THE EFFECT OF HIGH FRUCTOSE INTAKES IN THE RAT DIET ON SERUM GHRELIN AND BODY COMPOSITION

by

CAROLINE ELIZABETH COLQUITT

(Under the Direction of Silvia Giraudo)

ABSTRACT

This study investigates the effects of high fructose consumption in the rat diet on body composition and metabolic parameters, including serum glucose, insulin, and triglyceride concentrations. The effect of high fructose consumption on fasting and post-prandial ghrelin concentrations was also examined. Serum glucose concentrations were significantly higher in fructose-fed rats than dextrose-fed rats at weeks 1 (P = 0.047), 2 (P = 0.009), and 4 (P = 0.009). Greater increases in serum triglyceride concentrations were observed in the fructose-fed group at weeks 2 (P = 0.024), 3 (P = 0.024), and 4 (P = 0.032). Fasting ghrelin concentrations displayed trends toward significance for the treatment effect (P = 0.062) and the change over time (P = 0.076). Also, fructose-fed rats had significantly higher liver weights (P = 0.0004). This study demonstrates that high fructose consumption can affect metabolic parameters associated with obesity and may indicate an alteration in ghrelin activity.

INDEX WORDS: ghrelin, fructose, insulin resistance, body composition

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CAROLINE ELIZABETH COLQUITT

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CAROLINE ELIZABETH COLQUITT

Major Professor: Silvia Giraudo

Committee: Dorothy Hausman
Richard Lewis

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
University of Georgia
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DEDICATION

The completion of this thesis project would not have been possible without the love, support, and encouragement from my parents, to whom this thesis is dedicated.

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CHAPTER 1

INTRODUCTION

In the US, between 2007 and 2009, there was a 1.1% increase in the prevalence of obesity among adults. That means that during that time, an additional 2.4 million adults were classified as obese. Investigators studying this data have proposed that by 2050, the percentage of overweight (BMI ≥ 25) and obese (BMI ≥ 30) individuals will be approaching 100% [1]. The etiology of obesity is complex, therefore establishment of prevention and treatment measures for overweight and obesity on a population-wide basis is extremely difficult [2]. Parallel to the prevalence of obesity is the increase in fructose consumption, 25%, as well as high fructose corn syrup (HFCS) consumption, 1000%, over the past 30 years [3]. Although the causes of obesity are multi-factorial, the increased use of HFCS has been proposed as an important dietary factor contributing to the increase in obesity in the US [4]. Therefore, it is imperative to understand the metabolic effects that can result from high fructose consumption in attempts to reduce the rising prevalence of obesity.

Numerous studies have provided strong evidence that fructose consumption effects energy balance. These studies report associations between fructose consumption and increased food intake, weight gain, glucose intolerance, insulin resistance, and triglyceride concentrations[5-15]. These effects are suggested to be attributed to the differences in fructose and glucose metabolism. Differing from glucose, fructose metabolism does not

stimulate the secretion of insulin from the pancreas, a potential mechanism for weight gain associated with fructose consumption [10]. Also, fructose enters the glycolytic or gluconeogenic pathway at the triose-phosphate level, by-passing the rate-limiting step regulated by phosphofructokinase. Therefore, fructose serves as an unregulated source of acetyl-CoA production, enhancing lipogenesis [16, 17].

A greater understanding of the role of high fructose consumption on obesity can be established through identifying the effects that fructose elicits on hunger and satiety hormones. Ghrelin is a 28 amino peptide that is secreted by a number of endocrine cells within the body, primarily X/A oxyntic cells inside the stomach [18]. It is the only known circulating orexigenic gastrointestinal peptide[19]. Ghrelin is reported to display a diurnal pattern in lean subjects, with pre-prandial increases and post-prandial decreases; however studies have shown that obese individuals do not experience this post-prandial decline [20, 21]. Additionally, ghrelin activity is suggested to be regulated both directly and indirectly by insulin [22, 23]. The effect of high fructose consumption on ghrelin activity is still to be determined.

This thesis aims to investigate the effect of high fructose food intake in the rat diet on food intake, body weight, serum glucose, insulin, and triglyceride concentrations, as well as pre-prandial and post-prandial ghrelin concentrations over the course of four weeks. We will also examine the effect of high fructose consumption on body composition. The results of this study could be a beneficial addition to what is already understood about high fructose food consumption and obesity, as an alteration in ghrelin concentrations could indicate decreased satiety and ultimately overeating ensuing from high fructose food consumption.

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CHAPTER 2

LITERATURE REVIEW

The consumption of fructose has increased substantially over the past 40 years [24] and has paralleled the increasing prevalence of obesity in the US [25]. Fructose is associated with altered metabolic parameters, increased food intake, and weight gain [7, 10, 15, 26]. Although many studies provide strong preliminary data, the metabolic effects of fructose are measured through fructose-sweetened beverages and very few studies examine the relationship between fructose, ghrelin activity, and body composition. The purpose of this study is to investigate the relationship between fructose food intake, metabolic parameters and body composition, through the use of an animal model fed a high fructose diet (40% kcal fructose) and assessment of serum glucose, insulin, triglycerides, and ghrelin concentrations.

Fructose consumption and obesity

NHANES 1999-2004 data documented that from 1978 to 2004 total energy intake increased by 18% and total carbohydrate intake by 41% [27]. The US population, based on food survey data and food disappearance data, has increased its consumption of fructose 25% over the past 30 years, parallel to the increase in obesity [6, 16, 27]. In addition, HFCS consumption has increased 1000% during the past three decades [3]. Fructose has been suggested to increase palatability of food with the resulting consequence of overeating [26]. Currently, there has been no conclusive epidemiological studies that support the idea that fructose may be

causal of obesity in the general population [28]. However, increased use of high-fructose corn syrup as a sweetener over the last several decades has been proposed as one important dietary factor that may contribute to the widespread increase in obesity in the U.S. [4]. The extreme sweetness and widespread use in soft drinks and processed foods is argued to be one factor driving the consumption of increased energy which fuels the current obesity epidemic [6].

Pure fructose is present in significant quantities in fruits, honey, and some vegetables. However, the largest single source of fructose in the diet is added sugars, consumed in soft drinks and other sweetened beverages as well as candies and desserts. In the 1960s, the introduction of isomerase technology enabled the conversion of cornstarch to high fructose corn syrup (HFCS) for use in soft drinks and highly processed foods. In the U.S. food supply the primary source of added sweeteners is HFCS, which contains a mixture of fructose and glucose. The most common formulations contain either 55% fructose (HFCS-55) or 42% fructose (HFCS-42) [16]. Concentrated fructose syrup is also produced, consisting of 90% fructose, known as HFCS-90. In the U.S. from the early 1970s to mid 1990s, HFCS rapidly replaced sugar used in soft drinks and manufactured products due to its extreme sweetness, long shelf life, ability to maintain moistness, and low cost [6]. HFCS-55 is most commonly used in sweetened beverages whereas HFCS-42 is used primarily in processed food products [6, 16]. The per capita amount of fructose consumed in the United States ranges from 8 to 100 g/day, and the average is approximately 80 g/day. In addition, about 330-380 kcal/day of the energy intake of average Americans is derived from fructose (that is about 17-20% of daily energy intake, based on a 2000 kcal diet)[29]. Data from the NHANES 1999-2004 study documented that the consumption of HFCS accounted for 42% of total caloric sweetener consumption, compared to

16% in 1977-1978, and it is likely that today, fructose constitutes roughly 50% of the energy from added sweeteners [6, 16, 27]. Higher absolute amounts of fructose tend to be consumed by males than females, but the difference was not significant when intakes were reported as a percentage of the total energy intake. Sweetened beverages are the main dietary source of fructose and fructose consumption is highest among teenagers and young adults [27]. For example, some age groups such as adolescent males, who are heavy consumers of soft drinks, consume 2.2 times the average intake, and are likely to be consuming well over 100 g/d of fructose from added sweeteners [16].

Fructose absorption and metabolism

When fructose is ingested, either as pure fructose, HFCS, or sucrose, it is absorbed across the intestinal brush-border membrane by a fructose-specific transporter, GLUT5, into an enterocyte on the intestinal epithelium. Fructose absorption does not require the assistance from ATP hydrolysis or sodium absorption, unlike glucose. In addition, fructose absorption appears to be enhanced when consumed along with glucose, such as the usual consumption from beverages and processed foods [16]. Fructose in the enterocyte, diffuses into blood vessels on the basolateral pole of the enterocyte mediated by GLUT2 transporter [27]. Fructose is then transported from the enterocyte to the portal circulation via the GLUT5 transporter, which is primarily found in the jejunum, however it is present in smaller amounts in the kidney, skeletal muscle, adipocytes, and glial cells [16]. GLUT2, a low-affinity transporter of fructose is involved in fructose uptake across the hepatic membrane into the liver [29]. The liver readily extracts fructose from the portal circulation and very little fructose escapes hepatic metabolism, therefore the liver is the most important site of fructose metabolism. In the liver,

fructose is phosphorylated by ATP and rapidly metabolized to fructose-1-phosphate catalyzed by the highly fructose-specific enzyme fructokinase [27, 30]. The enzyme aldolase B then metabolizes fructose-1-phophate to glyceraldehyde and dihydroxyacetone phosphate. Triokinase, another enzyme specific for fructose metabolism, catalyzes the conversion of glyceraldehyde to glyceraldehyde 3-phosphate, an intermediate of the glycolytic pathway. In addition, dihydroxyacetone phosphate is reduced by glycerol 3-phosphate dehydrogenase to glycerol 3-phosphate, the significance of which will be discussed later [16, 27, 30]. Fructose conversion to triose phosphates (glyceraldehyde, dihydroxyacetone, and glyceraldehyde 3-phosphate) occurs rapidly because of the high affinity of fructokinase for fructose [27]. Thus, fructose metabolism provides increased substrate to the metabolic pathways that lead from the triose phosphates. These pathways include gluconeogenesis, glycolysis, glycogenesis, and lipogenesis and the major products that result are glucose, hepatic glycogen, and lactate[27, 30]. The triose phosphates are oxidized in liver cells to carbon dioxide and the major portion of the carbon atoms enters the gluconeogenic pathway and are released as glucose or glycogen [27]. Smaller, but significant amounts are converted to triacylglycerols through de novo lipogenesis, the process by which excess dietary carbohydrate is converted into fat [30, 31]. During de novo lipogenesis, fructose carbon enters glycolysis where it is metabolized to lactate, pyruvate, and acetyl-CoA. A portion of this lactate is also taken up by the liver where it enters gluconeogenesis and is converted to glucose or glycogen, or it can be metabolized to form acetyl-CoA [16, 27]. Acetyl-CoA joins glycerol-3-phosphate to form acyl glycerols, triglycerides and ultimately very-low-density-lipoprotein (VLDL)[16, 27, 30].

The metabolism of fructose greatly differs from that of glucose. Glucose is absorbed by a variety of different GLUT transporters depending on the tissue in which glucose uptake occurs. For example, in the liver, brain and red blood cells, glucose is absorbed in an insulin-dependent manner by GLUT4 transporters [32]. In adipose tissue and muscle, glucose is taken up in an insulin-dependent manner by GLUT2 transporters. In contrast, fructose is only absorbed in an insulin-independent manner through two GLUT transporters, GLUT5 and GLUT2 [17]. In addition, fructose is rapidly taken up by the liver because of the high affinity of fructokinase for fructose, where as the demand for glucose uptake is dependent upon the tissue involved and the physiological environment of the cell [16, 17]. Inside the cell, glucose metabolism stops when the liver cannot store any more glucose as glycogen. The inhibition of further glucose uptake, and ultimately glycolysis occurs through allosteric inhibition of phosphofructokinase by ATP and citrate [16]. Fructose enters the glycolytic or gluconeogenic pathway at the triosphosphate level, by-passing the rate-limiting step regulated by phosphofructokinase. Therefore, when large amounts of fructose are consumed, ADP and citrate do not exert a negative feedback regulation on fructokinase and aldolase B. Significant amounts of fructose thus continue to enter glycolysis increasing triglyceride production in the liver. Serving as an unregulated source of hepatic glyceraldehyde 3-phosphate and acetyl-CoA production, fructose thus enhances lipogenesis.

Fructose intake and metabolic effects

Understanding the metabolic effects that result from fructose consumption will provide further insight as to the roles of fructose in the current obesity epidemic. To determine this, we will measure certain metabolic parameters to determine how greatly fructose can influence the

risk for developing obesity. Metabolic parameters associated with the metabolic syndrome, which include abdominal obesity, dyslipidemia, insulin resistance, and glucose intolerance are of major concern in a number of other studies [16]. Thedevelopment of features of the metabolic syndrome including dyslipidemia, obesity, and insulin resistance have been observed after chronic fructose consumption in animal models [7, 8, 10]. There are no long-term, controlled studies in humans, but short-term studies have reported dyslipidemia as well as insulin resistance after consumption of fructose concentrations ranging from 7.5% to 25% of total kcal per day [13, 15, 32]. Bray (2008) reviewed the relationship between fructose consumption and body weight in humans through the evaluation of 12 cross-sectional and five longitudinal studies. Of the cross-sectional studies, 10 found a positive association between fructose-containing soft drink consumption and energy intake. An even stronger positive association was observed among the longitudinal studies. Similar results have been shown in animal studies. Light et al (2009) reports that after 8 weeks, rats drinking a HFCS solution had heavier final body weights compared to rats consuming the distilled water control and had heavier final body weights than rats consuming a glucose solution ($P < 0.02$). Additionally, they reported that the rats drinking the HFCS solution had higher absolute and relative retroperitoneal fat pad weights and gonadal fat pad weights compared to rats drinking either the glucose solution or distilled water solution.

The ingestion of food results in elevated blood glucose concentrations. As a result of food consumption and high blood glucose concentrations, the secretion of insulin, a key long-term regulator of food intake, is stimulated from pancreatic β-cells. Insulin functions to inhibit food intake and increase energy expenditure [10]. Insulin is considered a long-term regulator

of adiposity rather than a short-term regulator of satiety because the transport of insulin to the central nervous system (CNS) is not rapid, but rather occurs over a period of hours. Body adiposity has been correlated to both fasting plasma insulin concentrations as well as postprandial insulin concentrations[33]. There are very low concentrations of fructose-specific GLUT5 transporters in the pancreas, thus insulin is not secreted from the pancreas upon fructose ingestion [24]. The inability of fructose to promote insulin activity could be a potential mechanism by which fructose intake can result in over consumption and ultimately weight gain. An acute fructose feeding study in twelve normal-weight women reported that the excursion of plasma glucose was lower after high fructose meals compared to high glucose meals ($P < 0.001$). The mean insulin concentrations (both fasting and postprandial) were also markedly lower in subjects receiving a high fructose meal compared to a high glucose meal over the two-day feeding period. Additionally, the average peak insulin responses were reduced by approximately 50% ($P < 0.001$) [15]. These findings suggest the development of insulin resistance in women fed a high fructose diet. High fructose diets have also induced insulin resistance in animal models as well. Jurgens et al (2005) reported that in mice fed fructose-sweetened beverages with ad libitum access to chow, over the course of seventy-three days exhibited significantly higher plasma insulin concentrations compared with the water control group ($P < 0.05$). A study by Lindqvist et al (2008) reports that rats consuming a fructose solution for two weeks had lower serum glucose and increased concentrations of serum insulin compared to rats drinking a water solution ($P <0.005$).

Because fructose forms acetyl-CoA, chronic high fructose feeding promotes lipogenesis and can increase hepatic triglyceride production and output of VLDL particles [34]. In addition,

the lipogenic effects of fructose may promote insulin resistance indirectly because increased blood levels of fatty acids and triaclyglycerols and lipid deposition in the liver are implicated in the etiology of insulin resistance [10]. Lindqvist et al (2008) reported that all groups of rats offered sugar solutions (sucrose, glucose, and fructose) for two weeks had higher serum triglycerides and higher serum cholesterol concentrations compared to rats only offered water ($P < 0.05$). Using another animal model, Bezerra et al (2000), examined the effects of a high fructose (624 g/kg) diet versus a cornstarch control (527g/kg) diet versus for twenty-eight days in rats. Rats that were fed the high fructose diet exhibited significantly higher serum triacylglycerol concentrations when compared to the cornstarch fed control group ($P < 0.05$). This study also investigated the effect of fructose on the ability to stimulate glucose disposal by administering an intravenous insulin tolerance test (ITT). The ITT demonstrated a lower glucose disappearance rate in the fructose-fed rats as compared with the cornstarch control group ($P = 0.05$). Both findings of increased serum triacylglycerol concentrations and decreased glucose disappearance rate suggest the development of a moderate state of insulin resistance in the fructose fed rats [5]. Figlewicz et al (2009) examined the effects of sweetened solutions including stevia, hoodia, fructose, agave, HFCS, and HFCS/hoodia. Fasting plasma glucose did not differ among different sweetener groups, however serum cholesterol concentrations significantly increased ($P < 0.05$) and fasting insulin concentrations significantly decreased ($P < 0.05$) in rats fed a HFCS diet as compared to a water control diet. The effect of fructose consumption on increased triglycerides is well established in animal models, but human studies contain conflicting results. Nikkila and Ojala (1965) determined that both glucose and fructose solutions administered as supplementation to the diet over four weeks increased plasma

triglycerides, although this effect was markedly greater in the fructose-fed rats [12]. Bar-on and Stein (1968) examined the role of the liver in regard to fructose-induced hypertriglyceridemia. The study reports that after the administration of a 10% fructose solution, for 6 to 19 days, fructose was more readily converted to triglycerides in the liver, subsequently caused a greater secretion of triglycerides from the liver into the serum, and did not stimulate lipoprotein lipase activity in adipose tissue. Therefore, Bar-on and Stein (1968) concluded that because fructose does not stimulate lipoprotein lipase activity, the outflow of triglyceride from the serum is uninhibited and causes an elevated accumulation of triglyceride in the bloodstream [35]. Hallfrisch et al (1983) found that 7.5% and 15% fructose diets consumed for 5 weeks increased fasting plasma LDL cholesterol in healthy and hyperinsulinemic men and increased fasting serum triglycerides in hyperinsulinemic men [36]. A similar study by Reiser et al (1989) reported that a 20% fructose diet consumed for 5 weeks increased fasting plasma LDL cholesterol in healthy men and fasting plasma triglycerides in both healthy and hyperinsulinemic men [37]. In women fed high- fructose meals, (30% fructose from beverages over a 24 hour period), the result was an acute elevation in plasma triglycerides [15]. A study by Chong et al (2007) investigated the mechanisms for the acute effect of fructose on postprandial lipidemia by comparing fructose and glucose intake in a group of 14 healthy subjects. This study is believed to identify the most probable mechanism by which dietary fructose raises triglycerides. Chong et al (2007) reported that the fructose test meals showed a decrease in plasma insulin and plasma glucose and a greater increase in plasma triglycerides as compared to the glucose test meals. Also, only about 0.1% of fructose was converted to fatty acids at 240 minutes, therefore both results suggest that the lower insulin expression after

fructose ingestion results in less activation of adipose tissue lipoprotein lipase and impaired triglyceride clearance [38]. A study performed at Princeton University by Bocarsly et al (2010), examined the short-term and long-term effects of high fructose consumption in rats using a fructose-sweetened solution. Bocarsly et al (2010) identified relationships between fructose intakes and body weight as well as triglyceride concentrations. Rats fed ad libitum chow and an 8% HFCS solution 12 h/day for 2 months reported an increase in body weight greater than that of rats fed ad libitum chow and a 10% sucrose solution 12h/day. The ad libitum chow and 12-h or 24-h HFCS solution fed rats also showed an increase in circulating concentrations of triglycerides and an increase in fat accrual compared to controls over the course of 6 months.

Ghrelin and obesity

A greater understanding of the potential effects of high fructose consumption on obesity can be obtained by understanding the mechanism by which fructose effects satiety signals and hormones such as ghrelin. Ghrelin is a 28 amino peptide that is secreted by a number of endocrine cells within the body, primarily X/A oxyntic cells inside the stomach. It is just one of several peptide hormones secreted from the gastrointestinal tract that are involved with the coordination of eating behavior and weight regulation[18]. Ghrelin was first discovered as an endogenous ligand for growth hormone secretagogue receptor (GHS-R), but is now being researched in relation to its effects as an orexigenic (hunger-inducing) peptide [39]. Ghrelin is the only known circulating orexigenic gastrointestinal peptide. Other peripheral hormone signals involved in energy homeostasis are anorexigenic, promoting reductions in food intake and body weight. The biological actions of ghrelin, especially anabolic and endocrine effects require acylated ghrelin. Acylation involves a covalent bonding of the ghrelin

serine-3 residue to a medium-chain fatty acid during a post-translational modification in the stomach. This medium chain fatty acid typically comes from dietary sources of octanoic acid, because animals do not synthesize medium-chain fatty acids. Ghrelin, once activated, will subsequently bind to growth hormone secretagogue receptor 1a (GHS-R1a) to stimulate short-term food intake. The principle site of ghrelin production is the stomach, but very small amounts can also be synthesized in the lungs, pancreatic islets, gonads, placenta, adrenal cortex, kidney, and brain [19]. Once secreted, ghrelin travels via the circulation to the hypothalamus, the appetite regulating center of the brain and sensor of metabolic status for the body [11].

Secretion of ghrelin is down-regulated during positive energy balance and up-regulated in negative energy balance. Therefore, obese individuals, who are in positive energy balance, have decreased ghrelin concentrations, and in individuals with anorexia nervosa, ghrelin levels are elevated [20, 40]. In lean human subjects, circulating plasma ghrelin concentrations display a diurnal pattern (with pre-prandial increases and post-prandial decreases) during the day time and a maximum peak at 0200 h [20]. However, it is reported that obese individuals do not display a post-prandial decline in ghrelin levels as is seen with lean individuals [21]. This lack of ghrelin suppression after meals could potentially lead to increased food intake and ultimately obesity. Post-prandial suppression of ghrelin has been studied considerably more than the pre-prandial elevation. Although the importance of this effect has not yet been fully defined, it is suggested that the suppression of ghrelin plays a large role in the satiating effect of ingested nutrients [41]. Exogenous ghrelin has been shown to initiate feeding and increase food intake in animals and humans [40, 42]. Chronic ghrelin administration has been shown to increase

food intake, induce hyperphagia, increase body weight, and increase adiposity in rodents [42-44]. These findings all support the role of ghrelin in meal initiation and body weight regulation.

Fructose intake and ghrelin activity

It is suggested that both hormonal and nutritional factors regulate ghrelin activity [22, 23, 45]. Carbohydrates, protein, and fat differentially effect gastrointestinal hormones, such as ghrelin, and studies by Williams and Cummings (2005) report that ghrelin levels were most reduced by glucose and least suppressed by fat. In regards to hormonal regulation, it has been shown that ghrelin is both directly and indirectly affected by insulin [22, 23]. Oral and intravenous administration of glucose has been shown to decrease ghrelin concentrations [20]. Unlike glucose, fructose does not stimulate insulin secretion from the pancreas. Insulin-mediated glucose uptake and metabolism play a major role in regulation of leptin concentrations. Leptin blocks the desire to eat via the hypothalamus by energy expenditure through basal metabolism as well as inhibiting the effects of ghrelin. The inability of fructose to stimulate insulin secretion can inhibit this chain of events and indirectly lead to decreased suppression of ghrelin and ultimately decreased satiety [46]. Insulin has also been shown to directly affect ghrelin activity. Plasma ghrelin concentrations rise progressively before meals and then fall within an hour of eating, a pattern that is opposite to that of insulin. In lean individuals, fasting increases plasma ghrelin concentrations, which then decrease after feeding [22]. Insulin on the other hand, also responds to energy balance, but rises in response to feeding and decreases during fasting. Saad et al (2002) found that upon injection of insulin in humans, ghrelin concentrations decreased and stayed up to 64% suppressed until after the

insulin infusion was discontinued. Absolute insulin deficiency, in contrast, prevents prandial plasma ghrelin suppression until insulin deficiency is corrected [22].

Studies that examine the affect of fructose consumption on ghrelin activity are very limited. Human studies have shown acute effects of fructose consumption on ghrelin activity, but there are none reporting chronic effects. Akhavan and Anderson (2007) examined the effects of fructose consumption on ghrelin intake in adolescent men consuming sugar-sweetened solutions. These solutions included HFCS-55, sucrose, and solutions with glucose to fructose ratios (G:F) of G20:F80, G35:F65, G50:F50, and G80:F20. Akhavan and Anderson (2007) found that in all subjects consuming the sugar-sweetened solutions plasma ghrelin concentrations were significantly lower than the water control ($P < 0.005$)[47]. A study conducted by Teff et al (2004) reports that the suppression of ghrelin was significantly less in women after consuming meals with 30% fructose-sweetened beverages (in a 24 hour period) than after each meal consumed with 30% glucose-sweetened beverages, (in a subsequent 24 hour period) ($P = 0.01$). In addition, plasma ghrelin levels were elevated in the morning hours from 2300-0300 h of the high fructose feeding day but did not increase above baseline during this time on the glucose feeding day (P trend toward significance = 0.075). This supports our hypothesis that chronic high fructose feeding can result in an elevation in pre-meal serum ghrelin concentrations. There are fewer studies examining the effects in animals, however one study by Lindqvist et al (2008) reported that rats fed a fructose solution for two weeks had a 40% increase (1.4-fold) in fasting serum ghrelin concentrations compared to rats offered a water solution ($P < 0.05$). While research is well established on the metabolic effects of

fructose consumption in human and animal models, research is lacking concerning the effects of chronic high fructose feeding on serum ghrelin concentrations.

Summary

Several studies have established a relationship between high fructose consumption and indicators of obesity such as body weight, body composition, and insulin, glucose, and triglyceride concentrations. Although these studies have determined an existing link, the exact role of high fructose consumption in obesity remains to be determined. Furthermore, little is known regarding the impact of high fructose consumption and the satiety hormone, ghrelin. Because of ghrelin's ability to stimulate food intake, studies in this area are of vital importance to understand the relationship between high fructose consumption and the current obesity epidemic within the United States. Furthermore, if data report an alteration in pre- and post-prandial serum ghrelin concentrations, this could indicate that decreased satiety and subsequent overeating or even obesity may result from high fructose consumption.

Hypothesis

It is hypothesized that high fructose consumption will increase food intake, body weight, body fat, and liver weight. It is also hypothesized that high fructose consumption will elevate serum glucose, insulin, and triglyceride concentrations as well as both pre-prandial and post-prandial serum ghrelin concentrations.

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CHAPTER 3

THE EFFECT OF HIGH FRUCTOSE INTAKES IN THE RAT DIET ON METABOLIC PARAMETERS AND BODY COMPSOITION

Colquitt, C.E., Giraudo, S.Q., Wickwire, K. To be submitted to *Peptides*.

ABSTRACT

This study investigates the effects of high fructose consumption in the rat diet on body composition and metabolic parameters, including serum glucose, insulin, and triglyceride concentrations. The effect of high fructose consumption on fasting and post-prandial ghrelin concentrations was also examined. Serum glucose concentrations were significantly higher in fructose-fed rats than dextrose-fed rats at weeks 1 (P = 0.047), 2 (P = 0.009), and 4 (P = 0.009). Greater increases in serum triglyceride concentrations were observed in the fructose-fed group at weeks 2 (P = 0.024), 3 (P = 0.024), and 4 (P = 0.032). Fasting ghrelin concentrations displayed trends toward significance for the treatment effect (P = 0.062) and the change over time (P = 0.076). Also, fructose-fed rats had significantly higher liver weights (P = 0.0004). This study demonstrates that high fructose consumption can affect metabolic parameters associated with obesity and may indicate an alteration in ghrelin activity.

INTRODUCTION

A greater understanding of the role of high fructose consumption on obesity can be established through identifying the effects that fructose elicits on hunger and satiety hormones. Ghrelin is a 28 amino peptide that is secreted by a number of endocrine cells within the body, primarily X/A oxyntic cells inside the stomach [18]. Ghrelin was first discovered as an endogenous ligand for growth hormone secretagogue receptor (GHS-R), but is now being researched in relation to its effects as the only known orexigenic (hunger-inducing) gastrointestinal peptide [19, 39]. Once secreted, ghrelin travels via the circulation to the hypothalamus, the appetite regulating center of the brain and sensor of metabolic status for the body [11]. Secretion of ghrelin is down-regulated during positive energy balance and up-regulated in negative energy balance. Therefore, obese individuals have decreased ghrelin concentrations, and in individuals with anorexia nervosa ghrelin levels are elevated, indicating an inverse relationship between plasma ghrelin and body weight [20, 40]. Circulating plasma ghrelin concentrations display a diurnal pattern (with pre-prandial increases and post-prandial decreases) during the day time and a maximum peak at 0200 h [20]. Exogenous ghrelin has been shown to initiate feeding and increase food intake in animals and humans [40, 42, 44]. Chronic ghrelin administration has been shown to increase food intake, induce hyperphagia, increase body weight, and increase adiposity in rodents [40, 43, 44]. These findings all support the role of ghrelin in meal initiation and body weight regulation. Ghrelin activity is regulated both hormonally and by nutritional factors. It has been shown that ghrelin serum concentration is both directly and indirectly affected by insulin [22, 23, 45]. Unlike glucose,

fructose does not stimulate insulin secretion from the pancreas therefore the ability of insulin to suppress ghrelin concentrations is decreased with fructose feeding [23, 46].

The consumption of fructose has increased substantially over the past 40 years [24] and has paralleled the prevalence of obesity in the US [25]. Increased use of high-fructose corn syrup as a sweetener over the last several decades has been proposed as one important dietary factor that may contribute to the widespread increase in obesity in the US [4]. Several studies have reported data supporting the association between fructose consumption and altered metabolic parameters, increased food intake, and weight gain [10, 15, 26]. These studies provide strong evidence of the effect of fructose on energy balance, but the metabolic effects of fructose are often measured through fructose-sweetened beverage intake. While fructose-sweetened beverages are the main source of dietary fructose, a significant amount of fructose consumption (in the form of HFCS-42) is also derived from processed food products. In addition, few studies examine the relationship between fructose and hormones that control food consumption, such as ghrelin.

This research project aimed to examine the effect of high fructose food intakes in the rat diet on food intake, body weight, serum glucose, insulin, and triglyceride concentrations, as well as pre-prandial and post-prandial ghrelin concentrations over the course of four weeks. We also investigated the effect of high fructose consumption on body composition. We tested the hypothesis that high fructose consumption will increase food intake, body weight, body fat, and liver weight. In addition, we tested the hypothesis that high fructose consumption will elevate serum glucose, insulin, and triglyceride concentrations as well as both pre-prandial and post-prandial serum ghrelin concentrations. The results of this study could be a beneficial

addition to what is already understood about high fructose food consumption and obesity, as an alteration in ghrelin concentrations could indicate decreased satiety and ultimately overeating ensuing from high fructose food consumption.

METHODS

Animals

Twelve male Sprague-Dawley rats weighing 225-249 grams were obtained from Harlan (Prattville, AL). The animals were randomly assigned to two groups of 6 rats each, and receive either a dextrose diet or a fructose diet. The rats were individually housed in clear plastic shoebox cages attached to the BioDAQ Food Intake Monitoring System (Research Diets, New Brunswick NJ). The animals were acclimated to housing (21-22°C with lights on 12h/day from 7:00a.m. to 7:00p.m.) for seven days. The caloric content of the two diets was identical: 3.90kcal/g. The dextrose group (control) received a diet consisting of 68% kcal carbohydrate, 21% kcal protein, and 12% kcal fat. The carbohydrate portion of the diet includes 17% kcal cornstarch and 51% kcal dextrose (Research Diets, New Brunswick NJ, #D08082501). The fructose group (treatment) received a diet comprised of 68% kcal carbohydrate, 21% kcal protein, and 12% kcal fat. The carbohydrate portion of the fructose diet contains 17% kcal cornstarch, 42% kcal fructose, and 9% kcal dextrose (Research Diets, New Brunswick NJ, #D08082502). The guidelines for animal procedures were in accordance with the University of Georgia Institutional Animal Care and Use Committee.

Design

Rats were raised, by the breeder, on a regular chow diet until approximately 2 months of age. Upon receipt of the animals, 6 rats were assigned to a dextrose control diet and 6 rats to a fructose treatment diet. The experiment lasted four weeks and animals were given access to the diets between the hours of 5:00p.m. and 8:00a.m. during the dark cycle, their normal feeding time. Food intakes were measured using the BioDAQ Food Intake Monitoring System. In addition, the animals were weighed weekly at 8:00a.m. Final body weights were recorded on the day of sacrifice, day 31. The day of sacrifice, each animal was decapitated, a blood sample was collected, and livers, brains, and epididymal fat pads were removed, placed in aluminum foil, and kept on ice. Carcasses, blood samples, brains, livers, and fat pads were stored at -80°C until analysis.

Blood Sampling

Blood samples were collected by tail-bleed pre and postprandially each week at 5:00p.m. and the following morning at 8:00a.m., respectively, correlating with the beginning and ending of their feeding cycle. Prior to each pre-prandial tail bleed, animals were fasted approximately 8 hours. The blood samples were collected on day 2, 3, 9, 10, 16, 17, 23, 24, 30, and 31. The samples were centrifuged for 20 minutes at 1500G at 4°C (Sorvall RC5C Plus). Samples were then placed into microcentrifuge tubes by pipette and stored at -20°C until analysis. Serum glucose levels were measured by the glucose oxidase method with reagents from Pointe Scientific, Inc (Canton, MI). Serum triglyceride levels were measured by glycerol phosphate oxidase method with reagents from Point Scientific, Inc (Canton, MI). Serum ghrelin levels were measured by radioimmunoassay with a kit from Millipore Corporation (GHRT-89HK, Billerica

MA). Finally, serum insulin levels were analyzed by radioimmunoassay with a kit from Millipore Corporation (RI-13K, Billerica MA).

Body Composition

Body composition analysis was done using a procedure developed by Hartsook and Hershberger [48]. After sacrifice, carcasses were stored at -80°C. Frozen carcasses were autoclaved in individual sealed beakers for 45 minutes at 120°C. Once cool, each carcass was chopped with three times its own weight of distilled water in a Waring commercial blender. The homogenate was then transferred to a large plastic container to which a stir bar was added and the plastic container was placed on a stir plate. The homogenate was stirred for several minutes with a Corning stirrer/hot plate (model PC-420, Corning Inc, Lowell MA). Samples of homogenate for lipid and dry matter analyses were taken while homogenate is stirring. Carcass fat was determined in triplicate on 7mL samples of homogenate by chloroform-methanol extraction. Carcass dry matter was determined in triplicate on 7mL samples by drying at 85°C for 48hours [48].

Statistic Analysis

Statistical analysis including means, standard deviations, medians, confidence intervals, and correlations were performed with SAS, Inc 5.0 (Cary, NC). Repeated measures ANOVA were performed for within-group comparisons of body weight, food intake, and serum glucose, triglyceride, insulin, and ghrelin levels. A one-way ANOVA was conducted to detect differences when the between subjects effect was statistically significant. A P value < 0.05 was accepted as statistically significant. The results are represented as the mean ± SE M.

RESULTS

Body weight and food intake

At the beginning of the study, body weights of rats in both the fructose group and the dextrose group were similar, 255.98 ± 3.92g and 257.13 ± 5.61g, respectively. Body weight steadily increased over the 4 week period for rats in both the fructose and dextrose group. At the final week of the experiment, week 4, the average fructose group body weight was 333.73 ± 6.61g compared to 331.02 ± 9.31g for the dextrose group and this change in body weight over time was significant ($P < 0.0001$)(Table 1). No significant differences were seen among the two groups, however (Fig. 1). Rats in both the fructose and dextrose group increased their food intake over the 4 week period as well. Food intake was greater in the dextrose group as compared to the fructose group. The mean food intake of the fructose group at week 1 was 95.22 ± 2.47g compared to 100.54 ± 3.46g of the dextrose group (Table 2). At week 4, the mean food intake of the fructose group was 92.46 ± 1.90g compared to 103.95g ± 1.15g of the dextrose group (Table 2). The treatment effect for food intake was significant ($P < 0.0001$) as well as the effect for time ($P < 0.0001$) and the treatment effect over time ($P = 0.0482$) (Fig. 2). Statistically significant differences were found between the two groups at week 2 ($P = 0.0006$) and week 4 ($P = 0.0004$)(Table 2, Fig. 2). Although food intake was significantly greater in the dextrose-fed rats than the fructose-fed rats, the final body weights of the animals were not different. Upon analysis of the total body weight (grams) in comparison to total food intake (grams) over the course of the 4 weeks for both groups, we discovered that the fructose diet was more efficient (0.764 ± 0.032) than the dextrose diet (0.69 ± 0.016) at promoting weight gain. Although the difference in efficiency of the two diets in stimulating weight gain was not

significant (P = 0.08), the presence of a trend towards significance exists. Longer study duration could potentially allow for significance to be achieved.

Serum glucose, insulin, triglycerides

Serum samples were collected weekly, consisting of one pre-prandial and one post-prandial sample. Samples were then used to measure post-prandial serum glucose, insulin, and triglyceride concentrations and pre-prandial and post-prandial ghrelin concentrations. Serum glucose concentrations increased over time, and the change over time was significant (P = 0.000). Post-prandial serum glucose concentrations were consistently higher in the fructose fed rats than the dextrose fed rats. The treatment effect was significant for post-prandial serum glucose (P = 0.003) and differences among groups were seen at week 1 (P = 0.047), week 2 (P = 0.009) and week 4 (P = 0.009) (Table 3, Fig 3). Post-prandial serum insulin concentrations increased over time also with higher concentrations in the fructose group than the dextrose group. The treatment effect for pre-prandial serum insulin was significant (P = 0.014) as well as the change over time (P = 0.005). A significant difference between the two groups was seen at week 2 (P = 0.0515), indicating insulin resistance development after 2 weeks of feeding (Table 3, Fig. 4). Although significance criteria was not met, it is important to note the trend toward significance of insulin values at week 1 (P = 0.0976), week 3 (P = 0.1554), and week 4 (P = 0.0666)(Table 3, Fig. 4). Post-prandial serum triglyceride concentrations continually increased in both groups, and again, a greater increase was seen in the fructose group compared to the dextrose group at each measurement over the 4 week period (Table 3, Fig. 5). Significant differences among the groups were found at week 2 (P = 0.024), week 3 (P = 0.0235), and week 4 (P = 0.0318)(Table 3, Fig. 5). At week 2, average serum triglyceride concentrations in the

fructose group were 188.99 ± 19.14mg/dL compared to 94.40 ± 14.97mg/dL in the dextrose group. At week 3 average triglyceride concentrations were 212.40 ± 29.46mg/dL in the fructose group and 124.45 ± 14.76mg/dL in the dextrose group. Finally, at week 4, average serum triglyceride concentrations were 253.00 ± 20.07mg/dL in the fructose group and 176.72 ± 23.09mg/dL in the dextrose group (Table 3). The treatment effect for post-prandial triglyceride concentrations was significant ($P < 0.0001$) as well as the change over time ($P = 0.0041$)(Table 3).

Serum ghrelin

Fasting and post-prandial ghrelin concentrations were also measured once during each week. Fasting ghrelin concentrations showed a trend towards significance for the treatment effect ($P = 0.0619$) as well as the change over time ($P = 0.0759$). However, no significant differences were seen among the two groups. It is important to note that although significance criteria are not reached, there is a strong trend towards significance. A longer period of study could potentially allow for changes in fasting serum ghrelin concentrations related to fructose consumption to be seen. Post-prandial ghrelin concentrations were significant for the change over time ($P = 0.0036$). However, neither the treatment effect nor the differences among the two groups was significant (Table 4).

Body Composition

At the end of the 4 week period, on the day of sacrifice, the livers and epididymal fat pads of all animals were harvested and weighed. Rats in the fructose treatment group had a higher average liver weights when calculated as a percent of body weight (0.044 ± 0.001%) than those in the dextrose control group (0.038 ± .001%)(Fig. 6). The treatment effect was significant for

the differences among the two groups in liver weight (P = 0.0004). Rats in the fructose-fed group had a slightly higher average epididymal fat pad weight (1.81 ± .16g) compared to the dextrose-fed rats (1.56 ± .26g), although no significant differences were found between the two groups. Also, no significant differences were found among body fat percentage for animals in either of the two groups. The average body fat percentage for rats in the fructose-fed group was 7.24% ([% fat/% body weight] x 100) compared to 6.71% for the dextrose-fed group.

DISCUSSION

The primary findings of this study are the that high fructose food consumption in the rat diet significantly increases post-prandial concentrations of both serum glucose and insulin, opposed to rats fed a high dextrose diet. Serum glucose and insulin concentrations were both greater in the fructose-fed rats than the dextrose fed-rats at each time point over the course of four weeks. Significant differences in serum glucose were seen at week 1, week 2, and week 4 and in serum insulin at week 2. Although serum insulin concentrations were only significant at week 2, it is important to note the trend in significance for all other time points that were measured (week 1 P = 0.098, week 2 P = 0.155, week 3 P = 0.067). Zavaroni et al (1980) examined the effect of fructose feeding on insulin action and insulin secretion in the rat. To determine the effect of high fructose feeding on insulin secretion, plasma glucose and insulin responses were examined in response to an oral glucose and fructose load (180mg/100g body weight). The rats consuming a 66% fructose diet for seven days displayed a higher plasma insulin concentration at every time interval (30-180 minutes) following an oral glucose load (P = 0.05-0.01). In addition, after the oral fructose load, plasma insulin concentrations continually

rose in the fructose-fed rats and were significantly greater when compared to the control rats at every interval (P = 0.05-0.01). Plasma glucose concentrations in fructose-fed rats were higher than the controls after both the oral glucose and oral fructose loads ($P < 0.05$). In both the control and fructose-fed rats the steady state plasma insulin levels were similar, but the steady state plasma glucose levels were significantly higher in the fructose-fed rats ($P < 0.001$). This indicates the impaired ability of identical amounts of insulin to stimulate the disposal of glucose in the fructose-fed rats. The development of hyperglycemia and hyperinsulinemia in the fructose-fed rats is consistent with the development of insulin-resistance, as confirmed by the inability of insulin to dispose the glucose load. Zavaroni et al (1980) also reports that insulin resistance occurred in rats in comparable degrees when fed either 33% or 66% of total calories as fructose, indicating that insulin resistance can be seen in rats fed diets comprised of 33% calories from fructose [49]. Therefore, this study administered a 42% fructose chow diet as the treatment diet. A diet of 42% of total calories from fructose is similar to the composition of HFCS-42.

It has been established that the effect of fructose consumption on serum glucose differs markedly during acute versus chronic feeding. Acute fructose feeding results in less elevated plasma glucose concentrations than after an equal amount of glucose [50]. The same effect is seen regarding serum insulin. Therefore, fructose has been considered a preferable form of sugar for individuals with diabetes due to the fact that acute fructose feeding does not require insulin nor does it cause the elevation in serum glucose seen with acute glucose feeding. After a period of chronic feeding, however, the same results are not found. Periods of chronic high fructose consumption, at least two to three weeks results in dietary adaptations in which

enzymes and hormones adjust to the chronic diet [50]. Thorburn et al (1989) reported that rats fed 35% of calories from fructose or glucose over the course of 4 weeks showed higher fasting plasma glucose concentrations, higher fasting plasma triglyceride concentrations, and impaired insulin action in both the liver and peripheral tissues. This study is one of many reporting glucose intolerance as well as insulin resistance after chronic fructose feeding [11, 51, 52].

Multiple mechanisms have been proposed for insulin resistance resulting from high fructose consumption. Insulin resistance has been attributed to decreased hepatic glucose utilization, increased exposure of nonesterified fatty acid concentrations, and elevated triglyceride concentrations [10]. Animals which are deprived of dietary glucose must synthesize glucose through gluconeogenesis involving the hydrolysis of glucose-6-phosphate to glucose by glucose-6-phosphatase [53]. Thus, the consumption of fructose-containing diets leads to a decrease in glucose utilization due to increased glucose-6-phosphatase activity and decreased glucokinase activity. A study by Tobey et al (1982) compared glucose uptake in skeletal muscle and the liver to localize the major tissue sites of insulin resistance. Tobey et al (1982) found that the rats fed a 66% fructose diet developed insulin resistance and hyperinsulinemia and concluded that insulin resistance from chronic feeding results from a decrease in the ability of insulin to suppress hepatic glucose output, rather than a decrease in insulin-mediated uptake by skeletal muscle [54].

Our study found significant differences among post-prandial triglyceride concentrations between the two groups at week 2 ($P = 0.024$), week 3 ($P = 0.024$), and week 4 ($P = 0.032$), which are similar to the findings of other studies regarding high fructose consumption and elevated triglyceride concentrations [5, 11, 37, 51, 55]. Chronic positive energy balance and

increased body adiposity result in elevated nonesterified fatty acid concentrations which over time have can produce deleterious effects upon β-cell functioning in the pancreas [56]. An increase in VLDL triacylglycerol production can also result from elevated nonesterified fatty acid concentrations [10]. Thorburn et al (1989) investigated differences in body composition, post-prandial serum glucose and insulin concentrations, and insulin resistance in rats fed a 69% carbohydrate diet of which 34.5% of calories were from fructose (n = 6) or glucose (n = 6) and 34.5% of calories were from starch. The fructose-fed rats showed increased fasting triglyceride concentrations after 2 weeks of feeding (P < 0.001) and a decrease in the glucose infusion rate (GIR) required to maintain euglycemia after 4 weeks (P < 0.001). GIR, hepatic glucose production, and peripheral glucose disposal were then examined to identify correlations between the three measurements and triglyceride concentrations. Significance was found among all three relationships. The correlation of triglyceride with GIR (P < 0.01) and with peripheral glucose disposal (P < 0.05) were negatively associated, indicating that the higher the triglyceride concentration, the lower the insulin-mediated glucose disposal. The correlation of triglyceride concentrations and hepatic glucose production was positively associated (P < 0.05) indicating that the higher the triglyceride level the greater the impairment in insulin suppression of hepatic glucose output. Bezerra et al (2000) reported that after 28 days of treatment, serum triacylglycerol concentrations were significantly higher in fructose-fed rats than cornstarch-fed control rats. In addition, a moderate state of insulin resistance was demonstrated by the fructose-fed rats after 4 weeks of treatment.

This study also found significant changes in food intake between the two groups over the four week period. Food intake of rats in both the fructose-fed and dextrose-fed groups

increased over the course of the experiment. Surprisingly, however, the rats in the dextrose group had a significantly higher average food intake than the fructose-fed rats at week 2 (P = 0.0006) and week 4 (P = 0.0004). This could be related to the extreme sweetness of fructose, about 2 times as sweet as glucose, which could have adversely affected intakes in the fructose-fed rats [11]. Also, acute fructose feeding show smaller increases in plasma glucose and insulin, which is suggested to be the cause of increased food intake during short-term fructose feeding [57]. In our study, the body weights of the rats in both groups were similar and continually increased over the four week period. However, there were no significant differences noted among the two groups. Studies examining the long-term effects of fructose on food intake and body weight are extremely limited, although our findings for food intake[3, 7, 8, 51, 52, 55, 58] and body weight are consistent with other studies [3, 26, 52, 58] in these aspects.

Our study did not find any significant differences regarding high fructose consumption and pre-prandial or post-prandial ghrelin concentrations. These findings are similar to a study by Teff et al (2004) in which no significant differences were found regarding fasting ghrelin concentrations in humans consuming 30% of calories from a fructose sweetened beverage over a 24 hour period. Teff et al (2004) did, however, find that post-prandial suppression of ghrelin was significantly lower in the fructose-fed group. Also, Lindqvist et al (2008) conducted a study in rats given ad libitum access to chow and either a water solution, a 23% (w/v) sucrose, 23% glucose, or 23% fructose solution for 24 h, one week, or two weeks. The groups of rats offered sugar solutions for two weeks all reported increased serum triglyceride concentrations (sucrose $P < 0.05$, glucose $P < 0.01$, fructose $P < 0.01$). In addition, rats drinking the fructose solution for two weeks showed an approximately 1.4-fold (40%) increase in fasting serum ghrelin

concentrations compared to rats offered a water solution ($P < 0.05$). These results are interesting, considering that our study did not show significance for either of these measures. Teff et al (2004) and Lindqvist et al (2008) differed from our study design, however, because they examined the acute effects upon ghrelin concentrations using a fructose-sweetened solution. Our study did demonstrate a strong trend toward significance of pre-prandial serum ghrelin concentrations for the treatment effect as well as the change over time. This trend towards significance of elevated fasting ghrelin concentrations suggest that a failure of ghrelin suppression could result from high fructose consumption. Perhaps a longer duration of study would allow for a relationship to be discovered between high fructose consumption and fasting ghrelin concentrations. In addition, this study measured total serum ghrelin levels rather than the active form of ghrelin. Ghrelin must first be acylated to bind to GSH-R1a to stimulate short-term food intake [19].

Our study reported that body fat percentage of the fructose-fed rats were slightly higher than the dextrose-fed rats, 7.24% and 6.71%, respectively. The difference among the two groups was not significant, however. Thorburn et al (1989) similarly reports that no significant differences were seen among rats given 35% of calories from fructose over a four week period. Tuovinen and Bender (1975) discovered that after 12 weeks of fructose feeding, rats showed an increase in white adipose tissue, an enlargement in liver size, and an increase in fat content [59]. This suggests that a longer period of study or a greater percentage of calories derived from fructose may be necessary to see the effects of high fructose consumption on body composition. A previous study by Jurgens et al (2005) found liver weights in fructose-fed rats to be significantly higher than their water control counterparts over a 73 day period. In addition,

the fructose-fed rats displayed increased hepatic storage of lipids indicating the development of early hepatic steatosis. The mechanisms of fructose consumption and nonalcoholic fatty liver remain to be determined, but the increase in hepatic storage of lipids indicates that fructose consumption subsequently increases de novo lipogenesis. Our study also demonstrated greater liver weights as a percentage of body weight in the fructose-fed rats as opposed to the dextrose-fed control group. This is interesting considering there were no significant differences in food intake over the course of the 4 week period. This allows us to assume that high fructose consumption does in fact enhance de novo lipogenesis, in both the fructose-sweetened beverage and fructose food forms. When examining epididymal fat pad weight, our study found that the fructose-fed rats had slightly higher fat pad weights than the dextrose-fed group. These differences were not significant among the two groups however. Kanarek and Orthen-Gambill (1982) similarly found no differences in epididymal fat pad weight when comparing rats fed a standard diet or rats fed a standard diet and a 32% fructose solution for 50 days [60]. Light et al (2009) found that rats fed a 13% (w/v) HFCS-55 solution in addition to food had significantly greater retroperitoneal fat pad weights when compared to rats drinking a glucose or distilled water solution [9]. Bocarsly et al (2010) reports similar findings that female rats with 24 hour access to a HFCS solution and ad libitum chow for seven months had an increased overall fat pad weight compared to chow-fed controls, however there were no significant differences among gonadal fat pad weights. This finding illustrates that a high fructose-sweetened solution, in supplementation to the diet, can have an effect on fat deposition in the female rat. In this study male rats were used and retroperitoneal fat pads were not weighed, and interestingly there is no relationship between high fructose food

consumption and epididymal fat pad weight. It would be interesting to evaluate if the mechanism of fat deposition in females versus males could potentially affect fat pad deposition in relation to high fructose food consumption. At this time, more research is required to establish a relationship between high fructose food consumption and fat deposition.

CONCLUSION

The high fructose diet utilized in this study had no effect on body weight, serum ghrelin levels, or body composition over a four week period. The high fructose diet did affect metabolic parameters that are indicative of the metabolic syndrome, including an increase in post-prandial serum glucose, insulin, and triglyceride concentrations and an increase in liver weight. Future studies are needed to determine the relationship among chronic high fructose food consumption and ghrelin activity as well as body composition. The trend towards significance of high fructose food consumption and pre-prandial ghrelin concentrations could prove significant with longer study duration. The establishment of this significance could determine a link between decreased ghrelin suppression as a result of high fructose food consumption. Decreased ghrelin suppression resulting from long-term high fructose food consumption could induce decreased satiety and stimulate food intake, ultimately enhancing the development of obesity.

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Table 1. Effect of a fructose or dextrose diet on body weight.

Data are means ± SEM. No significant differences were seen among the two groups.

Time/Diet	Body Weight (g)
Week 1	
Dextrose	257.13 ± 5.61
Fructose	255.98 ± 3.92
Week 2	
Dextrose	300.65 ± 7.09
Fructose	301.85 ± 4.52
Week 3	
Dextrose	319.27 ± 9.09
Fructose	320.52 ± 5.59
Week 4	
Dextrose	331.02 ± 9.31
Fructose	333.73 ± 6.61

Table 2. Effect of a fructose or dextrose diet on food intake.

Data are means ± SEM. The asterisk indicates a significant difference between the two groups (week 2 *P* = 0.0006, week 4 *P* = 0.0004).

Time/Diet	Food Intake (g)
Week 1	
Dextrose	100.54 ± 3.46
Fructose	95.22 ± 2.47
Week 2	
Dextrose	114.64 ± 1.69
Fructose	100.28 ± 2.63*
Week 3	
Dextrose	109.39 ± 1.58
Fructose	106.28 ± 1.70
Week 4	
Dextrose	103.95 ± 1.15
Fructose	92.46 ± 1.90*

Table 3. Effect of a fructose or dextrose diet on post-prandial serum glucose, insulin, and triglycerides.

Data are means ± SEM. The asterisk indicates a significant difference between the two groups (glucose week 1 $P = 0.047$, week 2 $P = 0.009$, week 4 $P = 0.009$; insulin week 2 $P = 0.0515$; triglycerides week 2 $P = 0.0024$, week 3 $P = 0.0235$, week 4 $P = 0.0318$).

	Glucose mg/dL	Insulin ng/mL	Triglycerides mg/dL
Week 1			
Dextrose	104.14 ± 2.96	0.14 ± 0.04	138.23 ± 16.47
Fructose	115.03 ± 4.23*	0.30 ± 0.08	173.75 ± 16.23
Week 2			
Dextrose	111.31 ± 2.40	0.11 ± 0.03	94.40 ± 14.97
Fructose	123.23 ± 3.40*	0.22 ± 0.05*	188.99 ± 19.14*
Week 3			
Dextrose	130.56 ± 4.29	0.22 ± 0.04	124.45 ± 14.76
Fructose	139.75 ± 4.25	0.32 ± 0.06	212.40 ± 29.46*
Week 4			
Dextrose	120.43 ± 3.81	0.26 ± 0.04	176.72 ± 23.09
Fructose	142.86 ± 6.83*	0.45 ± 0.09	253.00 ± 20.07*

Table 4. Effect of a fructose or dextrose diet on fasting and post-prandial serum ghrelin.

Data are means ± SEM. No significant differences were seen among the two groups.

Time/Diet	Fasting Ghrelin (pg/mL)	Post-prandial Ghrelin (pg/mL)
Week 1		
Dextrose	836.46 ± 101.50	365.99 ± 29.54
Fructose	648.54 ± 112.20	381.60 ± 57.07
Week 2		
Dextrose	749.15 ± 111.08	713.29 ± 129.63
Fructose	621.48 ± 98.48	482.76 ± 121.82
Week 3		
Dextrose	1060.74 ± 124.45	688.30 ± 62.83
Fructose	845.25 ± 153.70	743.84 ± 95.02
Week 4		
Dextrose	734.05 ± 95.04	597.86 ± 69.87
Fructose	649.70 ± 97.78	465.22 ± 88.70

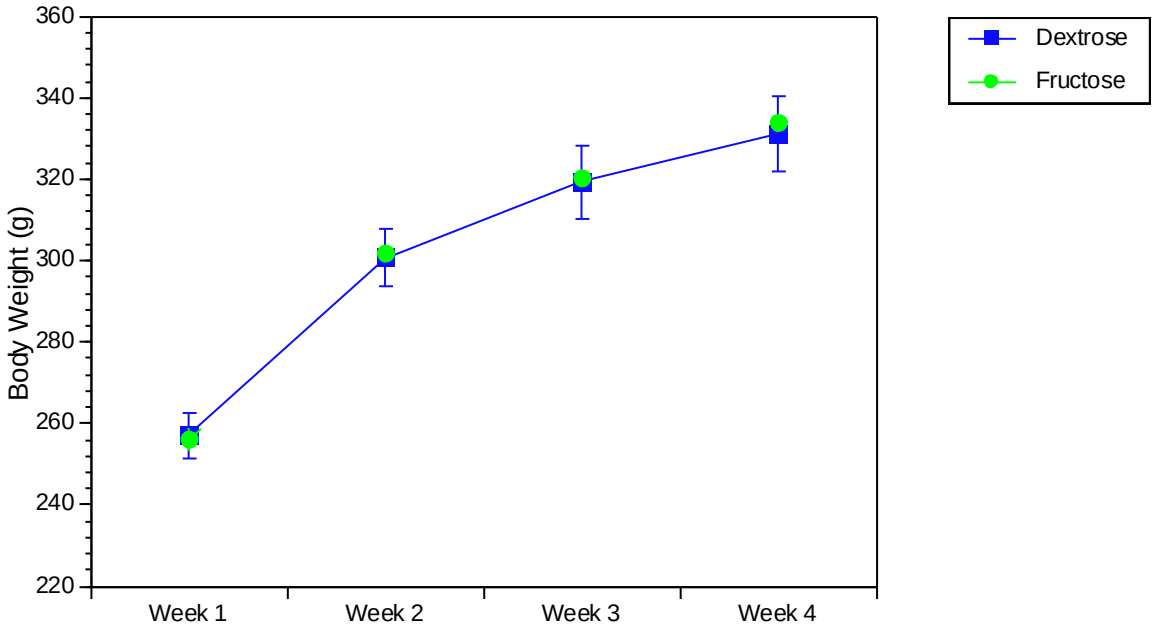

Figure 1. The change in body weights over a four week period. Data are means ± SEM. No significant differences were found between the two groups.

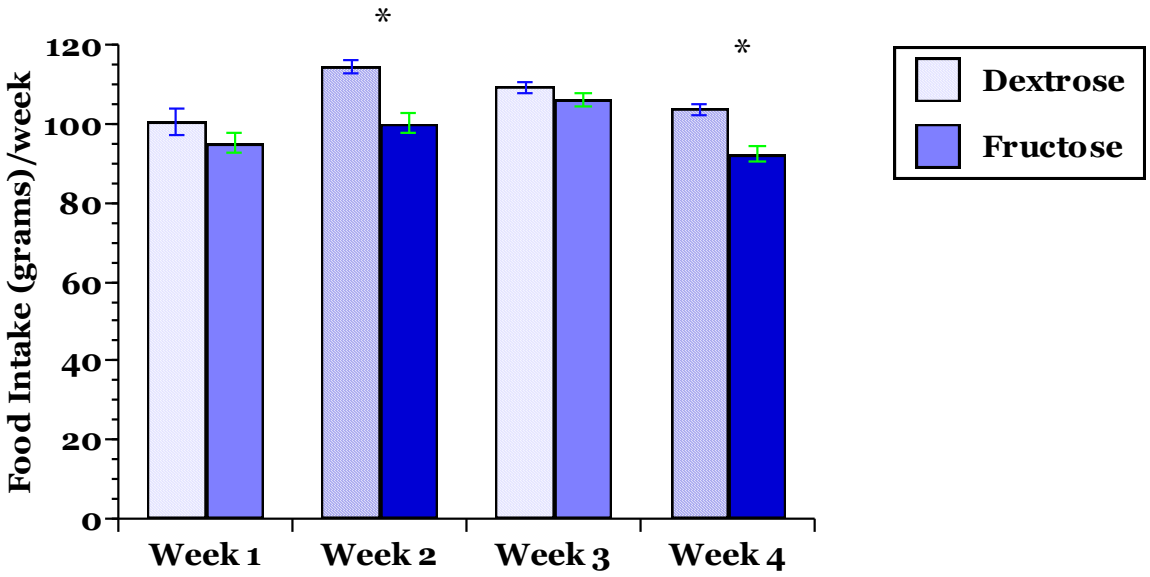

Figure 2. The change in food intake over a four week period. Data are means ± SEM. The asterisk denotes differences among the two groups at week 2 (P = 0.0006) and week 4 (P = 0.0004).

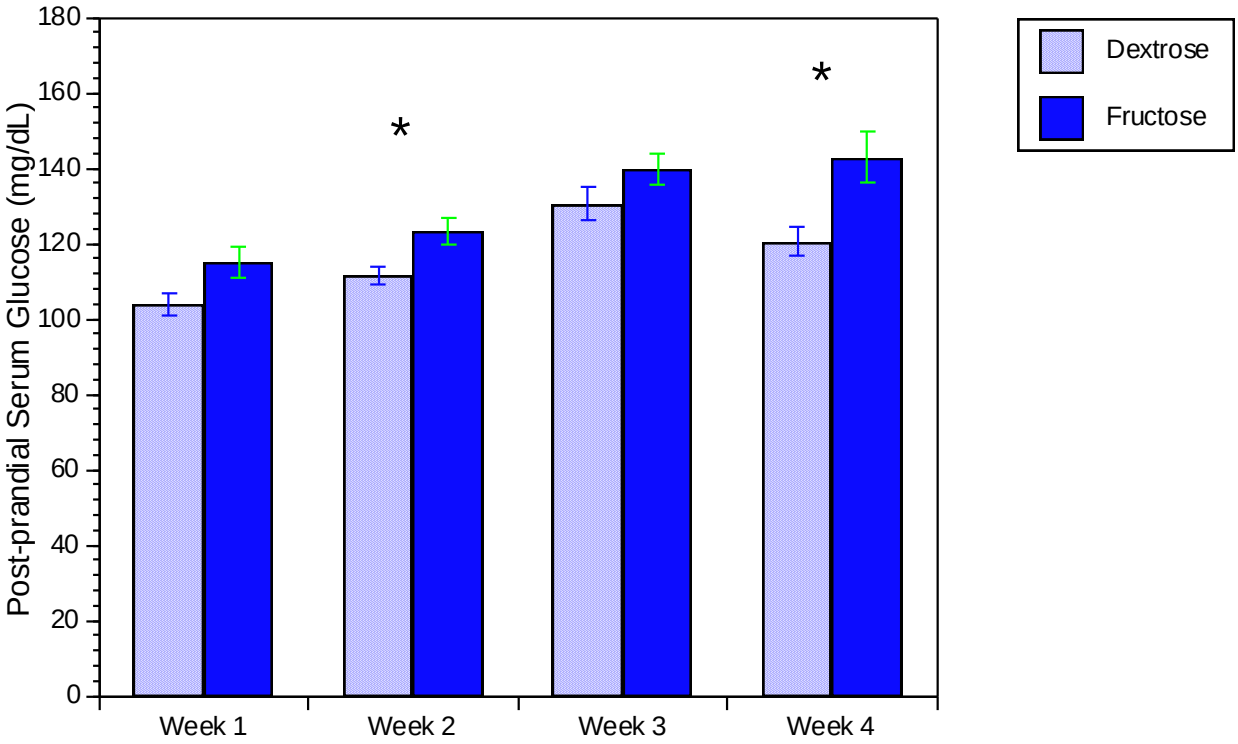

Figure 3. The change in serum glucose over a four week period.

Data are means ± SEM. The asterisk indicates a significant difference between the two groups (week 1 $P = 0.047$, week 2 $P = 0.009$, week 3 $P = 0.009$).

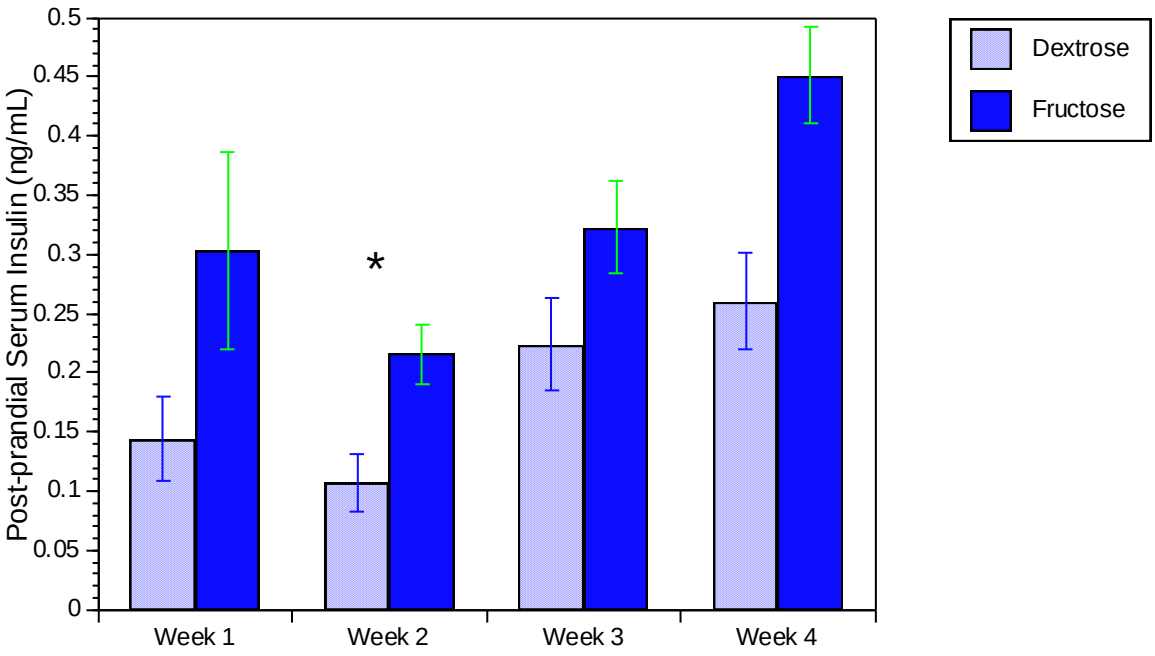

Figure 4. The change in serum insulin over a four week period.

Data are means ± SEM. The asterisk indicates a significant difference between the two groups (week 2 *P* = 0.0515).

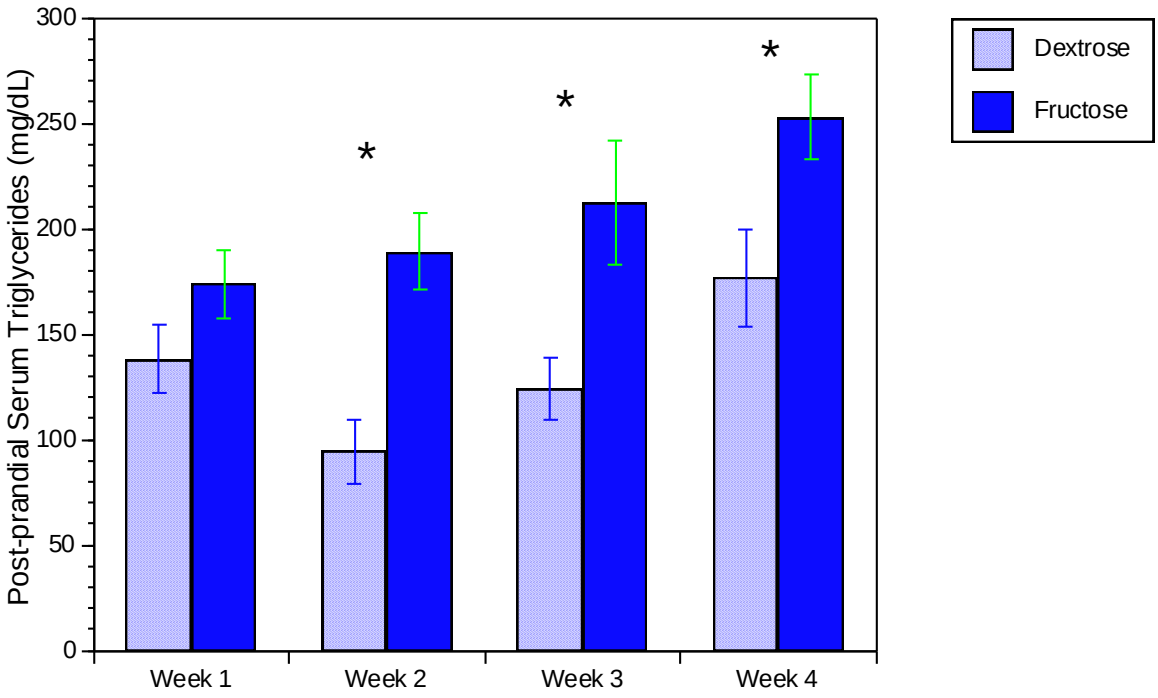

Figure 5. The change in serum triglycerides over a four week period.

Data are means ± SEM. The asterisk indicates a significant difference between the two groups (week 2 $P = 0.0024$, week 3 $P = 0.0235$, week 4 $P = 0.0318$).

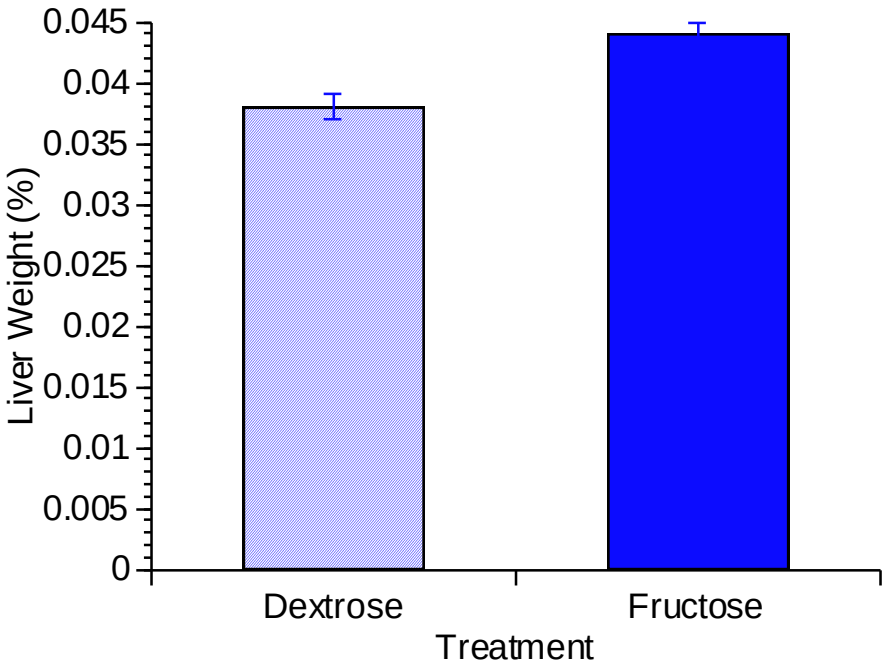

Figure 6. The effect of high fructose intake on relative liver weight.

Data are means ± SEM. The asterisk denotes differences among the two groups (*P* = 0.0004).

CHAPTER 4

CONCLUSION

This thesis project examined the effect of high fructose food intakes in the rat diet on food intake, body weight, serum glucose, insulin, and triglyceride concentrations, as well as pre-prandial and post-prandial ghrelin concentrations over the course of four weeks. Also examined was the effect of high fructose consumption on body composition. The high fructose diet did affect metabolic parameters that are indicative of the metabolic syndrome, including an increase in post-prandial serum glucose, insulin, and triglyceride concentrations and an increase in liver weight. However, no significant differences were reported in body weight, pre-prandial and post-prandial serum ghrelin levels, or body composition over a four week period.

This thesis demonstrates that the relationships between high fructose food intakes and ghrelin activity as well as body composition are very complex and require the investigation of a number of variables. This thesis did not find significant differences among pre-prandial or post-prandial serum ghrelin concentrations, which differed from two similar studies that did find high fructose consumption to be related to altered fasting and post-prandial ghrelin concentrations [11, 15]. These previous studies, however, used a high fructose-sweetened solution in addition to a standard chow diet. Differences in study design could therefore explain the differences in results between those studies and this thesis project. In addition, it is

important to note that this study did identify a trend towards significance of high fructose food consumption and pre-prandial ghrelin concentrations for the treatment effect ($P = 0.0619$) as well as the change over time ($P = 0.0759$). This trend towards significance of elevated fasting ghrelin concentrations suggest that a failure of ghrelin suppression could result from high fructose consumption and could prove significant with longer study duration.

The regulation of ghrelin activity is still being studied, but it is suggested that regulation occurs via hormonal and nutritional factors [22, 23, 45]. Insulin has been found to regulate ghrelin activity both directly and indirectly [22, 23]. Saad et al (2002) found that upon injection of insulin in humans, ghrelin concentrations decreased and stayed suppressed until after the insulin infusion was discontinued. Absolute insulin deficiency, in contrast, prevents prandial plasma ghrelin suppression until insulin deficiency is corrected [22]. Our present study is similar to existing research that reports glucose intolerance, insulin resistance, and elevated triglycerides as a result of high fructose intakes [5, 11, 37, 51, 52, 54, 55]. Insulin resistance has been attributed to decreased hepatic glucose utilization, increased exposure of nonesterified fatty acid concentrations, and elevated triglyceride concentrations, which is supported by the findings from this study [10].

Considering there was no significant difference in food intake over the course of the four weeks, it was interesting that our study also showed greater liver weights in the fructose-fed rats as opposed to the dextrose-fed control group ($P = 0.0004$). This is interesting considering there were no significant differences in food intake over the course of the 4 week period. Thorburn et al (1982) reported that the correlation of triglyceride with glucose infusion rate (GIR) ($P < 0.01$) and with peripheral glucose disposal ($P < 0.05$) were negatively associated,

indicating that the higher the triglyceride concentration, the lower the insulin-mediated glucose disposal. These findings allow us to reaffirm that high fructose consumption does in fact enhance de novo lipogenesis, in both the fructose-sweetened beverage and fructose food forms.

Future studies are needed to determine the effect of chronic high fructose food consumption on fasting and postprandial ghrelin concentrations as well as body composition. Decreased ghrelin suppression resulting from long-term high fructose food consumption could induce decreased satiety and stimulate food intake, ultimately enhancing the development of obesity.

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