CONSUMPTION OF DIFFERENT CALORIC SUGAR-SWEETENED SOLUTIONS ALTERS TISSUE FATTY ACID COMPOSITION AND LIPID METABOLISM IN YOUNG FEMALE SPRAGUE-DAWLEY RATS

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Abstract

High carbohydrate intake has been reported to contribute to obesity by promoting *de novo* lipogenesis and metabolic disorders by altering tissue fatty acid composition. The type of caloric sweeteners added to beverages has changed with some sugars suggested to be more lipogenic. Therefore, the study objective was to determine the effect of consuming different types of caloric sugar-sweetened solutions on tissue fatty acid composition and risk of metabolic disorders. Growing female Sprague-Dawley rats were randomly assigned (n=7-8 rats/group) to drink water or water sweetened with 13% (w/v) glucose, sucrose, fructose or high fructose corn syrup 55 (HFCS-55). The rats were provided their assigned sugar-sweetened solution and purified diet *ad libitum*. After 8 weeks, fat pads and liver were dissected and tissue fatty acid composition was determined by gas chromatography. Serum cholesterol, triglyceride (TG), lipoprotein profile, and liver function were determined by enzymatic colorimetric assays. The type of sugar appeared to have different effects on fatty acid composition and dyslipidemia. Rats drinking HFCS-55 solution had greater *de novo* lipogenesis indicated by higher (P=0.003) palmitoleic acid and lower (P=0.03) linoleic acid in the liver compared to rats drinking water. In the adipose tissue, linoleic acid and alpha-linolenic acid was lower (P<0.05) in rats drinking HFCS-55 solution compared to rats drinking water. Dyslipidemia in rats drinking HFCS-55 solution was indicated by increased hepatic lipid content, serum TG, and low density lipoprotein. This has important health implications since HFCS-55 has become the major caloric sweetener added to beverages.

Introduction

Consumption of caloric sweeteners has increased with the primary source of added sugars in the American diet being sugar-sweetened beverages (Block, 2004). The type of caloric sweeteners consumed has also changed. In the United States, high fructose corn syrup (HFCS) has replaced sucrose as the sugar added to beverages (Bray et al., 2004). Consumption of HFCS-55 has been suggested to lead to a higher fructose intake because HFCS-55 is comprised of 55% fructose and 42%

glucose as monosaccharides (White, 2008); whereas, sucrose is comprised of 50% fructose and glucose as a dissaccharide. According to the third National Health Examination Survey (NHANES) 1999–2004, fructose consumption has increased steadily with the highest consumers being young adults and the main source of fructose being soft drinks (Marriott et al., 2009). According to Popkin and Neilsen (2003), added sweeteners in soft drinks make a significant contribution to the human diet providing 318 kcals or 16% of total caloric intake.

The increase in fructose intake is a health concern because animal and humans studies have reported fructose to be a more potent inducer of hepatic *de novo* lipogenesis than glucose (Chong et al., 2007; Parks et al., 2008; Koo et al., 2008). Hepatic metabolism of fructose involves entry of fructose into glycolysis via fructose-1-phosphate which bypasses the rate controlling step of glycolysis catalyzed by phosphofructokinase. This allows fructose to provide an unregulated source of substrates for triglyceride (TG) synthesis (Havel, 2005). Fructose independent of insulin stimulates sterol receptor element binding protein 1c (SREBP 1c) which activates genes involved in *de novo* lipogenesis (Miyazaki et al., 2004). Since fructose does not stimulate insulin secretion and insulin signals the central nervous system to inhibit food intake (Elliot et al., 2002), fructose has also been suggested to contribute to weight gain by stimulating food consumption. In the present study, animals were provided free access to the liquid and solid diet to determine food regulation.

According to Schwarz et al. (1995), a high carbohydrate diet results in greater de novo lipogenesis than a high fat diet. TG synthesized from carbohydrates consists mainly of palmitic acid (16:0), palmitoleic (16:1n-7), and oleic acid (18:1n-9) (Segall et al., 1970). Hudgins (2000) reported that lean and obese adult subjects provided a very low fat (10%) and high-carbohydrate diet (75%) enriched with glucose, fructose or sucrose increased very low density lipoproteins (VLDLs) and palmitic acid (16:0) and decreased linoleic acid (18:2n-6). In turn, endogenously synthesized TGs transported in VLDLs to adipose tissue may contribute to weight gain and subsequent obesity.

Obesity is often accompanied by a cluster of metabolic abnormalities, which is collectively referred to as the metabolic syndrome. Metabolic syndrome is characterized by visceral obesity, hypertriglyceridemia, reduced high-density lipoprotein (HDL-C), raised fasting glucose levels, and elevated blood pressure (Potenza and Mechanick, 2009). In addition, metabolic syndrome is often accompanied by non-alcoholic fatty liver disease (NAFLD) (Alberti et al., 2009). NAFLD characterized by excessive lipid accumulation has been observed in rats consuming high fructose diets (Davail et al., 2005).

In a human study, subjects consuming beverages sweetened with fructose had increased *de novo* lipogenesis, visceral adiposity, dyslipidemia, and decreased insulin sensitivity (Stanhope et al., 2009). High carbohydrate intake has been reported to contribute to obesity by promoting *de novo* lipogenesis and metabolic disorders by altering tissue fatty acid composition. Iggman et al. (2010) reported that adipose tissue enriched with palmitic acid and depleted of essential fatty acids was associated with insulin resistance. In rats, long-term feeding of moderate amounts of fructose (15% of the diet by weight) impaired glucose tolerance while feeding high amounts of fructose (72% of the diet by weight) resulted in the development of diabetes mellitus (Blakely et al., 1981). Many animal studies reporting

adverse health effects have used doses of sweeteners that are not typically encountered in the human diet. Therefore, the objective of this study was to determine the effect of different caloric sugar-sweetened solutions provided at a dose typically added to soft drinks on tissue fatty acids composition and risk of metabolic abnormalities.

Materials and Methods

Animal and Diets

All animal procedures were approved by the Animal Care and Use Committee at West Virginia University and conducted in accordance with the guidelines set forth by the National Research Council for the Care and Use of Laboratory Animals (1996). Immature (age 28 days) female Sprague-Dawley rats (n=36) were purchased from Taconic Farms (Rockville, MD). The rats were individually housed in metabolic cages to measure liquid and food intake. Rats were kept in a room at a constant temperature of 21ºC with a 12 h light/dark cycle. During a 7 d acclimation period, animals were given *ad libitum* access to distilled water and purified AIN-93G diet (Harlan Teklad; Indianapolis, IN).

Following acclimation, animals were randomly assigned to one of five treatments (n=7-8 rats/group). Treatment groups consisted of either distilled water with no caloric sweetener added or distilled water sweetened with glucose, sucrose, fructose or HFCS-55. The different caloric sweeteners were added at the level of 13% (w/v) which is the dose reported in soft drinks (Jurgens et al, 2005). The rats were given *ad libitum* access to their assigned liquid solution and to purified AIN-93G diet throughout the 8 week study to assess food selection and regulation. The purified AIN-93G diet was used because it meets the nutritional requirements for growing rats as defined by the Nutritional Research Council (1995) and has a defined sucrose content and lipid source. The diet ingredients and fatty acid composition of the AIN-93G is shown in Table 1. Water and caloric sugar-sweetened solutions were measured and replaced daily. Food intake and body weights were measured weekly.

Organ Fatty Acid Composition

At the end of the 8 week feeding study, rats were fasted overnight then euthanized by CO2 inhalation. The liver and the major fat pads (gonadal and retroperitoneal) were excised, weighed, and immediately frozen in liquid nitrogen. Tissues were kept stored at -80oC until analyzed. Lipids were extracted from tissue samples according to the method by Bligh and Dyer (1959) and all samples were measured in duplicates. Briefly, samples were weighed and nonadecenoic (19:1) added as an internal standard, and samples were homogenized in a Tris/EDTA buffer (pH 7.4) and a chloroform:methanol:acetic acid (2:1:0.15 v/v/v) solution was added to the samples. Samples were centrifuged at 900 g for 10 min at 10oC and the bottom chloroform layer was then filtered through 1-phase separation filters. The extracted lipid was dried under nitrogen gas. Liver lipid content was calculated as lipid (g)/tissue wt (g) x 100.

Extracted lipid samples were transmethylated according to the method by Fritsche and Johnston (1990). Briefly, fatty acid methyl esters were generated by adding 4% H2SO4 in methanol to the dried lipid samples and incubated at 90oC for 60 min. Samples were cooled to room temperature and 3 mL of distilled water was added. Chloroform was added to the methylated sample, centrifuged at 900 g for 10 min at 10oC, and the bottom layer collected. Methylated samples were dried under nitrogen gas and resuspended in iso-octane to a concentration of 5 mg methylated lipid sample/mL of iso-octane.

The methylated lipid samples were analyzed by gas chromatography (CP-3800, Varian Inc, Walnut Creek, CA) using an initial temperature of 140oC held for 5 min and then increased 1oC per min to a final temperature of 220oC. A wall-coated open tubular fused silica capillary column (Varian Inc, Walnut Creek, CA) was used to separate fatty acid methyl esters with CP-Sil 88 as the stationary phase. Nitrogen was used as the carrier gas, and total separation time was 56 min. Quantitative 37 component fatty acid methyl esters Sigma Mix (Supelco, Bellefonte, PA) was used to identify and quantify fatty acids. Fatty acids were identified using retention time and peak area counts.

Serum Clinical Measurements

Trunk blood was collected and centrifuged at 1,500 g for 10 min at 4ºC and the serum collected and kept frozen at -80ºC until analyzed. Fasting serum cholesterol, TG, VLDL, low density lipoprotein (LDL-C), and HDL-C were determined by lipid test rotor enzymatic colorimetric assays and measured using a Hemagen Analyst automated spectrophotometer (Hemagen Diagnostics Inc., Columbia, MD). Serum lipid and lipoprotein values reported in this study were within the range reported for rats (DeMoura et al., 2008).

Liver function was assessed by measuring serum alanine transferase (ALT), Gamma-glutamyl transferase (GGT), bilirubin, and albumin. Serum liver function biomarkers were determined by Vet 16 veterinary test rotor enzymatic colorimetric assays measured using a Hemagen Analyst automated spectrophotometer. Serum liver function values reported in this study were within the range reported for rats.

Statistical Analysis

One-way ANOVA was used to determine differences among the treatment groups. Post-hoc multiple comparison tests were performed using Tukey's test with treatment differences considered significant at $P<0.05$. All statistical analyses were performed using the SigmaStat 3.1 statistical software program (Systat Software Inc, San Jose, CA).

Results

Animal Caloric and Macronutrient Intake

Rats provided glucose solution had the highest liquid intake among the treatment groups. Rats drinking sucrose solution had significantly higher liquid intake than rats drinking fructose solution. Food intake was lower (P=0.009) in rats drinking glucose, sucrose or HFCS-55 solution compared to rats drinking water. Additionally, food intake was significantly reduced in rats drinking glucose solution compared to rats drinking HFCS-55 or fructose solution. Food intake was also reduced in rats drinking sucrose compared to fructose solution. Differences in food and liquid consumption resulted in differences in total caloric intake. Rats drinking glucose solution had greater (P=0.03) total caloric intake compared to rats drinking HFCS-55 solution or water (Table 2).

Total sugar intake was highest in rats drinking glucose solution. Rats provided sucrose solution had significantly greater total sugar intake compared to rats provided fructose solution. Total lipid intake was lower (P=0.009) in rats drinking glucose, sucrose or HFCS-55 solution compared to rats drinking water. Additionally, total lipid intake was significantly lower in rats provided glucose compared to rats drinking HFCS-55 or fructose solution. Lipid intake was also significantly lower in rats drinking sucrose than rats drinking fructose solution (Table 2).

Animal Body and Organ Weights

There were no significant differences in final body weights, retroperitoneal or gonadal fat pad weights among the treatment groups. Absolute liver weight was higher (P=0.03) in rats drinking HFCS-55 solution compared to rats drinking water, but not when expressed as relative liver weight (Table 3).

Liver Fatty Acid Composition

The saturated fatty acid, myristic acid (14:0) was higher (P=0.02) in rats drinking HFCS-55 compared to rats drinking glucose solution. Hepatic palmitoleic acid (16:1n-7) was highest in rats drinking glucose solution. Rats drinking HFCS-55 solution had significantly greater hepatic palmitoleic acid compared to rats drinking water. Oleic acid (18:1n-9) was higher (P=0.02) in rats provided glucose solution compared to rats drinking fructose, sucrose solution or water. Additionally, hepatic oleic acid was significantly higher in rats drinking HFCS-55 solution compared to rats drinking fructose solution or water. Hepatic linoleic acid (18:2n-6) was lower (P=0.03) in rats drinking glucose or sucrose solution compared to rats drinking water. Only rats drinking glucose had decreased hepatic alpha-linolenic acid compared to rats drinking water (Table 4).

Adipose Tissue Fatty Acid Composition

In the gonadal fat pad, palmitoleic acid was higher (P=0.03) in rats drinking glucose solution compared to rats drinking water. Rats drinking glucose solution had the lowest linoleic acid among the treatment groups. Rats drinking HFCS-55 solution had significantly lower linoleic acid compared to rats drinking water. Alpha-linolenic acid and arachidonic acid (20:4n-6) were lowest in rats drinking glucose solution (Table 5).

In the retroperitoneal fat pad, palmitic acid (16:0) was higher (P=0.003) in rats provided glucose solution compared to rats drinking water, fructose or HFCS-55 solution. Fat pad palmitoleic acid was higher (P=0.01) in rats provided glucose solution compared to rats drinking fructose solution or water. Also, rats drinking sucrose had significantly higher palmitoleic acid compared to rats drinking water. Oleic acid was higher (P=0.008) in rats provided glucose solution compared to rats drinking solutions of fructose, HFCS-55 or water (Table 6). In the retroperitoneal fat pad, linoleic, alpha-linolenic, and arachidonic acid were significantly reduced in rats provided glucose or HFCS-55 solution compared to rats drinking water. Additionally, arachidonic acid was lower (P=0.03) in rats drinking glucose solution compared to rats drinking fructose solution (Table 6).

Serum Clinical Measurement

Liver lipid content was greater in rats drinking HFCS-55 solution (P=0.005) compared to rats drinking fructose solution. There were no significant differences in fasting serum glucose or insulin concentrations among the treatment groups. Rats drinking HFCS-55 solution had significantly higher serum TG concentration compared to rats drinking water. There was no significant difference in serum VLDL; however, rats drinking HFCS-55 solution had higher (P=0.02) serum LDL-C concentrations compared to rats drinking water. There were no significant differences in serum HDL-C or total cholesterol among the treatment groups. There were not differences in liver function indicated by no significant differences in serum ALT, GGT, bilirubin, and albumin concentration among rats drinking water or the different caloric sugar-sweetened solutions (Table 7).

Discussion

The results showed differences in tissue fatty acid composition and lipid metabolism among female rats drinking different caloric sugar-sweetened solutions. According to Segall et al. (1970), the most consistent effect of high carbohydrate induced de novo lipogenesis is an increase in palmitoleic acid and decrease in linoleic acid tissue content. In our study, rats provided HFCS-55, but not sucrose solution had increased hepatic *de novo* lipogenesis indicated by significantly higher liver palmitoleic acid compared to rats drinking water. In contrast, Fukuchi et al. (2004) reported that male Wistar King A rats provided a high (34%) sucrose liquid diet for 4 weeks increased hepatic palmitioleic acid compared to rats provided water. However, rats provided sucrose had greater sugar and total caloric

intake. In our study there were no significant differences in total calories, sugar or lipid intake between rats drinking HFCS-55 and sucrose solution. Based on our study results, HFCS-55 solution which provided slightly higher (55%) fructose as a monosaccharide appeared to be more lipogenic than fructose (50%) provided by sucrose as a disaccharide. Fructose and glucose consumed as monosaccharides rather than disaccharide may result in greater absorbability and the higher dietary fructose intake promotes hepatic *de novo* lipogenesis. In the liver, metabolism of fructose bypasses the major control point in glycolysis allowing fructose to serve as an unregulated source of precursors for *de novo* lipogenesis (Havel, 2005). Interestingly, in our study, rats provided HFCS-55, but not pure fructose solution had increased hepatic *de novo* lipogenesis. In a human study, healthy subjects administered equivalent amounts of pure fructose, glucose or HFCS-sweetened beverages resulted in similar increases in postprandial TGs (Stanhope et al., 2008). The results suggested that co-ingestion of glucose potentiates the lipogenic effect of fructose since HFCS contains approximately half the amount of pure glucose or fructose, yet produced similar lipid responses (Tappy and Le, 2009).

Another mechanism whereby dietary fructose has been suggested to contribute to weight gain is by stimulating energy intake (Elliot et al., 2002). Sugar consumption in liquid form has been reported to promote positive energy balance; whereas, comparable sugar in solid form resulted in dietary compensation (Mattes, 2006; DiMeglio and Mattes, 2000). In our study, rats provided caloric sweetened solutions *ad libitum* resulted in rats provided glucose solution drinking the most liquid and adjusted for increased calories obtained from liquid by reducing caloric intake from the solid diet. Lower (P=0.009) dietary lipid intake in rats drinking glucose solution resulted in significantly reduced hepatic essential fatty acid compared to rats drinking water. On the other hand, rats drinking glucose solution had significantly higher *de novo* lipogenesis indicated by significantly higher hepatic MUFAs, palmitoleic acid and oleic than rats drinking fructose solution. In contrast, studies in rats, mice, and humans have reported fructose consumption to be a more potent inducer of hepatic lipogenesis than glucose (Parks et al., 2008; Chong et al., 2007; Koo et al., 2008). In our study, higher hepatic MUFAs may be due to rat drinking glucose solution having the highest total sugar consumption due to their higher liquid intake. Our results demonstrated that sugar quantity consumed can over-ride the greater lipogenic effects of fructose.

Altered fatty acid composition in the liver may lead to fatty acid imbalances in the adipose tissues since adipose stores excess TGs. In the gonadal fat pad, essential fatty acids (linoleic acid and alpha-linolenic acid) were lowest in rats drinking glucose. Rats drinking glucose solution had the lowest lipid intake among the treatment groups. Additionally, arachidonic acid was lowest in rats drinking glucose solution. Arachidonic acid (20:4n-6) was not detectable in the purified AIN-93G diet and therefore, must be derived from the precursor linoleic acid (18:2n-6). The adipose tissue typically reflects dietary intake although the adipose tissue can perform de novo lipogenesis in response to changes in dietary intake. Rats drinking glucose solution had the highest sugar intake among the treatment groups and palmitoleic acid was higher (P=0.03) in rats provided glucose solution compared to rats drinking water.

Fatty acid composition in the gonadal fat pad differed from the retroperitoneal fat pad. In the retroperitoneal fat pad, palmitoleic acid was higher (P=0.01)

in rats drinking glucose or sucrose solution compared to rats drinking water. Additionally, rats drinking glucose showed significantly higher palmitic and oleic acid compared to rats drinking water, fructose or HFCS-55 solution. TG synthesized from carbohydrates consists mainly of palmitic acid, palmitoleic, and oleic acid (Segall et al., 1970). Similar to the gonadal fat pad, essential fatty acids and arachidonic acid were significantly lower in the retroperitoneal fat pad of rats drinking glucose solution compared to rats drinking water. Lipid intake was also reduced (P=0.009) in rats drinking sucrose or HFCS-55 solution compared to rats drinking water. However, decreased linoleic acid and alpha-linolenic acid occurred in the fat pads of rats drinking HFCS-55, but not sucrose solution. This may be due to fatty acid accumulation in the liver and decreased transport to the adipose tissue. Fatty acid accumulation in the liver was indicated by reduced hepatic linoleic acid in rats drinking sucrose or glucose solution, but not HFCS-55 solution. Additionally, liver lipid content was higher (P=0.005) in rats drinking HFCS-55 solution.

In humans, fat distribution (visceral fat) is an accepted risk factor for metabolic syndrome. However, the effect of different fat pads on metabolism in rats is uncertain. According to Fabbrini et al. (2009), the degree of intra-hepatic lipid is a better predictor of metabolic abnormalities than adipose tissue. Excessive *de novo* lipogenesis results in intra-hepatic lipid accumulation that may lead to non-alcoholic fatty liver disease (NAFLD) (Lim et al., 2010). Patients with NAFLD are more likely to have dyslipidemia (Le and Bortolotti, 2009). The earliest metabolic perturbation of increased *de novo* lipogenesis is postprandial hypertriglyceridemia (Teff et al., 2009). In our study, serum TG concentration was higher (P=0.02) in rats drinking HFCS-55 solution compared to rats drinking water. TG synthesized by the liver may be transported into the circulation as TG-rich VLDL. In turn, VLDL can be converted to LDL-C. In our study, female rats drinking HFCS-55 solution had no effect on fasting serum VLDL, but increased (P=0.02) serum LDL-C compared to rats drinking water.

Key substrates for TG synthesis are MUFAs (Flowers and Ntambi, 2010). Hepatic MUFAs, palmitoleic acid and oleic acid were significantly increased in rats drinking HFCS-55, but not sucrose solution compared to rats drinking water. In humans, increased plasma palmitoleic acid has been independently associated with hypertriglyceridemia (Paillard et al., 2007). In our study, rats drinking glucose solution had increased hepatic MUFAs, palmitoleic acid and oleic acid, but no effect on serum TGs or LDL-C. Unlike HFCS-55, hepatic linoleic acid was significantly lower in rats provided glucose or sucrose compared to rats drinking water. *De novo* lipogenesis that exceeds the oxidative capacity or needs of the liver results in excessive formation of malonyl-CoA which in turn, inhibits β-oxidation (Lim et al., 2009). We speculated that the combination of *de novo* lipogenesis and impaired β-oxidation resulted in the intra-hepatic lipid accumulation and dyslipidemia observed in rats drinking HFCS-55, but not glucose or sucrose solution. However, mechanistic studies are required to specifically address this observation. Also, of interest was the observation of altered liver total lipid content, fatty acid composition, and dyslipidemia in rats drinking HFCS-55 solution in the absence of adiposity. In a human study, sugar-sweetened beverage consumption was associated with intra-hepatic lipid accumulation independent of the degree of obesity (Assay et al., 2008).

Lipid accumulation in the liver has also been suggested to promote damage due to the oxidative susceptibility of fatty acids (Lim et al., 2010). In a human

study, healthy subjects administered a diet with 25% total energy as sucrose resulted in a rise in hepatic ALT and GGT concentrations (Porikos and Van Itallie, 1983). In our study, there was no effect on liver function indicated by no significant increase in serum ALT, GGT, bilirubin or albumin among rats drinking different caloric sugar-sweetened solutions.

Insulin resistance is another disorder characterizing the metabolic syndrome. Iggman et al. (2010) reported that adipose tissue enriched with palmitic acid and depleted of essential fatty acids was associated with insulin resistance. In our animal study, rats drinking glucose solution had significantly higher palmitic acid accompanied by depletion of essential fatty acids in the fat pads. However, there was no difference in fasting serum insulin or glucose among any of the treatment groups. In contrast, Fukuchi et al. (2004) reported increased plasma glucose, insulin, and adiposity after 20 weeks of sucrose loading of male rats. Our study duration was eight weeks and there were no significant increase in adiposity among the treatment groups. Also, several studies have suggested female rats are protected against fructose and sucrose induced changes in metabolism (Galipeau et al., 2002; Horton et al., 1997). However, a recent study reported that Sprague-Dawley female rats provided 10% w/v fructose solution for 14 d had higher incidence of glucose intolerance than their male counterparts. The authors suggested that this was due to the stronger induction of fructokinase in the liver of fructose-fed female rats than male rats (Vila et al., 2010).

In summary, among the different types of caloric sweeteners investigated, HFCS-55 consumption increased hepatic MUFAs and caused dyslipidemia. Although both HFCS-55 and sucrose provide glucose and fructose, only HFCS-55 increased hepatic palmitoleic acid. There were no significant differences in total calories, sugar or lipid intake between rats drinking HFCS-55 and sucrose, except for a slightly higher amount of fructose as monosaccharides. Based on the study, the type of sugar does appear to have an important effect on liver fatty acid composition and lipid metabolism. The relevance of the animal study findings to humans is important to investigate due to continuing controversy regarding the health implications of replacing sucrose with HFCS-55 in beverages and the role of caloric sweeteners in obesity and metabolic syndrome.

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Tables

Table 1. Diet nutrient and fatty acid composition.

Ingredients	**g/kg diet**
Corn Starch	397.5
Dextrinized cornstarch	132.0
Sucrose	100.0
Fiber	50.0
Casein	200.0
L-Cystine	3.0
Vitamin Mix**	10.0
Mineral Mix**	35.0
Choline bitartrate	2.5
Tert-butylhydroquinone	0.014
Soybean oil	70.0
Fatty acids	**mg/g diet**
Saturated fatty acids	
Myristic acid, 14:0	ND
Palmitic acid, 16:0	32.7 ± 0.8
Stearic acid, 18:0	7.3 ± 1.1
Monounsaturated fatty acids	
Palmitoleic acid, 16:1n-7	ND
Oleic acid, 18:1n-9	211.1 ±3.5
Polyunsaturated fatty acids	
Linoleic acid, 18:2n-6	234.3 ± 3.5
Alpha-linolenic acid, 18:3n-3	28.5 ± 0.4
Arachidonic acid, 20:4n-6	ND
Eicosapentaenoic acid, 20:5n-3	0
Docosahexaenoic acid, 22:6n-3	0
n-6/n-3	8.2 ± 0.2

*Results are expressed as Mean ± SEM of n=3 samples

**Based on the AIN-93G vitamin and mineral mixes (Reeves, 1997)

Abbreviation is ND, not detected.

Table 2. The effect of feeding different caloric sugar-sweetened so
growing rats.

Measurement	Water	Cl
Total liquid intake (mL)	1927±205[bc]	492
Total food intake (g)	982+30[a]	61
Total caloric intake (kcal)	3731±113[b]	467
Total sugar intake (g)	349+27[bc]	70
Total lipid intake (g)	687 ±21[a]	42

* Values are expressed as the mean ± SEM of n=7-8 rats/group. D
the same rows indicate significant differences at $P<0.05$ by one-w
Abbreviation is HFCS-55, high fructose corn syrup 55.

olution on caloric and macronutrient intake by

	Treatments*			
ucose	Fructose	Sucrose	HFCS-55	*P*-value
8±440[a]	1553±254[c]	3144±322[b]	2762±306[bc]	0.01
3+35[d]	899+19[ab]	698+39[od]	784+44[bc]	0.009
4±165[a]	4223±132[ab]	4184±129[ab]	4086±182[b]	0.03
2+55[a]	292+33[c]	479+38[b]	437+39[bc]	0.02
9 ±24[d]	629 ±16[ab]	488 ±27[od]	549 ±31[bc]	0.009

ifferent superscript letters a, b, c, d within
ay ANOVA followed by Tukey's test.

Table 3. Consumption of different caloric-sweetened solutions by

Measurement	**Water**	
Final body weight (g)	256±6	25
Absolute retroperitoneal adipose weight (g)	3.87±0.38	3.
Relative retroperitoneal adipose weight (mg/g bwt)	15.1±1.5	14
Absolute gonadal adipose weight (g)	7.85±0.75	8.
Relative gonadal adipose weight (mg/g bwt)	30.8±3.1	33
Absolute liver weight (g)	6.46±0.27[b]	6.
Relative liver weight (mg/g bwt)	25.1±1.0	27

*Values are expressed as the mean ± SEM of n=7-8 rats/group. Diff
significant differences at $P<0.05$ by one-way ANOVA followed by
55.

growing female rats on body weight, fat mass, and organ weights.

	Treatments*			
Glucose	**Fructose**	**Sucrose**	**HFCS-55**	***P*-value**
54±4	276±7	276±3	276±8	*0.09*
71+0.38	3.64+0.32	3.90+0.26	4.87+1.2	*0.57*
4.6±1.4	13.3±1.3	14.1±0.9	17.2±3.5	*0.65*
47±0.90	8.30±0.67	10.43±0.47	11.57±2.4	*0.15*
3.3±3.3	30.1±2.6	37.9±1.8	40.9±7.2	*0.25*
94±0.36^{ab}	7.37±0.36^{ab}	7.31±0.24^{ab}	7.95±0.25^{a}	*0.03*
7.3+1.4	26.6+0.8	26.5+0.7	28.9+1.3	*0.21*

ferent superscript letters a, b within the same rows indicate
Tukey's test. Abbreviation is HFCS-55, high fructose corn syrup-

Table 4. Consumption of different caloric-sweetened solutions by

Fatty acids (%)	Water	Trea Fruc
Saturated fatty acids		
Myristic acid, 14:0	0.70 ±0.15	0.62 ±
Palmitic acid, 16:0	23.4 ±3.0	20.1 ±
Stearic acid,18:0	13.8 ±1.3	12.0 ±
Monounsaturated fatty acids		
Palmitoleic acid, 16:1n-7	1.9 ±0.4[b]	2.6 ±0
Oleic acid, 18:1n-9	16.2 ±1.9[b]	17.3 ±
Polyunsaturated fatty acids		
Linoleic acid, 18:2n-6	26.2 ±3.0[a]	15.6 ±
Alpha-linolenic acid, 18:3n-3	1.9 ±0.3	1.0 ±0
Arachidonic acid, 20:4n-6	11.1 ±1.0	9.5 ±1
Eicosapentaenoic acid, 20:5n-3	ND	ND
Docosahexaenoic acid, 22:6n-3	ND	ND

*Values are expressed as the mean ± SEM of n=7-8 rats/group.
within the same rows indicate significant differences at $P<0.0$
Abbreviations are HFCS-55, high fructose corn syrup 55; ND,

growing female rats on liver fatty acid composition.

tments*			
tose	**Sucrose**	**HFCS-55**	***P*-value**
±0.12	0.80 ±0.16	1.1 ±0.26	*0.02*
±3.2	26.0 ±3.1	32.5 ±4.5	*0.15*
±1.8	11.6 ±1.6	12.0 ±1.5	*0.33*
0.5^{ab}	4.4 ±0.7^{ab}	5.8 ±0.8^{a}	*0.003*
±2.9^{b}	22.9 ±2.9^{ab}	35.9 ±4.0^{a}	*0.02*
±3.1^{ab}	13.3 ±2.3^{b}	17.5 ±4.2^{ab}	*0.03*
0.2	0.9 ±0.3	1.1 ±0.3	*0.006*
.2	10.0 ±1.7	10.0 ±1.1	*0.87*
	ND	ND	
	ND	ND	

Different superscript letters a, b, c
5 by one-way ANOVA followed by Tukey's test.
not detectable.

Table 5. Consumption of different caloric-sweetened solutions by

Fatty Acids (%)	Water	Glucose
Saturated fatty acids		
Myristic acid, 14:0	4.6 ±0.4	3.7±0.6
Palmitic acid, 16:0	79.6±4.3	89.7±10.4
Stearic acid,18:0	9.9±0.5	6.5±1.4
Monounsaturated fatty acids		
Palmitoleic acid, 16:1n-7	49.1 ±3.8[b]	89.1±9.9[a]
Oleic acid, 18:1n-9	392.1 ±16.8	429.2 ±51.1
Polyunsaturated fatty acids		
Linoleic acid, 18:2n-6	220.1 ±17.8[a]	52.3 ±8.7[c]
Alpha-linolenic acid, 18:3n-3	20.8 ±2.3[a]	3.5 ±0.7[b]
Arachidonic acid, 20:4n-6	3.8 ±0.5[a]	0.7 ±0.1[b]
Eicosapentaenoic acid, 20:5n-3	ND	ND
Docosahexaenoic acid, 22:6n-3	ND	ND

*Values are expressed as the mean ± SEM of n=7-8 rats/grou
within the same rows indicate significant differences at $P<0$.
Abbreviations are HFCS-55, high fructose corn syrup 55; NI

growing female rats on gonadal fat pad fatty acid composition.

Treatment*			
Fructose	**Sucrose**	**HFCS-55**	***P*-value**
5.1 ±0.8	5.9 ±0.7	3.9 ±0.5	*0.08*
90.3 ±13.9	104.5 ±11.9	76.9 ±7.3	*0.37*
10.6 ±1.0	10.0 ±2.0	7.4 ±1.3	*0.15*
65.3 ±10.8^{a,b}	73.7 ±10.9^{a,b}	70.8 ±9.1^{a,b}	*0.03*
457.1 ±70.3	526.5 ±62.5	385.6 ±30.8	*0.31*
175.8 ±18.1^{a,b}	164.8 ±11.5^{a,b}	147.9 ±22.1^{b}	*0.03*
16.2 ±1.9^{a}	14.0 ±1.9^{a}	14.8 ±2.4^{a}	*0.003*
3.0 ±0.3^{a}	3.1 ±0.3^{a}	2.7 ±0.6^{a}	*0.006*
ND	ND	ND	
ND	ND	ND	

up. Different superscript letters a, b, c
.05 by one-way ANOVA followed by Tukey's test.
D, not detectable.

Table 6. Consumption of different caloric-sweetened solutions by gr

Fatty acid (%)	Water	Glucose
Saturated fatty acids		
Myristic acid, 14:0	3.0 ±0.4	3.6 ±0.6
Palmitic acid, 16:0	73.0 ±6.9[b]	116.0 ±6.2[a]
Stearic acid,18:0	7.4 ±1.3	8.1 ±1.1
Monounsaturated fatty acids		
Palmitoleic acid, 16:1n-7	50.7 ±5.3[c]	104.1 ±7.9[a]
Oleic acid, 18:1n-9	339.0 ±47.9[b]	571.4 ±33.1[a]
Polyunsaturated fatty acids		
Linoleic acid, 18:2n-6	131.7 ±16.3[a]	57.1 ±9.5[b]
Alpha-linolenic acid, 18:3n-3	8.7 ±1.1[a]	3.4 ±0.7[b]
Arachidonic acid, 20:4n-6	1.5 ±0.2[a]	0.4 ±0.2[c]
Eicosapentaenoic acid, 20:5n-3	ND	ND
Docosahexaenoic acid, 22:6n-3	ND	ND

*Values are expressed as the mean ± SEM of n=7-8 rats/gr
within the same rows indicate significant differences at $P<$
Abbreviations are HFCS-55, high fructose corn syrup 55; I

owing female rats on retroperitoneal fat pad fatty acid composition.

	Treatment*			
	Fructose	**Sucrose**	**HFCS-55**	*P*-value
	2.5 ±0.4	3.9 ±0.7	3.3 ±0.5	*0.11*
	66.9 ±8.1^{b}	94.1 ±4.5^{ab}	76.2 ±10.0^{b}	*0.003*
	4.9 ±1.0	8.1 ±0.5	6.7 ±1.0	*0.19*
	60.7 ±8.3^{bc}	90.7 ±7.0^{ab}	74.9 ±10.3^{abc}	*0.01*
	311.6 ±42.8^{b}	431.1 ±23.7^{ab}	368.1 ±52.0^{b}	*0.008*
	84.5 ±7.6^{ab}	86.6 ±12.0^{ab}	73.9 ±12.0^{b}	*0.01*
	5.1 ±0.8^{ab}	5.2 ±1.0^{ab}	4.4 ±0.9^{b}	*0.02*
	1.1 ±0.2^{ab}	0.9 ±0.1^{abc}	0.7 ±0.1^{bc}	*0.03*
	ND	ND	ND	
	ND	ND	ND	

oup. Different superscript letters a, b, c
0.05 by one-way ANOVA followed by Tukey's test.
ND, not detectable.

Table 7. Consumption of different caloric sugar-sweetened solutions

Measurement	Water	Glucose
Liver lipid content (%)	6.75±1.55[ab]	8.89±0.42[ab]
Fasting glucose (mmol/L)	195.1±16.0	225.3±36.0
Insulin (pmol/L)	216.7±22.0	301.6±84.6
Serum lipids		
Cholesterol (mmol/L)	2.03±0.15	1.83±0.09
TG (mmol/L)	1.01±0.12[b]	1.13±0.16[ab]
VLDL (mmol/L)	0.72±0.12	0.60±0.11
LDL-C (mmol/L)	2.31±0.28[b]	2.57±0.37[ab]
HDL-C (mmol/L)	1.00±0.15	1.26±0.16
Liver function		
ALT (U/L)	82.3±12.1	49.7±14.1
GGT (U/L)	4.42±0.48	3.43±0.61
Bilirubin (µmol/L)	2.56±0.49	2.07±0.28
Albumin (g/L)	42.1±2.0	37.3±3.0

*Values are expressed as the mean ± SEM of n=7-8 rats/group. Di
rows indicate significant differences at $P<0.05$ by one-way ANOV
HFCS-55, high fructose corn syrup 55; TG, triglyceride; VLDL, v
density lipoprotein; HDL-C high density lipoprotein; ALT, alanine
transpeptidase

by growing female rats on serum clinical measurements.

Treatment* Fructose	Sucrose	HFCS-55	*P*-value
3.64±1.06[b]	6.40±1.34[ab]	10.60±1.55[a]	*0.005*
173.1±18.9	214.0±19.1	246.7±49.1	*0.51*
251.9±32.2	284.6±42.2	378.0±87.6	*0.35*
1.82±0.11	1.89±0.19	2.19±0.23	*0.50*
1.32±0.13[ab]	1.55±0.12[ab]	1.62±0.16[a]	*0.02*
0.64±0.05	0.65±0.07	0.76±0.07	*0.73*
3.01±0.30[ab]	3.55±0.27[ab]	3.69±35[a]	*0.02*
1.10±0.09	1.04±0.14	1.22±0.15	*0.66*
58.3±11.6	61.7±9.6	48.0±8.6	*0.24*
2.71±0.51	2.86±0.51	3.14±0.94	*0.34*
2.05±0.28	1.71±0.10	1.71±0.09	*0.20*
38.1±3.1	35.9±3.6	38.1±2.6	*0.64*

fferent superscript letters a, b within the same
VA followed by Tukey's test. Abbreviations are
ery low density lipoprotein; LDL-C, low
transaminase; GGT, gamma-glutamyl