

## TRANSLATIONAL PHYSIOLOGY |

# Pramlintide treatment reduces 24-h caloric intake and meal sizes and improves control of eating in obese subjects: a 6-wk translational research study

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**Smith SR, Blundell JE, Burns C, Ellero C, Schroeder BE, Kesty NC, Chen KS, Halseth AE, Lush CW, Weyer C.** Pramlintide treatment reduces 24-h caloric intake and meal sizes and improves control of eating in obese subjects: a 6-wk translational research study. *Am J Physiol Endocrinol Metab* 293: E620–E627, 2007. First published May 15, 2007; doi:10.1152/ajpendo.00217.2007.—Evidence from rodent studies indicates that the  $\beta$ -cell-derived neurohormone amylin exerts multiple effects on eating behavior, including reductions in meal size, intake of highly palatable foods, and stress-induced sucrose consumption. To assess the effect of amylin agonism on human eating behavior we conducted a randomized, blinded, placebo-controlled, multicenter study investigating the effects of the amylin analog pramlintide on body weight, 24-h caloric intake, portion sizes, “fast food” intake, and perceived control of eating in 88 obese subjects. After a 2-day placebo lead-in, subjects self-administered pramlintide (180  $\mu$ g) or placebo by subcutaneous injection 15 min before meals for 6 wk without concomitant lifestyle modifications. Compared with placebo, pramlintide treatment elicited significant mean reductions from baseline in body weight on day 44 ( $-2.1 \pm 0.3$  vs.  $+0.1 \pm 0.4\%$ ,  $P < 0.001$ ), 24-h caloric intake ( $-990 \pm 94$  vs.  $-243 \pm 126$  kcal on day 3,  $P < 0.0001$ ;  $-680 \pm 86$  vs.  $-191 \pm 161$  kcal on day 43,  $P < 0.01$ ), portion sizes, and caloric intake at a “fast food challenge” ( $-385 \pm 61$  vs.  $-109 \pm 88$  kcal on day 44,  $P < 0.05$ ). Pramlintide treatment also improved perceived control of eating, as demonstrated by a 45% placebo-corrected reduction in binge eating scores ( $P < 0.01$ ). The results of this translational research study confirm in humans various preclinical effects of amylin agonism, demonstrating that pramlintide-mediated weight loss in obese subjects is accompanied by sustained reductions in 24-h food intake, portion sizes, fast food intake, and binge eating tendencies.

obesity; weight loss; satiety; food intake; neuroendocrine hormones

IN RECENT YEARS, there has been growing recognition that peptide hormones secreted by the gut and endocrine pancreas play a key role in the regulation of energy homeostasis. Through humoral or vagal afferent pathways, these hormones provide signals to the hindbrain and/or hypothalamus as part of the integrated regulation of food intake. The effect of a given peptide hormone on eating behavior may be multifaceted, including changes in both the homeostatic and hedonic control of food intake. This effect may manifest itself as changes in

objective parameters such as meal size and composition, meal duration, and meal frequency as well as changes in subjective parameters such as in food cravings and binge eating tendencies (3, 8, 13, 19, 41, 42). To thoroughly understand the effect of a given peptide hormone on eating behavior, a careful and comprehensive assessment in both animal models of obesity and obese humans is required.

Amylin is a 37-amino acid neuroendocrine peptide hormone that is cosecreted with insulin by pancreatic  $\beta$ -cells in response to meals. In addition to the well-established role of amylin as a glucoregulatory hormone, studies in rodents indicate that amylin is also involved in the central regulation of food intake and body weight (14, 21, 22, 32, 33, 43). A major binding site for amylin is in the area postrema (4), a hindbrain region known to regulate feeding behavior in animals (9) and thought to serve an important role in the reception and integration of peripheral meal-related signals (3, 11). Peripheral administration of amylin has been shown (31) to induce neural activation (as measured by c-fos expression) in the area postrema as well as in limbic regions such as the central nucleus of the amygdala. Amylin agonism also modulates neuronal activity in the hypothalamus in that peripheral amylin administration to rats reversed fasting-induced neuronal activation in the lateral hypothalamus, similar to the response seen after feeding (28). Additionally, amylin binds to specific receptors in the nucleus accumbens (4, 35), a brain region implicated in the hedonic control of food intake (16).

In keeping with these neurobiological findings, Lutz (20) has argued that amylin fulfills the criteria for a satiating hormone in rodents. Peripheral amylin administration reportedly reduced food intake and meal size without increasing meal frequency (32, 40). Furthermore, amylin treatment has been reported to selectively decrease the intake of highly palatable foods (high fat and/or sucrose) (23) and to prevent stress-induced sucrose drinking (18).

Pramlintide is a soluble synthetic analog of human amylin that differs from amylin by only three amino acids and retains a broad range of the pharmacological actions of the native hormone, including amylin receptor binding (17). Pramlintide has been studied as an antihyperglycemic adjunct treatment for

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patients with type 2 or type 1 diabetes who use insulin, and it is also under investigation as a potential treatment for obesity. Chapman et al. (7) reported that a single dose of pramlintide administered to obese subjects prior to a buffet meal elicited a statistically significant 16% mean reduction in total caloric intake compared with placebo and enhanced prandial and postprandial satiation. In a recent 16-wk study of 204 obese subjects (2), administration of pramlintide prior to major meals resulted in a significant, placebo-corrected progressive reduction in body weight of  $-3.7\%$ .

To further assess the mechanism underlying pramlintide's weight-lowering effect in obese subjects, we conducted a 6-wk randomized, blinded, placebo-controlled multicenter study with two inpatient periods that encompassed a comprehensive assessment of eating behavior, including 24-h food intake, meal size and duration, intermeal intervals, hunger and fullness, caloric intake at a fast food challenge, and control of eating.

## STUDY DESIGN AND METHODS

### Subjects

A total of 179 subjects were enrolled in a study consisting of a pramlintide TID (3 times/day) arm, a placebo TID arm, and two exploratory continuous subcutaneous infusion arms (pramlintide and placebo). Herein, we report the results of the TID treatment arms, the route of administration relevant for further clinical development. Subjects [intent to treat (ITT);  $n = 88$ ] were obese males and postmenopausal females (not on hormone replacement therapy) between 25 and 60 yr, with a body mass index (BMI) of  $\geq 30$  to  $\leq 45$   $\text{kg}/\text{m}^2$ . Premenopausal females were excluded because of the confounding effect of the menstrual cycle on hunger and food intake (24). Other inclusion criteria included baseline clinical laboratory tests judged by the investigators to be not clinically significant, weight fluctuations of  $< 3$  kg and no major change in daily physical activity for 2 mo prior to screening, and typical consumption of three meals/day. Additionally, subjects were to be euthyroid, nonsmokers, and unrestrained eaters as defined by a response  $\leq 4$  on Question 1 and a score  $< 14$  on Factor 1 (cognitive restraint) of the Three-Factor Eating Questionnaire (36).

Exclusion criteria included the presence of diabetes or other endocrine disorders known to affect gastrointestinal motility or body weight; cardiac, hepatic, or renal disease; autoimmune disorders; gastrointestinal disorders; psychiatric illnesses; eating disorders (including anorexia, bulimia, and/or binge eating); untreated or poorly controlled hypertension (sitting blood pressure  $> 160/95$  mmHg); a medical history or characteristics suggestive of genetic or syndromic obesity; drug or alcohol abuse; current or planned enrollment in a weight loss program; and current or recent ( $\leq 2$  mo) treatment with antiobesity agents, psychotropic medications, or drugs that affect gastrointestinal motility.

The study protocol (including amendments) was reviewed and approved by the Independent Investigational Review Board; Chesapeake Research Review; the University of Kentucky Office of Research Integrity; the Human Subjects Committee, Scripps Clinic; and the Pennington Biomedical Research Center Institutional Review Board. The study was conducted in accordance with the principles described in the Declaration of Helsinki, including all amendments through the 1996 South Africa revision. All study participants gave written, informed consent prior to screening for the study. This clinical trial is registered (ClinicalTrials.gov Identifier No. NCT00444561).

### Study Design

This multicenter (10 investigational sites), randomized, blinded (subjects and investigators), placebo-controlled, 6-wk study included an initial 5-day inpatient period (days  $-1$  to 4), followed by an outpatient period of  $\sim 5$  wk (days 5–41), and a second inpatient period of 3 days (days 42–44) (Fig. 1).

Subjects were admitted to the clinical study site on day  $-1$  and randomized in a 2:1 ratio to either pramlintide (180  $\mu\text{g}$ ) or equivalent volume of placebo (see Fig. 1). On day 1, all subjects began a 2-day placebo lead-in period (days 1 and 2), during which they received placebo by subcutaneous injection 15 min prior to major meals. On day 3, subjects began the treatment period, during which they received either pramlintide or placebo by subcutaneous injection 15 min prior to major meals (breakfast, lunch, and dinner) for  $\sim 6$  wk (days 3–44). During the outpatient period (days 5–41), subjects continued study medication treatment and returned for visits on days 17 and 31 for body weight and safety assessments. To evaluate both acute and longer-term effects of pramlintide treatment, the baseline assessments (performed during the first 2 days of the initial inpatient period, days 1 and 2, when all subjects received placebo) were repeated on both days 3 and 4 (the first 2 days of treatment with randomized study medication) and again on days 43 and 44 (during the second inpatient period). No lifestyle intervention was introduced during the study, and subjects were asked to maintain their typical exercise and dietary regimens.

### Measurements

**Body weight.** Body weight was measured each day during the inpatient study periods and on the outpatient visits on days 17 and 31. Body weight on day  $-1$  was defined as baseline.

**Food intake.** On days 1, 3, and 43, subjects were offered ad libitum meals at regularly scheduled intervals for breakfast, lunch, dinner, and evening snack. At these meals, subjects were allowed to eat from a tray containing an excess of free-choice, preweighed items. Breakfast choices included bagels and cream cheese, muffins, cereal, fruit, orange juice, and coffee or tea. Lunch choices included assorted sandwiches, tortilla or potato chips, cookies, and a soft drink or juice. Dinner choices included casserole dishes, salad, bread, pudding cups, and a soft drink. Evening snack choices included peanut butter and jelly sandwiches, 2%-fat milk, chocolate chip cookies, and a soft drink. Food was weighed before and after each meal, and total caloric intake was calculated using nutritional analysis software (The Food Processor; ESHA Research). Prior to each meal, subjects at all study sites were given standardized instructions; they ate in private so as not to be influenced by other subjects, were encouraged to focus on their meal and avoid distractions, and were instructed to eat until comfortably full.

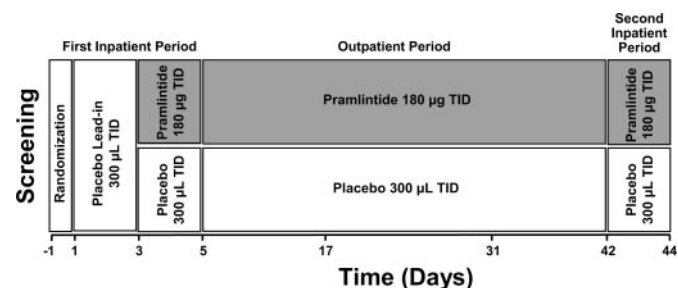


Fig. 1. Study Design. This study consisted of an initial 5-day inpatient period (day  $-1$  to 4), followed by an outpatient period of  $\sim 5$  wk (days 5–41), and a second inpatient period of 3 days (days 42–44). All subjects received placebo during the placebo lead-in period (days 1–2) and either placebo or pramlintide during the treatment period (days 3–44). Subjects returned for outpatient visits on days 17 and 31.

**Ratings of hunger, fullness, and nausea.** Subjective ratings of hunger, fullness, and nausea were obtained on *days 1, 3, and 43* using visual analog scale (VAS) measurements. Subjects used hand-held electronic diaries (CRF) with 101-point resolution ranging from 0 to 100 to make the self-reported assessments.

**Fast food challenge.** On *days 2, 4, and 44*, following a standardized breakfast (25% of individual total daily caloric requirements), subjects were told to request lunch when they were hungry. The lunch provided at the study site on these days was a more palatable, high-fat, high-sugar meal than the standard meal options; the lunch was comprised of deep-dish pizzas, ice cream, and high-fructose corn syrup-sweetened soft drinks. Subjects recorded their perception of the tastiness of the foods on a postmeal VAS.

In addition, the intermeal interval between the end time of breakfast and the time that a subject requested lunch was measured on *days 2, 4, and 44*.

**Meal duration.** Meal duration was measured for each meal on *days 1–4 and days 43 and 44*.

**Perceived control of eating.** The effect of pramlintide on perceived control of eating was evaluated using the Binge Eating Scale (BES) (10), a 16-item questionnaire that identifies different levels of binge eating severity by addressing both behavioral manifestations (e.g., eating large amounts of food) and feelings surrounding a binge eating episode (e.g., guilt, fear of being unable to stop eating). With higher scores (46 is the maximum score) indicating more severe binge eating tendencies, scores were categorized (based on previously reported thresholds) (10) into “mild-to-none” ( $\leq 17$ ), “moderate” ( $> 17$  to  $< 27$ ), and “severe” ( $\geq 27$ ). The BES was administered at admission on *day –1* and on *day 42*. Of note, subjects with a clinically significant history of an eating disorder (including binge eating syndrome) were excluded from study participation.

**Safety.** Safety and tolerability were assessed by evaluation of treatment-emergent adverse events, clinical laboratory measures, electrocardiograms, and physical examination findings.

#### Statistical Analysis

The ITT population included all randomized subjects who received at least one injection of pramlintide or placebo during the treatment period (*days 3–44*). The evaluable population included all ITT subjects remaining in the study through *day 44*, with no major protocol deviations. Safety and tolerability were summarized for the ITT population. Changes in body weight, total caloric intake by meal and over 24 h, meal duration, intermeal interval, VAS ratings of hunger, fullness, nausea and tastiness of meals, BES total scores, and distribution of binge eating severity were assessed in the evaluable population.

General linear models with factors for treatment, baseline BMI stratum, sex, and study site were used to analyze the changes in body weight, total caloric intake, meal duration, intermeal interval, and BES total scores. The *P* values ( $P < 0.05$  indicated statistical significance) were based on the least squares mean differences between treatments in the change from baseline (or placebo lead-in) to the subsequent study visit. Fisher’s exact test was used to analyze the proportion of subjects achieving  $\geq 2.5\%$  weight loss from baseline to *day 44*. The shift in binge eating severity was analyzed using a chi-square test. Data are reported as means  $\pm$  SE unless otherwise noted. Demographics data are means  $\pm$  SD.

## RESULTS

### Subject Baseline Demographics and Disposition

The demographics and baseline characteristics of the placebo and pramlintide treatment groups were generally well balanced (Table 1).

Completion rates for the 6-wk study were 86.2% for the placebo-randomized population and 96.7% for the pramlintide-

randomized population. During the treatment period, withdrawals included one pramlintide-treated subject (lost to followup) and three placebo-treated subjects [1 due to an adverse event (hypertension), 1 protocol violation, and 1 subject lost to followup]. Due to the high retention rate, results were very similar for the evaluable and ITT populations.

### Body Weight

Pramlintide treatment resulted in progressive weight loss, with a significant difference from placebo detected as early as *day 17* ( $P < 0.001$ ; Fig. 2). On *day 44*, pramlintide-treated subjects had a significant reduction in body weight from *day –1* of  $-2.07 \pm 0.3\%$  ( $-2.04 \pm 0.3$  kg) compared with a change of  $+0.11 \pm 0.43\%$  ( $0.00 \pm 0.43$  kg) in placebo-treated subjects ( $P < 0.001$ ). Furthermore, by *day 44* significantly more pramlintide- than placebo-treated subjects achieved weight loss  $\geq 2.5\%$  of baseline body weight (42 vs. 8%, respectively,  $P < 0.01$ ).

### Food Intake

On *day 1* (placebo lead-in), baseline 24-h caloric intake was  $3,932 \pm 159$  and  $3,780 \pm 178$  kcal in subjects randomized to subsequent treatment with pramlintide and placebo, respectively. Pramlintide treatment resulted in both acute and sustained reductions from baseline in total 24-h caloric intake. Compared with baseline, the reductions in food intake in pramlintide-treated subjects averaged  $-990 \pm 94$  kcal ( $-24 \pm 2\%$ ) vs.  $-243 \pm 126$  kcal ( $-5 \pm 3\%$ ) for placebo ( $P < 0.001$ ) on *day 3* and  $-680 \pm 86$  kcal ( $-16 \pm 2\%$ ) vs.  $-191 \pm 161$  kcal ( $-3 \pm 4\%$ ) for placebo ( $P < 0.01$ ) on *day 43* (Fig. 3A).

Table 1. Baseline demographics and disposition

Baseline Demographics (ITT)	Placebo ( <i>n</i> = 28)	Pramlintide ( <i>n</i> = 60)
Sex, female/male, %	50/50	50/50
Age, yr	51 $\pm$ 8	49 $\pm$ 9
Race (Caucasian/Black/Hispanic/other), %	61/14/25/0	63/13/20/3
Height, cm	168.4 $\pm$ 9.7	168.4 $\pm$ 9.0
Body weight, kg	103.2 $\pm$ 17.8	100.2 $\pm$ 14.3
BMI, kg/m <sup>2</sup>	36.3 $\pm$ 4.7	35.3 $\pm$ 3.6
Factor 1 (cognitive restraint) Three-Factor Eating Questionnaire score	4.7 $\pm$ 4.4	6.1 $\pm$ 4.3
Disposition		
Randomized population	29	61
ITT population*	28	60
Withdrawals from randomized population	4 (13.8%)	2 (3.3%)
Reason for withdrawal		
Withdrawal of consent	0 (0%)	1 (1.6%)†
Adverse event	1 (3.4%)	0 (0%)
Investigator decision	1 (3.4%)†	0 (0%)
Protocol violation	1 (3.4%)	0 (0%)
Lost to followup	1 (3.4%)	1 (1.6%)
Evaluable population‡	25 (86.2%)	59 (96.7%)

All data are means  $\pm$  SD unless otherwise indicated; numbers may not add up to 100% due to rounding. ITT, intent to treat; BMI, body mass index. \*ITT population included all randomized subjects who received at least 1 injection of study medication (pramlintide or placebo). †Subject not included in ITT population (did not receive at least 1 injection of study medication). ‡Evaluable population included all ITT subjects remaining in the study through *day 44*, with no major protocol deviations.

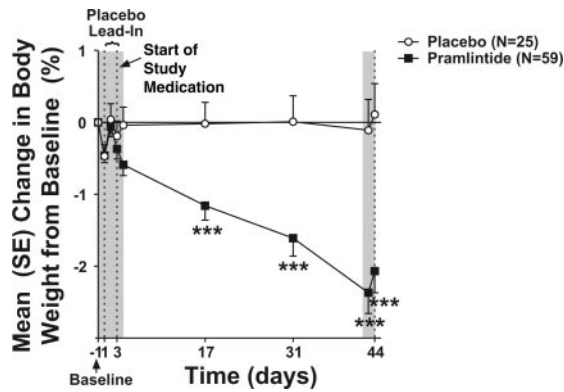


Fig. 2. Relative change in body weight. Mean relative change in body weight for pramlintide (■) and placebo (○) groups during the placebo lead-in (days 1–2) and treatment period (days 3–44). Inpatient periods are shaded. \*\*\* $P < 0.001$ .

On both *day 3* and *day 43*, pramlintide-mediated reductions in total 24-h caloric intake were cumulative over the course of the day (i.e., generally attributable to reductions in caloric intake at each major meal; Fig. 3B). Specifically, compared with placebo-treated subjects, pramlintide-treated subjects had a significant reduction from baseline in caloric intake at breakfast, lunch, and dinner on *day 3* and at breakfast and lunch on *day 43*. At the ad libitum evening snack, prior to which no study medication was administered, there were no statistically significant changes from *day 1* to either *day 3* or *day 43* in caloric intake between groups.

On both *day 3* and *day 43*, the reduction in mean total caloric intake was attributable to similarly proportionate reductions in calories derived from carbohydrate, protein, and fat such that the macronutrient composition of the meal was generally unaffected by treatment (data not shown).

There were no statistically significant changes in meal duration or intermeal intervals (data not shown).

#### VAS Ratings of Hunger, Fullness, and Nausea

In both treatment groups, mean VAS hunger ratings decreased markedly following ingestion of each meal and increased gradually between meals. Conversely, mean VAS fullness ratings increased after consumption of each meal and gradually decreased during the time between meals. Notably, the mean hunger and fullness rating profiles over the 12-h observation period were similar in pramlintide- and placebo-treated subjects on both *day 3* (Fig. 4, A and B) and *day 43* (data not shown) even though 24-h total caloric intake was significantly lower following pramlintide treatment (~19% lower on *day 3* and ~13% lower on *day 43*).

Mean VAS nausea ratings remained near baseline levels for both groups on both *day 3* and *day 43*. There were no major differences in nausea ratings between pramlintide and placebo-treated subjects throughout the 12-h observation period on either *day 3* (Fig. 4C) or *day 43*.

#### Fast Food Challenge

VAS ratings demonstrated that subjects rated the tastiness of the fast food-style lunch on *day 2* significantly higher ( $P < 0.001$ ; data not shown) than the standard lunch option served on *day 1*. At baseline (*day 2* of the placebo lead-in), mean total

caloric intake at the fast food challenge lunch averaged  $1,550 \pm 77$  and  $1,491 \pm 72$  kcal in subjects randomized to receive pramlintide and placebo, respectively. Upon active treatment, pramlintide-treated subjects experienced a significant reduction in mean caloric intake at the fast food challenge lunch compared with placebo on both *day 4* ( $-528 \pm 68$  vs.  $-273 \pm 80$  kcal,  $P < 0.05$ ) and *day 44* ( $-385 \pm 61$  vs.  $-109 \pm 88$  kcal,  $P < 0.05$ ) (Fig. 5).

#### Perceived Control of Eating

Pramlintide-treated subjects experienced a reduction from baseline in mean total BES scores compared with an increase in placebo-treated subjects at *day 42* ( $-25 \pm 8$  vs.  $+19 \pm 11$ ,  $P < 0.01$ ; Fig. 6A). There was also a significant shift in BES severity, with a greater proportion of pramlintide- than placebo-treated subjects shifting to a lower binge eating severity category (24.6 vs. 12.5%,  $P < 0.05$ ). At *day 42*, 83% of pramlintide- and 58% of placebo-treated subjects were categorized as having “mild-to-none” binge eating severity (compared with 67 and 54% at baseline; Fig. 6B).

#### Safety and Tolerability

Pramlintide was generally well-tolerated. The most frequent treatment-emergent adverse event reported was nausea, which

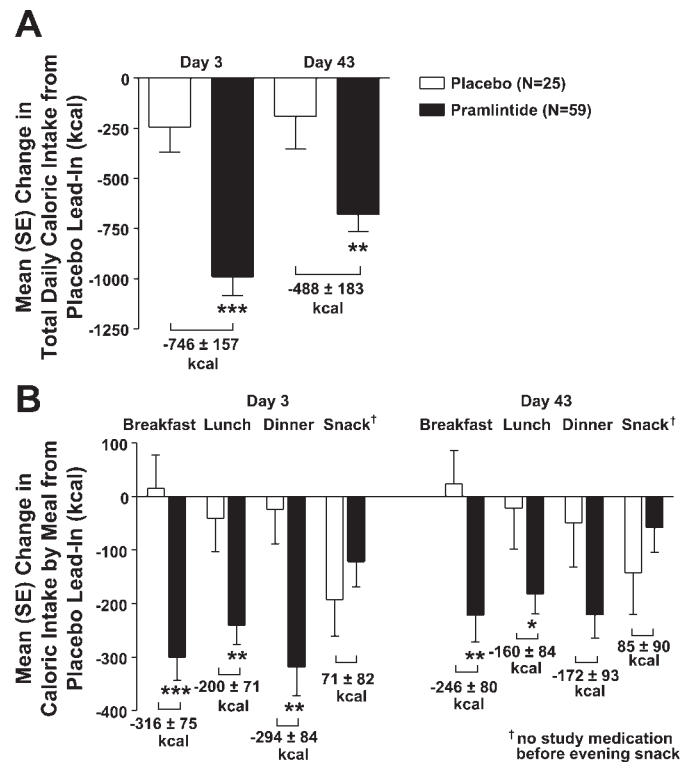


Fig. 3. Absolute change in total caloric intake. A: mean absolute change from *day 1* (placebo lead-in) in total daily caloric intake on *day 3* and *day 43* with either pramlintide or placebo. *Day 1* total caloric intake (means  $\pm$  SE, in kcal): pramlintide,  $3,932 \pm 159$ ; placebo,  $3,780 \pm 178$ . \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ . B: mean absolute change from *day 1* (placebo lead-in) in total caloric intake by meal on *day 3* and *day 43* with either pramlintide or placebo. *Day 1* total caloric intake (means  $\pm$  SE) by meal in pramlintide group (in kcal): breakfast,  $1,052 \pm 51$ ; lunch,  $1,035 \pm 50$ ; dinner,  $1,289 \pm 62$ ; evening snack,  $556 \pm 51$ . *Day 1* total caloric intake (means  $\pm$  SE) by meal in placebo group (in kcal): breakfast,  $949 \pm 59$ ; lunch,  $1,021 \pm 68$ ; dinner,  $1,192 \pm 74$ ; evening snack,  $618 \pm 71$ . \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

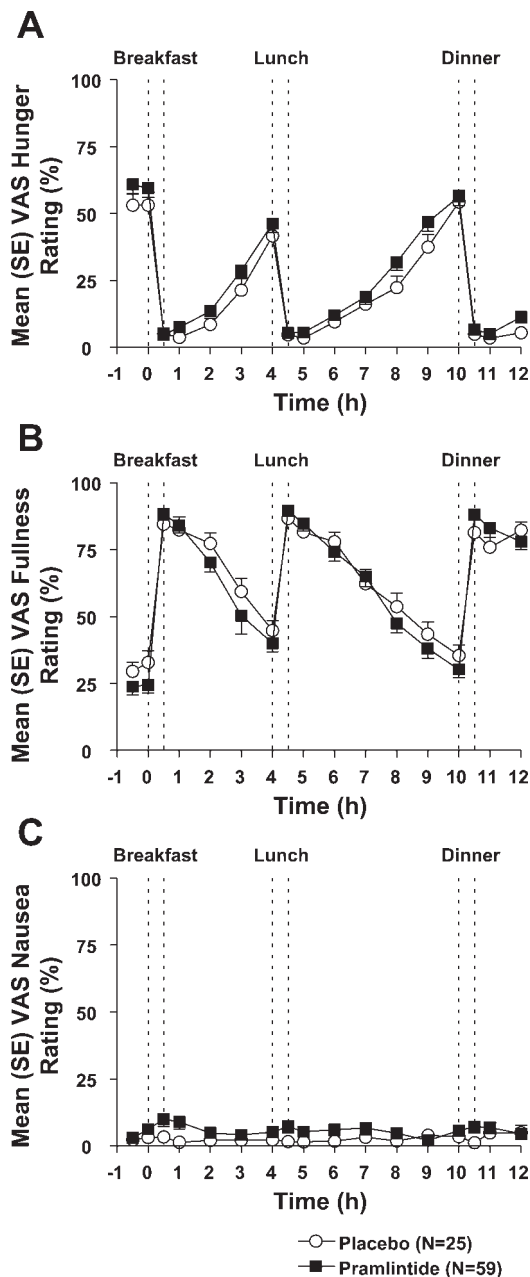


Fig. 4. Mean absolute visual analog scale (VAS) rating profiles of hunger (A), fullness (B), and nausea (C) in pramlintide (■) and placebo (○) groups on day 3. The dotted lines represent the time during which each buffet meal was offered.

occurred with a similar incidence in pramlintide- (23.3%) and placebo-treated (21.4%) subjects. Nausea was primarily mild in intensity (Table 2). There were no serious adverse events reported in either treatment group. No subjects treated with pramlintide withdrew from the study due to an adverse event.

## DISCUSSION

Treatment with the amylin agonist pramlintide has consistently elicited durable weight loss in obese subjects and insulin-using patients with diabetes (2, 12, 26, 27, 39). Preclinical and clinical studies (7, 20, 32, 40) have provided several lines

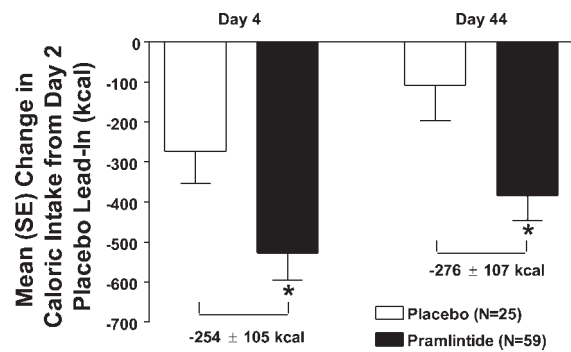


Fig. 5. Caloric intake at the "fast food challenge". Mean change from day 2 (placebo lead-in) in caloric intake at the fast food-style lunch on day 4 or day 44 in pramlintide- and placebo-treated subjects. \* $P < 0.05$ .

of evidence to support the hypothesis that the weight-lowering effect of pramlintide is mediated by a reduction in food intake, enhanced meal-related satiation, and improved appetite control. Herein, we provide the first detailed assessment of the anorexigenic mechanism of action underlying pramlintide's weight-lowering effect in obese humans. The present study provides important new insights, showing that the weight-lowering effect of pramlintide is accompanied by changes in several important aspects of appetite control and eating behavior.

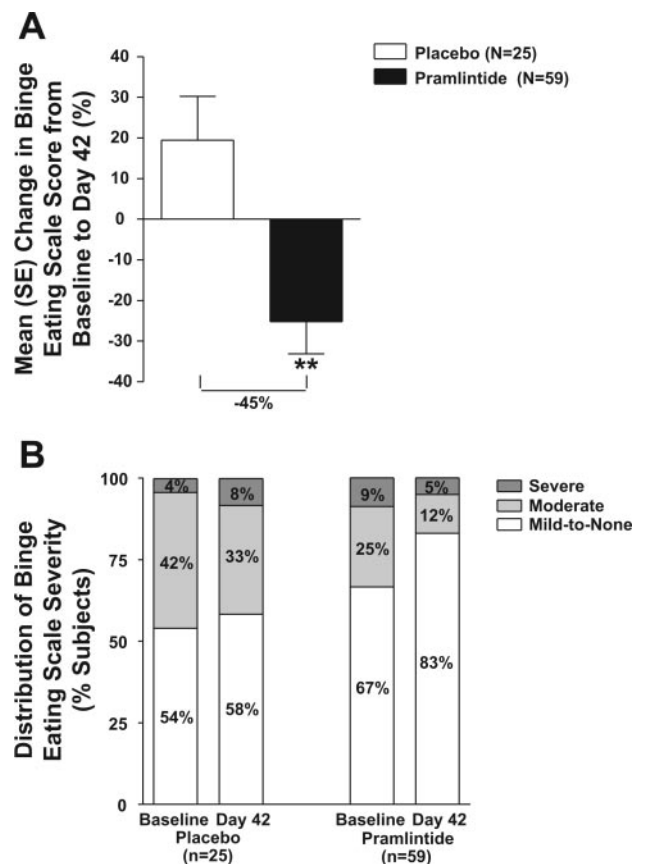


Fig. 6. Perceived control of eating. A: mean change in Binge Eating Scale score (%) from baseline to day 42. \*\* $P < 0.01$  B: distribution of Binge Eating Scale score severity at baseline and day 42 in pramlintide- and placebo-treated subjects. Percentages may not add up to 100% due to rounding.

Table 2. Adverse events with an incidence of  $\geq 5\%$  and with a higher incidence in pramlintide- than placebo-treated subjects (ITT;  $n = 88$ )

Adverse Event	Placebo ( $n = 28$ )		Pramlintide ( $n = 60$ )	
	$n$ (%)	Intensity* (x/y/z)	$n$ (%)	Intensity* (x/y/z)
Nausea	6 (21.4)	5/1/0	14 (23.3)	11/3/0
Injection site erythema	0	0/0/0	8 (13.3)	7/1/0
Injection site pruritus	0	0/0/0	3 (5.0)	2/1/0
Upper abdominal pain	0	0/0/0	3 (5.0)	3/0/0
Back pain	0	0/0/0	3 (5.0)	1/2/0

\*Where x/y/z refers to the number of subjects experiencing adverse events by intensity (mild/moderate/severe).

Treatment with pramlintide (180  $\mu\text{g}$  TID) for 6 wk resulted in progressive and statistically significant weight loss. This is consistent with results from previous studies (2) conducted in insulin-using subjects with diabetes and obese subjects with or without diabetes. The mean reduction ( $\sim 2\%$  of baseline body weight) achieved after 6 wk of treatment is consistent with the initial weight loss observed in an earlier study in obese subjects treated with pramlintide (2). In that study, weight loss was progressive beyond 6 wk, averaging  $-3.7\%$  after 16 wk.

Pramlintide treatment resulted in robust acute and sustained mean reductions from baseline for 24-h ad libitum food intake on *day 3* ( $-24\%$ ) and on *day 43* ( $-16\%$ ). These findings expand in several ways upon the results of a previously reported single-dose crossover study (7), which showed that a single 120  $\mu\text{g}$  injection of pramlintide significantly reduced ad libitum caloric intake and increased satiety compared with placebo at a buffet meal in obese subjects. First, the present study showed that the acute effect of pramlintide on food intake is maintained over a 6-wk treatment period. Although the mean placebo-corrected reduction in caloric intake at *day 43* ( $\sim 500$  kcal) was somewhat less than on *day 3* ( $\sim 750$  kcal), it is noteworthy that a robust reduction in 24-h food intake with pramlintide was still evident after significant initial weight loss, which under normal circumstances induces a compensatory increase in hunger (15). It would be interesting to conduct a longer-term study with measurements of food intake at weight loss plateau to ascertain whether a new food intake/body weight steady-state relationship emerges. Second, our study also demonstrates that the anorexigenic effect of pramlintide is sustained throughout the day in that the reductions in caloric intake occurred at each meal when study medication was administered.

Using VAS data, Chapman et al. (7) reported that pramlintide enhanced both satiation and satiety (i.e., hunger suppression intrameal and between meal, respectively). In the present study, VAS hunger and fullness ratings before, during, and after meals were similar between pramlintide- and placebo-treated subjects on *day 3* and *day 43* despite the significantly fewer calories consumed by the pramlintide treatment group on both days. This finding indicates that less food intake was required in pramlintide-treated subjects to produce similar levels of hunger suppression and fullness, providing further evidence that enhanced meal-related satiation is a dominant mechanism underlying the observed reductions in food intake and subsequent weight loss with pramlintide.

Although the daily caloric intake during the inpatient periods was higher than what might be observed under free-living

conditions (37), it likely represents a reliable estimate of an obese person's readiness to eat in an "obesigenic" environment. Moreover, it is likely that the placebo-corrected caloric intake suppression by pramlintide (500–750 kcal/day) during the inpatient periods reflects the average intake suppression for the whole of the outpatient period, since a caloric deficit of this magnitude would be expected to cause  $\sim 2$ – $2.5\%$  weight loss over 6 wk (1). Our study, therefore, provides strong support to the notion that food intake reduction is a primary mechanism underlying pramlintide's weight-lowering effect. This is consistent with pair-feeding experiments in obese rodents (30), which have shown that amylin-mediated weight loss is largely accounted for by reduced caloric intake.

A person's tendency to overeat and gain weight may be particularly evident upon exposure to an abundant supply of highly palatable foods (5). Intriguingly, in obese rodents, amylin has been shown to preferably reduce the intake of highly palatable food items (23). To explore this phenomenon in humans we specifically measured, on a separate test day, the effect of pramlintide on food consumption during a fast food challenge consisting of pizza, ice cream, and sugar-containing soft drinks. Although no definitive conclusions on food preferences can be drawn, the finding that pramlintide significantly reduced caloric intake compared with placebo in this fast food challenge suggests that pramlintide may help obese subjects better control their consumption of highly palatable, high-fat, and high-sugar foods.

Consistent with preclinical findings in amylin-treated rodents (40), no significant changes in intermeal interval were observed between pramlintide- and placebo-treated subjects in the present study. Although intermeal intervals were measured only on *days 2, 4, and 44* during the inpatient periods, these results indicate that pramlintide-mediated reductions in meal size are not compensated for by increased meal frequency, as has been reported (38) with other gastrointestinal satiety signals such as cholecystokinin. It is also clear from the profiles of hunger and fullness that the smaller meals consumed with pramlintide did not induce rapid return of the motivation to eat or a weakening of the sense of fullness.

Peripheral "satiety" hormones are commonly thought to act on the homeostatic control of food intake via binding in the hindbrain. These hindbrain regions also project upstream to limbic brain regions involved in the hedonic aspects of food intake. Amylin shows rich binding to the nucleus accumbens (16) and reduces stress-induced sucrose craving in rats (18). In the present clinical study, pramlintide significantly and substantially reduced consumption of highly palatable, high-fat, high-sugar fast foods. Although this may solely be due to the satiating effect of pramlintide, it is conceivable that amylin agonism may modulate hedonically-mediated eating in obese subjects possibly through an inhibitory action in the hedonic neural system. In addition, the finding of a significant, almost 50%, reduction in BES score also suggests that pramlintide may exert effects on food hedonics, leading to improved perceived control of eating. Further examination of this finding is warranted.

Because administration of gastrointestinal peptide hormones is associated with the occurrence of nausea in some subjects, it is crucial to control for tolerability when assessing the effect of these peptides on food intake. At the dosing regimen employed in the current study (180  $\mu\text{g}$  TID, without dose escalation), pramlintide-treated subjects' perceptions of nausea, as care-

fully assessed by hourly VAS ratings, were low and largely unchanged during the inpatient periods. Furthermore, the incidence of nausea-adverse events, primarily mild in intensity, was similar in pramlintide- (23.3%) and placebo-treated subjects (21.4%), and no subjects in the pramlintide group withdrew due to nausea. Collectively, these findings provide strong evidence that pramlintide-mediated reductions in caloric intake and body weight were dissociated from nausea. Again, this finding is consistent with animal experiments showing that amylin's anorexigenic effect is clearly distinguished from malaise (6, 25, 29, 34).

In conclusion, the present study demonstrates that pramlintide-mediated weight loss in obese subjects is accompanied by improvements in eating behavior, including reductions in 24-h food intake, portion sizes, fast food intake, and binge eating tendencies.

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