Negative Effect of Chronic High-fat Diet Consumption on Postprandial Plasma Peptide YY Levels

by

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ABSTRACT

Introduction Peptide YY (PYY) is a satiety hormone that is released from the small intestine following consumption of nutrients such as carbohydrates, protein, and dietary fats. Chronic excess energy consumption leads to suppressed satiety signaling. Therefore, we hypothesized that chronic consumption of high amounts of dietary fat stimulates PYY release while promoting rate of fat absorption. **Methods** In this study, male C57BL/6 mice (age 8 weeks) were given either chow or a high-fat diet (HF) *ad libitum* for twelve weeks (n = 6/group). Mice were weighed and their food intake was measured weekly. Mice were fasted and given a high-fat liquid meal challenge via oral gavage following the twelve-week trial. Postprandial tail vein blood was collected and PYY levels were measured by an ELISA assay. Mice were euthanized and tissues were collected for further analyses. **Results** As predicted, HF-fed mice gained significantly more weight than standard chow-fed mice (38.3+/-1.8 vs. 29.8+/-1.6; p=0.0002). To our surprise, HF-fed mice displayed significantly reduced basal and postprandial plasma PYY compared with chow mice (2808.9 ±290.5 pg/ml and 3640.7 ±605.5 pg/ml, respectively). Intriguingly, HF-fed mice displayed elevated intestinal PYY expression. **Conclusion** Our observations indicate that PYY levels are decreased in high-fat mice. Thus, this study provides evidence supporting the hypothesis that chronic high fat consumption promotes enhanced fat digestion and absorption, which ultimately leads to increased food intake and obesity.

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INTRODUCTION

Obesity is a leading preventable cause of death in the United States. Nearly two-thirds of adult Americans are overweight or obese (Marks, 2004a). While obesity is a significant cause of morbidity and mortality in developed nations, it is rapidly becoming a global issue. Obesity is linked with many diseases, including hypertension, dyslipidemia, cardiovascular disease, stroke, diabetes mellitus, and certain cancers (Cooper, 2014). Cooper (2014) attributes the growing epidemic of obesity "primarily to lifestyle factors, specifically by decreases in physical activity, increases in total energy and fat intake, and the interaction of these environmental factors with genetic susceptibility (p. 186)." This suggests that the growing epidemic of obesity is preventable. Despite an abundance of evidence indicating the benefits of maintaining a healthy weight and a physically active lifestyle, an increasing number of Americans continue to eat larger portion sizes than needed and remain less physically active than they should be (Marks, 2004a). According to the American Diabetes Association, sedentary adults in the United States eat an average of 500–800 calories more than needed per day to maintain their weight. In order to lose/gain one pound, 3500 calories must be burned or consumed, respectively. Therefore, without proper diet and exercise, the average American gains 2-5 pounds in a year with continued unwanted weight gain throughout adulthood as a result of excess energy consumption.

Hunger and satiety cues are natural physiological responses triggered by hormones in the human body; however, many individuals override their satiety cues leading to energy over-consumption. This is a major contributor to adult obesity in the United States, causing some to eat an extra 500-800 calories a day, and possibly more

(Marks, 2004a). Not only are most Americans consuming excess energy, but also much of the energy is consumed in the form of food that provide little nutrient density. Because energy intake is affected by both appetite and by the caloric content of the food consumed, many individuals eat food with high caloric content that satisfies their cravings (Nianhong et al, 2005).

Another contributing factor to energy overconsumption is the frequency at which Americans choose to eat out at restaurants. According to the United States Department of Agriculture, spending on food away from home as a share of household food expenditures has risen steadily, reaching an all-time high of 43.1 percent in 2012. Data from the USDA suggests that foods obtained in restaurants are generally higher in fat, saturated fat, and cholesterol than food prepared at home. Results from a study conducted in 2003 suggested that a higher frequency of eating away from home was associated with obesity (Ma et al, 2003). Furthermore, the frequency of consuming restaurant food is positively correlated with increased body weight in adults (McCrory et al, 1999). Because restaurant foods are higher in fats, people feel satiated longer and should require less frequent feedings (Ma et al. 2003). Dietary fat promotes satiety by delaying gastric emptying, causing the consumer to experience fullness for a longer period of time, allowing the body to spend additional time digesting and absorbing the calorically-dense meal. Another reason dietary fats promote the feeling of satiety is through the activation of satiety hormones, such as peptide YY (PYY), which is released after large, high-fat meals.

PYY is produced and released in the distal small intestine and proximal colon by endocrine cells called L-cells (Nianhong, Chongjian, Mingjia, Limei, Liegang, and Xiufa,

2005). PYY is released into the circulation following food intake, with levels rising to a plateau after one to two hours and remaining elevated for up to six hours (Nianhong et al. 2005). PYY has been studied for its effects on the body following consumption of starches and protein. However, only limited research has been conducted evaluating the influence of acute and/or chronic high-fat diets on PYY release or activity. Recently it was reported that higher levels of PYY were seen following fatty meals compared with meals containing high protein or carbohydrate, suggesting that PYY levels are influenced not only by caloric intake, but also by meal composition (Nianhong et al. 2005). Once researchers determine the effect that chronic high-fat diets have on PYY plasma levels, healthcare professionals will better understand the role of PYY in obesity, possibly contributing to future treatment protocols for obese individuals and even disease prevention. Not only would this be beneficial to Americans' health, but it would also lead to significant reductions in annual healthcare costs (Dall et al. 2009).

Previous observations have demonstrated that acute fat intake led to reduced PYY content, justifying a need for further investigation of the effects of chronic high-fat consumption on PYY response. The purpose of this study was to determine the effect of chronic high-fat diet consumption on intestinal PPY gene expression as well as plasma PYY levels.

LITERATURE REVIEW

Peptide YY Research Benefits

The majority of research on PYY has been published in the last decade, which has included several meaningful advances. What used to be a source of great controversy is now a topic upon which many scientists agree. More research will need to be conducted to further understand the effects of PYY; however, the research is headed in that direction, and most scientists are curious to find a way to use PYY as an anti-obesity treatment. The following literature review shows how far research in this field has come and how the research has led to the knowledge the scientific community has today.

Marks (2004a) and Cooper (2014) discuss how the majority of the population in the United States is overweight or obese, despite pressure from health professionals and the media to maintain a healthy body weight. Less than one-third of U.S. adults report regular leisure-time physical activity, and forty percent of adults report no leisure-time physical activity at all (Marks, 2004a). There is an abundance of available information about healthy living, but few Americans take the opportunity to be educated on this matter. Adults eat an average of 500-800 extra calories a day, and they are not exercising to burn these extra calories (Marks, 2004a). Therefore, those extra calories are causing the American people to keep gaining weight.

In particular, Americans of low socioeconomic status (SES) are at an increased risk of developing diet-related obesity because they are more likely to rely on fast food restaurants and high-fat calorically dense foods leading to excess energy consumption. Additionally, many of the healthy food choices that are available to this population are too expensive compared to other less nutrient-dense foods. Healthcare professionals are

encouraging their patients to eat more nutrient-dense foods and to increase their physical activity (Marks, 2004a). Successful weight loss is a lifetime commitment in which caloric intake and energy expenditure must be equal to maintain weight or just right to lose weight.

As rates of obesity have risen, research has been dedicated to understanding how obesity affects the body and strategies to improve disease outcome. Many contributing factors are associated with this growing problem, and satiety hormones (such as PYY) are just one of the many factors.

Peptide YY research is a relatively new area that has only been researched with intensity in the last decade. PYY was first reported in 1993 to decrease appetite and recently has shown to reduce food intake by thirty percent after an IV infusion of PYY 3-36 (Druce et al. 2004). Thereafter, in the mid-1990s, scientists began to conduct research that indicated the link between PYY, satiety, and obesity. After the emergence of the effect of PYY on satiety, interest in PYY as a potential therapeutic in metabolic diseases such as obesity has surged. Despite increasing research in obesity therapeutics, incidence of obesity continues to increase.

PYY research undoubtedly will result in safe and effective drug interventions in the future to prevent weight gain and assist with weight loss and maintenance for patients with weight problems (Marks, 2004b). Studying appetite-modulating hormones provides increased knowledge about the control of appetite, food intake, energy regulation, and weight control (Marks, 2004b). This knowledge can influence how our healthcare system addresses, prevents, and treats obesity.

Human Experiments

In 2003, Batterham et al. compared the effects of PYY infusion on appetite and food intake in twelve obese and twelve lean subjects in a double- blind, placebo-controlled, crossover study. Specifically, subjects consumed a buffet lunch two hours after the infusion of PYY. Calorie intake was decreased by thirty percent in the obese subjects and thirty-one percent in the lean subjects (Batterham et al. 2003). PYY infusion also caused a significant decrease in the cumulative 24-hour caloric intake in both obese and lean subjects (Batterham et al. 2003). Endogenous fasting and postprandial levels of PYY were significantly lower in obese subjects. Moreover, fasting PYY levels correlated negatively with the body-mass index (Batterham et al. 2003).

After this finding, Druce et al. (2004) authored a minireview explaining how gut hormones such as PYY, cholecystokinin (CCK), and glucagon-like peptide-1 (GLP-1) all play a role in satiety signals that inhibit appetite for a period after a meal. Cholecystokinin is a peptide hormone of the gastrointestinal system responsible for stimulating the digestion of fat and protein (Druce et al. 2004). Feeding releases GLP-1 from the gut and into the circulation and acts on the pancreas to cause insulin release but also has effects on appetite (Druce et al. 2004). Some reports have suggested that GLP-1 secretion is reduced in obese subjects, and weight loss normalizes the levels (Druce et al. 2004). The role for GLP-1 in physiological control of human appetite is not clear. Peripheral administration does inhibit food intake in normal individuals (Druce et al. 2004).

Degen et al. (2005) designed a study to examine PYY release in response to two meals differing in their calorie content and to relate the plasma levels to those obtained

after exogenous infusion. The effect of graded intravenous doses of synthetic human PYY 3-36 on food intake was also investigated in healthy male volunteers. The results showed that plasma PYY concentrations increased in response to food intake, reflecting the size of the calorie load. Graded PYY 3-36 infusions resulted in a dose-dependent reduction in food intake and a similar reduction in calorie intake. Nausea and fullness were the most common side effects produced by PYY, especially at the highest dose. Subjects experienced less hunger and early fullness in the pre-meal period during PYY 3-36 infusion at the highest dose.

In 2006, Ashby and Bloom reviewed the progress in research on peptide YY in response to controversy over PYY's ability to become an anti-obesity treatment (Ashby and Bloom, 2006). Ashby and Bloom show that many experts agreed that peptide YY is linked to obesity; however, they argued that more research is needed to elucidate the effects of PYY as an anti-obesity treatment. At the time, high levels of PYY had been reported in individuals with malabsorption or bowel infections, and low levels of PYY had been reported in individuals with obesity (Ashby and Bloom 2006). This finding led many people to assume that PYY deficiency could contribute to pathogenesis of obesity. An interesting finding that Ashby and Bloom mention is that when patients undergo gastric bypass (a surgical procedure that is associated with reduced intake), they exhibit increased levels of PYY. However, elevated PYY levels were also observed in patients with a colectomy, which is not a surgical procedure that is associated with reduced appetite. Ashby and Bloom refer to the controversy of the obesity-low PYY link and speculate how studies that failed to associate obesity or BMI (body mass index) with postprandial PYY were those that tested PYY levels with glucose stimulation instead of

lipid. High PYY levels have also been observed in individuals with cachexia, the elderly, and pre-term babies with low birth weight (Ashby and Bloom, 2006). By this point, most of the scientific community agreed that PYY is associated with energy intake and that, in conjunction with other peptides, it most likely mediates satiety.

In the same year, Guo et al. conducted a study in which fasting and postprandial plasma PYY concentrations were measured after an overnight fast, and 30 to 180 minutes after the consumption of a standardized meal. Postprandial changes in PYY were positively associated with postprandial changes in ratings of satiety and the maximal PYY concentrations achieved after the meal were negatively associated with 24-hour RQ (Guo et al, 2006). The peak PYY concentrations were negatively associated with changes in body weight and negatively correlated with various markers of adiposity during fasting PYY concentrations (Guo et al. 2006). This indicated that endogenous PYY may be involved in the long-term regulation of body weight and that this long-term effect was driven by the modulation of food intake but also by the control of energy expenditure and lipid metabolism (Guo et al. 2006).

In light of these new findings, in 2009, Marks and Austin explained PYY and its function. According to Marks and Austin (2009) and Cooper (2014), PYY is a peptide satiety hormone that is released from the L-cells of the small intestine and colon following a meal which peaks one-two hours after the meal is consumed. Marks and Austin (2009) discovered that PYY concentrations are proportional to the energy content of the meal consumed. Once the nutrients have reached the small intestine and PYY is released, the feeling of fullness occurs (Cooper 2014).

That same year in 2014, Karra, Chandarana, and Batterham explained an

interesting anomaly in the PYY research. Injection of PYY 1-36 into the third, lateral or fourth cerebral ventricle; paraventricular nucleus; or hippocampus stimulates food intake in rodents. Similarly, intracerebroventricular injection of PYY 3-36 increases food intake. This orexigenic action of PYY 3-36 is reduced when the Y1 and Y5 receptors are inhibited which suggests that the Y1 receptor and the Y5 receptor mediate the effects of administered PYY. Contrariwise, injection of PYY 3-36 directly into the hypothalamic arcuate nucleus, where the Y2 receptor is expressed, inhibited feeding (Karra et al. 2009). Therefore, the feeding effects of centrally administered PYY are dependent on which Y-receptor is activated (Karra et al. 2009).

In 2012, De Silva and Bloom explained how the gut is the largest endocrine organ in the body and secretes over 30 different regulatory peptide hormones. A number of these gut hormones are stimulated by gut nutrient content and affect short-term and intermediate-term feelings of hunger and satiety. Peptide YY and glucagon-like peptide 1 (GLP-1) are elevated following gastric bypass and have been shown to reduce food intake. Both hormones are released together following a meal to mediate postprandial satiety. Gastric bypass surgery is one of the most effective treatments for obese patients, where sustained weight loss results diminished appetite. These patients demonstrate increased levels of PYY and GLP-1. Inhibition of PYY and GLP-1 results in the return of appetite and increased food intake in these patients. Therefore, it is likely that elevated levels of PYY and GLP-1 play a key role in the sustained weight loss observed following gastric bypass surgery. Obesity does not appear to be associated with resistance to PYY (3-36) because there was a 30% reduction in food intake at a buffet meal served two hours after completion of a ninety minute infusion of PYY (3-36) delivered at an

unspecified dose based on body surface area. This compared with a 31% reduction in food intake in a group of lean subjects who also received a peripheral infusion of PYY (3-36). Additionally, there was also a comparable reduction in 24-hour energy intake in both lean and obese subjects following PYY 3-36 infusion.

The research on PYY 3-36 thus far leads to the prospect that subcutaneously delivered infusions may be used to treat obesity. During future drug development, monitoring the effect of PYY analogous in reducing food intake without inducing nausea will be a priority (De Silva and Bloom, 2012). Studies have shown that an intravenous infusion of PYY 3-36 to lean and overweight human subjects caused significant nausea which affected the study since more than half of the participants were not able to complete their infusion (De Silva and Bloom, 2012). De Silva and Bloom proposed that the goal of future research should be to reproduce the physiological consequences of a gastric bypass procedure solely by means of gut hormone-based therapy, thereby effectively creating a medical bypass (De Silva and Bloom, 2012).

PYY has many effects on the body, including delaying gastric emptying, inhibiting secretions from the pancreas and stomach, inhibiting gallbladder contraction, and increasing the absorption of fluid and electrolytes from the ileum (Marks & Austin, 2009). Marks and Austin (2009) reviewed how a PYY deficiency easily leads to obesity and causes less satiety, defined by increased food intake in mice. When PYY was administered in obese and lean adults, food intake decreased over a 24-hour period, while the obese adults were more resistant to the PYY administered than the lean adults (Marks & Austen 2009). Interestingly, obese patients that complete gastric bypass surgery experience an increase in PYY release, which contributes to weight loss (Marks &

Austen 2009).

Following these observations, Duca, Sakar, and Covasa (2013) investigated how the gastrointestinal (GI) tract is affected by dietary fat (p. 378). Following exposure to high fat content, the GI tract can be physiologically, metabolically, and morphologically altered to better manage elevated dietary lipids (Duca, Sakar, and Covasa, 2013). Moreover, the longer a high-fat diet continues, the more efficient the body becomes at absorbing and using fat as its major energy source (Duca, Sakar, and Covasa, 2013). This often causes a decreased sense of satiety and an increased obesity rate. Duca, Sakar, and Covasa (2013) examined the signaling hormones that are responsible for the detection of and response to consumed lipids. They observe several satiation signals, such as glucagon-like peptide 1 (GLP-1), Cholecystokinin (CCK), and PYY, which are all part of the intestinal sensing of fats (Duca, Sakar, and Covasa, 2013). They determined that chronic fat exposure changes the way the GI tract handles fat consumption throughout the entire digestive system.

In 2013, Kozimor, Chang and Cooper revealed the different effects of a high-fat meal, based on type of fat the high-fat meal contained, on the body. The study consisted of fifteen normal weight women between the ages of 18-45 years old that were asked to make three visits for the three different treatments (Kozimor, Chang, and Cooper, 2013). On all three visits, the women were given high-fat meals with differing fat composition (Kozimor, Chang, and Cooper, 2013). During each visits, their blood was drawn eight times over a period of five hours (Kozimor, Chang, and Cooper, 2013). Their results indicated that PYY release was greater in meals that were composed of fats high in PUFAs and SFAs, as compared to meals that were composed of fats high in MUFAs.

This also revealed that meals high in MUFAs might not have as high of a satiety response as the PUFA and SFA have (Kozimor, Chang, and Cooper, 2013). The research also showed that the subjects felt fuller when they were given meals high in SFAs in comparison to MUFAs and PUFAs (Kozimor, Chang, and Cooper, 2013). Additionally, Kozimor, Chang, and Cooper, (2013) studied clearance of polyunsaturated fatty acids (PUFA) from plasma and found a slower rate when compared with saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA). Thus MUFAs release less PYY than SFA and PUFA (p. 42). Therefore, there are many factors that affect PYY release, including the composition of fatty acids.

In 2014, Cooper reviewed the regulation of satiety hormones by their mechanisms of feeding behavior, which affects energy homeostasis. Cooper (2014) reviewed how there has been results showing that increased PYY plasma levels cause decreased food intake. There are also studies that show that injecting subjects with PYY causes them to lose weight; however, there were several side effects, such as nausea and vomiting, that prevented its continued use (Cooper, 2014). It was not until recently that research has been conducted to see how these PYY plasma levels are affected by the dysregulation of metabolism. Cooper explains that studies have shown that exercise can increase PYY levels, while age and sex do not affect the release of PYY (Cooper 2014).

Mouse Experiments

In 2004, Tovar, Seoane, Caminos, Nogueiras, Casanueva, nd Dieguez assessed the influence of age, sex, thyroid status, growth hormone (GH), pregnancy, and food restriction on PYY levels in rats. PYY levels were influenced by age, with the highest hormone levels achieved in early postnatal life and decreasing thereafter (Tovar et al.

2004). PYY levels were also dependent on thyroid hormone status being decreased in hyperthyroid rates (Tovar et al. 2004). Exogenous GH administration led to a decrease in PYY levels in both normal and GH-deficient rats (Tovar et al. 2004). The PYY levels observed in acute and chronic food-restricted rats indicate that, in situations of decreased energy intake, the lower PYY levels could serve to disinhibit central pathways and facilitate food intake (Tovar et al. 2004).

A study conducted by Chelikani, Haver, and Reidelberger (2005) demonstrated that intravenous infusions of PYY (3–36), which are more likely than intraperitoneal injections to mimic postprandial increases in plasma PYY (3–36), potently inhibit food intake in a dose-dependent manner. Their study also showed that PYY (1–36) is an order of magnitude less potent than PYY (3–36) and that PYY (3–36) reduces food intake by decreasing meal size and increasing the satiety ratio. Lastly, their research revealed that PYY (3–36) can inhibit food intake independently of its action to inhibit gastric emptying. These results support the hypothesis that PYY (3–36) acts as a hormonal signal from the gut to the brain to inhibit food intake. It remains to be determined whether intravenous doses of PYY (3–36) that reproduce postprandial increases in plasma PYY (3–36) are sufficient to inhibit food intake (Chelikani et al. 2005).

In 2005, Le Roux et al. reviewed several different studies that, when taken together, suggest that the observed lower postprandial PYY levels in obese individuals may result in an increase in food intake to achieve the same level of satiety as seen in normal-weight subjects. Obesity does not seem to cause a peripheral resistance to PYY, unlike the marked resistance observed for leptin and insulin. PYY release from the intestinal tract may be inhibited in the obese, thus leaving obese subjects with a

functional deficiency in PYY-induced satiety. Low plasma PYY may therefore reinforce obesity (Le Roux, et al. 2005). The definitive role of PYY in the pathogenesis of obesity and the mechanisms that contribute to the reduced plasma levels of PYY in obese humans and rodents remains to be determined. This study also addressed the question as to whether low plasma PYY is a cause or consequence of obesity. Following randomization of mice into high-fat fed (HF) or low fat fed (LF) groups, plasma PYY was found to be lower in the diet-induced obese mice suggesting low plasma PYY is more likely to be a consequence rather than a cause of obesity. The study supports previous findings that obese subjects have reduced plasma PYY levels. Greater meal calorie content was required to increase plasma PYY concentrations in obese to similar levels seen in normal-weight subjects (Le Roux et al. 2005).

The following year, Boey et al. described the two endogenous forms of PYY in the body, PYY 1-36 and PYY 3-36. Both PYY 1-36 and PYY 3-36 suppress appetite and food intake in rodents (Boey et al. 2006). Research studies had primarily focused on PYY's effect on feeding and body weight and less on how PYY influences body composition. Even less was known on how hormonal parameters influenced PYY. PYY has been shown to slow the transit of food through the gastrointestinal tract, delay gastric and gallbladder emptying, and delay pancreatic and intestinal secretion via direct actions on target tissues or indirect actions via vagal pathways (Boey et al. 2006). PYY also has an important function in regulating insulin release and glucose homeostasis. Previous studies demonstrated that PYY acts to inhibit insulin secretion and glucose-stimulated insulin release from isolated pancreatic islets. In obese rodents, PYY3-36 reinforced insulin action of glucose disposal independently of changes in food intake and

bodyweight. Therefore, it is possible that PYY may influence long-term energy balance via these mechanisms.

Boey et al. (2006) studied the role of PYY in the regulation of multiple hormonal and metabolic determinants of lean and fat mass. This study demonstrated that PYY-mediated pathways are important for regulation of body composition, serum insulin levels and glucose homeostasis. PYY deficiency resulted in late-onset obesity. Mice that had PYY ablation had significant increases in bodyweight and fat mass. PYY ablation was associated with significant increases in basal and/or glucose-induced serum insulin levels. Because hyperinsulinaemia is causally linked to the etiology of obesity, it is possible that hyperinsulinaemia contributes to the development of increased adiposity. The absence of PYY was found to have a significant effect on body composition and on basal and glucose-induced serum insulin levels. This finding suggested that low circulating PYY concentrations may causally contribute to the development of hyperinsulinemia and obesity, during ageing or under conditions of long-term consumption of a high-fat diet, respectively (Boey et al. 2006).

In 2007, Chelikani, Haver, and Reidelberger determined the dose-dependent effects of 3-hour IV infusions of PYY (3-36) and PYY (1-36) at dark onset on food intake in non-food deprived rats. IV infusions of PYY (3-36) potently inhibit food intake in a dose-dependent manner. PYY (1-36) is an order of magnitude less potent than PYY (3-36) and PYY (3-36) reduced food intake by decreasing meal size and increasing the satiety ratio. PYY (3-36) can inhibit food intake independently of its action to inhibit gastric emptying. This study supports the hypothesis that PYY (3-36) acts as a hormonal signal from the gut to the brain to inhibit good intake. This did not, however, determine

whether IV doses of PYY (3-36) reproduced postprandial increases in plasma PYY levels to sufficiently inhibit food intake (Chelikani, Haver, and Reidelberger, 2007).

In 2008, Reidelberger et al. attempted to identify an intermittent dosing strategy for intraperitoneal infusion of PYY 3-36 that produces a sustained reduction in daily food intake and adiposity in diet-induced obese rats. Reidelberger et al. (2008) wanted to study whether intermittent intraperitoneal administration of PYY 3-36 can produce a sustained reduction in daily food intake in diet-induced obese rats when body weight and adiposity are decreasing in response to administration of the substance. The results demonstrated that reducing body weight and adiposity was more difficult to accomplish than offsetting weight gain and fat deposition (Reidelberger, Haver, Chelikani, and Buescher, 2008). The inability of the infusion to produce sustained reduction in daily food intake has been seen in many previous studies. Reidelberger et al. found evidence that transient feeding responses to intermittent PYY 3-36 administration were likely due to redundancy and plasticity in the energy regulatory system, rather than to downregulation of PYY receptors. This was supported by the findings that food intake significantly increased on each of the five occasions when PYY 3-36 treatments were discontinued for one day following apparent loss in treatment efficacies. If loss of PYY 3-36 receptors was primarily responsible for loss in PYY 3-36 efficacy, then discontinuing PYY 3-36 treatment should have had little effect on food intake. Reidelberger et al. 2008 concludes that an important early step in discovery of anti-obesity drugs is defining methods of administration of anorexigenic agents that can produce a sustained reduction in daily food intake and body weight in obese experimental animals.

In 2013, Duca, Swartz, Sakar, and Covasa conducted an experiment in which

twelve mice were treated with a high-fat/ high-energy diets for 10 weeks after being selected for diet-induced obesity (DIO) or diet- induced obesity resistant (DR) (Duca, Swartz, Sakar, and Covasa, 2013). Six of the mice were DIO mice that did not become obese during the study because of their resistance to the disorder, and six mice were DR mice that did become obese during the study because of their lack of resistance (Duca, Swartz, Sakar, and Covasa, 2013). The mice were selected into each category based on weight gain. Their food intake and weight were measured every week during the entire experiment (Duca, Swartz, Sakar, and Covasa, 2013). The rodents were anesthetized and tissues were collected in order to do further testing (Duca, Swartz, Sakar, and Covasa, 2013). The rats that were given high-fat/ high-energy diets stimulating obesity displayed decreased fat-induced satiation and also decreased release of PYY (Duca, Swartz, Sakar, and Covasa, 2013).

Most of the research already conducted has tested the effect of a high-fat diet on the amounts of PYY circulating in the plasma during a short amount of time averaging about six days. Long-term effects, however, remain unknown. Thus, this study tested the effects of a chronic high-fat diet on PYY postprandial plasma levels in mice over the course of twelve weeks.

METHODS

The Texas Tech University Animal Care and Use Committee has approved all procedures in the care, use, and treatment of these animals. This project began with twelve C57BL/6 Male C57BL/6 mice (age 8 weeks) in a 12-hour light-dark cycle and temperature-controlled vivarium. Mice were group-caged (three mice per cage), with a total of four cages with food and water *ad libitum*. Two of the cages (n = 6/group) were given standard rodent chow and two of the cages (n = 6/group) were given a high-fat diet for 12 weeks. Measurements of body weight and food intake were taken weekly. Following the feeding trial, mice were given a high-fat liquid meal challenge via oral gavage. Tail vein blood was collected immediately before and up to 2 hours following the oral gavage. Plasma triglyceride and PYY levels were quantified using a colorimetric assay and ELISA procedures, respectively (ELISA technical guide and protocols, 2010). Following the feeding trails and high-fat meal challenge, mice were sacrificed via isoflurane inhalation, and tissues were collected and stored at -80°C for further analysis. Lastly, distal portions of the small intestine were used to detect PYY gene expression. Briefly, intestines were homogenized and the RNA was recovered. Gene expression of PYY and CCK was quantified via quantitative real time-polymerase chain reactions (qRT-PCR) (Kanmogne, 2013). Weekly body weights and food intake were analyzed via two-way repeated measures ANOVA with Bonferonni's pair-wise comparisons. Plasma PYY content and relative gene expression was analyzed using student's *t*-test. All of the data is represented as group mean ± SEM.

RESULTS

Chronic high-fat diet significantly increased body weight of mice and significantly increased average daily energy intake (Fig 1A and B, respectively). High-fat fed mice gained significantly more weight than the standard chow fed mice (Fig 1A) The chow mice began with an average weight of about twenty-four grams and weighted about twenty-seven grams at the end of the study with total weight gain aproximating three grams (Fig 1A) The high-fat fed mice began with an average weight of about twenty-five grams and weighted an average of roughly thirty-seven grams at the end of the experiment (total weight gain was about twelve grams) (Fig 1A) The difference between the weight of the standard chow fed mice and the high-fat fed mice was significant from weeks three through twelve (Fig 1A). Energy intake for high-fat fed mice was elevated when compared to chow fed mice (Fig 1C). The standard chow fed mice ate an average of about 12.2 kcal/g/day and the high-fat fed mice ate an average of about 13.1 kcal/g/day (Fig 1C).

Plasma PYY levels were decreased after oral gavage in chronic high-fat fed mice compared to chow fed mice (Fig 2A). The standard chow fed mice's PYY levels were significantly higher at time zero during the PYY response trial and simply higher than the high-fat fed mice during the rest of the time points for the PYY response trial (Fig 2A). The PYY response trial summary represented by area-under-the-curve (AUC) was significantly lower for the high fat fed mice when compared to the standard chow fed mice (Fig 2B). The AUC for high fat fed mice was about 355000 and the AUC for the standard chow fed mice was about 400000 (Fig 2B).

HF-fed mice displayed elevated intestinal gene expression of PYY and CCK (Fig 3). The intestinal gene expression of PYY for the standard chow fed mice was one while the intestinal gene expression of PYY for the high fat fed mice was about 2.3 (Fig 3). The intestinal gene expression of CCK for the standard chow fed mice was one and the intestinal gene expression of CCK for high fat fed mice was significantly higher at about four (Fig 3).

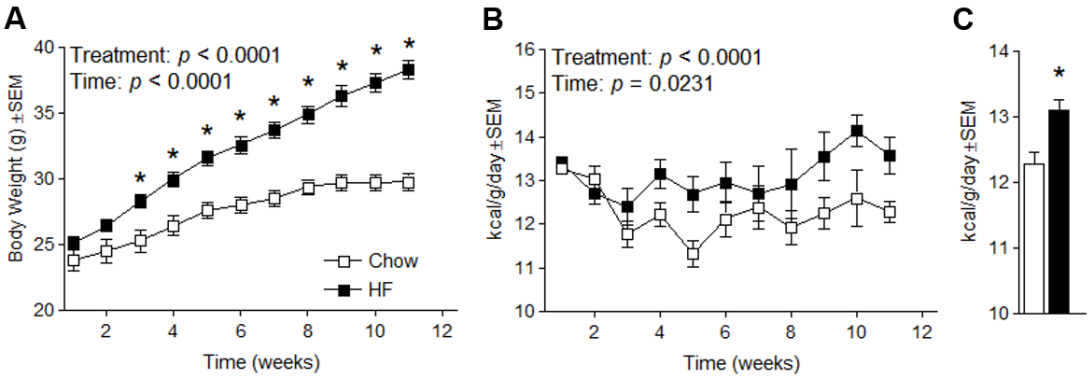

Fig 1. Body Weight and Energy Intake (A) Body weight (g) of mice fed either chow or high-fat hyper-caloric feed (HF) for 12 weeks. (B) Energy intake (kcal/g body weight/day) for each week of the 12 week feeding trial. (C) Average daily energy intake (kcal/g body weight/day).

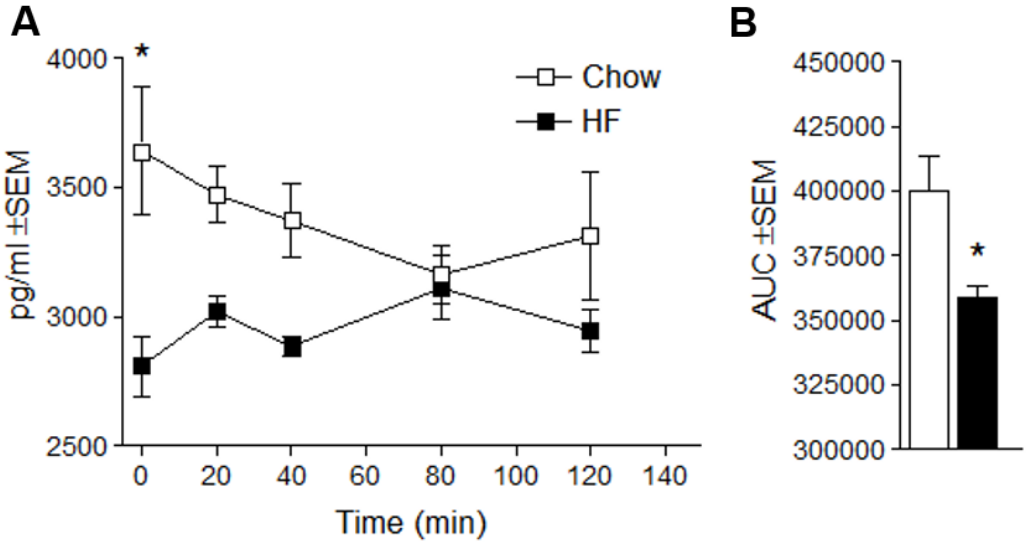

Fig 2. Plasma PYY of mice fed either chow or high-fat hyper-caloric diets for 12 weeks. (A) PYY response trial following ingestion of a high-fat meal. (B) PYY response trial summary represented by area-under-the-curve (AUC).

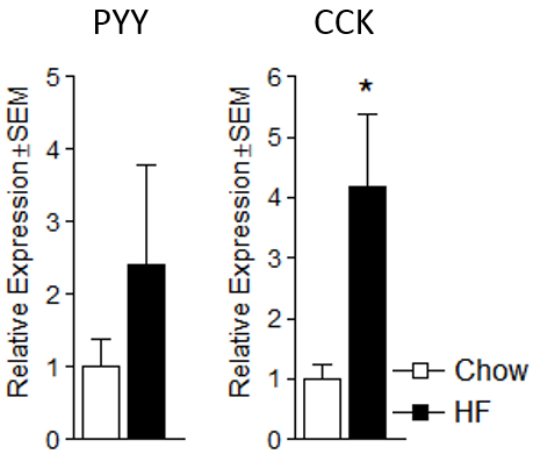

Fig 3. Intestinal gene expression of Peptide YY (PYY) and Cholecystokinin (CCK) of mice fed either chow or high-fat hyper-caloric feed (HF) for 12 weeks of genes associated with triglyceride synthesis and lipid storage.
Data are presented as group mean ±SEM. * indicates $p < 0.05$.

DISCUSSION

Great progress in understanding the role of PYY as a satiety hormone and its role in regulating energy balance has markedly increased over the last thirty years. Past studies describe the effects of PYY in the body, as well as how exercise and various nutrients affect PYY levels. Past observations also summarize the influence of age, sex, ethnicity and weight status on PYY levels. However, the regulation of PYY synthesis and release, and especially the mechanisms that describe circulating PYY's effect on decreasing appetite and obesity, continue to be elucidated.

Interestingly, the results of this study show that the high-fat mice had an increased intestinal gene expression of peptide YY, suggesting high-fat fed animals may be attempting to express PYY; however, increased intestinal expression may not translate into enhanced PYY or proper satiety cues. This observation suggest that high-fat fed mice may develop a defect in the up-regulation of PYY content thereby suppressing the satiation generated by a high-fat meal. The mice fed the chronic high-fat diet group also consumed significantly more energy throughout the trial than the chronic chow-fed mice group. Further studies are needed to address the biological mechanism leading to the observed insensitivity and if it is caused by chronic high-fat diet or chronic low levels on PYY in the plasma after high-fat meals. Also, more research is needed to determine if the intestinal gene expression of mice fed high-fat diets returns to normal following the cessation of high-fat consumption. It would also be interesting to investigate if intestinal gene expression returns to chow levels following the administration of exogenous PYY.

It remains unclear why chronic high-fat consumption lowers plasma levels of PYY and causes insensitivity to the PYY gene expression. Batterham et al. (2003)

reported that endogenous fasting and postprandial levels of PYY were significantly lower in obese than in lean individuals, despite obese subjects consuming more calories during the buffet meal, suggesting that a deficiency in the peptide could partly explain why some people are prone to obesity (p. 944).

These findings support the hypothesis that chronic high-fat consumption alters fasting and postprandial PYY content, a link that may prove valuable for future anti-obesity efforts. Now that the correlation between chronic high-fat consumption and PYY plasma levels has been found to be negative, scientists can conduct research to find out the mechanism by which this phenomenon occurs. These results provide some support for the notion that a deficiency in the PYY gene expression and circulating PYY may lead to obesity.

The scientific community has been passionately researching the causes and effects of obesity and trying desperately to find a treatment for those that have not been able to avoid this disease. More research needs to be conducted to see whether PYY injections could be safely administered to patients suffering with obesity without the negative side effects is warranted. However, as established in past studies, there are challenges facing the therapeutic potential of PYY given that the common side effect of PYY administration is nausea and that there are a number of hunger and satiety signals that probably influence overall energy intake (Cooper, 2014). Future studies should focus on the identification of other pharmacological, nutrient and alimentary options as a potential way to alter PYY levels, and possibly energy intake.

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