Leptin does not directly regulate the pancreatic hormones, amylin and pancreatic polypeptide: interventional studies in humans

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ABSTRACT

Objective: Leptin and the pancreatic hormones, amylin and pancreatic polypeptide (PP), 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 PP 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 PP after: 1) a 75-gram glucose load in 28 healthy, normal-weight women; 2) 72-hour complete energy deficiency (severe hypoleptinemia) with administration of either placebo or replacement-dose recombinant 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 (HA) before and after r-metHuLeptin administration for 3 months.

Results: Amylin and PP levels increased 15 minutes after a 75-gram glucose load and remained elevated at 60 and 120 minutes (P<0.0001). Fasting for 72 hours decreased leptin (to 21%) and amylin (to 67%) of baseline, but not PP levels. Normalizing leptin levels with r-metHuLeptin did not alter the fasting-induced decrease in amylin and had no effect on PP levels. Neither amylin nor PP levels were different in leptin-deficient HA women compared to weight-matched controls, and normalization of leptin levels with r-metHuLeptin treatment did not alter amylin or PP 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.
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The endocrine pancreas, in addition to producing insulin and glucagon, also secretes several other hormones important in energy homeostasis, including amylin and pancreatic polypeptide (PP). Amylin, or islet amyloid polypeptide, is co-secreted with insulin from pancreatic \( \beta \)-cells and plays a complementary role to insulin in the regulation of glucose homeostasis (1). PP is secreted from pancreatic F-cells and is also released in response to food intake (2). It has been proposed that both amylin and PP 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 like insulin and leptin, amylin and PP appear to play important roles in body weight regulation and energy homeostasis. In rats, amylin decreases food intake, body weight, and fat mass, while inhibition of amylin signalling has the opposite effect (5,6). Pramlintide, an FDA-approved synthetic amylin analogue for the treatment of diabetes, induces weight loss in individuals with (7) and without diabetes (8). Transgenic mice over-expressing PP are leaner than controls (9), and chronic peripheral administration of PP to mice reduces body weight (10). Although observational studies of PP levels in humans are conflicting (11-14), intravenous infusion of PP in normal-weight subjects has been shown to reduce 24-hour energy intake (15).

As accumulating evidence suggests that amylin and PP may contribute to the regulation of body weight, understanding whether “crosstalk” 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 (18,19), specifically decreasing fat mass (19), and a recent clinical trial in humans involving administration of amylin and leptin suggests a similar synergy (www.amylin.com). Even fewer data are available on the interaction between leptin and PP, but it has been shown that PP 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 PP 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 PP response to a 75-gram 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 human leptin (r-met-HuLeptin) to normalize the fasting-induced hypoleptinemia would alter amylin and/or PP levels in healthy, normal-weight subjects. Finally, we tested whether amylin and PP levels are different in women with hypothalamic amenorrhea (HA), who have mild chronic energy deficit resulting in relative leptin deficiency, compared to weight-matched controls, and whether r-met-HuLeptin administration for up to 3 months to normalize circulating leptin levels would alter circulating amylin and PP levels.

**RESEARCH DESIGN AND METHODS**

**Human Subjects.** The oral glucose tolerance test (OGTT) study was carried out at Harokopio University, 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 recombinant methionyl human leptin (r-metHuLeptin) was supplied by Amgen, Inc. (Thousand Oaks, California) and administered under an Investigational New Drug application submitted to the Food and Drug Administration by the investigators.

a. OGTT Study. 28 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; non-smoker; no history of hypertension, endocrine, or metabolic diseases; sedentary lifestyle; fasting glucose <126 mg/dL; body mass index (BMI) <27 kg/m²; stable body weight ≥ 6 months before the study; not pregnant; not on medications (including birth control or hormone replacement therapy). Subjects abstained from alcohol or structured exercise for 24 hours prior to the study. After a 12-hour overnight fast and collection of a fasting blood sample, subjects ingested a solution containing 75 grams of anhydrous glucose, and blood samples were obtained at 15, 30, 60, 90, and 120 minutes for glucose, insulin, amylin (except in 2 subjects), and PP. Area under the curve (AUC) was calculated.

b. Short-term Energy Deprivation Study. Eight men (age 23.3±1.2 yr, mean ± SE) and 7 women (age 23.7±1.5 yr) with BMI < 25 kg/m² were studied during 3 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-hr fasting with administration of placebo; and 72-hr 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 6 men are presented (age 23.5±1.5 yr). 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 were not on oral contraceptives for at least 6 months prior to 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 8am on day 1 (men and women) and 8am on day 3 (men) or 4 (women) for measurement of leptin, amylin, PP, and insulin levels. During the baseline fed condition, subjects received a standardized isocaloric diet: 20% calories from breakfast (8am), 35% from lunch (2pm), 35% from dinner (6pm), 10% from a snack (10pm). 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/day on the first day and 0.1 (men) or 0.2 (women) mg/kg/day on the second and third days. The total daily r-metHuLeptin dose for each day was divided into 4 equal doses given every 6 hours by subcutaneous (s.c.) injection. Placebo (a buffer solution) was administered according to the same schedule as r-metHuLeptin.

c. Chronic Energy Deprivation Study. Seven normal-weight women (age=25.0±2.2 years) with chronic energy deficit, HA 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-
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MetHuLeptin (0.08 mg/kg/day for 2 months, then 0.2 mg/kg/day for the third month) s.c. twice daily with 40% of the daily dose at 8am and 60% at 8pm to mimic the normal diurnal variation of leptin levels. Blood samples for measurement of leptin, amylin, and PP were obtained at an initial screening visit one month prior to the study and after 1, 3, 7, and 11 weeks of r-metHuLeptin treatment. Two subjects completed the study at 8 weeks due to achieving the primary outcome of ovulation; thus these two subjects did not have data collected at 11 weeks and data for only 5 subjects is available at the 11th week. The 6 women from the short-term energy deficit study were used as controls.

Hormone Measurements. All samples used were stored at –80°C. We evaluated whether freeze-thaw cycles would significantly degrade amylin and PP levels in test human plasma. There were no changes in amylin or PP with up to 4 freeze-thaw cycles (amylin: 14.6 pM, 15.6 pM, 15.5 pM, 16.5 pM after 1 to 4 freeze-thaw cycles, respectively; PP: 101.7 pg/ml, 96.8 pg/ml, 101.3 pg/ml, 104.8 pg/ml after 1 to 4 freeze-thaw cycles, respectively). Amylin and PP levels were measured by ELISA (Millipore, Billerica, MA) with sensitivities of 3.9 pg/ml (1 pM) and 12.3 pg/ml, respectively. Leptin levels were measured by RIA (Millipore, Billerica, MA) with sensitivity of 0.5 ng/ml. Glucose was determined by enzymatic calorimetric method (Roche Diagnostics, Mannheim, Germany and Randox Laboratories Ltd., Co. Antrim, Ireland). Insulin was measured by IRMA using a radiolabeled mouse monoclonal anti-insulin and solid phase guinea pig anti-insulin (supplied by 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 mean±SEM. Statistical analyses were conducted using SPSS 11.5 (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. AUC of amylin and PP between relatives and controls and between lower and higher BMI groups was assessed using Mann-Whitney U test. For the short-term energy deficit study, nonparametric Wilcoxon rank sum and paired t-tests were used to assess change in hormone levels for each condition, with similar results obtained except where noted. To determine whether changes in hormone levels varied between conditions, we conducted a comparison of mean final to initial day differences using one-way ANOVA and Kruskal-Wallis tests, 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 HA subjects vs. controls, 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 PP levels rise in response to oral glucose load and remain elevated for at least 120 minutes.

We first characterized the amylin and PP response to a 75-gram glucose load in 28 healthy women (age=30.5±1.1 years; BMI=22.4±0.4 kg/m²) (Figure 1). Glucose levels increased significantly at 15 minutes, peaked to 1.5 fold of baseline at 30 minutes, declined after 60 minutes, but remained significantly higher than baseline at 120 minutes (overall P<0.0001). Insulin levels peaked to 10-fold of baseline at 30 minutes and declined after 60 minutes, but still remained higher than baseline after 120 minutes (overall P<0.0001). Amylin levels increased significantly at 15 minutes, peaked to nearly 1.5 fold of baseline after 60 minutes,
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and then plateaued and remained higher than baseline at 120 minutes (overall P<0.0001). PP levels increased 4-fold at 15 minutes (P<0.05), and levels remained significantly elevated above baseline at 60 and 120 minutes (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 to those without (P=0.04 by Wilcoxon, P=0.12 by t-test), but PP AUC did not differ based on family history (P=0.75). When subjects were divided according to the median BMI value into lower and higher BMI sub-groups (20.7±0.3 kg/m² vs. 24.1±0.4 kg/m², P=0.001), there was no significant difference in amylin and PP AUC between sub-groups (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 PP levels and whether potential fasting-induced changes are mediated by leptin in normal-weight subjects (BMI 23.5±0.4 kg/m² [men], 21.7±0.8 kg/m² [women]. During the baseline fed condition, amylin (56.0±25.8 vs. 53.2±23.8 pg/ml, P=0.29) and PP (238.0±138.7 vs. 226.0±138.8 pg/ml, P=0.46) levels remained stable (Figure 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 72-hour complete fasting, 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 pg/ml 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) due to differences between the fed condition and each fasting state, but not between fasting alone vs. fasting with r-metHuLeptin administration (P=0.99).

Because amylin and insulin are co-secreted, 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 day of each condition (data not shown). PP 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 PP 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 that of other subjects, accounting for the large standard error. Another subject had PP levels that were ~10 times greater than that of other subjects, again accounting for the large standard error. 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 PP levels were affected by chronic leptin deficiency and leptin replacement in women with HA and relative leptin deficiency. Leptin levels were significantly lower in HA women compared to weight-matched eumenorrheic controls (3.9±0.8 ng/ml vs. 11.4±1.6 ng/ml, P=0.007), despite similar BMI (HA, 20.6±0.8 kg/m² vs. controls, 21.7±0.8 kg/m², P=0.46). Despite the difference in leptin levels, levels of amylin and PP in HA subjects were not
different from controls (amylin, 45.2±12.8 pg/ml [HA] vs. 36.6±23.0 pg/ml [controls], P=0.26; PP, 249.6±100.1 pg/ml [HA] vs. 366.4±255.2 pg/ml [controls], P=0.95). Over 11 weeks of r-metHuLeptin treatment, leptin levels increased significantly to physiologic levels during the first two months (baseline, 3.9±0.8 ng/ml; week 1, 8.9±1.4 ng/ml; week 3, 10.0±1.8 ng/ml; 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; week 11, 43.5±12.4 pg/ml, P=0.30) or PP 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; 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 PP by caloric ingestion as well as acute and chronic states of energy deficit and show that changes in amylin and PP levels induced by energy deficit are not mediated by leptin. In lean individuals, amylin and PP levels increase in response to oral glucose intake and remain elevated for up to 2 hours. Complete fasting for 72 hours significantly decreases amylin levels, but this effect is not mediated by leptin. In contrast, PP levels are not significantly affected by fasting or leptin replacement. Finally, amylin and PP levels in women with hypothalamic amenorrhea (a model of chronic but milder energy deficit associated with hypoleptinemia) are not different from weight-matched controls with higher leptin levels nor altered by r-metHuLeptin for up to three months.

Because both amylin and PP 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 decreased amylin-to-insulin ratio (26). Although earlier studies found no difference in the amylin response to glucose between relatives of patients with type 2 diabetes and controls (29) and no correlation with markers of glucose metabolism (29,30), a more recent study found 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, sub-group analysis suggested a trend for amylin levels to be higher in offspring of patients with type 2 diabetes vs. controls. However, subjects in our study were generally younger, leaner, and comprised of all women compared to the prior study in which the average age was ~40 years and average BMI was ~29 kg/m² (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 to 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 to 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 to amylin/pramlintide alone (8.4%) (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. Consistent with the idea that amylin is co-secreted 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. Since 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 (AP) of the hindbrain, which lacks a functional blood brain barrier, is a critical site for amylin’s anorectic barrier, is a critical site for amylin’s anorectic actions (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 controls, suggesting that amylin may play some role as a lipostatic signal when leptin signalling 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 PP as a regulator of body weight remains unclear with observational cross-sectional studies in humans showing no difference in PP levels between lean and obese subjects (11,12) or lower PP levels in obese individuals (13,14). Patients with Prader-Willi syndrome have a blunted PP response to oral intake (37). Infusion of PP reduced food intake in Prader-Willi patients (38) and reduced feeding at a buffet meal in normal-weight subjects with anorectic effects persisting for up to 24 hours (15), suggesting a role for PP in the treatment of obesity. However, longitudinal prospective evaluation of Pima Indians over 5 years indicate that PP’s role in regulating energy balance may be complex, as higher fasting PP levels were associated with greater risk of weight gain, but higher postprandial PP levels
were associated with decreased risk of weight gain (39). In our study, we found that that short-term fasting tended to increase PP levels, whereas PP levels were not significantly changed by fasting with r-metHuLeptin treatment. Although the fasting-induced change did not reach statistical significance after adjusting for multiple comparisons, the findings from the short-term fasting study suggest an effect of leptin to regulate PP. However, more chronic energy deficit and r-metHuLeptin replacement had no effect on PP levels. Thus, the role of PP in regulating energy homeostasis and body weight requires further evaluation, but our data do not support a role of PP 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 PP. Relatively small sample size of these studies is one potential limitation. However, we were able to demonstrate statistically significant findings that fasting-induced change in amylin is independent of leptin, whereas there may be an effect of normalizing leptin levels on PP levels during short-term fasting. Based on our data, ~80-100 subjects would be required for 80-90% power to detect a statistically significant effect of leptin on PP.

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 amylin nor PP levels are 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 likely not due to alterations of amylin levels by leptin, but related to central mechanisms. These findings are consistent with our previous findings that leptin and gastrointestinal-secreted hormones (e.g. ghrelin (40) and PYY (41)) are independently regulated and support the existence of redundancy in the systems that regulate energy homeostasis.

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REFERENCES


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
Figure 1. (A) Glucose, (B) Insulin, (C) Amylin, and (D) Pancreatic polypeptide (PP) levels following a 75-gram oral glucose load (n=28 normal-weight women). P-values by repeated measures ANOVA with post hoc tests. *P<0.05, **P<0.01 vs. baseline.

Figure 2. Levels of (A) Leptin, (B) Amylin, and (C) Pancreatic polypeptide (PP) at the beginning (day 1) and end (day 3 or 4) of a baseline fed condition, 72-hour complete fasting with administration of placebo, and 72-hour complete fasting with administration of r-metHuLeptin (N=6 normal-weight men and 7 normal-weight women). Black bar = Day 1, White bar = Final day. *P<0.0167
Figure 1

(A) Glucose (mmol/L)

(B) Insulin (pmol/L)

(C) Amylin (pg/ml)

(D) PP (pmol/L)
Figure 2

(A) Leptin (ng/ml)

(B) Amylin (pg/ml)

(C) PP (pg/ml)