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Effects of a High Fructose Diet on Physiology and Cognition in Male Sprague-Dawley Rats

Amy Patricia Ross

EFFECTS OF A HIGH FRUCTOSE DIET ON PHYSIOLOGY AND COGNITION IN MALE SPRAGUE-DAWLEY RATS

by

AMY PATRICIA ROSS

Under the Direction of Dr. Marise B. Parent

ABSTRACT

Fructose consumption has increased exponentially during the past four decades. The physiological effects of a high fructose diet include obesity and insulin resistance. In animal models, the effects of a high fructose diet on fat distribution are inconclusive in that some studies find increases in body mass and lipids while others find no effect. Recent findings indicate that a high fructose diet causes hippocampal insulin resistance in hamsters, raising the possibility that the diet causes impairments in cognition. The following experiments tested the hypotheses that a high fructose diet alters fat distribution rather than total body mass and impairs hippocampal-dependent memory. Results indicated that the high fructose diet did not affect fat distribution, but did increase plasma triacylglycerides. Interestingly, the diet also impaired spatial reference memory in the Morris water maze, and this effect was correlated with plasma triacylglycerides. These results indicate that a high fructose diet impairs brain function.

INDEX WORDS: Fructose, Diet, Memory, Hippocampus, Triacylglycerides, Free fatty acids, Morris water maze

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AMY PATRICIA ROSS

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DEDICATION

I would like to dedicate this work to my loving family and friends. Their unending support and encouragement has been incredibly important to my success.

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I would like to thank my mentor Marise and committee members Tim and Kim for their guidance, support and patience. I would also like to thank fellow lab members for their assistance.

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Introduction

Consumption of simple sugars (monosaccharides) such as fructose has risen markedly in the American diet since the 1970s. This is due in large part to the development of high fructose corn syrup (HFCS), which is produced by the enzymatic conversion of glucose (also known as dextrose) to fructose (Hanover and White 1993). The most popular form of HFCS is comprised of 55% fructose (Hanover and White 1993; Park and Yetley 1993). Compared to sucrose, fructose is preferred by food manufacturers for several reasons: fructose is sweeter, and HFCS can be easily produced at a low cost. It has also been found to enhance flavors of foods such as caramel and chocolate (Park and Yetley 1993; Bray, Nielsen et al. 2004).

Fructose comprises approximately 8% of the total daily energy intake in the United States (Park and Yetley 1993; Havel 2005). This percentage includes both naturally occurring sources, such as fruits and vegetables, and added sources such as HFCS, sucrose (50% fructose), concentrated fruit juices, and crystalline fructose. Food consumption surveys conducted in 2006 indicate that teenagers (ages 12-18 years) consume the most fructose. Approximately 12.17% of their total energy intake is from fructose, the highest percentage of which comes from non-alcoholic, non-100% juice beverages (NHANES, unpublished). The per capita use of HFCS increased from 0.23 kg in 1970 to 28.4 kg in 1997 (Putnam, Allshouse et al. 2002).

Consuming a high fructose diet produces numerous physiological effects.

Increased consumption of HFCS is positively correlated with the increase in obesity in the United States (Elliott, Keim et al. 2002; Bray, Nielsen et al. 2004). Ingesting a high amount of fructose can produce obesity and related problems for a number of reasons. The main organ in the body that metabolizes fructose is the liver (Topping and Mayes 1972). This metabolism produces glyceraldehyde and dihydroxyacetone phosphate, which enter the gluconeogenic

pathway. Phosphofructokinase (PFK) is the rate-limiting enzyme that determines the level of glucose production. Fructose metabolites enter the pathway after the PFK step, which results in uncontrolled production of triacylglyceride (TG) precursors and glucose (Kelley, Allan et al. 2004; Basciano, Federico et al. 2005; Havel 2005). Consequently, the liver produces TGs, which are in turn metabolized into free fatty acids (FFA). Another product of fructose metabolism, acetyl-CoA, is a precursor for FFA (Havel 2005). Accumulation of these fats in the liver leads to a number of hepatic diseases, including nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH) (Cave, Deaciuc et al. 2007). This increase in FFA in the liver also results in the elevation of blood levels of TGs and FFA (Sleder, Chen et al. 1980; Zavaroni, Chen et al. 1982; Bantle, Raatz et al. 2000). Circulating FFA stimulate insulin release (Crespin, Greenough et al. 1969). Insulin, in turn, perpetuates the buildup of FFA, as insulin reduces oxidation (lipolysis) of FFA (Cave, Deaciuc et al. 2007). Increases in FFA can cause insulin insensitivity by escalating intramyocellular lipids (Elliott, Keim et al. 2002).

There are several other consequences of fructose-induced insulin resistance. These include impaired glucose tolerance (Tobey, Mondon et al. 1982; Elliott, Keim et al. 2002). Dietary fructose metabolism leads to the creation of glucose. In addition, the increased concentration of FFA in the liver increases hepatic glucose production (Elliott, Keim et al. 2002). Fructose consumption, however, does not directly promote insulin secretion from pancreatic cells, which is necessary for glucose metabolism (Bezerra, Ueno et al. 2000; Elliott, Keim et al. 2002; Teff, Elliott et al. 2004). Glucose produced as a result of fructose metabolism stimulates insulin release, but the fructose-induced insulin resistance prevents the insulin from effectively metabolizing glucose. As a result, increased amounts of glucose circulate throughout the body. Insulin resistance can also lead to compensatory hyperinsulinemia, where the body attempts to

balance the reduced effects of insulin by producing and releasing more insulin (Zavaroni, Sander et al. 1980; Suga, Hirano et al. 2000). Insulin also is important for leptin gene expression and leptin secretion (Elliott, Keim et al. 2002). Leptin is one of a number of hormones that signals the brain that enough food has been consumed. Plasma leptin levels in fructose-fed rats are increased 2-fold compared to control rats in response to oral glucose loads (Lee, Ko et al. 2006), suggesting that leptin resistance may be present in these animals. Triacylglycerides promote leptin resistance by preventing leptin from crossing the blood brain barrier (Banks, Coon et al. 2004). Consequences of leptin resistance include an increase in caloric intake due to decreased satiety signals.

Numerous studies have found that high fructose diets also cause hypertension in animals (Hwang, Ho et al. 1987; Elliott, Keim et al. 2002; Catena, Giacchetti et al. 2003; Delbosc, Paizanis et al. 2005). In fact, fructose-fed rats are used as a model for hypertension (Elliott, Keim et al. 2002). Decreased glucose metabolism increases aldehyde levels, which in turn affects calcium channels, elevates oxidative stress and results in vascular resistance and hypertension (MacLeod, Vasdev et al. 1997).

Despite the previous evidence for fructose-induced obesity, the effects of a high fructose diet on body mass are inconsistent in animal models. Some studies suggest that diets high in fructose augment overall body and lipid mass (Kanarek and Orthen-Gambill 1982; Williams and Szepesi 1983; Jurgens, Haas et al. 2005), whereas other research finds no effect on body mass (Vrana, Fabry et al. 1973; Reiser and Hallfrisch 1977; Blakely, Hallfrisch et al. 1982). It is possible that instead of an overall gain in adipose tissue, the distribution of fat is shifted so total body mass is not affected. As a result, Experiment 1 tested the hypothesis that a high fructose diet causes a shift in fat distribution.

Consuming a high fructose diet may cause cognitive deficits.

In addition to physiological effects, a diet high in fructose may also affect cognition. The idea that diet can affect brain function is supported by the finding that rats fed a diet high in saturated fatty acids exhibit impaired performance on a number of hippocampal-dependent memory tasks (Greenwood and Winocur 1990; Greenwood and Winocur 1996; McNay, Herzog et al. 2005). Given that fructose is preferentially metabolized into lipids, a high fructose diet is essentially a high fat diet. The mechanism underlying the high fat-induced memory impairment is currently under investigation, but it is hypothesized to involve insulin resistance (Greenwood and Winocur 2001). Interestingly, recent research found that a diet high in fructose produces insulin resistance in the brain. Specifically, Syrian hamsters fed a diet of 60% fructose for six weeks had impaired neuronal insulin signaling in the hippocampus and cortex (Mielke, Taghibiglou et al. 2005).

Insulin signaling in the hippocampus is important for learning and memory. In fact, studies in healthy individuals indicate that insulin can enhance hippocampal-dependent memory. Specifically, administration of intravenous and intranasal insulin in humans enhances declarative memory in a dose-dependent manner (Benedict, Hallschmid et al. 2004; Stockhorst, de Fries et al. 2004). In addition, intraventricular and intrahippocampal injections of insulin in rats improve memory in an avoidance task (Park, Seeley et al. 2000; Babri, Badie et al. 2007) and in a spatial memory task (Moosavi, Naghdi et al. 2006). Moreover, participation in hippocampal-dependent memory tasks increases activation of insulin receptors in the hippocampus (Zhao, Chen et al. 1999; Dou, Chen et al. 2005).

Correspondingly, disrupted insulin signaling, such as that caused by insulin resistance, may impair learning and memory. In fact, type 2 diabetes, which is characterized by peripheral

insulin resistance, is associated with hippocampal-dependent memory loss in humans (Gold, Dziobek et al. 2007). At least two possible reasons for this memory impairment exist. First, insulin resistance leads to impaired glucose tolerance, as stated earlier. Circulating glucose levels are important for cognitive functioning, as glucose is readily transported across the blood brain barrier and used as fuel in the brain (Dwyer 2002). Even in individuals without type 2 diabetes, mildly impaired glucose tolerance is correlated with hippocampal atrophy and cognitive decline (Convit et al 2003). Reductions in hippocampal volume also are found in patients with type 2 diabetes (Gold, Dziobek et al. 2007). Second, the disease may cause central insulin resistance in addition to the known peripheral insulin resistance. Insulin resistance in the hippocampus significantly reduces the ability of insulin to stimulate long-term depression (LTD) (Mielke, Taghibiglou et al. 2005). LTD is a cellular mechanism of plasticity that underlies learning and memory (Malenka and Bear 2004; Massey and Bashir 2007). In addition, experimentally diabetic rats show deficits in spatial-working memory and impaired LTP (Biessels, Kamal et al. 1996; Biessels, Kamal et al. 1998; Artola, Kamal et al. 2005), another cellular mechanism of learning and memory (O'Dell, Kandel et al. 1991). Though this is a type 1 diabetes model, it is relevant because it is characterized by a decrease in circulating insulin.

Originally, it was thought that fructose was a safe alternative to dietary sucrose for individuals with type 2 diabetes, mainly because fructose metabolism is not dependent on insulin (Cohen, Teitelbaum et al. 1977). In contrast, fructose-fed rats now are viewed as an animal model of type 2 diabetes; rats fed a high fructose diet exhibit glucose intolerance and insulin resistance as seen in humans with type 2 diabetes (Cohen, Teitelbaum et al. 1977; Elliott, Keim et al. 2002). Interestingly, new treatments for type 2 diabetes include oral medications that target the enzyme fructose-1,6 bisphosphatase. Specifically, inhibition of this rate-limiting enzyme in

the gluconeogenesis pathway results in a significant decrease in the production and therefore circulation of glucose (Erion, van Poelje et al. 2005; Dang, Kasibhatla et al. 2007). This enzyme is involved in the process of utilizing fructose metabolites for the production of glucose. In effect, these medications that treat type 2 diabetes inhibit glucose production from dietary fructose.

In summary, rats fed a high fat diet exhibit hippocampal-dependent memory impairments, possibly caused by the development of insulin resistance (Greenwood and Winocur 2001). Hamsters fed a high fructose diet exhibit insulin resistance in the hippocampus (Mielke, Taghibiglou et al. 2005), though cognitive functioning in these animals has not been examined. Rats fed a high fructose diet develop glucose intolerance as seen in type 2 diabetes (Cohen, Teitelbaum et al. 1977), and humans with type 2 diabetes display hippocampal-dependent memory loss (Gold, Dziobek et al. 2007). Based on this evidence, the following experiments tested the hypothesis that a high fructose diet causes hippocampal-dependent memory deficits.

Experiment 1

The goal of the first experiment was to test the hypothesis that a high fructose diet alters fat distribution and impairs hippocampal-dependent learning and memory.

Methods

Subjects

Male Sprague-Dawley rats (Charles River, Wilmington, MA), aged 53 days upon arrival, were habituated for 1 week before the experiment started. The rats were weighed the day they arrived and again during the 3 days before the diet change. They were then matched and assigned to either the Control (n = 14) or Fructose (n = 15) group based on absolute body mass and percent body mass change during the habituation week. The animals were housed in suspended

cages with wire mesh bottoms (Hazelton Systems, Aberdeen, MD). All procedures involving the rats were approved by Georgia State University's Institutional Animal Care and Use Committee (IACUC).

Diets

The Fructose group was fed a diet of chow consisting of 60% fructose (Research Diets, New Brunswick, NJ) *ad libitum.* This fructose concentration was used because it is the concentration that produced brain insulin resistance in hamsters (Mielke, Taghibiglou et al. 2005). The Control group was fed a diet of standard rat chow (60% vegetable starch; Research Diets, New Brunswick, NJ) *ad libitum*. Both diets contained equal percentages of carbohydrates (70%), proteins (20%), and lipids (10%), and both diets were also equal in kilocalories (kcal) per gram.

Body Mass and Food Intake

Rat body mass and food intake were recorded daily. To measure food intake, pellets in each hopper and spillage from under each cage were weighed. These weights were subtracted from the amount placed in the hopper the previous day to yield an estimate of the amount of food ingested by each animal. Daily kcal consumption was calculated by multiplying the average grams of food consumed daily by kcal per gram of food. Feed efficiency was calculated for each rat by dividing the overall change in body mass by the total kcal consumed.

Insulin Tolerance Test

Insulin resistance was assessed using an insulin tolerance test (ITT) before (week 11) and after (week 14) cognitive testing. For the ITT, each rat was given an intraperitoneal (IP) injection of insulin (Eli Lilly, Indianapolis, IN) following a 4-hour fast. A dose of 0.75 U/kg was given during week 11 and a dose of 1.25 U/kg was given during week 14. The higher dose was given

the second time because the lower dose did not produce a sufficient decrease in blood glucose levels in Control rats. A drop of blood from the tail was collected 0, 15, 30, 45, 60, 90 and 120 minutes after the injection. Blood glucose levels were measured using a One Touch Basic System (Lifescan, Milpitas, CA).

Hippocampal-dependent Memory Tasks

Rats were tested for memory using two tasks that involve the hippocampus: spontaneous alternation (SA) and continuous multiple-trial inhibitory avoidance (CMIA). Hippocampal lesions impair performance on these tasks (Roberts, Dember et al. 1962; Douglas 1966; Deacon, Bannerman et al. 2002; Broadbent, Squire et al. 2004; Clark, Broadbent et al. 2005); whereas hippocampal infusions of insulin improve performance in these tasks (McNay, Herzog et al. 2005; Babri, Badie et al. 2007). Memory was assessed during the thirteenth week on the diet. The tasks were not counterbalanced to avoid memory assessment at varying diet durations. SA was tested first because it is the least stressful of the two tasks.

Spontaneous Alternation

During SA, each rat was allowed to move freely through a 4-arm maze for 8 minutes. The number and sequence of arms entered were recorded by the experimenter, and an alternation score was calculated by dividing the number of alternations (an alternation is when the rat enters 4 different arms consecutively) by the number of arms entered minus 3 (the degrees of freedom) and multiplying this quotient by 100. Rats were not included in the statistical analyses if they did not enter at least 10 arms during the testing period.

SA is assumed to be a measure of spatial-working memory, because in order to successfully alternate rats must remember which arms they previously explored. This interpretation is supported by evidence showing that alternation performance declines when

extra-maze cues are altered or when the interval between arm entries is increased (Richman, Dember et al. 1987; Dember and Richman 1989; Lalonde 2002).

Continuous Multiple-trial Inhibitory Avoidance

For CMIA training, each rat was placed into the lighted portion of an alley. Upon entering the adjacent dark compartment, they were given a 1.2 mA shock. Shocks were given until the rat remained in the lighted compartment for 100 consecutive seconds or a maximum of 5 shocks was given. Forty-eight hours later, the rats were tested for retention. They were placed in the lighted chamber and allowed to cross into the darkened compartment. Latency (600 seconds maximum) to enter the darkened compartment was recorded. Longer latencies were assumed to reflect better memory of the training. CMIA is a measure of long-term emotional memory (McGaugh 1989).

Post-mortem Measures

At least 2 days following the second ITT, the rats were anesthetized with isoflurane gas (5% in 95% oxygen) and decapitated. Trunk blood was immediately obtained in heparanized tubes and centrifuged for the collection of plasma. The liver of each rat was extracted, weighed and snap frozen in liquid nitrogen. Plasma and livers were stored at -80º C until the assays and hepatic lipid analysis were performed. Inguinal, epididymal, retroperitoneal, and mesenteric fat pads were extracted and weighed. Following replacement of the fat pads into the carcass, a total carcass weight was recorded. Carcasses were stored at -20º C until the carcass composition analysis was performed.

For the hepatic lipid analysis, livers were thawed and homogenized (Fisher Scientific, Pittsburgh, PA). Petroleum ether (Acros, Geel, Belgium) was added to each sample of homogenate, which was then vortexed (Henry Troemner, Sparks, MD), shaken (Eberbach, Ann

Arbor, MI) and centrifuged (Eppendorf, Westbury, NY) until the supernatant was clear. The supernatant was collected after each round and left to evaporate overnight in a fume hood. The next morning, the remaining sample was weighed. This amount was the total lipid in the sample. Total lipid in the liver was then calculated by dividing the total grams of lipid in the sample by the total sample mass, which is proportional to the total grams of lipid in the liver divided by the total liver mass.

For the carcass composition analysis, the carcasses were thawed and placed in an oven (Grieve, Round Lake, IL) set at 76.6º C for 2 weeks. Carcasses were then weighed daily until a constant weight was achieved for 3 consecutive days. A measure of the total carcass water (TCW) was then calculated by subtracting the post-oven carcass weight from the pre-oven carcass weight. Carcasses were then homogenized using a blender. Petroleum ether was added to each sample of homogenate, which was then vortexed, shaken and centrifuged until the supernatant was clear. The remaining sample (fat free dry mass) was left overnight in a fume hood and weighed the next morning. Total lipid in the sample was then calculated by subtracting this fat free dry mass from the weight of the original homogenate. A total carcass lipid (TCL) was extrapolated for each sample by dividing the total grams of lipid in the sample by the total sample mass, which is proportional to the total grams of lipid in the carcass divided by the total carcass mass.

Plasma samples were assayed for TG (Sigma, St. Louis, MO), FFA (Wako Chemicals, Richmond, VA), leptin (ELISA, St. Charles, MO), and insulin (ELISA, St. Charles, MO) using spectrophotometry. Samples were run in duplicate with standards. Glucose was measured using an Accu Chek glucose meter (Roche, Indianapolis, IN). All assays were performed according to the manufacturer’s instructions.

Data Analysis

The data were stored and analyzed using Microsoft Excel, Version 5.0 and Statistical Package for the Social Sciences (SPSS), Version 15.0. A two-tailed Student's t-test was performed to determine whether there were differences between the means of the Control rats and the Fructose rats for percent change in body mass, kcal consumed, feed efficiency, number of arms entered and percent alternation during SA, baseline blood glucose levels, plasma assays (TG, FFA, leptin, insulin, glucose), liver mass, fat pad masses, hepatic lipids, and carcass lipids. The number of trials to reach criterion and latency to enter the darkened compartment during CMIA were not evenly distributed due to the limits on the maximum number of training trials and the maximum retention latency. As a result, the non-parametric Mann-Whitney U-test was used to analyze these scores. A mixed ANOVA was computed to determine whether there was a difference in the insulin-induced percent change in blood glucose levels over time (within factor) between the Control and Fructose groups (between factor). To determine if there was an association between the peripheral and cognitive effects of a high fructose diet, Pearson correlations were run between the peripheral measures and the statistically significant memory task scores. An alpha level of .05 was used as the criterion of significance for all tests.

Results

Body Mass and Food Intake

The average daily kilocalorie consumption was higher in the Fructose rats than in the Control rats [$t(27) = -2.45$, $p < 0.05$]; however, the groups did not significantly differ in percent change in body mass [$t(27) = -0.84$, $p > 0.05$] (see Figure 1A and B). Interestingly, feed efficiency did not significantly differ between the Fructose and Control rats, [$t(27) = 0.80$, $p > 0.05$] (see Figure 1C).

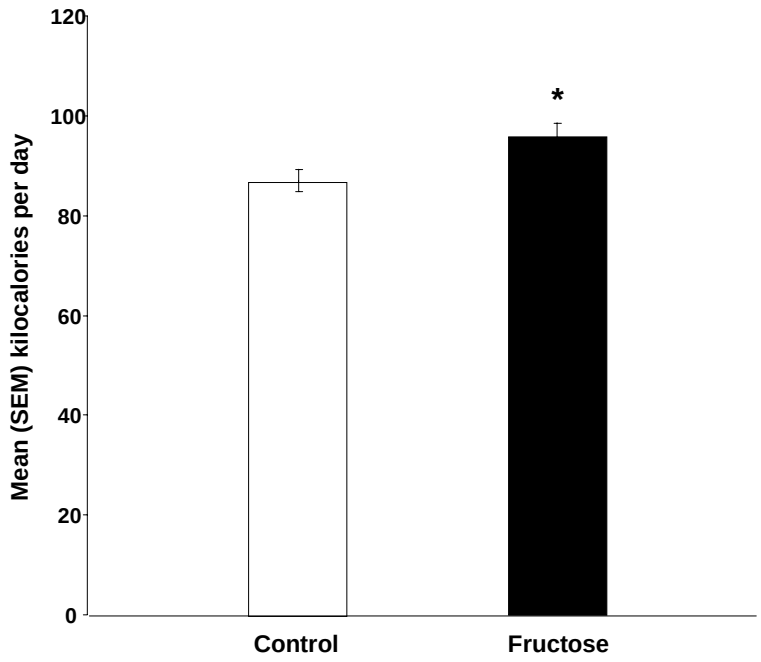

Figure 1A. Mean (+/- SEM) kilocalories of chow consumed per day by rats fed a control or high fructose (60%) diet for 97 days (*$p < 0.05$ vs. Control rats).

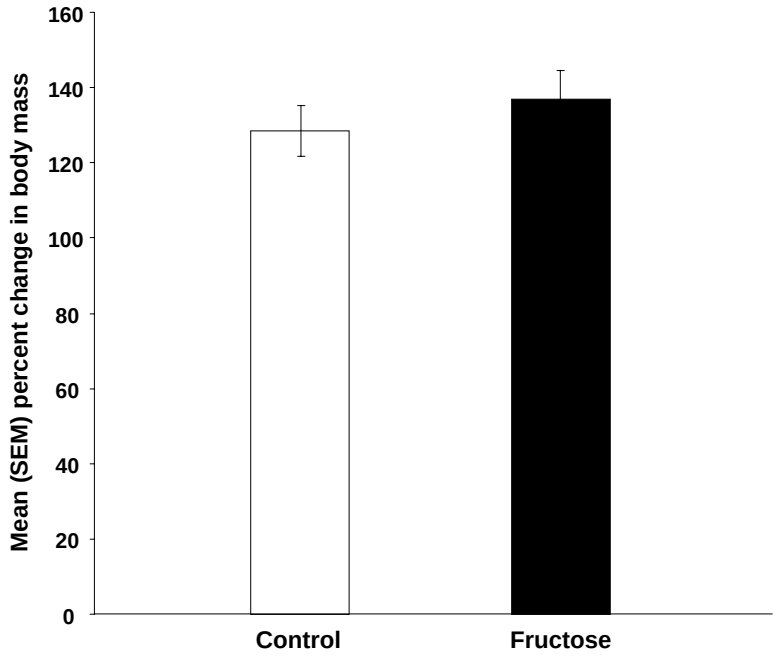

Figure 1B. Mean (+/- SEM) percent change in body mass in rats fed a control or high fructose (60%) diet for 97 days.

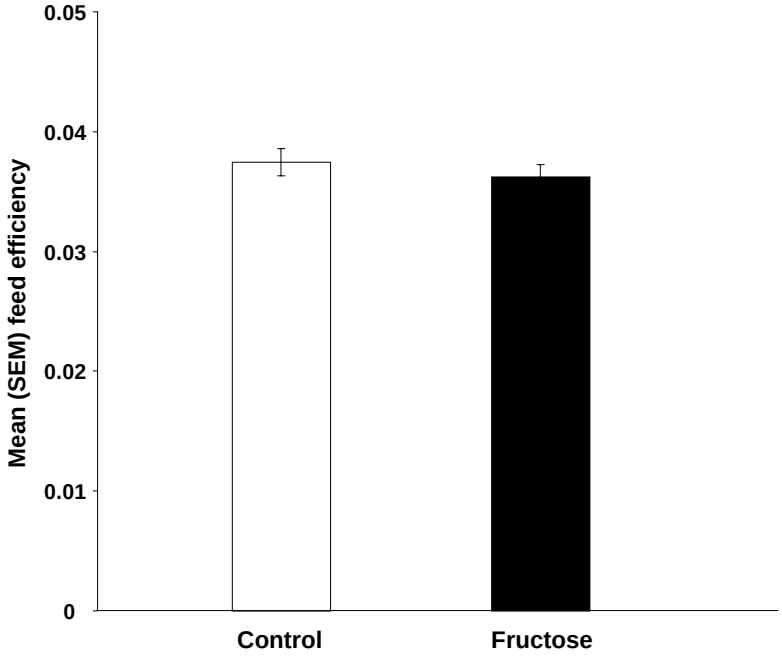

Figure 1C. Mean (+/- SEM) feed efficiency (change in body mass divided by kilocalories consumed throughout the experiment) in rats fed a control or high fructose (60%) diet for 97 days.

Insulin Tolerance Test

Week 11

The high fructose diet did not affect baseline blood glucose levels [$t(27) = 1.84$, $p > 0.05$] (see Figure 2A). There was a significant main effect of time [$F(1, 5) = 17.15$, $p < 0.05$] (see Figure 2B), indicating that insulin decreased blood glucose levels over time. The diet appeared to produce insulin insensitivity; specifically, there was a trend toward a significant main effect of diet [$F(1, 5) = 4.01$, $p = 0.055$] (see Figure 2B insert). That is, there was a tendency for insulin to not produce as big a decrease in blood glucose levels in the Fructose rats as it did in the Control rats. There was not a significant interaction between time and diet [$F(1,5) = 0.74$, $p > 0.05$].

Week 14

By week 14, the diet significantly increased baseline blood glucose levels [$t(25) = 3.09$, $p < 0.05$] (see Figure 3A). As in week 11, there was a significant main effect of time [$F(1, 5) = 57.25$, $p < 0.05$] (see Figure 3B), indicating that insulin decreased blood glucose levels. The diet produced insulin resistance; specifically, there was a significant main effect of diet [$F(1, 5) = 7.32$, $p < 0.05$] (see Figure 3B insert). That is, the ability of insulin to decrease glucose was attenuated in Fructose rats. There was not a significant interaction between time and diet [$F(1,5) = 1.22$, $p > 0.05$].

Hippocampal-dependent Memory Tasks

Spontaneous Alternation

Seven rats in the Control group and six rats in the Fructose group did not enter at least 10 arms and were therefore excluded from the statistical analyses. Analyses of the remaining scores ($n = 7$ Control, $n = 9$ Fructose) indicated that the diet did not affect SA behavior. That is, the

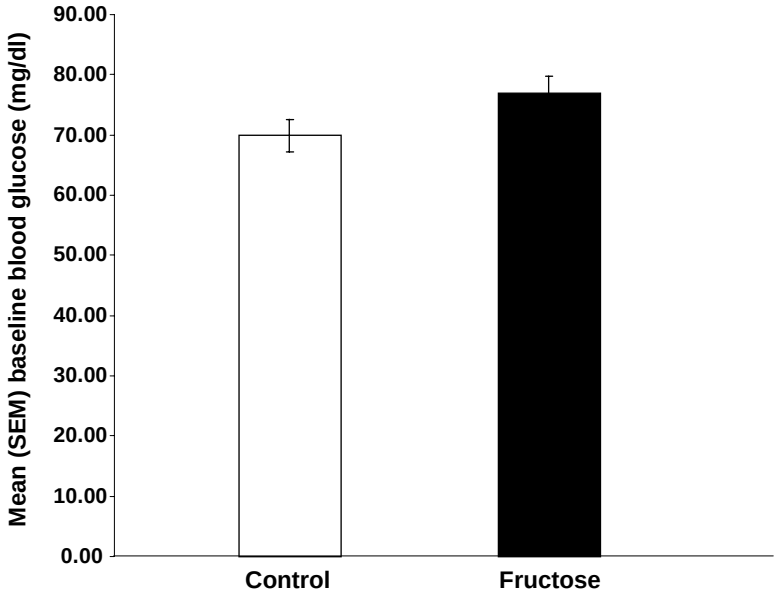

Figure 2A. The effects of eating a control or high fructose (60%) diet on mean (+/- SEM) baseline blood glucose levels during the week 11 ITT.

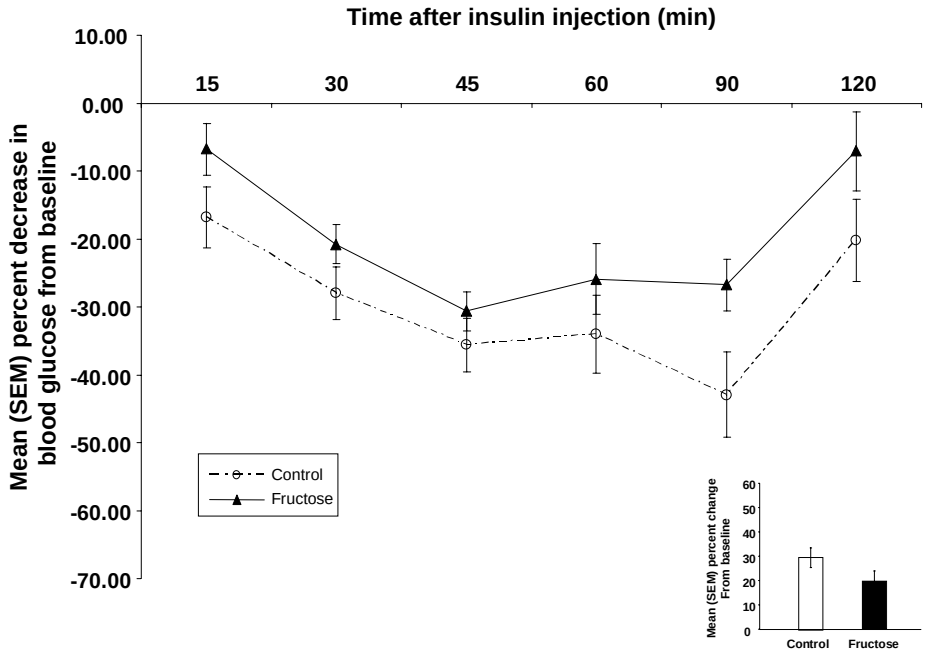

Figure 2B. The effects of eating a control or high fructose (60%) diet on the mean (+/- SEM) percent decrease in blood glucose levels from baseline over time and on the mean (+/- SEM) percent change in blood glucose levels from baseline during the week 11 ITT (inset).

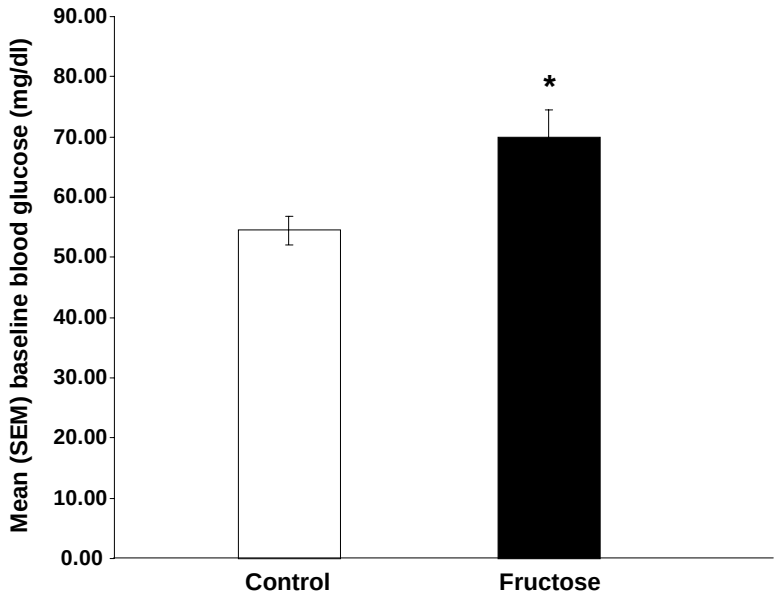

Figure 3A. The effects of eating a control or high fructose (60%) diet on mean (+/- SEM) baseline blood glucose levels during the week 14 ITT (*$p < 0.05$ vs. Control rats).

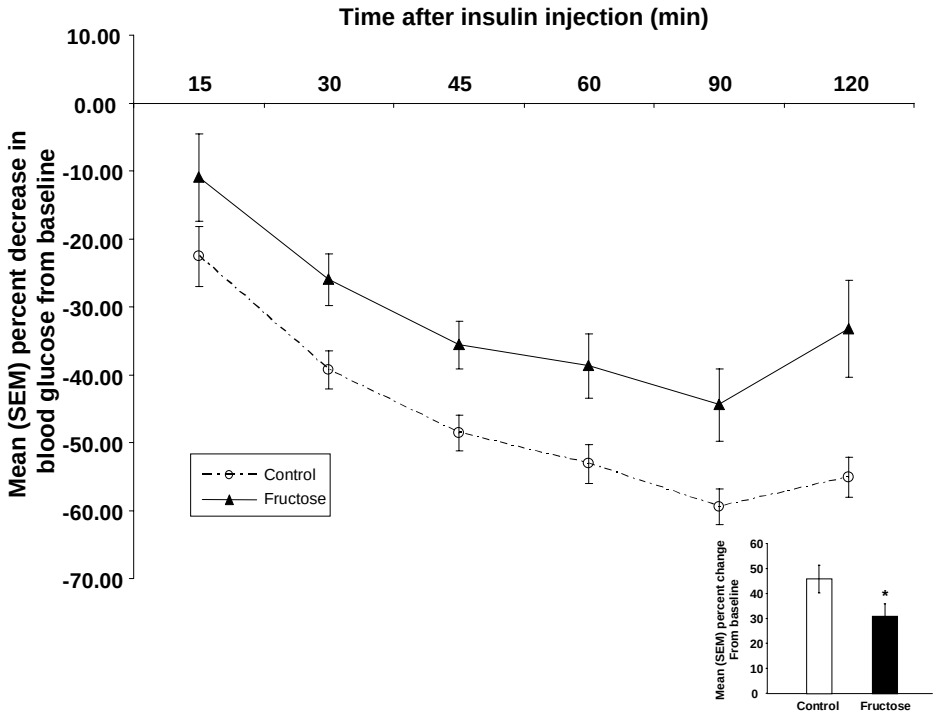

Figure 3B. The effects of eating a control or high fructose (60%) diet on the mean (+/- SEM) percent decrease in blood glucose levels from baseline over time and on the mean (+/- SEM) percent change in blood glucose levels from baseline during the week 14 ITT (inset; *$p < 0.05$ vs. Control rats).

percent alternation scores of the Fructose rats were not significantly different from those of the Control rats [t (14) = 0.52, p > 0.05] (see Figure 4A). The diet did not affect the ability of the rats to move in the maze, because the number of arms entered by the Fructose group was not significantly different from that of the Control group [t (14) = -1.61, p > 0.05] (see Figure 4B).

Continuous Multiple-trial Inhibitory Avoidance

The high fructose diet did not significantly impair inhibitory avoidance behavior. During training, latency to enter the darkened compartment [U (14, 15) = 89.50, p > 0.05] and the number of trials to reach learning criterion [U (14, 15) = 91.00, p > 0.05] did not significantly differ between the Control and Fructose rats (see Figures 5A and B). Interestingly, there was a tendency for the diet to impair retention performance, although retention latencies did not significantly differ between the two groups [U (14, 15) = 84.00, p = 0.08] (see Figure 5C).

Post-mortem Measures

Liver and Fat Pad Masses

The high fructose diet significantly increased liver mass. Compared to the Control rats, the Fructose rats had heavier livers [t (27) = -2.98, p < 0.05] (see Figure 6A). This effect remained significant after correcting for body mass. That is, the liver mass to body mass ratio was greater in the Fructose rats than in the Control rats [t (27) = -5.13, p < 0.05] (see Figure 6B). The high fructose diet did not significantly affect fat distribution. Retroperitoneal [t (27) = -0.96, p > 0.05], epididymal [t (27) = -0.96, p > 0.05], mesenteric [t (27) = 0.57, p > 0.05], and inguinal [t (27) = -1.18, p > 0.05] fat pad masses did not significantly differ between the Control and Fructose rats (data not shown).

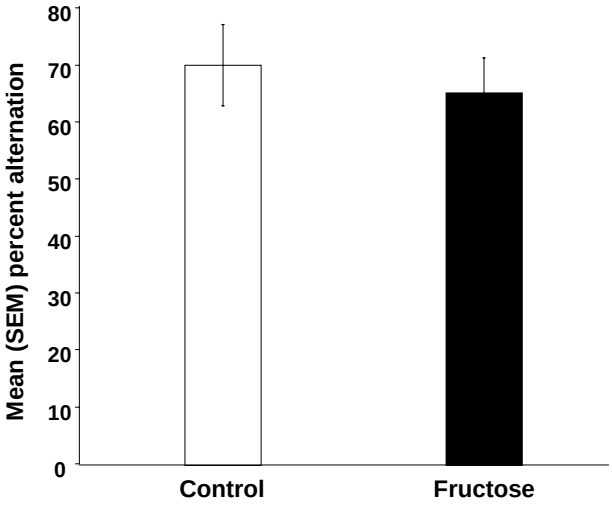

Figure 4A. The effects of eating a control or high fructose (60%) diet on mean (+/- SEM) percent alternation in a plus maze.

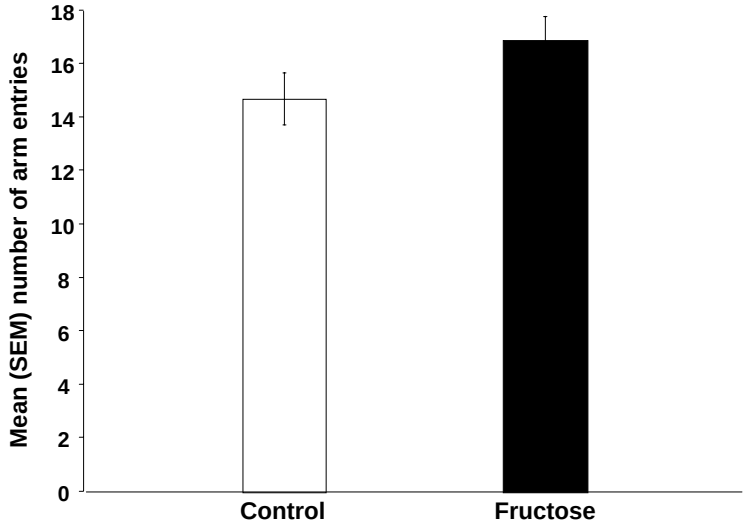

Figure 4B. The effects of eating a control or high fructose (60%) diet on mean (+/- SEM) number of arms the rats entered in the plus maze in 8 minutes.

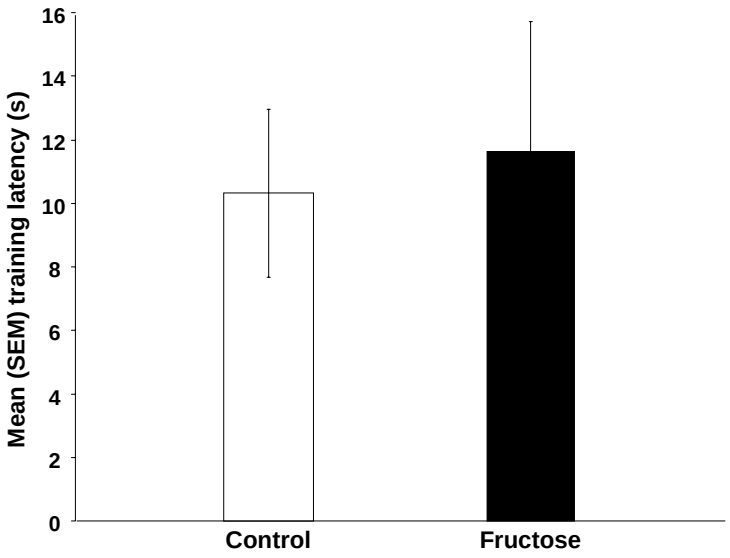

Figure 5A. The effects of eating a control or high fructose (60%) diet on the mean (+/- SEM) latency to enter the darkened compartment during CMIA training.

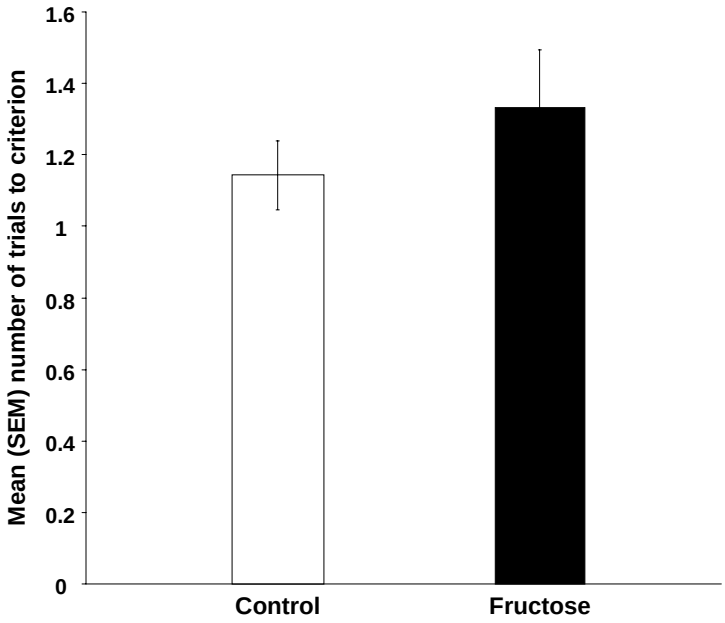

Figure 5B. The effects of eating a control or high fructose (60%) diet on the mean (+/- SEM) number of trials to reach learning criterion during CMIA training.

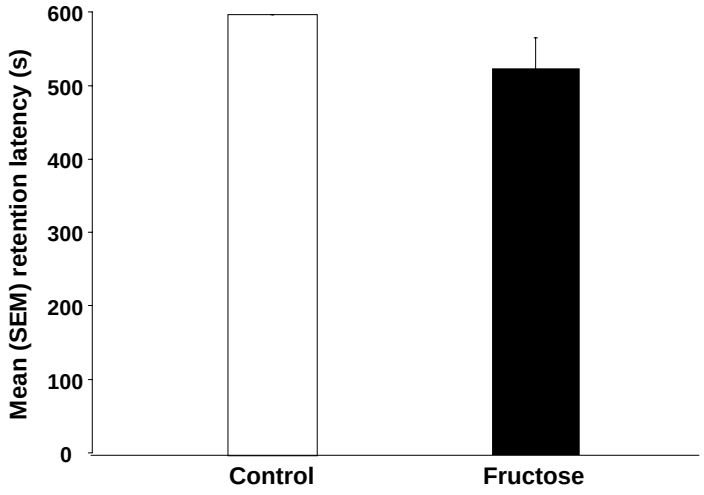

Figure 5C. The effects of eating a control or high fructose (60%) diet on the mean (+/- SEM) latency to enter the darkened compartment during CMIA retention.

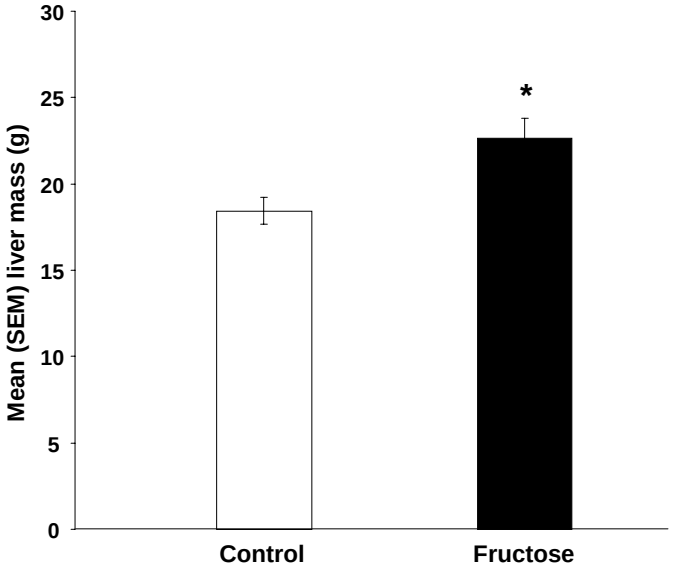

Figure 6A. Mean (+/- SEM) liver mass of rats fed a control or high fructose (60%) diet for 97 days (*p < 0.05 vs. Control rats).

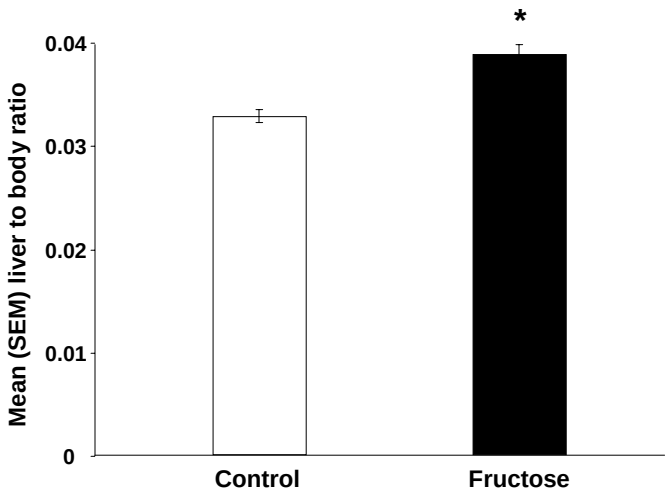

Figure 6B. Mean (+/- SEM) liver mass to body mass ratio of rats fed a control or high fructose (60%) diet for 97 days (*$p < 0.05$ vs. Control rats).

Carcass Composition and Hepatic Lipids

The high fructose diet did not affect the amount of hepatic lipids. Lipids in the livers of the Fructose rats were not significantly different from those of the Control rats [$t(27) = -1.70$, $p > 0.05$]. The diet did not affect the amount of overall amount of lipids in the body. TCL, which included lipids from the liver, were not significantly different between Control and Fructose rats [$t(27) = -0.24$, $p > 0.05$] (data not shown).

Plasma Assays

The high fructose diet increased circulating lipids. Compared to the Control rats, the Fructose rats had significantly higher plasma TG [$t(27) = -3.91$, $p < 0.05$] and FFA [$t(27) = -2.36$, $p < 0.05$] levels (see Figures 7 and 8). The diet did not significantly affect other plasma measures. Specifically, plasma leptin [$t(27) = 0.11$, $p > 0.05$], insulin [$t(27) = -0.02$, $p > 0.05$], and glucose [$t(27) = -1.35$, $p > 0.05$] did not significantly differ between the Fructose and Control rats (data not shown).

Correlations

Given that there was a tendency for the diet to affect CMIA retention scores, Pearson correlations were run using these scores and the *post-mortem* measures. The results of this analysis showed that CMIA retention scores did not significantly correlate with any of these measures.

Discussion

The behavioral testing results from Experiment 1 indicate that the high fructose diet did not affect spatial working memory, as the SA scores of the Fructose and Control rats did not differ. This effect was not likely due to the small sample size, as there was no trend toward significance. The results of CMIA suggest that the high fructose diet may impair emotional

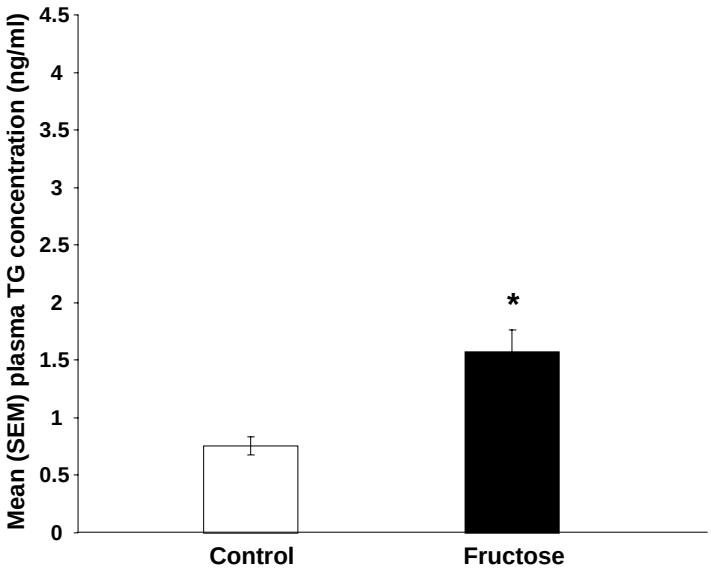

Figure 7. Mean (+/- SEM) plasma TG concentrations of rats fed a control or high fructose (60%) diet for 97 days (*$p < 0.05$ vs. Control rats).

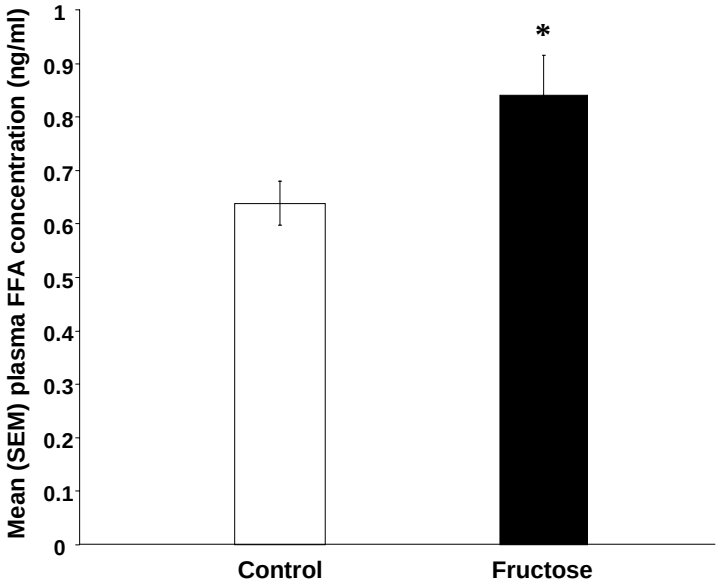

Figure 8. Mean (+/- SEM) plasma FFA concentrations of rats fed a control or high fructose (60%) diet for 97 days (*$p < 0.05$ vs. Control rats).

memory. It is unlikely that this effect was not statistically significant due to a small sample size, because our sample size consisted of 15 Fructose and 14 Control rats. This is usually more than sufficient for observing deficits in this task. Another possibility is that the diet did impair the ability of the hippocampus to participate in the memory of the task, but that other brain areas such as the striatum and the amygdala mediated the spared retention that was observed. It is well known that, in addition to the hippocampus, successful performance in this task can be mediated by the stiatum or the amygdala (McDonald and White 1994; Packard and Teather 1998). Consequently, the effects of the diet on memory in the Morris water maze (MWM) were assessed in a subsequent experiment, as this type of memory is more solely dependent on the hippocampus for successful performance (McDonald and White 1994). Hippocampal lesions impair performance on this task (Morris, Garrud et al. 1982; Broadbent, Squire et al. 2004; Clark, Broadbent et al. 2005); whereas infusions of insulin improve performance on this task (Moosavi, Naghdi et al. 2006).

Experiment 2

The goal of the second experiment was to determine whether a high fructose diet would impair learning and memory in the spatial water maze task, which is more dependent on the hippocampus than is CMIA.

Methods

The same procedures were used as in Experiment 1, with the following exceptions:

Body Mass and Food Intake

In an effort to reduce work load and because weight gain and food consumption patterns were established during Experiment 1, rat body mass and food intake were recorded for 1 week out of every 3 weeks until behavioral tests were performed.

Insulin Tolerance Test

Insulin resistance was assessed using an ITT during weeks 11 and 12. Results of the ITT during week 11 were not similar to those seen in week 11 of Experiment 1; specifically, the diet did not appear to produce insulin resistance. As a result, we opted to keep the rats on the diet longer that in the first experiment in order to determine whether the diet would eventually produce signs of insulin resistance. Given that we were approved by IACUC to keep the rats on the diet for a total of 20 weeks, the second ITT was postponed until weeks 16 and 17, and cognitive function was tested during weeks 19 and 20.

Hippocampal-dependent Memory Tasks.

Morris Water Maze

For water maze acquisition, the rats were trained to locate a submerged platform in a pool. The procedures used were designed to measure spatial reference memory, which is dependent on the hippocampus (Morris, Garrud et al. 1982; Broadbent, Squire et al. 2004; Clark, Broadbent et al. 2005). Acquisition consisted of 8 training trials per day for three consecutive days. Trials had 30 second intertrial intervals (ITI). At the beginning of the first trial on each of the 3 days, the rats were placed on the platform for 30 seconds. Then they were placed in the pool in one of three quadrants, the order of which was randomly determined. The fourth quadrant contained the platform and was considered the “target quadrant.” Latency to locate the platform was recorded. If the rats did not reach the platform within 60 seconds, they were guided by hand to the platform. The rats remained on the platform for 15 seconds at the end of each trial. Retention of the training was tested 48 hours after the last training day using one-20 second probe trial in which the platform was not present. Time spent in the target quadrant and latency

to cross the platform location or “target” were recorded. Number of target approaches was recorded, and swim speed was calculated.

Post-mortem Measures

Animals were sacrificed at the conclusion of MWM. Given that there were no differences between the fat pad masses, hepatic lipids or the total carcass lipids in Experiment 1, these variables were not measured in Experiment 2.

Data Analysis

A two-tailed Student’s t-test was performed to determine whether there were differences between the means of the Control rats and the Fructose rats for time spent in the target quadrant and swim speed during MWM. Latency to cross the target and the number of target approaches were not normally distributed; as a result, a Mann-Whitney U-test was used to analyze these scores. A mixed ANOVA was performed to determine whether there was a difference in time to reach the platform during acquisition of MWM over time (within factor) between the Control and Fructose rats (between factor).

Results

Body Mass and Food Intake

As in Experiment 1, the Fructose rats consumed more kilocalories than did the Control rats [$t(39) = -2.33$, $p < 0.05$], but the groups did not significantly differ in average body mass [$t(39) = 0.59$, $p > 0.05$] (see Figures 9A and B). Again, feed efficiency was not statistically different between the Fructose and Control rats, [$t(38) = 1.72$, $p > 0.05$] (see Figure 9C).

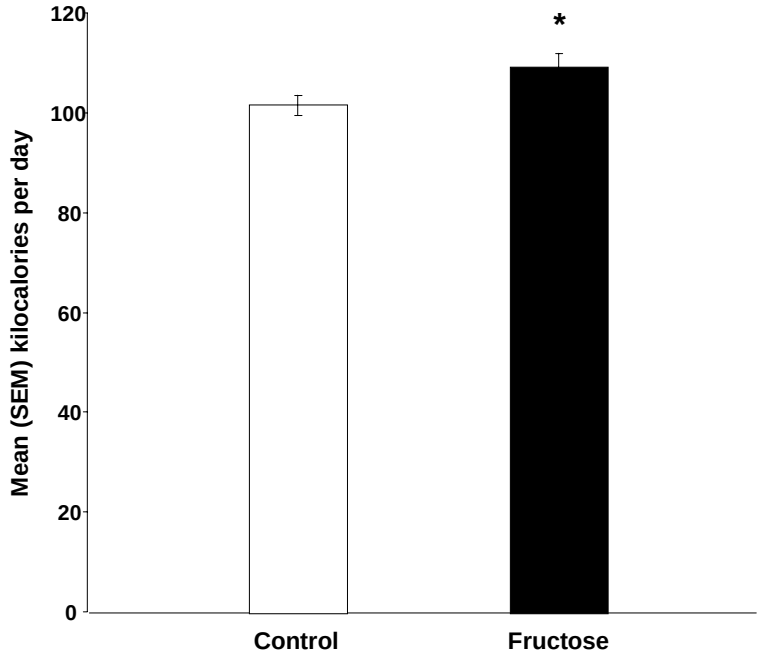

Figure 9A. Mean (+/- SEM) kilocalories of chow consumed per day by rats fed a control or high fructose (60%) diet for 138 days (*$p < 0.05$ vs. Control rats).

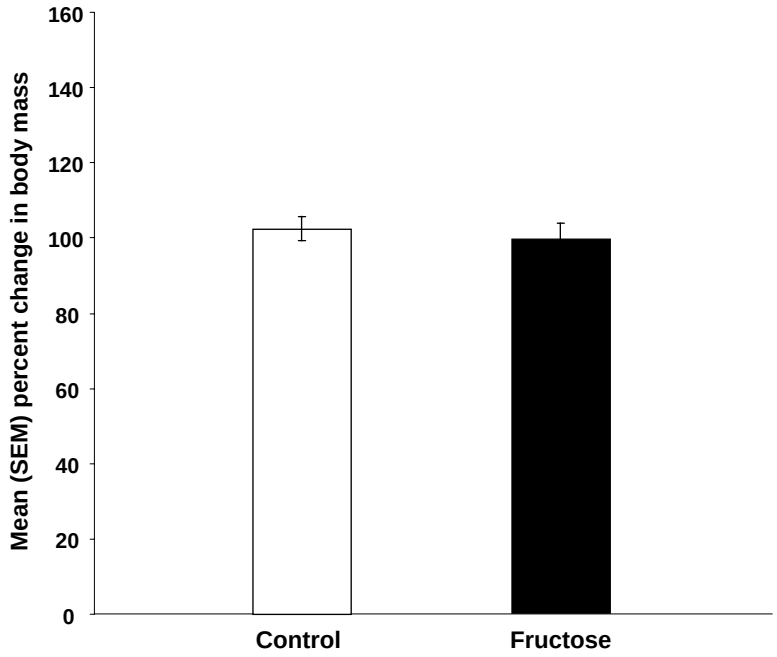

Figure 9B. Mean (+/- SEM) percent change in body mass in rats fed a control or high fructose (60%) diet for 138 days.

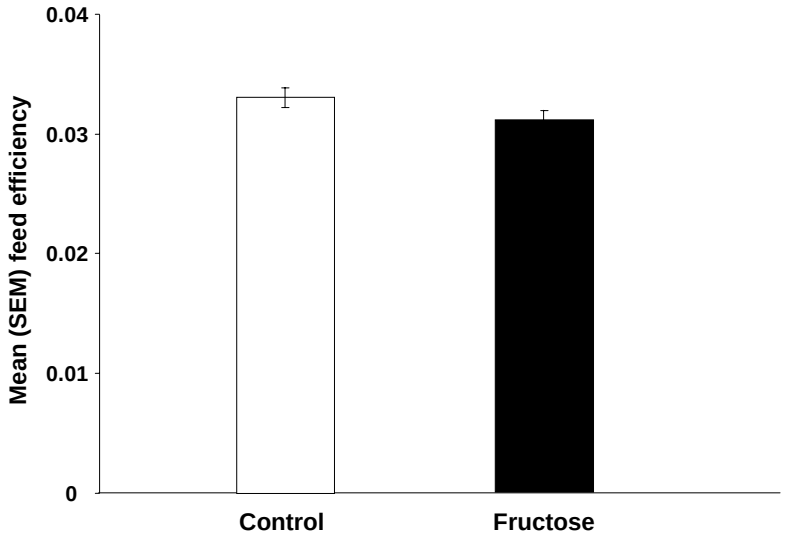

Figure 9C. Mean (+/- SEM) feed efficiency (change in body mass divided by kilocalories consumed throughout the experiment) rats fed a control or high fructose (60%) diet for 138 days.

Insulin Tolerance Test

Week 11

The high fructose diet did not affect baseline blood glucose levels [t (39) = -0.22, p > 0.05] (see Figure 10A). There was a significant main effect of time [F (1, 5) = 52.94, p < 0.05] (see Figure 10B), indicating that insulin decreased blood glucose levels over time. There was not a significant main effect of diet [F (1, 5) = 0.05, p > 0.05] (see Figure 10B insert), indicating that the diet did not induce insulin insensitivity. That is, insulin produced as big a decrease in blood glucose levels in the Fructose rats as it did in the Control rats. There was not a significant interaction between time and diet, [F (1, 5) = 1.24, p > 0.05].

Week 16

The diet did not affect baseline blood glucose levels [t (38) = -0.74, p > 0.05] (see Figure 11A). There was a significant main effect of time [F (1, 5) = 79.47, p < 0.05] (see Figure 11B), indicating that insulin decreased blood glucose levels over time. There was not a significant main effect of diet [F (1, 5) = 0.74, p > 0.05] (see Figure 11B insert), indicating that the high fructose diet did not produce insulin insensitivity. That is, insulin produced as big a decrease in blood glucose levels in the Fructose rats as it did in the Control rats. There was not a significant interaction between time and diet group [F (1, 5) = 0.47, p > 0.05].

Memory Tasks

Morris Water Maze

The high fructose diet did not affect water maze acquisition, but it did impair retention. During acquisition, there was a significant main effect of time [F (1, 23) = 23.92, p < 0.05], indicating that the latencies to reach the platform decreased over time. There was not a significant main effect of diet [F (1, 23) = 0.81. p > 0.05], indicating that there were no

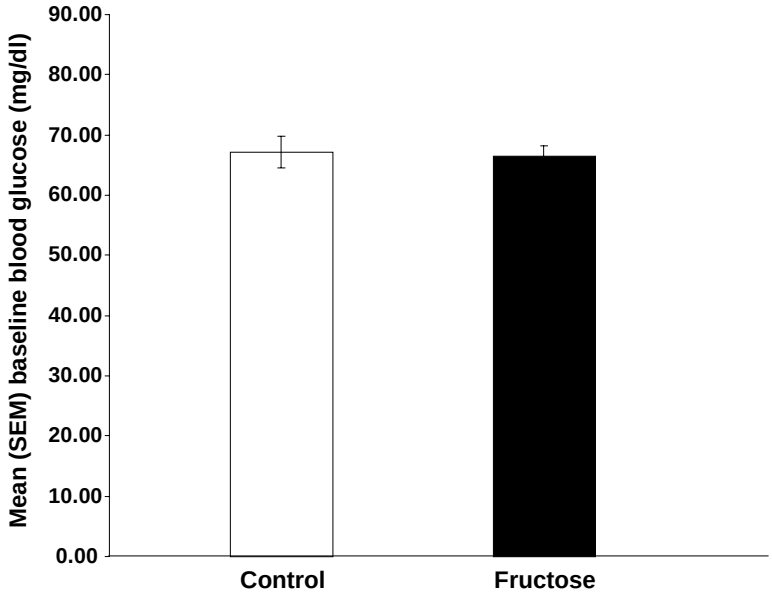

Figure 10A. The effects of eating a control or high fructose (60%) diet on mean (+/- SEM) baseline blood glucose levels during the week 11 ITT.

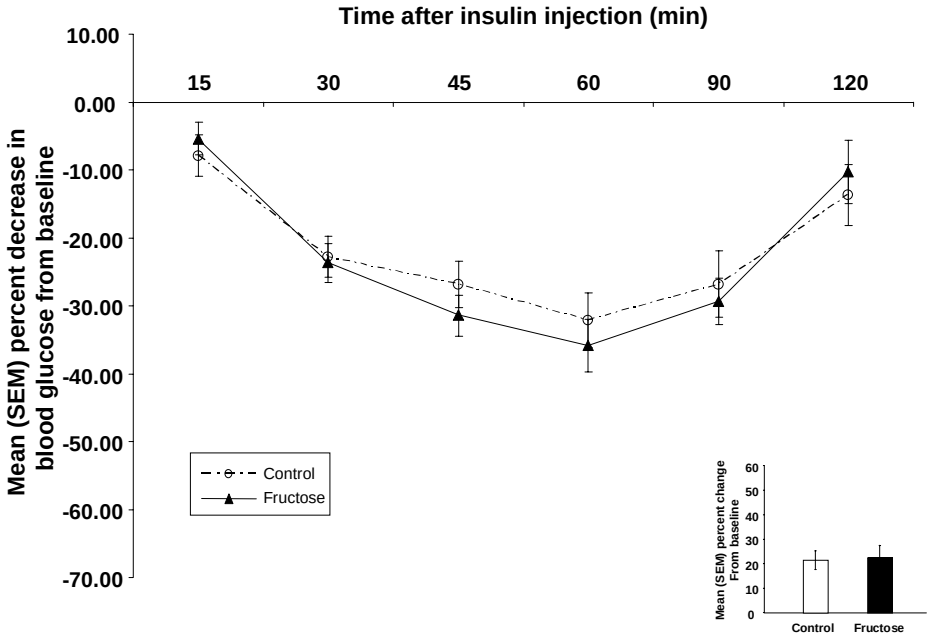

Figure 10B. The effects of eating a control or high fructose (60%) diet on the mean (+/- SEM) percent decrease in blood glucose levels from baseline over time and on the mean (+/- SEM) percent change in blood glucose levels from baseline during the week 11 ITT (inset).

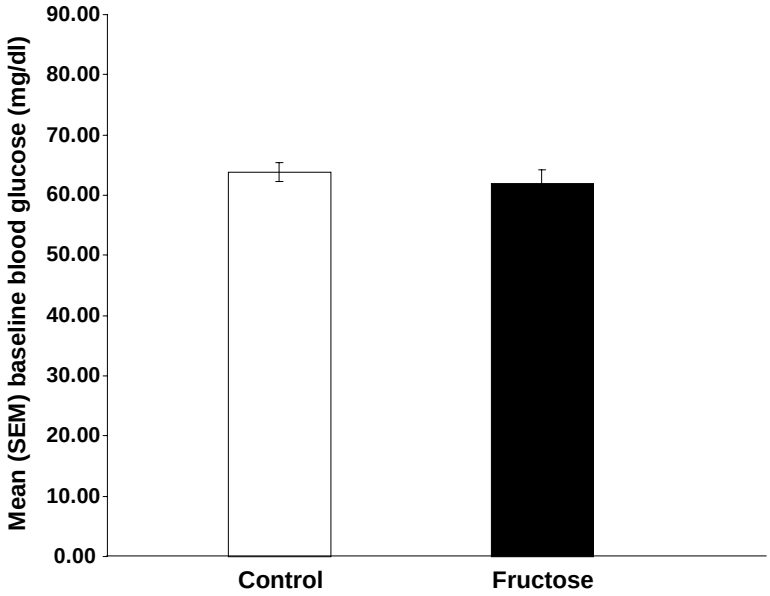

Figure 11A. The effects of eating a control or high fructose (60%) diet on mean (+/- SEM) baseline blood glucose levels during the week 16 ITT.

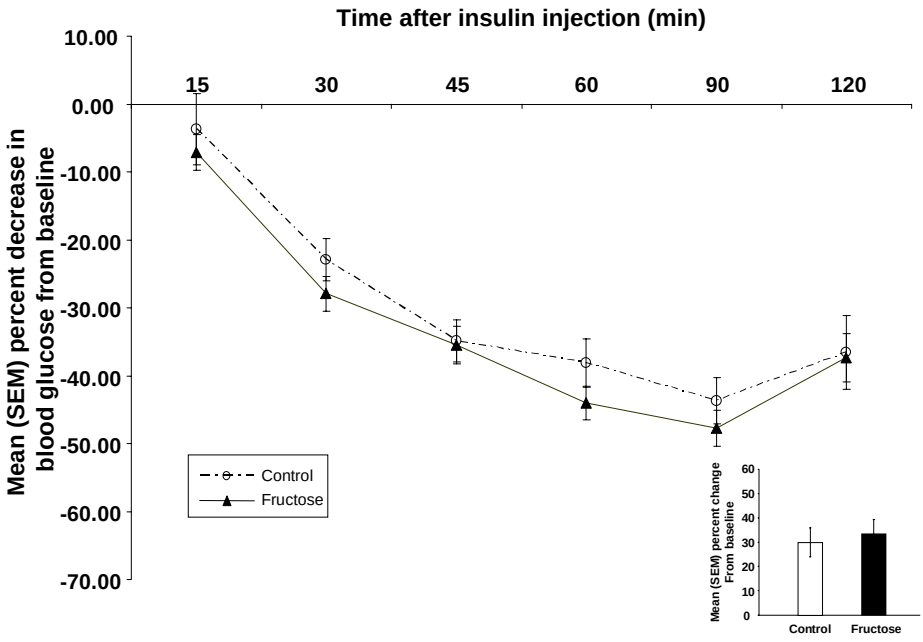

Figure 11B. The effects of eating a control or high fructose (60%) diet on the mean (+/- SEM) percent decrease in blood glucose levels from baseline over time and on the mean (+/- SEM) percent change in blood glucose levels from baseline during the week 16 ITT (inset).

differences in latencies between the two groups (see Figure 12A). During the probe test, the Fructose rats had significantly longer latencies to reach the target [$U (16, 19) = 72.00$, $p < 0.05$] and made significantly fewer target approaches than did the Control rats [$U (16, 19) = 64.00$, $p < 0.05$] and (see Figures 12B and C). In addition, the Fructose rats spent significantly less time in the target quadrant than did the Control rats [$t (33) = 2.79$, $p < 0.05$] (see Figure 12D). The diet did not appear to affect the ability of the rats to move in the maze, because swimming speed did not significantly differ between the Fructose and Control rats [$t (33) = 0.68$, $p > 0.05$] (see Figure 12E).

Post-mortem Measures

Liver Mass

The high fructose diet significantly increased liver mass [$t (39) = -4.21$, $p < 0.05$] (see Figure 13A). This effect remained significant after correcting for body mass [$t (39) = -6.14$, $p < 0.05$] (see Figure 13B).

Plasma Assays

As in Experiment 1, the high fructose diet increased circulating lipids. Compared to the Control rats, the Fructose rats had significantly higher plasma TG [$t (34) = -3.53$, $p < 0.05$] (see Figure 14). The diet also increased circulating glucose. Plasma glucose levels were also significantly higher in the Fructose rats compared to the Control rats [$t (39) = -2.29$, $p < 0.05$] (see Figure 15). The diet did not significantly affect other plasma measures. Plasma leptin [$t (38) = -0.36$, $p > 0.05$], insulin [$t (39) = 1.48$, $p > 0.05$], and FFA [$t (39) = -0.92$, $p > 0.05$] levels did not significantly differ between the Fructose and Control rats (data not shown).

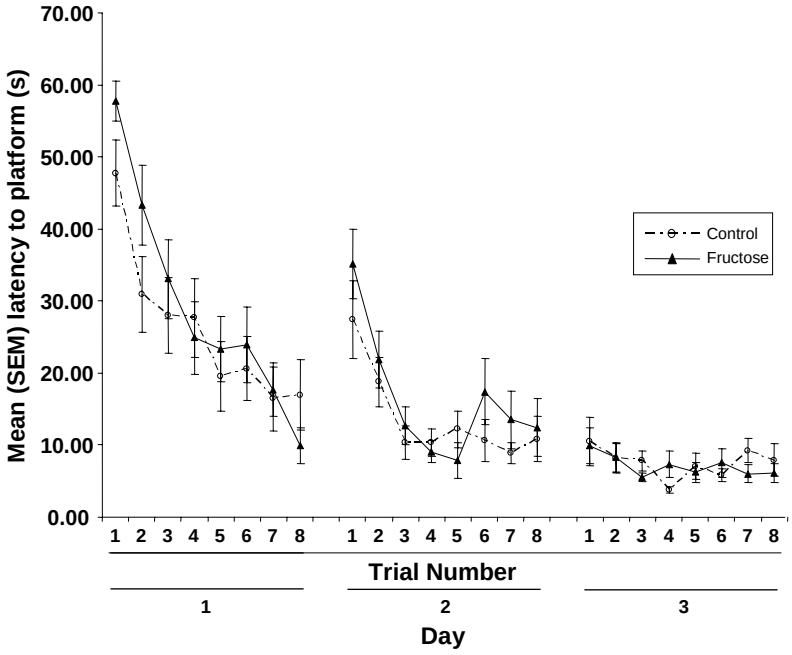

Figure 12A. The effects of eating a control or high fructose (60%) diet on the mean (+/- SEM) latency to reach the platform during MWM acquisition.

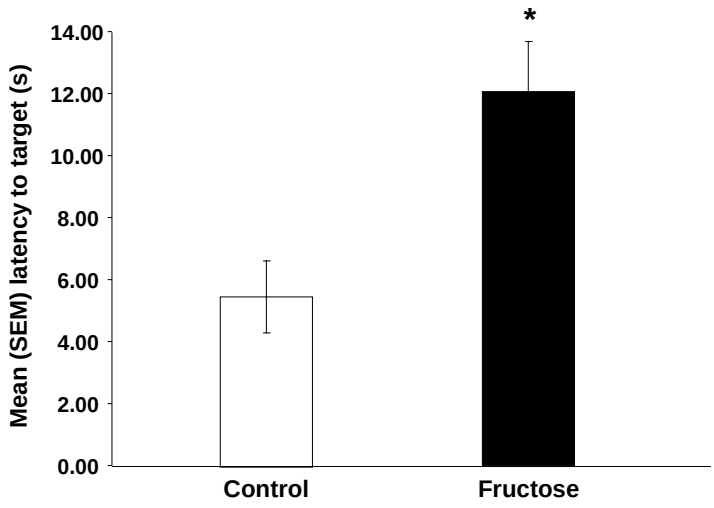

Figure 12B. The effects of eating a control or high fructose (60%) diet on the mean (+/- SEM) latency to reach the target during MWM retention (*$p < 0.05$ vs. Control).

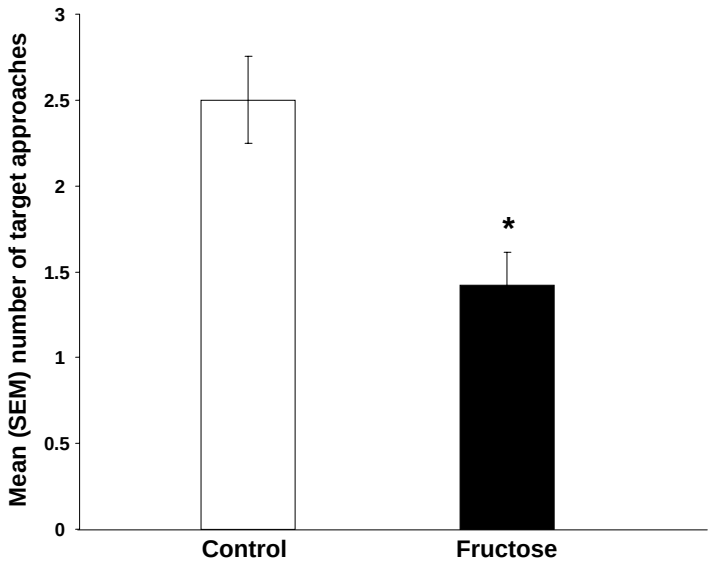

Figure 12C. The effects of eating a control or high fructose (60%) diet on the mean (+/- SEM) number of target approaches made during MWM retention (*$p < 0.05$ vs. Control rats).

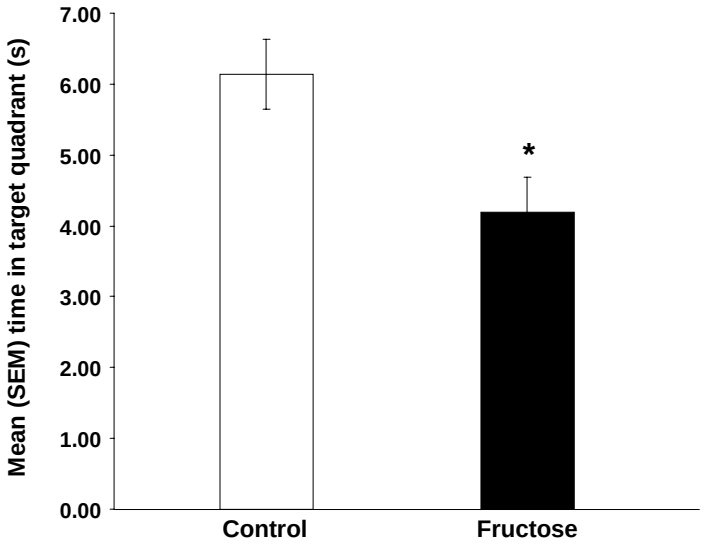

Figure 12D. The effects of eating a control or high fructose (60%) diet on the mean (+/- SEM) amount of time spent in the target quadrant during MWM retention (*$p < 0.05$ vs. Control rats).

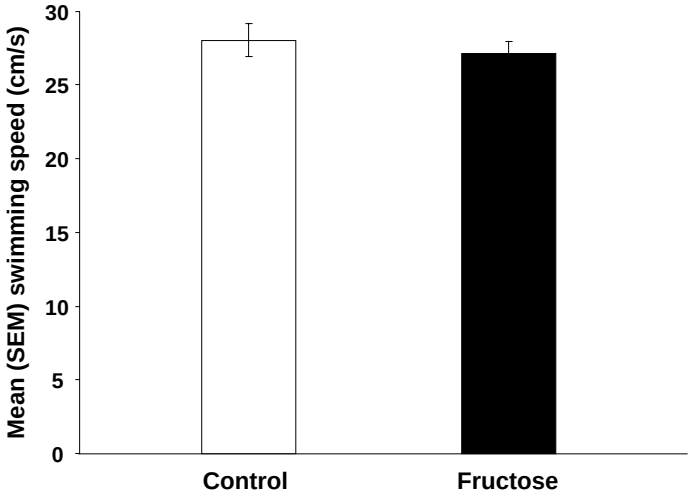

Figure 12E. The effects of eating a control or high fructose (60%) diet on the mean (+/- SEM) swimming speed of the rats during MWM retention.

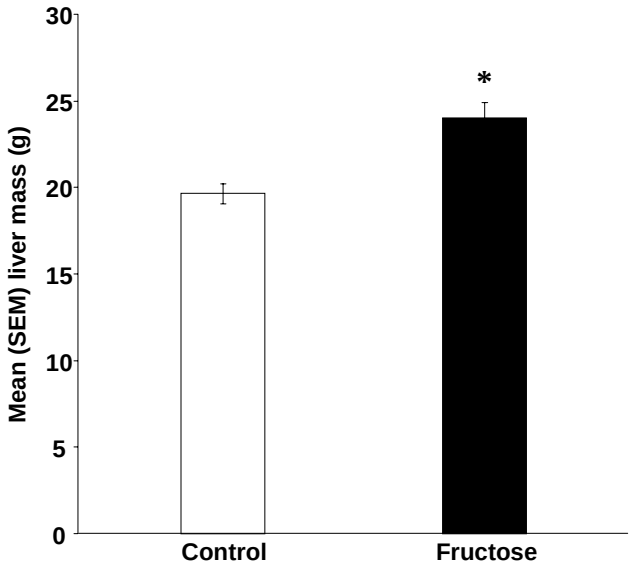

Figure 13A. Mean (+/- SEM) liver mass of rats fed a control or high fructose (60%) diet for 138 days (*p < 0.05 vs. Control rats).

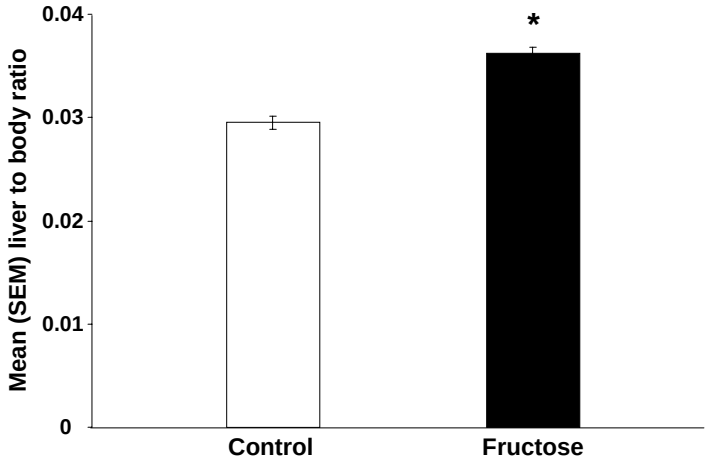

Figure 13B. Mean (+/- SEM) liver mass to body mass ratio of rats fed a control or high fructose (60%) diet for 138 days (*p < 0.05 vs. Control rats).

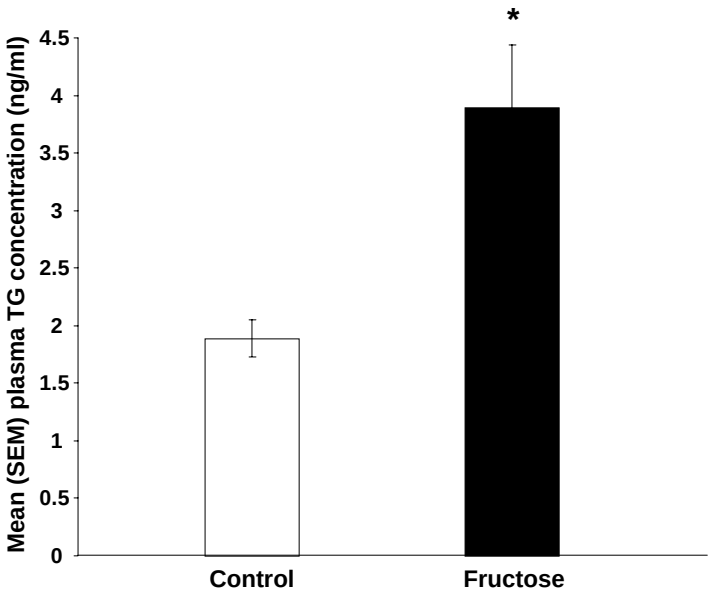

Figure 14. Mean (+/- SEM) plasma TG concentrations of rats fed a control or high fructose (60%) diet for 138 days (*$p < 0.05$ vs. Control rats).

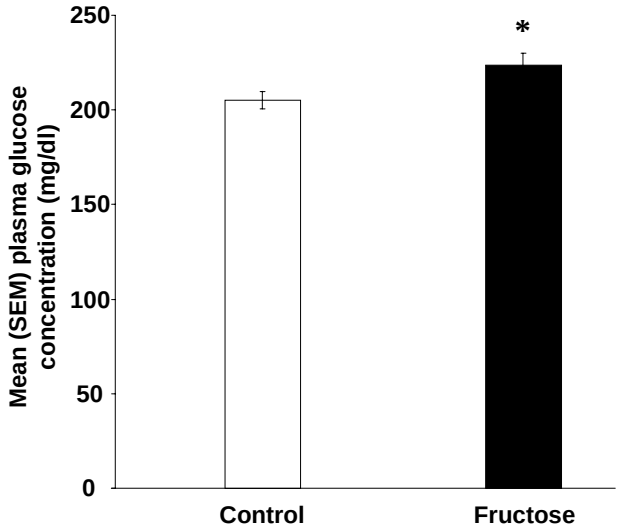

Figure 15. Mean (+/- SEM) plasma glucose concentrations of rats fed a control or high fructose (60%) diet for 138 days (*$p < 0.05$ vs. Control rats).

Correlations

Interestingly, increases in plasma TG were associated with the MWM retention deficits. Specifically, plasma TG were positively correlated with latencies to reach the target [$r(28) = 0.53$, $p < 0.05$], and negatively correlated with target approaches [$r(28) = -0.34$, $p < 0.05$] and liver mass [$r(33) = -0.44$, $p < 0.05$] (see Figures 16A and B).

Discussion

The behavioral testing results from Experiment 2 indicate that a high fructose diet impaired memory in a task that relies heavily on the hippocampus for successful performance. We were unable to detect peripheral insulin resistance as seen in Experiment 1, despite administration of the ITT at later point in the experimental timeline. We were able to replicate another physiological effect, elevated plasma TG. Plasma FFA were not increased, however, in Experiment 2.

General Discussion

The present results demonstrate that a high fructose diet does not impair acquisition, but does impair memory in a hippocampal-dependent task. Specifically, on the probe test of the MWM, the diet increased the latency for the rats to reach the target, decreased the number of target approaches, and decreased the time spent in the target quadrant. Theses results were not likely due to motor disturbances, as swimming speed was not affected by the diet. In both of the experiments, the high fructose diet elevated plasma TG levels; moreover, TG levels were correlated with retention scores in the MWM. As TG levels increased, the latency to reach the target increased and the number of target approaches decreased. These results are consistent with a recent finding that TG induce deficits in obese mice in hippocampal-dependent tasks, including the MWM, and that these impairments are most likely caused by disturbances in hippocampal

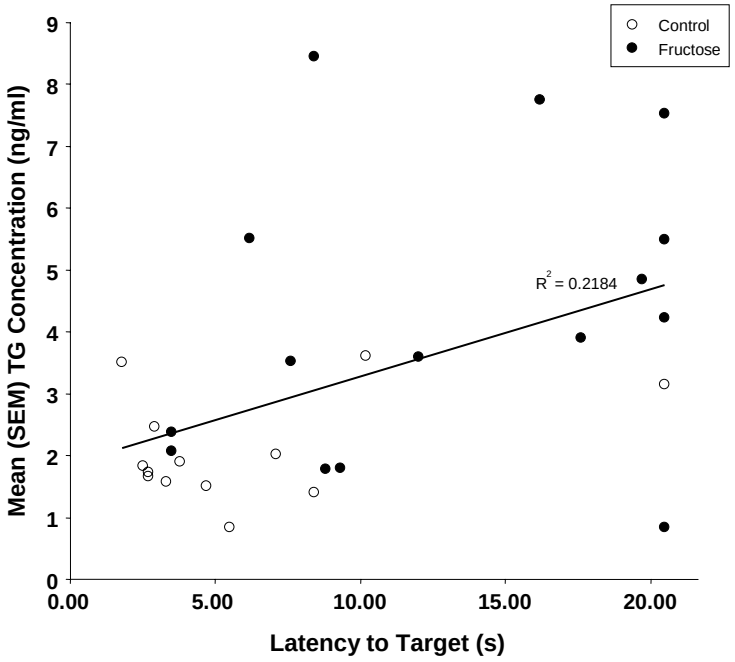

Figure 16A. The association between *post-mortem* plasma TG concentration and latency to reach the target during MWM retention.

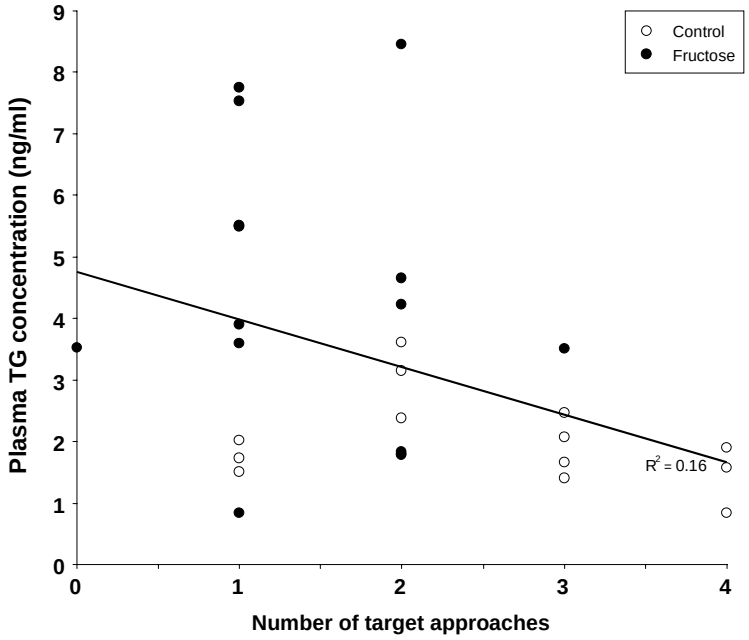

Figure 16B. The association between *post-mortem* plasma TG concentration and number of target approaches during MWM retention.

LTP (Farr, Yamada et al. 2008). The present results also are in line with with previous research showing that feeding rats high fat diets impairs performance in hippocampal-dependent memory tasks (Greenwood and Winocur 1990; Greenwood and Winocur 1996; McNay, Herzog et al. 2005). In addition, given that a high fructose diet is used as a model of type 2 diabetes, the present results are consistent with evidence from rodents and humans showing that type 2 diabetes is associated with deficits in memory mediated by the hippocampus (Gold, Dziobek et al. 2007).

The patterns of behavioral results suggest the cognitive deficits produced by the high fructose diet were restricted to memory storage and that learning and consolidation were intact. The fact that the diet did not affect acquisition in the MWM suggests that the animals were able to form spatial associations and maintain them for short periods of time. In addition, it appears that the diet did not impair consolidation, as the rats were able to recall the location of the platform across acquisition days. The high fructose diet-induced deficits appeared specific to memory storage. That is, deficits were seen in MWM after 48 hours, indicating that after a long delay, the rats were unable to recall the location of the platform. The results of CMIA are consistent with this idea; specifically, there was a tendency for the diet to impair retention tested 48 hours after the training, but there was no apparent effect of the diet on CMIA acquisition.

The behavioral results of the present experiments further suggest the fructose-induced deficits were specific to spatial reference memory, as no deficits were seen during the SA task, which is a measure of spatial working memory. The present results also support the idea that a high fructose diet impairs emotional memory, because CMIA and MWM are both tasks that release stress hormones (Mabry, Gold et al. 1995; Galvez, Mesches et al. 1996). Finally, these results also support the idea that the diet-induced impairments are restricted to long-term

memory, given that MWM and CMIA are measures of long-term memory and SA, which was unaffected by the diet, is a measure of short-term memory.

In the present experiments, the diet did not increase body mass nor did it cause shifts in fat distribution. We had hypothesized that the diet caused alterations in the distribution of fat in various fat pads, because the effects of fructose on body mass are inconsistent in animal models. Some studies have found increases in body mass and lipids (Kanarek and Orthen-Gambill 1982; Williams and Szepesi 1983; Jurgens, Haas et al. 2005), while others find fructose-induced dyslipidemia without increases in body mass (Vrana, Fabry et al. 1973; Reiser and Hallfrisch 1977; Blakely, Hallfrisch et al. 1982). Surprisingly, the results of Experiment 1 indicated that the diet did not significantly affect fat pad mass, overall body mass or lipids in muscle mass or liver. The high fructose diet did induce dyslipidemia, as the diet elevated plasma TG levels in both experiments and increased plasma FFA in Experiment 1.

The fact that the high fructose diet did increase FFA in Experiment 1 but not in Experiment 2 could explain why insulin resistance was found in Experiment 1 but not in Experiment 2. As previously mentioned, elevated levels of circulating TG and FFA can have numerous consequences. One such consequence is the initiation and perpetuation of insulin resistance. FFA stimulate the release of insulin, and insulin, in turn, perpetuates the buildup of FFA because of its antilipolytic property. It is possible that insulin resistance did not develop in Experiment 2 because FFA were not elevated. On the other hand, the rats may not have had increased FFA because they did not develop insulin resistance. In addition, it is possible that our ITT may not have identified insulin resistance but that it was indeed present. This is possible given that the diet did increase plasma glucose in Experiment 2 and the fact that plasma TG levels in Experiment 2 were increased 2-fold over those observed in Experiment 1. Other

measures of insulin resistance, particularly the euglycemic clamp, are more sensitive to changes in glucose in response to insulin; unfortunately, they are also much more invasive. Implantation of cannulae into the jugular vein and carotid artery increase the risk of death, a risk which we preferred not to take on rats that had been fed fructose for a significant amount of time.

In both experiments, more kcal of the high fructose diet were consumed per day than kcal of the control diet. Most other studies do not find increases in food intake in animals fed a fructose diet (MacRae, Nickel et al. 1974; Blakely, Hallfrisch et al. 1982; Jurgens, Haas et al. 2005; Sanchez-Lozada, Tapia et al. 2007). It is not likely that the higher consumption of fructose was due to a lack of leptin-signaled satiety, because the diet did not affect plasma insulin or leptin. This suggests that fructose-feeding did not influence the insulin-stimulated production of leptin. The diet did not likely affect the ability of leptin to effectively reach the brain, because there was not an overproduction of the hormone, as is normally found when leptin resistance is present (Ferezou-Viala, Roy et al. 2007). It is possible that the physiological effects of the diet are attributable to increased caloric intake as opposed to fructose. This is not likely because the increased intake of the high fructose diet was not enough to increase body mass or affect feed efficiency. In order to definitively rule out increased caloric intake as the cause of the fructose diet-induce effects, on future experiments we would need to yoke the amount of food consumption in fructose and control diet rats.

The present results did not reveal the mechanism(s) underlying the ability of fructose to produce memory deficits. There are not many studies that have examined the effects of a high fructose diet on the brain; however, recent research found that a diet high in fructose produces central insulin resistance (Mielke, Taghibiglou et al. 2005). Specifically, hamsters fed a diet of 60% fructose for six weeks had impaired neuronal insulin signaling in the hippocampus and

cortex, as indicated by decreased levels of phosphorylated proteins in the insulin signaling cascade. The diet also significantly reduced the ability of insulin to stimulate LTD in hippocampal slices (Mielke, Taghibiglou et al. 2005). Several lines of evidence indicate that insulin signaling in the hippocampus is important for learning and memory. For instance, administration of intravenous and intranasal insulin in humans enhances hippocampal declarative memory in a dose-dependent manner (Benedict, Hallschmid et al. 2004; Stockhorst, de Fries et al. 2004). In addition, intraventricular and intrahippocampal injections of insulin in rats improve memory in an avoidance task (Park, Seeley et al. 2000; Babri, Badie et al. 2007) and in a spatial memory task (Moosavi, Naghdi et al. 2006). Moreover, participation in hippocampal-dependent memory tasks increases activation of insulin receptors in the hippocampus (Zhao, Chen et al. 1999; Dou, Chen et al. 2005).

There are many ways through which hippocampal insulin signaling could affect memory. Hippocampal LTP requires calcium influx provided by NMDA receptors (O'Dell, Kandel et al. 1991), which are regulated by protein-tyrosine kinases (PTK) (Wang and Salter 1994). Insulin binding induces the PTK phosphorylation of proteins. P13K is another molecule involved in the insulin signaling cascade and in the recruitment of AMPA receptors to the cell surface (Man, Wang et al. 2003). Increases in AMPA receptors play a critical role in the maintenance of LTP (Lee, Takamiya et al. 2003). Disruptions of the insulin signaling cascade also could produce impairments in learning and memory by reducing LTD, another mechanism of synaptic plasticity. As noted, a high fructose diet significantly reduces the ability of insulin to stimulate LTD in hamsters (Mielke, Taghibiglou et al. 2005). Fructose-induced insulin resistance could also impair memory by causing hippocampal cell death. Normally, P13K also stimulates phosphor-kinase B (PKB), which subsequently inactivates proteins essential for programmed cell

death (Ryu, Ko et al. 1999; Yamaguchi, Tamatani et al. 2001; van der Heide, Ramakers et al. 2006).

The TG produced by fructose metabolism could be the mechanism through which fructose produces hippocampal insulin resistance. The present results showed that the memory-impairing effects of a high fructose diet were correlated with increases in TG. TG administration impairs memory in a t-maze avoidance task, most likely by reducing hippocampal LTP (Farr, Yamada et al. 2008). Application of TG to liver cells decreases the ability of insulin to activate the signaling cascade in the periphery (Kim, Jeong et al. 2007). In addition, a high fat diet induces hippocampal insulin resistance (McNay, Herzog et al. 2005) and impairs memory (Greenwood and Winocur 1990; Greenwood and Winocur 1996; McNay, Herzog et al. 2005; Farr, Yamada et al. 2008).

Future research goals may include determining whether different diet durations or fructose concentrations alter the fructose-induced impairments on hippocampal-dependent memory. Such experiments will indicate when exactly the diet affects cognition and how much fructose can be consumed before memory impairments are observed. At a concentration of 66%, fructose can induce elevations in plasma TG in as soon as 3 days and increase plasma FFA in 4 days (Zavaroni, Chen et al. 1982). A 60% fructose diet causes peripheral insulin resistance after 4 weeks (Bezerra, Ueno et al. 2000), and central insulin resistance is seen after 6 weeks (Mielke, Taghibiglou et al. 2005). In addition, the results may provide evidence concerning the permanence of these diet-induced memory deficits. For example, if fructose is consumed for a given amount of time and then removed from the diet, are the cognitive deficits reversed? Another question that can be addressed is whether pharmacological treatments can prevent or reverse the cognitive effects of the high fructose diet. Anti-diabetic drugs such as metformin can

reverse fructose-induced insulin resistance and hypertension (Verma, Yao et al. 2000) and promote lipid metabolism (Anurag and Anuradha 2002). These drugs may be able to reverse the memory impairments by reversing the peripheral effects of a high fructose diet.

In conclusion, the results of the present experiments show that a high fructose diet causes hippocampal-dependent memory impairments. In addition, these impairments are associated with diet-induced increases in plasma TG. These results are consistent with previous studies demonstrating that diet can influence cognition.

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