Neural Integration of Peripheral Signals Implicated in the Control of Energy Homeostasis and Metabolism

Leptin extends the anorectic effects of chronic PYY(3-36) administration in ad libitum-fed rats

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Unniappan S, Kieffer TJ. Leptin extends the anorectic effects of chronic PYY(3-36) administration in ad libitum-fed rats. Am J Physiol Regul Integr Comp Physiol 295: R51–R58, 2008. First published April 16, 2008; doi:10.1152/ajpregu.00234.2007.—Acute administration of peptide YY(3-36) [PYY(3-36)] results in a reduction in food intake in several different vertebrates. However, long-term continuous administration of PYY(3-36) causes only a transient reduction in food intake, thus potentially limiting its therapeutic efficacy. We hypothesized that a fall in leptin levels associated with reduced food intake could contribute to the transient anorectic effects of continuous PYY(3-36) infusion and thus that leptin replacement might prolong the anorectic effects of PYY(3-36). Seven-day administration of 100 μg/kg body wt \(^{-1}\) day \(^{-1}\) PYY(3-36) using osmotic minipumps caused a significant reduction in food intake of ad libitum-fed rats, but only for the first 2 days postimplantation. Circulating levels of leptin were reduced 1 day following continuous infusion of PYY(3-36), and combined leptin infusion at a dose of leptin that had no anorectic effects on its own (100 μg·kg body wt \(^{-1}\) day \(^{-1}\)) prolonged the anorectic actions of PYY(3-36) in ad libitum-fed rats for up to 6 days postimplantation and yielded reduced weight gain compared with either peptide alone. The inhibitory effects of 100 μg·kg body wt \(^{-1}\) day \(^{-1}\) PYY(3-36) on food intake were absent in rats refed after a 24-h fast and substantially reduced at a dose of 1,000 μg·kg body wt \(^{-1}\) day \(^{-1}\) PYY(3-36). Leptin replacement was unable to recover the anorectic effects of PYY(3-36) in fasted rats. Our results suggest that an acute fall in leptin levels is not solely responsible for limiting duration of action of chronic PYY(3-36) infusion, yet chronic coadministration of a subanorectic dose of leptin can extend the anorectic effects of PYY(3-36).

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and a temporary decrease in plasma leptin levels; 2) fasting attenuates the satiety effects of PYY(3-36) and leptin; and 3) an acute reduction in leptin levels does not appear to be critical in limiting the anorectic actions of continuous PYY(3-36) administration, yet coadministration of leptin, at a dose that alone is ineffective in reducing food intake can extend the anorectic action of PYY(3-36).

MATERIALS AND METHODS

Animals. Fischer 344 rats were purchased from Charles River Laboratories (Saint-Constant, Quebec, Canada). Age- and weight-matched (mean = 224.70 ± 3.77 g) male rats were used for all studies, and unless otherwise mentioned were individually housed in grid cages in a 12:12-h light (7 AM-7 PM)-dark (7 PM-7 AM) photoperiod at 23 ± 1°C and controlled humidity in the animal care facility of University of British Columbia or York University (for the study shown in Fig. 3). Unless otherwise mentioned, animals had ad libitum access to tap water and rat chow (Purina Mills, St. Louis, MO). Fresh rat chow was provided daily between 5 and 7 PM on the floor of grid cages, and this allowed us to collect residual food particles on a tray kept beneath cages to monitor food intake. For studies described in Fig. 3, food was provided in the feeder of regular rat cages. Research protocols used in this study adhered to the guidelines of the Canadian Council for Animal Care and were approved by the Animal Care Committee of the University of British Columbia or York University.

We have previously shown that continuous administration of PYY(3-36) using osmotic minipumps inhibits food intake of acclimatized rats (37). Therefore, in this study, acclimatization of rats, subcutaneous implantation of pumps, and monitoring of food intake and body weight were conducted as described previously (37). Each study used a different cohort of acclimatized rats, and in each study a group of rats that received saline infusing pumps served as controls. Rats were housed in grid cages or regular cages for 3 days from the day of arrival and then were acclimatized for 7 days. Starting on day 1 of acclimatization, animals were transferred to the procedure room on a cart, anesthetized using 3% isoflurane using oxygen as gaseous carrier, and shaved in the area where the incision was to be made and weighed. All of the above acclimatization procedures except shaving were repeated for the next 6 days. On the surgery and implantation day (day 7 of acclimatization), rats were anesthetized, a small subcuticular incision was made, and osmotic minipumps were implanted subcutaneously. Following implantation, wounds were immediately sealed using wound clips, and antiseptics were administered using a cotton swab. Rats were then removed from the anesthetic machine, weighed, and returned to their cages and allowed to recover from anesthesia. Animals were returned to the animal care facility, preweighed quantities of food were given, and food intake was measured by deducting the quantity of food recovered after 24-h feeding from the initial amount of food given.

Materials. Rat PYY(3-36) was purchased from Phoenix Pharmaceuticals (Belmont, CA). Human PYY(3-36) was synthesized at the Peptide Synthesis Unit, Biomedical Research Centre, University of British Columbia, Vancouver, Canada. Recombinant mouse leptin was purchased from Dr. A. F. Parlow (National Hormones and Peptides Program, Harbour-UCLA Medical Center, Los Angeles, CA). All peptides were HPLC purified to >95% purity. Peptides were lot matched when multiple vials were required in a study and were freshly prepared in 0.9% saline for each study. We found that both rat PYY(3-36) and human PYY(3-36) are equipotent in reducing food intake and weight gain of rats (S. Unniappan and T. J. Kieffer, unpublished results), and hence we used rat or human PYY(3-36) in our studies as indicated. One-day (model 2001D), 7-day (model 2ML1), and 14-day (model 2ML2) Alzet osmotic minipumps were purchased from Durect (Cupertino, CA). Rat/mouse leptin ELISA kit (cat. no. 022-LEP-E06) was purchased from Linco Research (Windham, NH).

Effects of continuous infusion of PYY(3-36) on food intake of ad libitum-fed rats. Fourteen-day pumps infusing saline or rat PYY(3-36) (100 μg·kg body wt⁻¹·day⁻¹ = 25 nmol·kg body wt⁻¹·day⁻¹) were implanted into acclimatized rats, and daily food intake was monitored for 7 days. Our previous results (37) indicated that continuous infusion of PYY(3-36) for 7 days yields only a transient reduction in food intake during the treatment period. To eliminate the possibility that the transient effects were due to pump failure or loss of activity of the PYY(3-36), in this study we removed both saline and PYY(3-36) pumps on day 7 and reimplanted them into two new groups of acclimatized rats. These new recipients were then monitored for daily food intake for 7 days (to day 14 of the pump).

Effects of continuous infusion of PYY(3-36) on plasma leptin levels of ad libitum-fed rats. We conducted this experiment to test the hypothesis that administration of PYY(3-36) results in a reduction in circulating levels of leptin and that this fall in leptin may attenuate the effects of continuous PYY(3-36) administration on daily food intake of rats. Since the primary aim of this study was to collect plasma samples, ad libitum-fed rats were housed three rats per cage. Seven-day pumps infusing saline or rat PYY(3-36) (100 μg·kg body wt⁻¹·day⁻¹) were implanted into acclimatized ad libitum-fed rats, blood samples (250 μl) were collected daily by tail bleeding from all animals at 3:30–4:15 PM, and plasma was separated by centrifugation at 10,000 g for 9 min and stored at −20°C until assay for leptin was conducted following the manufacturer’s protocol.

Fig. 1. Effects of continuous infusion of saline (○) or 100 μg·kg body wt⁻¹·day⁻¹ peptide YY(3-36) [PYY(3-36)] (●) on daily food intake (A) of male Fischer 344 rats when infused using 14-day osmotic minipumps. Pumps were recovered from the animals after 7 days and reimplanted into another group of rats, and their daily food intake was measured (B). Data are presented as means ± SE. *Statistically significant difference compared with saline controls; n = 6 rats/group.
Determination of a subanorectic dose of leptin in ad libitum-fed rats. To find a subanorectic dose of leptin in rats, saline or leptin at 100, 200, 400, or 800 μg·kg body wt⁻¹·day⁻¹ was infused for 24 h using 1-day osmotic minipumps. Twenty-four-hour cumulative food intake and body weight were monitored. In a second study, leptin levels were determined in plasma samples from saline and leptin (100 μg·kg body wt⁻¹·day⁻¹) infused ad libitum-fed animals at 0 (9 AM), 12 (9 PM), and 24 h (9 AM next day) postpump implantation.

Effects of coadministration of leptin and PYY(3-36) on food intake and body weight of ad libitum-fed rats. To test whether coadministration of leptin can prolong the ability of PYY(3-36) to inhibit food intake of ad libitum-fed rats, saline, leptin (100 or 200 μg·kg body wt⁻¹·day⁻¹), human PYY(3-36) (100 or 1,000 μg·kg body wt⁻¹·day⁻¹), or a combination of both leptin and human PYY(3-36) [100 μg·kg body wt⁻¹·day⁻¹ PYY(3-36) + 100 μg·kg body wt⁻¹·day⁻¹ leptin, 100 μg·kg body wt⁻¹·day⁻¹ PYY(3-36) + 200 μg·kg body wt⁻¹·day⁻¹ leptin, or 1,000 μg·kg body wt⁻¹·day⁻¹ PYY(3-36) + 100 μg·kg body wt⁻¹·day⁻¹ leptin] was infused. In one study, the leptin administration was for 1 day (n = 4 per group); in other studies it was for 7 days (n = 6 per group). Daily food intake and body weight were monitored for 7 days. In the coadministration studies, a single pump was used to deliver both PYY(3-36) and leptin when both peptides were administered for 7 days, while separate pumps were used when leptin was delivered only 1 day.

Effects of leptin replacement on satiety actions of PYY(3-36) in rats refed after a 24-h fast. We hypothesized that the anorectic actions of PYY(3-36) may be attenuated in fasting rats as a result of a fasting-induced fall in leptin levels that might override the actions of PYY(3-36). We tested this via leptin replacement during PYY(3-36) treatment in fasted rats. Rats were fasted for 24 h, and osmotic minipumps infusing saline, leptin (100, 200, 400 or 800 μg·kg body wt⁻¹·day⁻¹), human PYY(3-36) (100 or 1,000 μg·kg body wt⁻¹·day⁻¹), or a combination of leptin and human PYY(3-36) [100 μg·kg body wt⁻¹·day⁻¹ PYY(3-36) + 400 μg·kg body wt⁻¹·day⁻¹ leptin or 1,000 μg·kg body wt⁻¹·day⁻¹ PYY(3-36) + 400 μg·kg body wt⁻¹·day⁻¹ leptin] were implanted at the end of fasting. Twenty-four-hour cumulative food intake and body weight were monitored in all studies. In the coadministration study, a single pump was used to deliver both PYY(3-36) and leptin.

Statistical analyses. All data are presented as means ± SE. Data were analyzed using t-test or ANOVA followed by Student-Newman-Keuls or Tukeys multiple comparison post hoc test as indicated. P < 0.05 was considered statistically significant. Graphing and statistical analyses were conducted using GraphPad Prism version 4 (GraphPad Software, San Diego, CA).
RESULTS

Effects of continuous infusion of PYY(3-36) on food intake of ad libitum-fed rats. A significant reduction in food intake was seen in 100 μg·kg body wt⁻¹·day⁻¹ PYY(3-36)-treated rats on day 1 (control, 17.58 ± 0.99 g vs. treatment, 13.58 ± 0.27 g; P < 0.01) and day 2 (control, 17.44 ± 0.87 g vs. treatment, 14.62 ± 0.49 g; P < 0.01) postimplantation of osmotic minipumps; but food intake reached control levels by day 3 postimplantation (Fig. 1A). However, reimplantation of PYY(3-36) pumps recovered from these rats into a new group of rats again resulted in a significant reduction in food intake for 2 days postimplantation of the pumps (day 1 = control, 18.58 ± 0.62 g vs. treatment, 15.64 ± 0.50 g; day 2 = control, 18.25 ± 0.62 g vs. treatment, 15.94 ± 0.21 g; P < 0.05; Fig. 1B), with food intake returning to control levels on day 3 postimplantation.

Effects of continuous infusion of PYY(3-36) on plasma leptin levels of ad libitum-fed rats. A significant reduction in plasma leptin levels was found in 100 μg·kg body wt⁻¹·day⁻¹ PYY(3-36)-treated rats on day 1 postimplantation (control, 5.28 ± 0.6 vs. PYY, 3.9 ± 0.42 ng/ml, P < 0.05, Fig. 2A), but leptin levels were normal on day 2 postimplantation and were significantly increased in PYY(3-36)-treated rats on day 3 postimplantation (control, 5.79 ± 0.45 vs. PYY, 7.28 ± 0.3 ng/ml, P < 0.05, Fig. 2A).

Determination of a subanorectic dose of leptin in ad libitum-fed rats. Compared with food intake of saline-treated rats (19.22 ± 0.63 g), leptin (200, 400, and 800 μg·kg body wt⁻¹·day⁻¹) significantly reduced food intake (16.22 ± 0.86 g, P < 0.05; 14.70 ± 0.18 g, P < 0.01; 16.18 ± 0.63 g, P < 0.05, respectively, Fig. 2B), while the leptin dose of 100 μg·kg body wt⁻¹·day⁻¹ did not. Among the doses tested, only 400 μg·kg body wt⁻¹·day⁻¹ leptin caused a significant reduction in body weight (control, 2.26 ± 0.94 g vs. treatment, 0.06 ± 0.32 g; P < 0.05, Fig. 2C). Circulating levels of leptin in 100 μg·kg body wt⁻¹·day⁻¹ leptin-treated rats were significantly higher than control rats at 12 h (control, 16.13 ± 2.03 ng/ml vs. treatment, 59.23 ± 4.81 ng/ml, P < 0.0001) and 24 h (15.06 ± 2.03 ng/ml vs. 39.67 ± 3.19 ng/ml, P < 0.0001) postimplantation of pumps (Fig. 2D).

Effects of coadministration of both leptin and PYY(3-36) on food intake and body weight of ad libitum-fed rats. PYY(3-36) alone at 100 μg·kg body wt⁻¹·day⁻¹ (Figs. 3A and 4, A and C) and 1,000 μg·kg body wt⁻¹·day⁻¹ (Fig. 4E) caused a transient reduction in food intake of rats, compared with saline controls. Leptin alone at 100 μg·kg body wt⁻¹·day⁻¹ did not cause any effects on food intake (Fig. 4A), while 200 μg·kg body wt⁻¹·day⁻¹ leptin caused a significant reduction in food intake compared with saline-treated controls during the study period (Fig. 4C). Coadministration of 100 μg·kg body wt⁻¹·day⁻¹ leptin on day 1 during the 7-day infusion of 100 μg·kg body wt⁻¹·day⁻¹ PYY(3-36) did not prolong the anorectic effects of PYY(3-36) compared with saline-treated rats or rats that received PYY(3-36) alone (Fig. 3A), although food intake was significantly (P < 0.05) reduced on day 2 by the coadministration with leptin compared with PYY(3-36) alone. However, coadministration of this same dose of leptin for the full 7 days with either 100 μg·kg body wt⁻¹·day⁻¹ PYY(3-36) (Fig. 4A) or 1,000 μg·kg body wt⁻¹·day⁻¹ PYY(3-36) (Fig. 4E) prolonged the anorectic effects of PYY(3-36) compared with saline-treated rats or rats that received either peptide alone.

Relative to saline, administration of 100 μg·kg body wt⁻¹·day⁻¹ PYY(3-36) significantly reduced 7-day cumulative weight gain (17.15 ± 0.76 g vs. 10.88 ± 1.96 g, P < 0.01, Fig. 4B; 21.16 ± 0.63 g vs. 17.6 ± 1.02 g, P < 0.05, Fig. 4D and 26 ± 0.77 g vs. 21.48 ± 1.32 g, P < 0.05, Fig. 4F) as did leptin alone at 100 μg·kg body wt⁻¹·day⁻¹ (26.77 ± 0.27 g vs. 20 ± 1.79 g, P < 0.05, Fig. 4F) and 200 μg·kg body wt⁻¹·day⁻¹ (21.16 ± 0.63 g vs. 11.31 ± 1.25 g, P < 0.001, Fig. 4D). A combination of 100 μg·kg body wt⁻¹·day⁻¹ leptin and PYY caused a significant reduction in body weight (10.0 ± 2.28 g; P < 0.01; Fig. 4B) compared with saline controls (17.15 ± 0.76 g; Fig. 4B), but this reduction was not greater than that caused by PYY or leptin alone. Weight gain of rats was further diminished relative to controls when either the leptin or PYY doses was increased [100 μg·kg body wt⁻¹·day⁻¹ PYY(3-36) + 200 μg·kg body wt⁻¹·day⁻¹ leptin; 8.11 ± 1.37 g vs. 21.16 ± 0.63 g, P < 0.001, Fig. 4D] and 1,000 μg·kg body wt⁻¹·day⁻¹ PYY(3-36) + 100 μg·kg body wt⁻¹·day⁻¹ leptin (11.7 ± 1.61 g vs. 26 ± 0.77 g, P < 0.001, Fig. 4F). These decreases caused by the coadministration of leptin and PYY(3-36) were also greater compared with the

Fig. 3. Effects of saline, PYY(3-36) (100 μg·kg body wt⁻¹·day⁻¹) or a combination of PYY(3-36) and leptin (100 μg·kg body wt⁻¹·day⁻¹) on 24-h cumulative food intake (A) and 7-day cumulative weight gain (B) of ad libitum-fed rats. Arrow indicates the day of pump implantation. In the coadministration group, PYY(3-36) was infused using a 7-day pump and leptin was administered using a separate 1-day pump. Data are presented as means ± SE.

*Statistically significant difference in the food intake or body weight of the PYY(3-36) and PYY(3-36)+leptin groups compared with saline controls.

³Statistically significant difference in the food intake of the PYY(3-36)+leptin group compared with the PYY(3-36) alone group, n = 4 rats/group.
weight gain of rats that received leptin or PYY(3-36) alone (Fig. 4, D and F).

Effects of leptin replacement on satiety actions of PYY(3-36) in rats fed after a 24-h fast. Infusion of 100 μg·kg body wt⁻¹·day⁻¹ PYY(3-36), an anorectic dose of PYY(3-36) in ad libitum-fed rats, was ineffective in reducing food intake in fasted rats (control, 23 ± 1.61 g vs. treatment, 21.7 ± 1 g, Fig. 5A). Administration of 1,000 μg·kg body wt⁻¹·day⁻¹ PYY(3-36) only caused ~30% reduction in food intake in fasted rats (control, 22.78 ± 1.01 g vs. treatment, 15.76 ± 0.77 g, $P <
0.01, Fig. 5C), whereas the same dose reduced day 1 food intake ~42% reduction in fed rats (control, 17.26 ± 0.59 vs. treatment, 10.11 ± 0.46 g, P < 0.001, Fig. 4E). Leptin alone reduced food intake in the fasted rats when used at a dose of 800 μg·kg body wt⁻¹·day⁻¹ (control, 23 ± 1.61 g vs. leptin, 19.44 ± 1.34 g, P < 0.05, Fig. 5A) but not at 400 μg·kg body wt⁻¹·day⁻¹. Moreover, in contrast to the results obtained with fasted rats (Fig. 4, D and F), coadministration of this relatively high dose of leptin (400 μg·kg body wt⁻¹·day⁻¹) and 1,000 μg·kg body wt⁻¹·day⁻¹ PYY(3-36) did not cause a greater reduction in weight gain compared with fasted rats that received PYY(3-36) alone (Fig. 5D). Leptin administration did not restore the anorectic effects of 100 μg·kg body wt⁻¹·day⁻¹ PYY(3-36) in fasted rats (Fig. 5A).

**DISCUSSION**

Our results provide further evidence for the anorectic effects of PYY(3-36) in lean rats. The magnitude of reduction in food intake we found during PYY(3-36) administration was similar to that seen in other studies that delivered PYY(3-36) by osmotic pumps (4, 5). Despite the transient reduction in food intake we observed with chronic administration of PYY(3-36), the explant and reimplant studies indicate that PYY(3-36) remained biologically active while in the pumps for at least 9 days. The results of the present study are in agreement with our own previously published data (37) and results of others (19), which indicate that chronic administration of PYY(3-36) causes a transient reduction in food intake in rats. Concomitantly with reduced food intake, 7-day continuous infusion of PYY(3-36) resulted in a significant reduction in circulating leptin levels of ~35%. Unexpectedly, the reduction in plasma leptin levels was exclusive to 1-day postimplantation, reaching control levels on day 2. Interestingly, plasma leptin levels were significantly higher in the PYY(3-36)-treated rats compared with saline-treated controls on day 3 postimplantation of the pumps. This unanticipated increase might be a delayed compensatory response to the initial fall in leptin or caused by other physiological processes. Further studies are required to explore the possibility that PYY(3-36) modulates leptin release.

Chelikani et al. (21) found that daily intermittent infusion of PYY(3-36) caused a more prolonged decrease in food intake and body weight of lean rats than our findings with continuous PYY(3-36) delivery. These findings suggest that receptor desensitization or downregulation are two possible reasons why chronic administration of PYY(3-36) fails to cause a sustained reduction in food intake and body weight. However, it is also possible that the transient reduction in leptin levels during PYY(3-36) administration may have contributed to the lack of sustained anorectic effects of PYY(3-36) during chronic infusion. We hypothesized that the anorectic effects of
PYY(3-36) could be prolonged by preventing this transient fall in leptin. First, we found a dose of leptin (100 g kg body wt\(^{-1}\cdot\text{day}^{-1}\)) that on its own does not reduce food intake, despite yielding vastly increased circulating levels. Then, we coinfused this subanorectic dose of leptin for 1 day during a 7-day PYY(3-36) administration and found that preventing the transient fall in leptin by exogenous administration was unable to extend the anorectic effects of PYY(3-36). However, in a series of experiments shown in Fig. 4, we found that the coinfusion of this subanorectic dose of leptin for the entire duration of the PYY(3-36) infusion caused a prolonged reduction in food intake in ad libitum-fed rats. The reduction in body weight was greater in rats coinfused with certain doses of leptin and PYY(3-36) compared with rats treated with saline, leptin, or PYY(3-36) alone. While the prolonged reduction in food intake during coadministration of PYY(3-36) and leptin undoubtedly contributed to a greater reduction in body weight, effects of this peptide combination on other aspects of metabolism including energy expenditure, energy conversion, and physical activity might also have contributed.

The anorectic effects of PYY(3-36) are believed to be mediated through the distinct regions of the brain (1, 2). PYY(3-36) stimulates c-fos mRNA expression in a subset of neurons in the nucleus tractus solitarius of mice (25), and PYY(3-36) administration to humans modulates neural activity within both corticolimbic and higher cortical areas as well as homeostatic brain regions including the hypothalamus (13). It is possible that leptin modulates the actions of PYY(3-36) in one or more of these locations. Leptin action in the forebrain has been demonstrated to regulate the hindbrain responses to the satiety effects of CCK (32). This finding led the authors to propose that forebrain signaling by leptin limits food intake on a meal-to-meal basis by regulating the hindbrain response to short-acting satiety signals. Further investigations are required to unravel the precise mechanisms by which leptin and PYY(3-36) interact.

Fasting results in a reduction in plasma leptin levels (16, 26, 29). We found the satiety effects of PYY(3-36) were attenuated in fasted rats during a 24-h refeeding period. Previously it was demonstrated that the fasting-induced fall in leptin results in attenuation of the satiety effects of acute administration of CCK (29). However, unlike the response to CCK in that study, we were unable to recover the effects of PYY(3-36) on food intake in fasted rats after leptin replacement. This suggests that in fasted rats, factors in addition to leptin are involved in regulating the satiety effects of PYY(3-36). The fasting-induced fall in plasma leptin levels also does not appear to alter PYY levels (18). Fasting causes several other neuroendocrine changes, including changes in the expression of several appetite regulatory peptides and their receptors in the brain (26). Our results indicate that anorectic effects of both PYY(3-36) and leptin are blunted during fasting and exogenous leptin fails to reinstate the satiety effects of PYY(3-36) in fasted rats.

**Perspectives and Significance**

Our studies demonstrate the enhanced anorectic and weight-reducing effects of coadministration of leptin and PYY(3-36) and suggest this hormone combination could represent a promising approach to promote weight loss. However, the appetite regulatory effects of several peptides are species specific, and it has been shown that even PYY(3-36) itself displays varying degrees of anorectic potency among rodents (33). Therefore, further studies will be required to examine the effects of coadministration of PYY(3-36) and leptin in other animals. The weight loss effects of this therapy are predicted to be less in obese subjects who are leptin resistant. However, as recently demonstrated by Shapiro et al. (35), even a modest amount of physical activity may be able to reverse leptin resistance and thus would be anticipated to enhance the efficacy of a leptin-PYY combination therapy. Future studies are warranted to elucidate the mechanisms by which leptin prolongs the anorectic effects of PYY(3-36) along with the neuroendocrine factors responsible for attenuating the satiety effects of PYY(3-36) and leptin during fasting.

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