrinol Metab* 52:1264–1266, 1981
38. Berntson GG, Zipf WB, O'Dorisio TM, Hoffman JA, Chance RE: Pancreatic polypeptide infusions reduce food intake in Prader-Willi syndrome. *Peptides* 14: 497–503, 1993
39. Koska J, DelParigi A, De Court, Weyer C, Tataranni PA: Pancreatic polypeptide is involved in the regulation of body weight in Pima Indian male subjects. *Diabetes* 53: 3091–3096, 2004
40. Chan JL, Bullen J, Lee JH, Yiannakouris N, Mantzoros CS: Ghrelin levels are not regulated by recombinant leptin administration and/or three days of fasting in healthy subjects. *J Clin Endocrinol Metab* 89:335–343, 2004
41. Chan JL, Stoyneva V, Kelesidis T, Raciti P, Mantzoros CS: Peptide YY levels are decreased by fasting and elevated following caloric intake but are not regulated by leptin. *Diabetologia* 49:169–173, 2006