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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.

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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 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

Abbreviations: AgRP, agouti-related protein; ARC, arcuate nucleus; GAL, galanin; HiSat, diet with relatively high levels of saturated fatty acids; NPY, neuropeptide Y; *ns*, 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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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 for the Care and Use 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 (Experiments 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 was 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-