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Effect of Dietary Fatty Acid Composition on Food Intake, Triglycerides, and Hypothalamic Peptides

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Abstract

While a high-fat diet when compared to low-fat diet is known to produce overeating and health complications, less is known about the effects produced by fat-rich diets differing in their specific composition of fat. This study examined the effects of a high-fat diet containing relatively high levels of saturated compared to unsaturated fatty acids (HiSat) to a high-fat diet with higher levels of unsaturated fatty acids (USat). A HiSat compared to USat meal caused rats to consume more calories in a subsequent chow test meal. The HiSat meal also increased circulating levels of triglycerides (TG) and expression of the orexigenic peptides, galanin (GAL) in the hypothalamic paraventricular nucleus (PVN) and orexin (OX) in the perifornical lateral hypothalamus (PFLH). A similar increase in TG levels and PVN GAL and PFLH OX was also seen in rats given chronic access to the HiSat compared to USat diet, while neuropeptide Y (NPY) and agouti-related protein (AgRP) in the arcuate nucleus showed decreased expression. The importance of TG in producing these changes was supported by the finding that the TG-lowering medication gemfibrozil as compared to vehicle, when peripherally administered before consumption of a HiSat meal, significantly decreased the expression of OX, while increasing the expression of NPY and AgRP. These findings substantiate the importance of the fat composition in a diet, indicating that those rich in saturated compared to unsaturated fatty acids may promote overeating by increasing circulating lipids and specific hypothalamic peptides, GAL and OX, known to preferentially stimulate the consumption of a fat-rich diet.

Keywords

saturated fat; unsaturated fat; triglycerides; galanin; hypothalamus

1. Introduction

Consumption of a fat-rich diet can lead to obesity and its attendant health complications [1]. In both male and female rodents, access to a high-fat diet compared to low-fat diet stimulates weight gain [2, 3], due largely to an increase in caloric intake [4, 5], with females generally showing a stronger preference for fat compared to males [6]. In addition, this diet

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raises levels of circulating triglycerides (TG), after chronic consumption [2, 3] or a single meal [4, 7]. These TG, once broken down into fatty acids, can lead to a number of detrimental effects, including obesity [8], cardiovascular disease [9], and even cognitive impairment [10].

Part of the hyperphagia that occurs with a high-fat diet may be due to alterations in the expression of orexigenic peptides in the hypothalamus. Consumption of a high-fat diet compared to a low-fat diet has been shown to stimulate the expression of galanin (GAL) in the paraventricular nucleus (PVN) [7, 11] and of orexin (OX), also known as hypocretin, in the perifornical lateral hypothalamus (PFLH) [3, 7, 12]. The involvement of these two peptides in the overeating induced by fat is supported by the finding that they preferentially stimulate the ingestion of a high-fat diet when injected directly into the hypothalamus [13, 14]. This evidence suggests that GAL and OX each operate within a positive feedback circuit, whereby consumption of a high-fat diet stimulates the endogenous peptides in the PVN or PFLH that, in turn, promote further consumption of the fatty diet. This positive relationship, however, is not evident with two other orexigenic peptides, neuropeptide Y (NPY) and agouti-related protein (AgRP), which coexist in neurons of the arcuate nucleus (ARC) [15, 16]. These peptides in the ARC are unaffected or suppressed by consumption of a high-fat diet [17–19] and, when injected, can preferentially stimulate the intake of a low-fat, carbohydrate-rich diet as shown with NPY [20, 21]. This evidence reveals marked differences between the various peptides, with GAL in the PVN and OX in the PFLH positively regulated by fat and acting to increase intake of a high-fat diet that extends beyond nutritional requirements and with NPY and AgRP in the ARC negatively regulated by fat and generally acting to stimulate carbohydrate intake.

Further evidence suggests that the hyperphagia and neuropeptide changes induced by a high-fat diet may be attributed, in part, to changes in circulating levels of lipids. After consumption of a high-fat meal, rats with higher levels of TG consume more chow during a subsequent test meal [22]. This greater caloric intake is preceded by an increase in gene and protein expression of OX in the PFLH [22], which is somewhat reversed by medications such as gemfibrozil (Lopid) that reduce levels of TG when given acutely or chronically [23, 24]. Clinically, gemfibrozil compared to placebo enhances weight loss and reduces weight gain [25]. Further confirming the role of circulating lipids in stimulating neuropeptide expression, direct peripheral administration of a lipid emulsion has been shown to increase GAL mRNA in the PVN and OX in the PFLH, while having no effect on NPY or AgRP in the ARC [26]. Whereas the precise mechanisms mediating these changes have yet to be determined, the evidence strongly implicates TG as a mediator of the behavioral and neurobiological effects of high-fat diet consumption.

In addition to these effects of a high-fat diet compared to low-fat diet, there is some evidence that the specific composition of the high-fat diet may also be an important factor in determining the effects of this diet. Currently, adults in the United States consume about 35% of their total daily fat from saturated fat and 20% from polyunsaturated fat [27, 28], although the recommended intake is closer to 30% and 40%, respectively [29]. Although there are relatively few studies in animals, the evidence that exists shows that chronic consumption of diets high in saturated fatty acids, such as those containing lard, compared to those high in unsaturated fatty acids, such as those with fish oil, can promote hyperphagia and weight gain while raising TG levels, even when these diets contain similar concentrations of overall fat [30, 31]. To understand possible mechanisms that underlie these disturbances, the present study examined under both acute and chronic conditions the behavioral, physiological and neurochemical effects of a diet containing relatively high levels of saturated fatty acids (HiSat), as compared to one containing higher levels of unsaturated fatty acids (USat). The questions being addressed are whether the composition

of fat in a high-fat diet is an important factor in positively linking changes in eating and TG levels to specific orexigenic peptides in the hypothalamus that are stimulated by a high-fat diet and whether the expression of these particular fat-related peptides can be reduced by a drug, gemfibrozil, that is known to lower TG levels.

2. Materials and Methods

2.1. Animals

Adult, male and female Sprague–Dawley rats (220–240 g upon arrival) (Charles River Breeding Labs, Wilmington, MA) were individually housed in plastic cages, in a fully accredited AAALAC facility (22 °C, with a 12:12-h light–dark cycle with lights off at 2 pm), according to institutionally approved protocols as specified in the NIH Guide to the Use and Care of Animals and also with the approval of the Rockefeller University Animal Care and Use Committee. All animals were given one week to acclimate to the lab conditions before the start of the experiments. Standard laboratory chow (Purina 5001 rat chow; 4.00 kcal/g) and water were available *ad libitum*, except where indicated in the Test Procedures. In female rats, estrus cycle stage was determined through daily microscopic examination of vaginal cytology, and those in proestrus were selected for study.

2.2. Diets

The high-fat diets used in the experiments consisted of 50% fat, 25% carbohydrate, and 25% protein (Bioserv, Frenchtown, NJ, USA). The carbohydrate component was composed of 30% dextrin, 30% cornstarch (ICN Pharmaceuticals, Costa Mesa, CA, USA) and 40% sucrose (Domino, Yonkers, NY, USA). The protein component was composed of 100% casein (Bio-Serv, Frenchtown, NJ). The fat component for the HiSat diet (5.15 kcal/g) was composed of 82% lard (Armour, Omaha, NE, USA) and 18% soybean oil (Wesson Vegetable Oil, Omaha, NE, USA), approximating the fatty acid profile of the diet currently consumed in the United States (see Section 1), while the fat component of the USat diet (5.06 kcal/g) was composed of 20% lard and 80% fish oil (Eskimo-3 Fish Oil Liquid, Enzymatic Therapy, Green Bay, WI), approximating the fatty acid profile of the diet recommended for adults in the United States (see Section 1). Both diets were supplemented with 4% minerals (USP XIV Salt Mixture Briggs; ICN Pharmaceuticals) and 3% vitamins (Vitamin diet fortification mixture; ICN Pharmaceuticals). They were presented in round metal jars inside the cage.

2.3. Test procedures

Rats in all acute experiments were first adapted to a 10 kcal meal of their test diet over several days until they consumed all of the meal for at least two consecutive days. In Experiment 1 with a within-subject design, rats were adapted to HiSat, USat or chow meals on alternate days in counterbalanced order such that they received three adaptations to each diet. In all other experiments, rats were divided into two groups and given either a HiSat or USat meal in a between-subject design. Six adaptation days were used in Experiment 2 and three were used in Experiment 4. On test days (Experiment 1 and 2), chow was removed and the test meal given 90 min later for 30 min. Rats that failed to consume the test meal were removed from the experiment. Chow was then returned to the cage at the completion of the experiment. Test diets in all experiments were presented in round metal jars inside the cage, and intake was assessed by briefly removing and weighing each jar.

In Experiment 1, using a within-subject design, male rats (N=10) were given a small chow versus HiSat versus USat meal, and subsequent caloric intake and TG levels were then assessed. Each rat first received a 10-kcal HiSat, USat or chow meal for 30 min. One hour following the end of this meal, a test meal of chow was presented, starting at dark onset,

with intake measurements taken at 15 min, 30 min and at 24 h. This was repeated on the following two days such that each rat was tested with each type of diet. To obtain TG levels, the same procedures were repeated for an additional three days except that tail vein blood was collected at the time that the test meal would have been given, at dark onset, one hour following the end of the HiSat, USat or chow meal.

In Experiments 2A and 2B, using a between-subject design with weight-matched groups, male rats (Exp 2A) and female rats (Exp 2B) (n=5/group) were given a small HiSat versus USat meal, and peptide mRNA and TG levels were then assessed. Following the adaptation days, on the test day, a 10-kcal HiSat- or USat meal was given for 30 min. One hour following the end of the meal, at dark onset, animals were sacrificed by rapid decapitation. Trunk blood was collected for TG analysis, and brains were dissected for quantitative real-time polymerase chain reaction (qRT-PCR) analysis of GAL, OX, NPY, and AgRP mRNA.

In Experiment 3, using a between-subject design with initially weight-matched groups, male rats (n=5/group) were given as their only food source either the HiSat or USat diets *ad libitum* for 30 days, and hypothalamic gene expression and TG levels were then assessed. Body weight and 24-h food intake were measured every 7 days. At the end of the experiment, rats were sacrificed 1 h before dark onset by rapid decapitation. Trunk blood was collected for analysis of TG as well as leptin, insulin, glucose, and corticosterone levels. Brains were dissected for qRT-PCR analysis of GAL, OX, NPY, and AgRP mRNA.

In Experiment 4, using a between-subject design with weight-matched groups, male rats (n=6/group) were given a TG-lowering drug prior to a HiSat meal, and hypothalamic gene expression and TG levels were then assessed. Rats were first adapted both to a 10-kcal HiSat meal and to the oral gavage procedure using water. Chow was removed, and 30 min later, animals were gavaged with 0.5 ml of water via a feeding needle, followed by a 30-min lard meal given starting 120 min after that, at dark onset. For the following 6 days, the same procedure was followed, except that one group was gavaged intragastrically with gemfibrozil (50 mg/kg i.g.; Schering-Plough, Kenilworth, NJ) and the other with vehicle (Tween 20, Sigma-Aldrich Co., St. Louis, MO) prior to the same 10-kcal HiSat meal. On the final day, the animals were gavaged with gemfibrozil or vehicle and then sacrificed 60 min after completion of the meal. Trunk blood was collected for analysis of TG and brains dissected for qRT-PCR analysis of GAL, OX, NPY, and AgRP mRNA.

2.4. Blood sampling procedures

For Experiment 1, tail vein blood was collected during testing for measurements of TG levels. For Experiments 2–4, trunk blood was collected at the time of sacrifice for measurements of TG levels and hormones.

2.5. Hormone and metabolite determinations

Serum was assayed for insulin and leptin using RIA kits from Linco Research Inc, MO and for corticosterone using an RIA kit from MP Biomedicals, NY. Triglycerides and glucose were measured with an E-Max Microplate Reader using, respectively, a Triglyceride Assay Kit or glucose Trinder Reagent Kit from Sigma, St Louis, MO.

2.6. Real-time quantitative PCR

Immediately after sacrifice, the brains removed for peptide measurements using qRT-PCR were placed in a matrix on ice with the ventral surface facing up. A total of five coronal cuts, yielding four slices, were made using gem razor blades (American Safety Razor Co., Verona, VA). The first cut was made in the anterior middle optic chiasm (Bregma −0.8 mm), according to the atlas of Paxinos and Watson [32]. The second cut was made 1.0 mm

caudal to this, yielding a slice used for microdissection of the PVN (Bregma -0.8 to -1.8 mm). A third slice, made 0.5 mm rostral to this, yielded a slice that was discarded. The fourth slice, 0.5 mm thick, was then used for microdissection of the ARC (Bregma -2.3 to -2.8 mm). The fifth slice, 1 mm thick, was then used for microdissection of the PFLH (Bregma -2.8 to -3.6 mm). Each slice was placed on a microscope slide on top of an ice-filled petri dish (Fisher Scientific, Pittsburgh, PA). The brain regions were then dissected from the slices under a dissecting microscope using a #11 scalpel (BD Bard Parker, Franklin Lakes, NJ). The PVN was dissected as an inverted isosceles triangle, 1.0 mm bilateral to the ventricle and between the fornix structures. For the PFLH, the dissection was taken from the area surrounding the fornix, within a target range of 0.2 mm medial and ventral to the fornix, 0.3 mm dorsal and 0.1 mm lateral. For the ARC, the area adjacent to the bottom of the third ventricle was dissected parallel to the border of the ventricle, with the width of 0.3 mm at the bottom gradually narrowing to 0.1 mm at the top. These dissections were immediately placed into RNAlater (Ambion, Inc., Austin, TX) until processing.

As previously described [26], total RNA from pooled microdissected PVN, PFLH or ARC samples was extracted with TRIzol reagent. RNA was treated with RNase-free DNase I before RT. For qRT-PCR, cDNA and minus RT were synthesized using an oligo-dT primer with or without SuperScript II Reverse Transcriptase. The SYBR Green PCR core reagents kit (Applied Biosystems, CA) was used, with β -actin as endogenous control. PCR was performed in MicroAmp Optic 96-well Reaction Plates (Applied Biosystems) on an ABI PRISM 7700 Sequence Detection system (Applied Biosystems), with the condition of 2 min at 50°C , 10 min at 95°C , then 40 cycles of 15 s at 95°C and 1 min at 60°C . Each study consisted of 4 independent runs of PCR in triplicate, and each run included a standard curve, non-template control, and negative RT control. The levels of GAL, OX, NPY, and AgRP gene expression were quantified relative to the level of β -actin, using standard curve method. The primers, designed with ABI Primer Express V.1.5a software based on published sequences, were: 1) β -actin: $5'$ -GGCCAACCGTGAAAAGATGA- $3'$ (forward) and $5'$ -CACAGCCTGGATGGCTACGT- $3'$ (reverse); 2) GAL: $5'$ -TTCCCACCACTGCTCAAGATG- $3'$ (forward) and $5'$ -TGGCTGACAGGGTTGCAA- $3'$ (reverse); 3) OX: $5'$ -AGATACCATCTCTCCGGATTGC- $3'$ (forward) and $5'$ -CCAGGGAACCTTTGTAGAAGGA- $3'$ (reverse); 4) NPY: $5'$ -CACAGAAAATGCCCCAGAA- $3'$ (forward) and $5'$ -GTCAGGAGAGCAAGTTTCATTTC- $3'$ (reverse); and 5) AgRP: $5'$ -GCAGAGGTGCTAGATCCACAGAA- $3'$ (forward) and $5'$ -AGGACTCGTGCAGCCTTACAC- $3'$ (reverse). The concentrations of primers were 100 to 200 nM, and all reagents, unless indicated, were from Invitrogen.

2.7. Data analysis

All values are expressed as mean $\pm$ SEM. For Experiment 1, TG levels were analyzed by a one-way repeated measures ANOVA, using first meal type as the within-subject factor, followed by pairwise comparisons using a Bonferroni correction. Chow intake during the test meal was analyzed by a two-way repeated measures ANOVA, with first meal type and time as within-subject factors. Testing for the simple main effects at each time point was conducted with a one-way repeated measures ANOVA and pairwise comparisons using a Bonferroni correction. For Experiment 3, weekly diet intake and body weight were first analyzed by a two-way repeated measures ANOVA, with time as the within-subject factor and diet as the between-subject factor, and were then followed up by unpaired two-tailed t -tests when appropriate. In all other cases, statistical analyses of the different measures for the groups in each experiment were performed using paired or unpaired two-tailed t -tests where appropriate.

3. Results

3.1. Effect of a HiSat versus USat or chow meal on lipid levels and chow intake

With evidence closely linking TG to certain neuropeptides that promote overeating (see Section 1), Experiment 1 was designed to test, in the same animals, whether the HiSat diet as compared to the USat or a chow diet, when given acutely in a 10-kcal meal, differentially stimulates circulating levels of these lipids in conjunction with subsequent caloric intake. In blood collected via the tail vein, measurements of serum TG showed that levels were stimulated differently depending on the diet consumed ($F(2, 18) = 9.49, p < 0.01$) (Figure 1, left panel). Specifically, TG levels after the HiSat meal were 45% higher than after an equicaloric USat meal ($p < 0.05$) and 55% higher than after an equicaloric chow meal ($p < 0.01$), with no difference seen after the USat compared to chow meal (not significant, *ns*). When animals were given *ad libitum* access to lab chow following the 10-kcal HiSat, USat or chow meal, their intake differed depending on the diet consumed ($F(2, 18) = 4.19, p < 0.05$) (Figure 1, right panel). This was due to a significant difference during the first 15 minutes of chow access ($F(2, 18) = 5.83, p < 0.05$). At this time, intake after the HiSat meal was 55% higher than after the USat meal ($p < 0.05$) and 57% higher than after the chow meal ($p < 0.05$) while intake after the USat or chow meal was remarkably similar (-1% , *ns*). There was no longer any difference in intake after 30 min ($F(2, 18) = 3.51, ns$) or 24 h ($F(2, 18) = 2.72, ns$). Thus, acute consumption of a fat-rich diet containing relatively high levels of saturated fat has an initial stimulatory effect on subsequent caloric intake in association with an increase in circulating lipids both when compared to a diet with higher levels of unsaturated fat as well as to a standard chow diet.

3.2. Effect of a HiSat versus USat meal on TG levels and expression of orexigenic peptides

In separate groups of rats, Experiment 2 tested whether a single HiSat versus USat meal differentially stimulates the expression of orexigenic peptides in the hypothalamus and whether males and females differ in this response. These rats consumed the entire, 10-kcal meal during 30 min. In Experiment 2A, one hour after the end of the meal, TG levels were 42% higher ($p < 0.05$) in the male rats that consumed the HiSat meal compared to those consuming the equicaloric USat meal (Figure 2A, left panel). In these same rats, the expression of GAL mRNA in the PVN and OX mRNA in the PFLH was also significantly elevated after the HiSat meal ($+268\%$, $p < 0.001$ and $+111\%$, $p < 0.05$, respectively) (Figure 2A, right panel). In contrast, NPY and AgRP expression in the ARC was slightly but not significantly decreased (-21% , *ns* and -3% , *ns*, respectively). Similarly, in the female rats, TG levels one hour after the meal were 69% higher ($p < 0.05$) in those that had consumed the HiSat meal compared to the USat meal (Figure 2B, left panel). Like the males, expression of GAL in the PVN and OX in the PFLH was significantly elevated after the HiSat meal ($+194\%$, $p < 0.01$ and $+104\%$, $p < 0.05$, respectively) (Figure 2B, right panel), while NPY and AgRP in the ARC was slightly but not significantly decreased (-17% , *ns* and -3% , *ns*, respectively). These results provide the first evidence that the fatty acid composition of a high-fat diet is an important factor in stimulating endogenous expression of this specific orexigenic peptides through its effect on circulating TG levels and that this effect is not sex-dependent.

3.3. Effect of a chronic HiSat versus USat diet on expression of orexigenic peptides

In a separate set of rats, Experiment 3 tested whether chronic consumption of a HiSat diet compared to a USat diet is similar to an acute meal in stimulating the expression of GAL and OX while having little effect on NPY or AgRP in the hypothalamus. With 30 days of access to these diets, the rats showed no difference in their body weight ($F(1, 8) = 0.20, ns$), either before (USat: 335 ± 2 g; HiSat: 331 ± 3 g) or after (USat: 501 ± 17 g; HiSat: 525 ± 4 g) completion of the experiment. Analysis of caloric intake revealed increased intake of the

HiSat versus USat diet during the first week of access (83% more calories, $p < 0.01$) but not during the second, third or fourth weeks (Figure 3). While the rats on the HiSat diet consumed more overall calories than those on the USat diet ($F(1, 8) = 7.39$, $p < 0.05$), there was a significant interaction between diet and time ($F(3, 24) = 7.07$, $p < 0.001$), due to the significant increase in HiSat diet consumption during the first week of the experiment. At the time of the sacrifice, when all rats were similar in body weight and food intake, rats chronically consuming the HiSat diet as compared to USat diet exhibited a significant increase in their circulating TG levels (+115%, $p < 0.05$) (Figure 4, left panel) and also in the expression of GAL in the PVN (+38%, $p < 0.05$) and OX in the PFLH (+20%, $p < 0.05$) (Figure 4, right panel). These differences between the diet groups were similar to those observed in the Experiment 2 with acute diets. In addition to these changes, measurements of NPY and AgRP showed that chronic consumption of the HiSat compared to USat diet significantly suppressed their expression in the ARC (−12%, $p < 0.05$ and −24%, $p < 0.01$, respectively) (Figure 4, right panel). These peptide changes together with elevated TG levels occurred in the absence of any change in circulating levels of the adiposity hormones, leptin and insulin, or to levels of glucose or corticosterone (Table 1). These results show that diets with higher saturated fatty acid content, when compared to diets with higher unsaturated fatty acid content, can significantly affect orexigenic peptide gene expression as well as circulating TG under chronic conditions, without any changes in body weight or adiposity-related hormones.

3.4. Effect of a TG-lowering drug on hypothalamic gene expression after a HiSat diet

The objective of Experiment 4 was to determine whether peptide gene expression induced by the HiSat diet, in association with elevated TG, can be prevented by a compound that lowers TG levels. Separate groups of weight-matched rats were intragastrically administered the TG-lowering drug, gemfibrozil (50 mg/kg i.g.), or vehicle prior to a 10-kcal meal of the HiSat diet. Without affecting intake of this small meal, gemfibrozil compared to vehicle markedly reduced TG levels by 53% ($p < 0.01$) following HiSat exposure (Figure 5, left panel). In addition, it produced a gene expression pattern which was generally opposite to that induced by a HiSat diet that elevates TG. Compared to vehicle, administration of gemfibrozil caused a decrease in expression of OX in the PFLH (−35%, $p < 0.05$) and elevated NPY (+85%, $p < 0.05$) and AgRP (+76%, $p < 0.05$) expression in the ARC (Figure 5, right panel). On the other hand, this compound had little effect on the expression of GAL in the PVN (−7%, *ns*) (Figure 5, right panel). Thus, the lowering of TG has effects on orexigenic peptides that are generally opposite to those produced by a HiSat diet that increases TG.

4. Discussion

The present study demonstrates that a high-fat diet relatively high in saturated fatty acids elevates levels of circulating lipids significantly more than a diet relatively high in unsaturated fatty acids, even when the total concentration of fat is identical between the two diets. This result is consistent with previous reports involving similar diets provided over 2-day to 7-week periods [30, 31, 33] and is the first to show this difference with a much shorter period of intake, lasting only 30 min. Although the source of unsaturated fat in the HiSat (soybean oil) and USat (fish oil) diets differed, the finding that TG levels are similar after 1 week of a soybean versus fish oil-based high-fat diet [34] suggests that the difference in TG observed with the diets of the present study more likely reflects their different level of saturated fat. The increase in lipid levels also appears to reflect the well-known stimulatory effect of a diet high in saturated fat, rather than the ability of the fish-oil ingredient of the USat diet to reduce TG levels [35]. Consistent with published studies revealing the TG-lowering effects of fish oil only in patients with hypertriglyceridemia or after extended

periods of fish oil consumption [35, 36], the present study in normal-weight rats showed no difference in lipid levels after consumption of the USat meal as compared to the equicaloric lab chow meal. As plasma TG reflect the lipid component of dietary fat circulating after a meal and the HiSat and USat meals contained the same total concentration of fat, this difference in circulating lipid levels likely reflects a less efficient hydrolysis of the TG in the HiSat compared to USat diet.

In agreement with the finding that an acute elevation of TG by a fat-rich diet can stimulate hyperphagia in a subsequent meal [7, 22], the higher levels of TG after the 10-kcal HiSat meal, as compared to the 10-kcal USat or chow meal, were followed by a larger chow test meal. To our knowledge, this finding represents the first report that the type of fat in the diet can determine subsequent caloric intake. While the manual weighing of the food dishes could be a factor in differentially affecting subsequent intake in a diet-dependent manner, this appears unlikely since hyperphagia with the HiSat versus the USat diet was also observed during the first week of the 30-day test when food dishes were only handled every few days. The animals with 30 days of access to these diets showed significantly elevated overall caloric intake with a HiSat versus USat diet, due to the large increase of intake during the first week of access. These results are consistent with one study reporting increased intake of a lard diet compared to fish oil diet with 6 weeks of access [30], although there are two others showing no difference with 3 or 7 weeks of diet access [37, 38], possibly due to differences in the concentrations of fatty acids in the diets. These effects of a HiSat diet compared to a USat diet, involving increased lipid levels along with hyperphagia, are likely to be major contributors to the obesity and other health complications associated with saturated fatty acids in the diet [39].

Another result of the current study is that a HiSat compared to USat diet significantly alters the expression of hypothalamic orexigenic peptides, in a region-specific manner. The HiSat diet compared to USat diet produced a significant increase in the expression of GAL in the PVN and of OX in the PFLH. These effects were demonstrated after a single, 10-kcal meal and also after 30 days of diet consumption. These results, which are new for GAL and consistent with a published study of OX after 6 weeks of diet access [30], suggest that these particular orexigenic peptides, which are enhanced by consumption of a high-fat compared to low-fat diet [3, 11], are similarly stimulated by ingestion of a HiSat compared to USat diet. With evidence that fat-preferring Brattleboro rats compared to control Long Evans rats exhibit higher GAL mRNA and lower GAL concentrations in the PVN [40, 41] and that injection of GAL antagonists into this brain area significantly decreases fat consumption [42], it is likely that the elevated GAL expression in the present experiment, particularly as it occurred after consumption of a single meal, may contribute to the initial hyperphagia produced by the HiSat diet. A similar conclusion regarding increased intake may be drawn for OX, which is thought to play a major role in seeking out rewarding substances in general [43]. The stimulatory effect of hypothalamically injected OX on fat intake [13], along with elevated levels of this peptide in rats prone to overconsuming a fat-rich diet [44], suggest that, similar to GAL, this peptide may also play a role in an increased food intake following a HiSat meal as well as during chronic access to this diet. The lack of change in the body weight may also be the result of changes in hypothalamic peptides. While there is a general lack of relationship observed between GAL and bodyweight in studies with GAL mutant mice [45] or obese children and adolescents [46], OX may be directly involved in the lack of body weight difference between rats consuming HiSat and USat diet. This idea is supported by a study where transgenic overexpression of OX, perhaps by increasing energy expenditure, can actually facilitate resistance to fat-induced obesity [47], which may explain the similar body weights of the HiSat- and USat-consuming rats in the present study.

The peptide changes in the ARC are generally opposite to those observed with the orexigenic peptides in the PVN and PFLH. It has previously been demonstrated that a saturated fat diet compared to an unsaturated fat diet can suppress NPY and AgRP mRNA in the ARC when given to mice for 7 days or 7 weeks [48]. Although this effect was not seen in rats in another study after 6 weeks of diet consumption [30] or in the current study after a single meal, it was evident here with 30 days of HiSat compared to USat diet consumption. Previous studies of the NPY system after a high-fat compared to a chow diet have shown that fat, in addition to decreasing NPY expression after several weeks of intake, increases the animals' sensitivity to the hyperphagic effects of NPY administration [49, 50]. Whereas this increased sensitivity to NPY may help to explain the initial hyperphagia in the present study, the decrease in NPY and AgRP expression may then contribute to the normalization of the HiSat diet intake occurring after the first week of access. This gradual decline in the orexigenic signals from these peptides in the ARC suggests that they are reacting in a homeostatic manner to counteract excessive intake and weight gain on a high-fat diet.

The diet-induced changes in the expression of these hypothalamic orexigenic peptides, as well as levels of TG, were very similar between male and female rats. Previous studies have shown females to exhibit a stronger preference for dietary fat than males [6, 51], in association with higher expression of OX and GAL [52, 53], which are stimulated by estrogen [51, 52, 54]. In contrast, this gender difference is not apparent with NPY in the arcuate nucleus [55]. The present study failed to reveal significant gender differences in responsiveness to the HiSat fat diet, showing females to be similar to males in their peptide changes. This is consistent with published evidence showing females and males to be comparable in their increased energy intake with a high-fat compared to low-fat diet [5]. In the present study, the comparable changes in hypothalamic peptide expression likely reflect a similar orexigenic response in female and male rats after consuming the HiSat compared to the USat diet. Thus, the females appear to respond to different fatty diets in largely the same way as males despite different baselines in fat preference and peptide expression.

The importance of circulating lipids in these effects on the peptides is supported by the results obtained with the TG-lowering drug, gemfibrozil. Acute administration of this compound, in rats given a 10-kcal meal of the HiSat diet, produced generally opposite effects to those of the TG-stimulating HiSat diet. The expression of OX mRNA in the PFLH was lower and NPY and AgRP mRNA in the ARC was higher after gemfibrozil compared to vehicle, strengthening the idea that circulating TG are involved in the diet-induced changes in peptide expression. Whereas leptin, insulin, glucose, and corticosterone are also known to modulate the expression of these peptides [56–58], they were not significantly altered by chronic consumption of the HiSat compared to USat diet, in agreement with previous reports [30, 37]. The elevated TG on the HiSat diet likely affect the different orexigenic peptides through their fatty acid constituents in the circulation, which can pass through the blood brain barrier to reach the hypothalamus [59, 60]. Previous research has shown that certain fatty acids, specifically the long-chain unsaturated fatty acid oleic acid, can decrease expression of both NPY and AgRP [61] but that this same fatty acid differentially affects hypothalamic neurons, stimulating the activity of some while inhibiting others [59]. These distinct responses are very likely due to differences in fatty acid receptor expression throughout the hypothalamus. For example, GPR40, a receptor for polyunsaturated fatty acids, has been detected in the PVN [62], while fatty acid translocase CD36, which mediates the cellular uptake of very long chain fatty acids, has been localized in both the anterior PVN and the lateral hypothalamus [63]. The HiSat and USat diets in the present experiment were not only composed of different fatty acids, but as suggested by the resulting TG levels, these fatty acids likely crossed into the brain over different time-courses. The evidence suggests that the fatty acids affected different hypothalamic neurons by binding to distinct receptors and through this mechanism differentially affected peptide expression.

One unexpected finding was that GAL in the PVN, which was strongly stimulated by the HiSat compared to USat diet, was unaffected by administration of gemfibrozil. Although PVN GAL expression is found to be closely, positively associated with TG levels [4], the present finding suggests that this peptide can also be modulated by other factors related to dietary fat. The taste of fat may be one such factor that is sufficient to maintain an elevation of PVN GAL expression. Research indicates that rats and mice can detect fat in the mouth directly through taste receptors as mediated by CD36 [64, 65]. Interestingly, both GAL and the GalR2 receptor are expressed in the rat taste bud-containing epithelium [66], and mice lacking the GAL gene are found to strongly avoid eating fat [67]. Thus, even in the absence of higher TG, PVN GAL expression may still remain elevated after consumption of a fat-rich meal.

Chronic access to a high-fat diet generally leads to overconsumption, which can ultimately lead to serious health complications. The present results underscore the importance of the composition of the fat in the diet in terms of its behavioral and neurobiological consequences, with diets relatively higher in saturated fatty acids producing greater initial hyperphagia and higher lipid levels compared to diets higher in unsaturated fatty acids. Although certain peptides in the ARC are eventually downregulated by the HiSat diet, perhaps to maintain homeostasis, GAL in the PVN and OX in the PFLH are upregulated by this diet. These peptides are likely to have a role in mediating the increased drive toward excessive consummatory behavior produced by saturated fat, which in turn contributes to the rise in circulating lipids.

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List of abbreviations

AgRP	agouti-related protein
ARC	arcuate nucleus
GAL	galanin
HiSat	diet with relatively high levels of saturated fatty acids
NPY	neuropeptide Y
<i>ns</i>	not significant
OX	orexin
PFLH	perifornical lateral hypothalamus
PVN	paraventricular nucleus of the hypothalamus
qRT-PCR	quantitative real-time polymerase chain reaction
TG	triglycerides
USat	diet with relatively high levels of unsaturated fatty acids

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Research Highlights

- A meal higher in saturated vs. unsaturated fat stimulates circulating triglycerides
- A meal higher in saturated vs. unsaturated fat stimulates subsequent food intake
- A meal higher in saturated vs. unsaturated fat stimulates hypothalamic peptides
- A diet higher in saturated vs. unsaturated fat has similar effects to acute meals
- Lowering triglycerides has effects opposite those of a meal higher in saturated fat

Figure 1.

A 10-kcal HiSat meal stimulates circulating triglyceride levels and subsequent chow intake more than a 10-kcal USat or chow meal. Data are mean $\pm$ S.E.M., * $p < 0.05$ vs. chow or USat meal.

Figure 2A.

Figure 2B.

Figure 2.

Figure. 2A. A 10-kcal HiSat compared to USat meal stimulates circulating triglyceride levels and differentially affects peptide gene expression in various hypothalamic areas 60 min later. The HiSat diet stimulates galanin (GAL) mRNA expression in the paraventricular nucleus (PVN) and orexin (OX) in the perifornical lateral hypothalamus (PFLH), while it does not significantly affect neuropeptide Y (NPY) or agouti-related protein (AgRP) in the arcuate nucleus (ARC). Data are mean $\pm$ S.E.M., * p < 0.05, ** p < 0.01 vs. USat meal. Figure. 2B. As in male rats, a 10-kcal HiSat compared to USat meal in females stimulates circulating triglyceride levels and differentially affects peptide gene expression in various hypothalamic areas 60 min later. The HiSat diet stimulates galanin (GAL) mRNA expression in the paraventricular nucleus (PVN) and orexin (OX) in the perifornical lateral hypothalamus (PFLH), while it does not significantly affect neuropeptide Y (NPY) or agouti-related protein (AgRP) in the arcuate nucleus (ARC). Data are mean $\pm$ S.E.M., * p < 0.05, ** p < 0.01 vs. USat meal.

Figure 3.

A HiSat compared to USat diet presented over a 30-day period increases 24-h caloric intake in the first week of access but not in subsequent weeks. Data are mean $\pm$ S.E.M., $**p < 0.01$ vs. USat diet.

Figure 4.

A HiSat compared to USat diet for 30 days increases circulating triglyceride levels and differentially affects peptide gene expression in various hypothalamic areas. The HiSat diet stimulates galanin (GAL) mRNA expression in the paraventricular nucleus (PVN) and orexin (OX) in the perifornical lateral hypothalamus (PFLH), while it reduces neuropeptide Y (NPY) and agouti-related protein (AgRP) mRNA expression in the arcuate nucleus (ARC). Data are mean $\pm$ S.E.M., * p < 0.05, ** p < 0.01 vs. USat diet.

Figure 5.

Lowering triglyceride levels with gemfibrozil (50 mg/kg i.g.) after a 10-kcal HiSat meal affects hypothalamic gene expression. Gemfibrozil compared to vehicle decreases orexin (OX) mRNA in the perifornical lateral hypothalamus (PFLH), while having no effect on galanin (GAL) mRNA in the paraventricular nucleus (PVN), and it increases neuropeptide Y (NPY) and agouti-related protein (AgRP) mRNA expression in the arcuate nucleus (ARC). Data are mean $\pm$ S.E.M., ** $p < 0.05$, ** $p < 0.01$ vs. vehicle.

Table 1

A HiSat compared to USat diet for 30 days had no significant effect on levels of leptin, insulin, glucose or corticosterone.

	Leptin (ng/ml)	Insulin (ng/ml)	Glucose (mg/dl)	Corticosterone (ng/ml)
USat	17.1 ± 1.8	3.7 ± 0.5	182.0 ± 13.2	49.0 ± 6.3
HiSat	16.2 ± 0.5	4.0 ± 1.2	179.3 ± 10.3	40.3 ± 4.6

Data are mean ± S.E.M.