Effects of prior or concurrent food restriction on amylin-induced changes in body weight and body composition in high-fat-fed female rats

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Roth JD, Hughes H, Coffey T, Maier H, Trevaskis JL, Anderson CM. Effects of prior or concurrent food restriction on amylin-induced changes in body weight and body composition in high-fat-fed female rats. Am J Physiol Endocrinol Metab 293: E1112–E1117, 2007. First published August 14, 2007; doi:10.1152/ajpendo.00395.2007.—Amylin infusion reduces food intake and slows body weight gain in rodents. In obese male rats, amylin (but not pair feeding) caused a preferential reduction of fat mass with protein preservation despite equal body weight loss in amylin-treated (fed ad libitum) and pair-fed rats. In the present study, the effect of prior or concurrent food restriction on the ability of amylin to cause weight loss was evaluated. Retired female breeder rats were maintained on a high-fat diet (40% fat) for 9 wk. Prior to drug treatment, rats were either fed ad libitum or food restricted for 10 days to lose 5% of their starting body weight. They were then subdivided into treatment groups that received either vehicle or amylin (100 μg·kg⁻¹·day⁻¹ via subcutaneous minipump) and placed under either a restricted or ad libitum feeding schedule (for a total of 8 treatment arms). Amylin 1) significantly reduced body weight compared with vehicle under all treatment conditions, except in always restricted animals, 2) significantly decreased percent body fat in all groups, and 3) preserved lean mass in all groups. These results indicate that amylin’s anorexigenic and fat-specific weight loss properties can be extended to a variety of nutritive states in female rats.

Amylin; diet-induced obese rats; food restriction; weight; body composition

IN RODENTS AND HUMANS, weight loss induced by reduced caloric intake has clearly been shown to trigger the activation of powerful compensatory mechanisms aimed at maintaining the initial body weight (13, 17). In animals these mechanisms have been shown to include an increased drive to eat, increased feed efficiency, decreased energy expenditure, and decreased fat oxidation, collectively resulting in rapid and efficient weight regain. These adaptations might also be expected to counteract the efficacy of anorexigenic weight loss agents. For example, the abilities of the norepinephrine/serotonin reuptake inhibitor sibutramine (10) and the melanocortin agonist MT-II to induce weight loss were somewhat diminished in weight-reduced rats relative to ad libitum-fed rats (23).

Amylin, a 37-amino acid peptide hormone that is cosecreted with insulin from pancreatic β-cells in response to food intake (28), has been shown to decrease food intake and weight in rodents (12, 19, 21, 22). Similarly, pramlintide, a synthetic analog of human amylin, has been shown to increase satiety, resulting in decreased food intake and weight loss in obese subjects and subjects with type 1 and type 2 diabetes (3, 8, 9, 16). To date, all studies investigating the body weight-reducing effects of amylin in rats have been conducted only in male animals with ad libitum access to food (19, 21). To determine the effects of food restriction on the ability of amylin to induce weight loss and to extend our previous findings, we tested the effects of amylin vs. vehicle treatment on a variety of dietary conditions in high-fat-fed female rats.

MATERIALS AND METHODS

Animal models. All studies were approved by the Institutional Animal Care and Use Committee at Amylin Pharmaceuticals, in accordance with Animal Welfare Act guidelines. Retired female breeder rats (Sprague-Dawley, n = 120) were fattened on high-fat food (TD 95217, 40% fat; Teklad, Madison, WI) at Harlan Sprague Dawley (Indianapolis, IN) for 7 wk, and the 58 rats that either gained the most weight or were the heaviest overall (mean body weight 316 g at start of testing) were selected for the study. These animals were acclimated at Amylin Pharmaceuticals for 2 wk prior to study initiation, and during that time all rats had ad libitum access to high-fat food. For gene expression analyses, male obesity-prone C57/6DIOBR Levin rats (Charles River Laboratories, Wilmington, MA) maintained on a moderately high-fat diet (32% kcal from fat; Research Diets D1226B) were utilized (mean body weight 545 g at start of testing). All rats were housed individually in shoebox cages at 22°C in a 12:12-h light-dark cycle.

Food restriction and drug delivery. Figure 1 illustrates the experimental procedure. Ad libitum food intake and body weight were measured during the last week of acclimation (day 17 to day 11) to establish mean baseline values. Rats were then divided into treatment groups counterbalanced for baseline food intake and body weight. One-half of the rats were placed on a food restriction schedule and were allowed access to 75% of their individual ad libitum mean daily food intake each day for 10 days (day −10 to day −1). On day 0, minipumps (Durect, Cupertino, CA) containing either amylin (100 μg·kg⁻¹·day⁻¹ or 25.3 nmol·kg⁻¹·day⁻¹) or vehicle (50% DMSO in sterile water) were surgically implanted (using aseptic techniques) in isoflurane-anesthetized rats subcutaneously between the shoulder blades, and food access was either ad libitum or restricted to 75% of individual baseline mean daily food intake for the next 17 days. For gene expression profiling, rats were divided into two weight-matched groups (n = 7/group) and implanted with pumps containing either amylin (100 μg·kg⁻¹·day⁻¹) or vehicle (50% DMSO in water) for the next 7 days, at which point animals were killed and tissues harvested for gene expression analyses.

Body composition measurements. Animals were briefly (measurements took less than 1 min) placed in a ventilated Plexiglas tube that was then inserted into a specialized rodent NMR machine (Echo Medical Systems, Houston, TX). Animals were scanned prior to minipump implantation (day 0) and on the final day of the experiment.

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Changes in percent body composition for fat and dry lean tissue were calculated.

\( \text{Plasma analyses.} \) Plasma was obtained from blood collected by cardiac puncture in deeply anesthetized rats that had been fasted for 3 h (postabsorptive, bled during the light cycle). Plasma leptin and amylin were measured using the Multiplex Luminex Assay (Millipore, Billerica, MA).

\( \text{Gene expression.} \) Liver tissue was removed after the animals had been euthanized and was immediately snap-frozen in liquid nitrogen and stored at \(-80\,^\circ \text{C}.\) Tissue was later homogenized using a FastPrep instrument (Qbiogene, Irvine, CA), and total RNA was extracted using Tri Reagent according to the manufacturer’s directions (Ambion, Austin, TX). Quantitative real-time PCR for genes of interest and 18S ribosomal RNA housekeeping gene was performed using premade Taqman gene expression assays and Taqman One-Step PCR Master Mix (Applied Biosystems, Foster City, CA) on an ABI PRISM 7900 sequence detection system (Applied Biosystems).

\( \text{Data analysis.} \) Graphs were generated using Prism 4 for Windows (GraphPad Software, San Diego, CA). Data points are expressed as means ± SE. Mean differences between amylin and vehicle for all end points were evaluated using one-way analysis of variance followed by contrasts. The contrasts were adjusted for multiple comparisons by using Hochberg’s method (7). The significance level of each test was \( \alpha = 0.05. \) All analyses were performed using SAS v. 8.2 (SAS Institute, Cary, NC). For gene expression analyses, direct comparisons between amylin-treated and vehicle control groups were assessed using a two-tailed Student’s \( t \)-test, and significance was assumed for \( P < 0.05 \) (GraphPad Software).

\( \text{RESULTS} \)

\( \text{Effects of amylin on food intake with or without food restriction.} \) To determine the effects of prior or concurrent food restriction on the ability of amylin to decrease food intake, diet-induced obese (DIO) female rats were either food restricted or fed ad libitum for 10 days prior to receiving a continuous amylin or vehicle infusion via minipump. Food-restricted animals lost an average of 5% of their baseline body weight. Following minipump implantation on day 0, animals were either food restricted or fed ad libitum until the end of the study 17 days later (Fig. 1). Food intake initially decreased in all amylin-treated animals and then gradually increased to reach levels similar to, but never surpassing, those observed in vehicle-treated animals by study end (Fig. 2). Cumulative food intake was significantly reduced in amylin-treated vs. vehicle-treated animals in all groups (Table 1).
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Cumulative food intake and changes in body weight, fat mass, and dry lean mass

<table>
<thead>
<tr>
<th>Food Access Before Treatment</th>
<th>Food Access During Treatment</th>
<th>Treatment (n)</th>
<th>Cumulative Food Intake, g*</th>
<th>Change In Body Weight, g</th>
<th>Change In Body Weight, %</th>
<th>Change In %Fat</th>
<th>Change In %Dry Lean Mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ad-lib</td>
<td>Ad-lib</td>
<td>Vehicle (7)</td>
<td>149.3 ± 5.1</td>
<td>−10.7 ± 1.2</td>
<td>−3.4 ± 0.3</td>
<td>−5.9 ± 0.4</td>
<td>0.4 ± 0.1</td>
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<tr>
<td></td>
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<td>Amylin (7)</td>
<td>101.9 ± 4.4</td>
<td>−29.6 ± 3.0</td>
<td>−9.7 ± 0.8</td>
<td>−10.1 ± 0.5</td>
<td>0.9 ± 0.1</td>
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<td></td>
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<td></td>
<td></td>
<td>*P &lt; 0.0001</td>
<td>*P = 0.0006</td>
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<td>*P &lt; 0.0001</td>
</tr>
<tr>
<td>Restricted</td>
<td>Restricted</td>
<td>Vehicle (7)</td>
<td>124.9 ± 4.8</td>
<td>−19.7 ± 3.3</td>
<td>−6.4 ± 0.8</td>
<td>−8.5 ± 0.5</td>
<td>0.5 ± 0.1</td>
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<tr>
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<td></td>
<td>Amylin (8)</td>
<td>101.2 ± 3.7</td>
<td>−26.0 ± 3.0</td>
<td>−8.7 ± 0.9</td>
<td>−11.1 ± 1.0</td>
<td>1.0 ± 0.1</td>
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<td>*P = 0.019</td>
<td>*P = 0.09</td>
<td></td>
<td>*P = 0.008</td>
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<tr>
<td>Restricted</td>
<td>Ad-lib</td>
<td>Vehicle (8)</td>
<td>172.5 ± 7.0</td>
<td>1.6 ± 4.0</td>
<td>0.5 ± 1.2</td>
<td>−3.6 ± 0.6</td>
<td>0.3 ± 0.1</td>
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<tr>
<td></td>
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<td>Amylin (7)</td>
<td>106.9 ± 6.5</td>
<td>−23.9 ± 3.7</td>
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<td>*P &lt; 0.0001</td>
<td>*P &lt; 0.0001</td>
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<td>*P &lt; 0.0001</td>
</tr>
<tr>
<td>Restricted</td>
<td>Restricted</td>
<td>Vehicle (7)</td>
<td>122.8 ± 2.4</td>
<td>−22.3 ± 1.9</td>
<td>−7.0 ± 0.5</td>
<td>−7.6 ± 0.3</td>
<td>0.6 ± 0.1</td>
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<tr>
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<td>Amylin (6)</td>
<td>93.3 ± 5.6</td>
<td>−34.7 ± 4.3</td>
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<td>*P = 0.0004</td>
<td>*P = 0.021</td>
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<td>*P = 0.014</td>
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*Cumulative food intake values are means ± SE from day 1 to day 17; all other values are means ± SE from day 0 to day 17. Hochberg’s correction was used to adjust P values for multiple comparisons.

Effects of amylin on body weight. Sustained infusion of amylin resulted in greater weight loss (measured from pump implantation on day 0 to study termination on day 17) compared with vehicle in all treatment groups, except in animals that were always food restricted (Table 1 and Fig. 3). In always-food-restricted amylin-treated animals, an initial decrease in weight, presumably due to a relatively large drop in food intake on days 1 and 2 of treatment (Fig. 2B), was followed by continuous weight loss paralleling the rate of weight loss observed in the vehicle-treated group.

Effects of amylin on body composition. NMR imaging was performed on days 0 and 17 to measure body fat and dry lean mass. In all groups, amylin treatment resulted in a greater reduction in body fat percentage than vehicle treatment (Table 1). Of particular note, the always-restricted amylin-treated group exhibited greater body fat reduction than the vehicle control group despite comparable weight loss between the two treatment groups. In contrast, the proportion of dry lean mass significantly increased in all amylin-treated groups vs. vehicle, except in animals fed ad libitum and then restricted (Table 1).

Plasma amylin and leptin levels. Plasma amylin levels were elevated ~10-fold compared with vehicle controls (Table 2). Importantly, a similar level of plasma amylin was achieved across all amylin-treated groups. To examine the relationship between amylin treatment and leptin, a hormone secreted by adipose tissue in direct correlation with adipose tissue mass (14), plasma leptin levels were measured at study end. Amylin treatment resulted in lower plasma leptin levels in animals fed ad libitum during the treatment period, regardless of food access prior to treatment, correlating with lower body fat percentages (Table 2).

Gene expression profiling. To begin to elucidate possible mechanisms whereby amylin exerts its weight- and adipose-reducing effects, the expression levels of key hepatic genes involved in fatty acid oxidation and synthesis were examined following continuous infusion of amylin in obese male rats. After 1 wk of continuous amylin infusion, which significantly reduced body weight (by 6% relative to vehicle, P < 0.001; data not shown), and food intake (by 43% relative to vehicle, P < 0.001; data not shown), hepatic expression of the key lipogenic gene stearoyl coenzyme-A desaturase-1 (Scd1) was significantly reduced relative to vehicle controls (Fig. 4A). Expression of another biosynthetic gene, fatty acid synthase (Fas), however, was not affected by amylin therapy (Fig. 4B).

Additionally, uncoupling protein-2 (Ucp2) mRNA in the liver was modestly, though not significantly, increased compared with vehicle (P = 0.069; Fig. 4C). Overall, the expression profile of these genes supports a role for amylin therapy in reducing fatty acid synthesis and preserving metabolic function, which could explain, at least in part, the observation of enhanced loss of adipose tissue with amylin treatment.

DISCUSSION

This study demonstrates the ability of amylin to reduce body weight with an associated reduction in adiposity and preservation of lean tissue under a variety of dietary conditions in intact female rats. Specifically, 1) the anorexigenic effects of amylin were evident regardless of prior and/or concurrent food restriction; 2) the weight loss effects of amylin were evident across all conditions, with the exception of always-restricted conditions (although amylin still tended to reduce body weight); and 3) body weight loss was always accompanied by a significant reduction in body fat, with a preservation of lean mass relative to total body weight. The significance of these findings is discussed below.

In agreement with the known actions of amylin (22, 28), cumulative food intake was significantly reduced in amylin-treated animals compared with vehicle controls in all food access groups. Strikingly, decreases in food intake were most pronounced during the first week of amylin treatment, when one might have predicted that the effects of amylin on food intake would have been blunted due to increased hunger following food restriction. Although food intake rebounded somewhat toward the end of the treatment period, intake never surpassed that of vehicle controls. Cumulative food intake was surprisingly similar among all amylin-treated animals, suggesting that amylin may somehow reset food intake to a particular value regardless of food access prior to and during treatment. The effects of other anorexigenic peptides, such as cholecystokinin (CCK), on food intake have been shown to be influenced by changes in endogenous estrogen (2, 6). As the present studies did not control the phase of the estrous cycle, the potential impact of estradiol levels on the efficacy of amylin therapy remains to be elucidated.
Reductions in food intake correlated with weight loss, supporting previous observations that the weight loss effect of amylin is primarily due to its anorexigenic properties (19). There was an unexplained trend for vehicle controls to decrease food intake and body weight over the course of the study, possibly due to the age of the animals used. However, it is important to note that amylin treatment had a clear experimental effect above and beyond that of vehicle treatment. Under always-ad libitum conditions, amylin treatment resulted in a sustained decrease in body weight, consistent with previous studies that also showed that the weight loss effect of amylin is of similar magnitude in lean and DIO rats (19, 21). Food restriction prior to treatment caused animals to lose ~5% of their initial body weight, and amylin treatment clearly prevented weight regain when these rats were allowed to feed ad libitum. The percent weight loss achieved when amylin was administered to ad libitum-fed animals with prior food restriction (~8.0%) was not significantly different from that observed in amylin-treated animals always fed ad libitum (~9.7%). When amylin was administered concurrently with food restriction to rats previously fed ad libitum, weight loss was more dramatic than in vehicle controls. These preclinical data suggest that amylin agonists could be effective in both the induction of weight loss and the prevention of weight regain following calorie-restricted dieting. It was only when rats were restricted prior to and during amylin treatment that effects on body weight were not significantly different from those of vehicle treatment, suggesting that, at an already reduced body weight, amylin treatment concomitant with food restriction may not override counterregulatory mechanisms.

Corresponding to the aforementioned results, pramlintide, a synthetic analog of amylin, significantly reduced weight in obese subjects compared with placebo when administered over 4 mo in the absence of lifestyle intervention (26, 27). Another clinical study showed that pramlintide administered to obese subjects in conjunction with lifestyle intervention geared toward weight loss (including diet and exercise) resulted in significantly greater weight loss over 4 mo compared with placebo plus lifestyle intervention (1, 25). Additionally, continuation of pramlintide administration in conjunction with lifestyle intervention geared toward weight maintenance enabled subjects to maintain the initial weight loss. In contrast, the initial weight loss was largely regained in subjects receiving placebo during this extension phase despite receiving the same lifestyle intervention (24).

An examination of body composition found that amylin-treated animals under all food access conditions had a larger reduction in body fat percentage compared with vehicle controls, even when body weight loss was similar. This implies that the effects of amylin on adiposity may be additive to the effects of food restriction. In male DIO rats, pair-fed controls exhibited weight loss similar to that of amylin-treated animals, but they lost dry lean mass and did not lose as much fat as the amylin-treated animals (19). However, no notable changes in genes involved in lipid metabolism or energy balance were observed after 3 wk of amylin infusion in DIO rats, possibly because they were measured when weight loss had already reached a plateau (19). Here, we measured changes in hepatic genes during the first week of amylin administration (during active weight loss). Gene expression analysis in liver implied that reduced fatty acid synthesis (reduced Scd1) as well as an increase in, or maintenance of, metabolism (modestly increased Ucp2) may at least partly explain the amylin-induced reduction in adiposity. Furthermore, amylin-dependent effects on body composition are unlikely to be due to direct effects of

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**Fig. 3.** Body weight was measured on each day of the study (day −16 to day 17), and the change in body weight from the day of pump implantation (day 0) is graphically depicted as the mean change ± SE for animals fed always ad-lib (A), always restricted (B), restricted then ad-lib (C), and ad-lib then restricted (D). Mean change in weight (±SE) from day 0 to day 17 is shown to the right of each treatment line.
amylin on adipose tissue (11, 19). As Fas expression was not altered by amylin therapy, further examination of the possible role of amylin in the regulation of fatty acid biosynthesis is required. The present results extend the observations of amylin’s effects on body composition to several nutritive conditions in female rats and begin to build a link to amylin-induced modulation of hepatic gene expression during active weight loss.

Leptin, a hormone that suppresses food intake and enhances energy expenditure (5), is secreted by adipose tissue in proportion to adipose tissue mass (14). Thus, a decrease in body fat percentage would be expected to result in a reduction in the amount of leptin circulating in the bloodstream. Indeed, amylin-treated animals fed ad libitum had lower serum leptin concentrations than vehicle-treated animals, correlating with reduced body fat percentages. Interestingly, amylin-treated animals had similar body fat percentages and leptin concentrations regardless of food access. Considering the differences in terminal body fat percentage between amylin and vehicle-treated animals under food-restricted conditions at study end, leptin concentrations were higher than might be expected in amylin-treated animals. One possible explanation for this is that amylin may have prevented a reduction in serum leptin in order to guard against counterregulatory effects, including increased food intake. Amylin may also have restored sensitivity to leptin, ultimately translating into additional fat-specific body weight loss. This notion is consistent with the observations that 1) leptin-deficient animals show reduced responsiveness to the amylin agonist salmon calcitonin (4); 2) the acute anorexigenic properties of amylin are amplified by intracerebroventricularly applied leptin (15); and 3) combined chronic administration of amylin and leptin synergistically decreased food intake and body weight in DIO leptin-resistant rats (18, 20).

In clinical trials studying pramlintide in obese subjects (1, 24–27) and insulin-treated subjects with diabetes (8), pramlintide treatment resulted in significant reductions in body weight (9). On the basis of these findings, the potential utility of amylinomimetics as antiobesity agents is currently being evaluated. Obese patients taking an amylinomimetic as a weight loss agent might 1) already be dieting, 2) be asked to use the drug in conjunction with dieting, or 3) be asked to use the drug in conjunction with dieting but not conform to the diet. The results of the current preclinical study suggest that amylinomimetics may elicit a weight loss effect under any of these conditions, providing additional support for their evaluation as weight loss agents.

**DISCLOSURES**

All of the coauthors of this paper were employed by and held stock in Amylin Pharmaceuticals, Inc. Heather Hughes and Christen Anderson are no
REFERENCES


