The Type of Caloric Sweetener Added to Water Influences Weight Gain, Fat Mass, and Reproduction in Growing Sprague-Dawley Female Rats

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Caloric sweetened beverages have been suggested to be a major dietary contributor to weight gain, particularly among adolescents. Dietary recommendations are for moderating intakes of added sugars; however, the question remains whether certain types of sugars should be limited. The objective of this study was to determine the effect of drinking different caloric sweetened beverages on the development of adiposity, metabolic, and endocrine disorders. Young (age 28 days) female Sprague-Dawley rats (n = 8–9 rats/group) were randomly assigned to drink either deionized distilled water (ddH_2O) or ddH_2O sweetened with 13% (w/v) glucose, sucrose, fructose or high fructose corn syrup 55 (HFCS-55) for 8 weeks. Rats drinking caloric sweetened solutions failed to completely compensate for liquid calories ingested by reducing their consumption of solid food. This resulted in greater total energy intake compared to the ddH_2O control; however, there was no significant difference in total energy intake between rats drinking sucrose, fructose or HFCS-55. Of the different caloric sweeteners, only rats drinking HFCS-55 had greater ($P < 0.05$) final body weights and fat mass compared to the rats drinking ddH_2O or glucose solution. This may have occurred because drinking HFCS-55 solution promoted a faster body weight gain. Adiposity induced by caloric sweetened water was not accompanied by metabolic disorders indicated by the absence of dyslipidemia and no differences in fasting serum glucose, insulin or C-peptide among the treatment groups. However, rats drinking HFCS-55 showed lengthened estrous cycles due to prolonged estrus. Based on this study, the type of caloric sweetener added to beverages should be considered when making dietary recommendation for reducing excess body weight and related health risk. Exp Biol Med 234:651–661, 2009

Key words: sugar beverages; adiposity; reproduction; metabolic disorders

Introduction

The prevalence of being overweight among children and adolescents continues to rise (1). One of the major factors influencing weight gain is excess calories from the diet. A significant proportion of the total energy intake may be attributed to the consumption of caloric sweetened beverages (2). In the United States, soft drink consumption has increased by 300% in the past 20 years with 56–85% of children in school consuming at least one soft drink daily (3). In general, high consumption of caloric sweetened beverages has been associated with excess body weight (4). Therefore, dietary recommendations are for moderating intakes of total added sugars. However, questions have arisen as to whether certain types of sugars should be limited more than others (5). This is important because the type of caloric sweetener added to soft drinks and other beverages has changed from sucrose consisting of 50% fructose and 50% glucose to high fructose corn syrup 55 (HFCS-55) comprised of 55% fructose, 42% glucose, and 3% higher saccharides (6). The slightly higher amount of fructose associated with HFCS-55 has been suggested to promote fat gain due to various factors such as: differences in metabolism, reduced satiety, greater caloric intake, and incomplete compensation for higher energy intakes (5, 7–8).

Animal studies have already demonstrated that feeding diets high in caloric sweeteners promotes weight gain and adiposity (9–11). However, most studies have used fructose to represent HFCS and few studies have compared the effects of sucrose and HFCS consumption on weight gain.

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Our treatment groups included HFCS-55, fructose, and sucrose-sweetened solutions. Also, previous animal feeding studies used high doses (≥30% v/w) of caloric sweeteners that are unlikely to be physiologically relevant to the amount of sugar consumed by humans. In the present study, caloric sweeteners were added to water at 13% v/w which is in the range added to soft drinks (12). Different caloric sweeteners were added to the water rather than solid diet due to reports that liquid sugar consumption promoted positive energy balance, whereas comparable solid sugar consumption resulted in dietary compensation (13).

Increased fat mass has been associated with health risks including: insulin resistance, dyslipidemia, and reproductive dysfunction. Studies have reported that being obese or even overweight increased the incidence of anovulation and subfertility in adult women (14) and menstrual disorders in adolescent girls (15). As the prevalence of obesity continues to rise worldwide in adolescents, reproductive dysfunction is posed to become a major health issue.

The development of effective dietary strategies towards the prevention of increased body fat and associated health consequences requires a better understanding of the effects of consuming different types of caloric sweetened beverages on adiposity. Therefore, the objective of this study was to determine the effect of drinking different caloric sweetened beverages on the development of adiposity, metabolic disorders, and reproductive dysfunction. The main source of added caloric sweeteners is through the consumption of soft drinks with the highest consumption by adolescents (16). Therefore, in our animal feeding study, we provided adolescent female rats different caloric sweeteners in the drinking water at the concentration found in soft drinks.

Materials and Methods

Animal and Experimental Design. All animal procedures were conducted in accordance with the guidelines set forth by the National Research Council for the Care and Use of Laboratory Animals (17) and approved by the Animal Care and Use Committee at West Virginia University. Immature (age 28 days) virgin female outbred Sprague-Dawley rats ($n = 44$) were purchased from Taconic Farms (Rockville, MD). Upon arrival, the rats were individually housed in metabolic cages for the duration of the experiment to determine liquid intake and food consumed. The room was kept at a constant temperature of 21°C with a 12 h light/dark cycle. During a 7 d acclimation period, animals were given *ad libitum* access to deionized distilled water (ddH_2O) and purified AIN-93G diet (Harlan Teklad, Indianapolis, IN).

Following acclimation, animals ($n = 8$–9/group) were randomly assigned to one of five treatments. Treatment groups consisted of ddH_2O with no caloric sweetener added or ddH_2O sweetened with glucose, sucrose, fructose or HFCS-55. The different caloric sweeteners were added at a level of 13% w/v which is in the concentration range reported for soft drinks (12). Throughout the 8 week study, rats were given *ad libitum* access to ddH_2O or their assigned liquid solution. Rats were also given free access to a standard purified diet. The AIN-93G was used because its nutrient composition does not vary, ingredients are precisely defined with a sucrose content of 10% (100 g/kg diet) (18) and was formulated to meet the nutritional requirements for growing rats as defined by the Nutritional Research Council (19). Providing food and liquid *ad libitum* allowed us to evaluate whether greater caloric intake or incomplete compensation for higher energy intakes played a role in weight gain with sugar consumption. Food and liquid consumption were measured twice a week. The use of metabolic cages allowed calculations of food consumption and water intake to be corrected for spillage. Fresh diet and ddH_2O or the assigned caloric sweetened solutions were replaced twice weekly. Body weights were measured weekly.

Estrous Cycles. All rats attained puberty indicated by vaginal canalization at age 34 ± 0.6 d and a body weight of 98 ± 2 g. The estrous cycles were assessed by monitoring vaginal cytology for 35 consecutive days (~7 cycles) by performing daily vaginal smears. The tip of a saline dampened cotton swab was gently introduced into the vagina with care being taken not to insert to a depth of >1.0 cm to avoid pseudopregnancy. The vaginal smear was transferred onto a microscope slide and examined under a light microscope (×20) to determine the stage of the estrous cycle. The estrous cycle stages are: i) proestrus (mainly epithelial cells), ii) estrus (mainly cornified cells), iii) metestrus (cornified cells and leukocytes), and iv) diestrus (mainly leukocytes). A normal estrous cycle in rats was defined as 4–5 d. Rats were considered acyclic if the rat remained in one phase for >15 d (20). Each slide was assessed independently by two different experimenters who were blinded to the identity of the treatment groups.

RNA Isolation and Gene Expression. At the end of the 8 week feeding study, rats were fasted for 15 h then euthanized by CO_2 inhalation. Retroperitoneal and gonadal fat pads were excised, weighed, and then immediately frozen in liquid nitrogen. Tissues were kept frozen at –80°C until analyzed. Total RNA was extracted from the fat depots using the RNeasy lipid tissue mini kit (Qiagen, Valencia, CA). A tissue sample (100 μg) was homogenized in 1 mL of Qiazol for 20 sec using a Polytron homogenizer. After standing for 5 min at room temperature, chloroform (100 μL) was added to each tissue homogenate sample followed by vigorous shaking for 15 sec. The samples were then centrifuged at 4°C at 16,000 *g* for 15 min. The top aqueous phase was transferred into an RNase-free microcentrifuge tube with the addition of an equal amount of 70% RNase-free ethanol and vortexed. The samples were transferred into the Qiagen spin column and Total RNA eluted from the column using 30 μL of RNase-free water. The concentration and purity of RNA in each sample was determined using a NanoDrop 1000 spectrophotometer (NanoDrop Technolo-

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gies, Wilmington, DE). The quality of the RNA was further confirmed by running 1.5 μg of RNA per sample on a 1.5% agarose gel.

From aliquots of each fat depot, mRNA expression of β-actin (housekeeping gene), leptin, PPARγ, and LPL were quantified using real time RT-PCR. Real time RT-PCR was performed using SYBR Green on an iCycler Thermal Cycler (BioRad Laboratories, Hercules, CA). Forward and reverse primers were designed using PRIMER3 primer design. Primer sequences were as follows (forward, reverse). β-actin (5′-ATGTACGTAGCCATCCAGGC-3′, 5′-ACCCTCATAGATGGGCACAG-3′), leptin (5′-AGCTGCAAGGTCCAAGAAGA-3′, 5′-TGATGAGGGTTTTGGTGTCA-3′), PPARγ (5′-ACCACGGTTGATTTCTCCAG-3′, 5′-GCAGGCTCTACTTTGATCGC-3′), LPL (5′-CTTCAACCACAGCAGCAAAA-3′, 5′-GGCCCGATACAACCAGTCTA-3′).

The samples were run in duplicate in a reaction volume of 50 μL. The reaction mixture consisted of 25 μL of SYBR Green, 5 μL of cDNA, 1.25 μL (β-actin, leptin, or PPARγ), and 0.625 μL (LPL) of forward and reverse primers. A 3-min hot start at 95°C started the reaction, followed by 40 cycles of amplification. Each cycle included three steps: i) 15 sec at 95°C denaturation, ii) 30 sec at 55°C annealing, and iii) 30 sec at 72°C elongation.

Determination of Clinical Chemistry. At the end of the 8 week feeding study, rats were fasted for 15 h and then euthanized by CO_2 inhalation. Trunk blood was centrifuged at 1,500 *g* for 10 min at 4°C and serum was collected. Serum was kept frozen at –80°C until analyzed. Fasting serum cholesterol, triglyceride, very low density lipoprotein (VLDL), low density lipoprotein (LDL), and high density lipoprotein (HDL) were determined by Lipid test rotor enzymatic colorimetric assays measured using a Hemagen Analyst automated spectrophotometer (Hemagen Diagnostics Inc., Columbia, MD). Using this assay all serum lipid and lipoprotein values were within the range reported for rats (21–22). Biomarkers of liver function (i.e. serum aspartate amino transferase, alanine transferase, and bilirubin) were determined by Vet 16 veterinary test rotor enzymatic colorimetric assays measured using a Hemagen Analyst automated spectrophotometer.

Fasting serum glucose was determined by the Vet 16 veterinary test rotor hexokinase/glucose-6-phosphate dehydrogenase assay measured by Hemagen Analyst automated spectrophotometer. Using this assay serum glucose values were in the range reported for rats (23). Serum insulin was measured using a commercially available rat specific enzyme immunoassay (EIA) (Biomedical Technologies, Stoughton, MA). Optical densities of the samples were determined at wavelength 450 nm using a Spectramax Plus microplate reader (Molecular Devices, Sunnyvale, CA). Serum C-peptide was measured by rat specific EIA (Alpco Diagnostics, Salem, NH) and optical densities of the samples determined at wavelength 490 nm using a Spectramax Plus microplate reader.

Reproductive Organ Weights and Hormones Assays. To avoid hormonal fluctuations, only rats confirmed to be in the estrus phase on the morning of dissection were used in the analysis. Therefore, of the $n = 44$ rats in the study, only $n = 31$ rats were used in the analysis of reproduction. The uterus and ovaries were excised and weighed. Trunk blood was collected, centrifuged at 1,500 *g* for 10 min at 4°C, and the serum kept frozen at –80°C until analyzed. Serum estradiol was determined using a commercially available double antibody estradiol radioimmunoassay (RIA) kit and serum total testosterone was determined using a Coat-a-Count RIA kit (Siemens Healthcare Diagnostics, Los Angeles, CA). Radioisotope counting was performed using a Wallac 1470 Wizard Gamma Counter (PerkinElmer, Waltham, MA). The intra-assay coefficient of variation was 8% for serum estradiol and 10.5% for serum testosterone.

Serum leptin was measured by a rat specific EIA (Assay Designs, Ann Arbor, MI). The optical densities of the samples were determined at wavelength 450 nm by Spectramax Plus microplate reader.

Statistical Analysis. One-way ANOVA was used to determine differences between the treatment groups. Post-hoc multiple comparison tests were performed using Tukey's test with treatment differences considered significant at $P < 0.05$. The relationship between food intake and liquid consumption was determined by a simple regression of the individual animals in each treatment group. One-way ANOVA and correlation analyses were performed using the SigmaStat 3.1 statistical software program (Systat Software Inc., San Jose, CA).

Repeated Measures in a mixed model procedure was used to analyze body weight gain and estrous cycle lengths during the 8 week feeding study. Repeated measures calculations were performed using SAS 9.1 (SAS Institute Inc., Cary, NC). All differences were considered significant at $P < 0.05$.

Results

Liquid, Food and Caloric Intake. Liquid intake was in the order of glucose > sucrose > HFCS-55 = fructose = ddH_2O. Among the rats provided the caloric sweetened solution, the group drinking the glucose solution consumed the greatest ($P = 0.001$) amount of liquid kilocalories (kcals). Rats drinking the sucrose solution consumed greater ($P = 0.03$) liquid kcals compared to the rats provided the HFCS-55 or fructose solution. There were no significant differences in liquid calorie intake between the rats drinking the HFCS-55 or fructose solution (Table 1).

There was negative correlation ($r^2 = –0.76$, $P = 0.01$) between solid food intake and liquid consumption (Fig. 1). Rats drinking ddH_2O consumed the most ($P = 0.001$) kcals from the solid food. According to Table 1, among the rats provided the different caloric sweetened solutions, the group drinking the glucose solution had reduced ($P = 0.001$) kcal

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Table 1. The Effect of Providing Young Female Rats Different Caloric Sweetened Solutions on Liquid, Food, and Caloric Intake, Body Weight, and Fat Mass

Measurement	Treatments*				
	ddH_2O	Glucose	Sucrose	Fructose	HFCS-55
Liquid intake (mL)	1844 ± 196^{a}	5154 ± 368^{c}	3403 ± 312^{b}	2267 ± 633^{a}	2795 ± 236^{ab}
Food intake (g)	968 ± 29^{d}	597 ± 29^{a}	681 ± 34^{ab}	876 ± 17^{cd}	785 ± 34^{bc}
Energy intake					
From liquid (kcal)	0.0 ± 0.0^{a}	2452 ± 175^{d}	1659 ± 152^{c}	870 ± 130^{b}	1119 ± 94^{b}
From food (kcal)	3678 ± 111^{d}	2267 ± 110^{a}	2588 ± 129^{ab}	3354 ± 68^{cd}	2982 ± 128^{bc}
From food + liquid (kcal)	3678 ± 111^{a}	4719 ± 130^{c}	4247 ± 143^{b}	4224 ± 114^{b}	4101 ± 141^{b}
Feed efficiency (g bw gain/g intake)	0.18 ± 0.01^{b}	0.14 ± 0.01^{a}	0.17 ± 0.01^{b}	0.17 ± 0.01^{b}	0.17 ± 0.01^{b}
Body weight					
Initial body weight (g)	80 ± 2	82 ± 4	88 ± 3	81 ± 3	86 ± 4
Final body weight (g)	256 ± 6^{a}	256 ± 3^{a}	274 ± 3^{ab}	276 ± 5^{ab}	284 ± 10^{b}
Retroperitoneal adipose (g)	2.9 ± 0.2^{a}	3.1 ± 0.2^{a}	4.2 ± 0.2^{ab}	4.0 ± 0.2^{ab}	5.0 ± 0.8^{b}
Relative retroperitoneal adipose (mg/g bw)	12.0 ± 0.7^{a}	12.8 ± 0.8^{a}	16.0 ± 0.7^{ab}	15.4 ± 0.8^{ab}	18.1 ± 2.0^{b}
Gonadal adipose (g)	6.3 ± 0.5^{a}	7.9 ± 0.5^{a}	9.8 ± 0.6^{ab}	9.5 ± 0.4^{ab}	11.6 ± 1.6^{b}
Relative gonadal adipose (mg/g bw)	26.2 ± 1.8^{a}	32.5 ± 1.9^{ab}	37.5 ± 1.9^{b}	36.5 ± 1.5^{b}	42.0 ± 4.1^{b}

* Values are expressed as the mean ± SEM of $n = 8$–9 rats/group. Different superscript letters a, b, c, d within the same rows indicate significant differences at $P < 0.05$ by one-way ANOVA followed by Tukey's test. Same superscript letters ab, bc, cd indicate no significant differences. HFCS-55, high fructose corn syrup 55; ddH_2O, deionized distilled water.

intake from solid food compared to animals drinking the fructose or HFCS-55 solution. Rats drinking the sucrose solution consumed less ($P = 0.001$) kcals from solid food than rats drinking the fructose, but not the HFCS-55 solution. There were no significant differences in kcal intake from solid food between the rats drinking the HFCS-55 or fructose solution (Table 1).

As shown in Table 1, differences in liquid and solid food intake resulted in differences in total energy intake among the treatment groups. The rats provided ddH_2O had the lowest ($P = 0.01$) total energy intake. Among the rats provided caloric sweetened solution, rats drinking the glucose solution had the greatest ($P < 0.001$) total energy intake, but the lowest ($P = 0.001$) feed efficiency. There were no significant differences in total energy intake between the rats provided the sucrose, HFCS-55 or fructose solution.

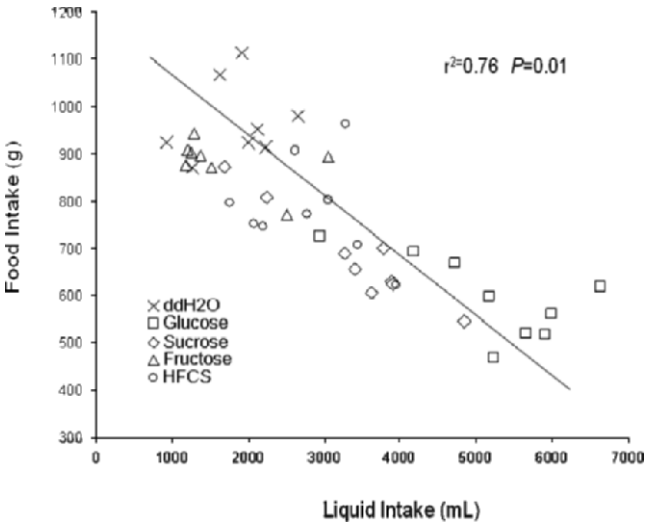

Figure 1. The relationship between beverage consumption and food intake. Values are a simple regression of individual animals ($n = 8$–9 rats) in the different treatment groups.

Body Weight and Fat Mass. Initial body weights among the treatment groups were not significantly different (Table 1). After 8 weeks, only rats drinking the HFCS-55 solution had heavier ($P = 0.02$) final body weights than the ddH_2O control. Among rats provided the caloric sweetened water, rats drinking the HFCS-55 solution had heavier ($P = 0.02$) final body weights than rats drinking the glucose solution. There were no significant differences in the final body weights among the rats drinking HFCS-55, sucrose or fructose solution.

Body weight showed a time ($P < 0.001$), a treatment ($P = 0.001$), and a treatment × time effect ($P < 0.001$). In Figure 2, the body weights of rats drinking the HFCS-55 solution increase more rapidly starting at ~3–4 weeks compared to rats drinking the glucose solution ($P = 0.004$) or ddH_2O ($P = 0.02$). Similarly, the body weights of rats drinking the fructose solution increase more rapidly starting at ~4–5 weeks compared to rats drinking the glucose solution ($P = 0.004$) or ddH_2O ($P = 0.02$). Linear regression of body weights indicated slope coefficients of 16.13 for HFCS-55, 15.97 for fructose, 14.48 for sucrose, 14.06 for ddH_2O, and 13.29 for the glucose sweetened solution.

Shown in Table 1, rats drinking HFCS-55 solution had higher ($P = 0.004$) absolute and relative retroperitoneal fat pad weights compared to rats drinking glucose solution or ddH_2O. Absolute gonadal fat pads weights were also higher ($P = 0.002$) in rats drinking the HFCS-55 solution compared to rats drinking the glucose solution or ddH_2O. Expressed as

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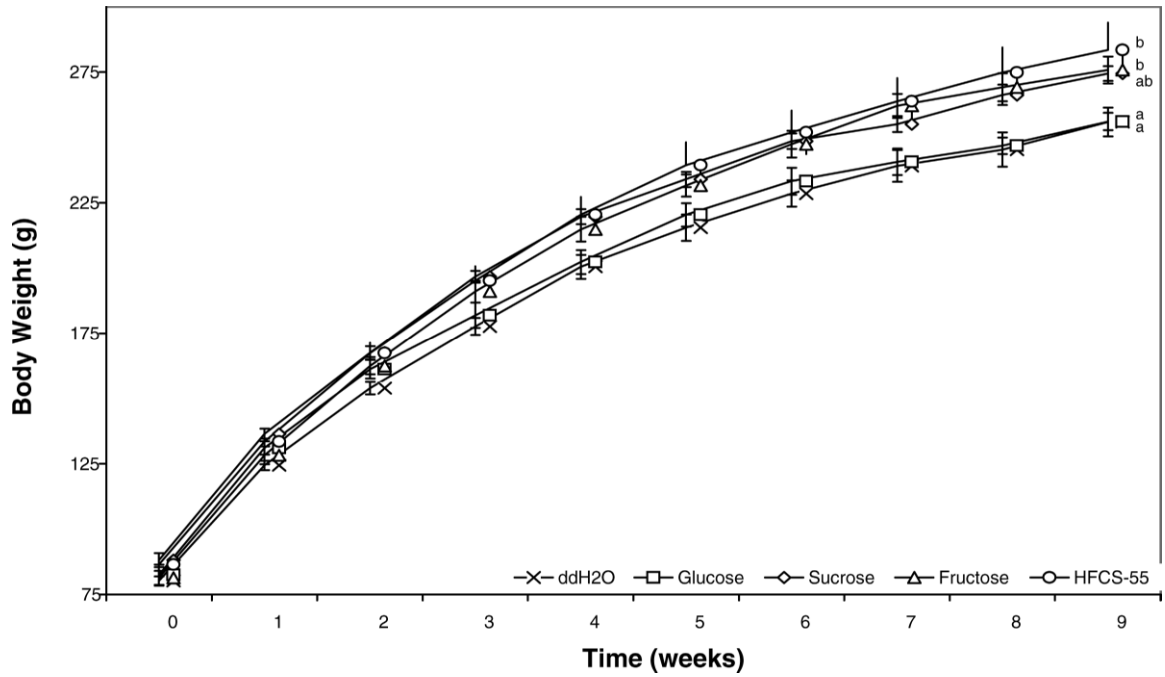

Figure 2. The effects of feeding different caloric sweetened solutions to young female rats on body weight over an 8 week period. Repeated Measures in a mixed model procedure was used to analyze body weight gain during the 8 week feeding study. Values are the means ± SEM of the different treatments ($n = 8$–9 rats/group) each week. Different superscript letters a, b indicate significant differences at $P < 0.05$ by one-way ANOVA followed by Tukey's test. Same superscript letters ab indicate no significant differences.

relative weights, the gonadal fat pad weight of rats drinking HFCS-55, sucrose or fructose solution was greater ($P = 0.001$) than the ddH_2O control. There were no significant differences in gonadal or retroperitoneal fat pad weights among the rats drinking HFCS-55, sucrose or fructose solution (Table 1).

Leptin, PPARγ and LPL. There were no significant differences in serum leptin concentrations among any of the treatment groups (Fig. 3A). Leptin mRNA abundance in the gonadal fat pad was elevated ($P = 0.02$) in rats drinking HFCS-55 solution compared to the rats drinking the sucrose or glucose solution and the ddH_2O control (Fig. 3B). Gene expression of leptin was not detectable in the retroperitoneal fat depot. There were no significant differences in PPARγ (Fig. 4A) or LPL (Fig. 4B) mRNA abundance in the gonadal fat pad among any of the treatment groups. Gene expression of PPARγ and LPL was not detectable in the retroperitoneal fat depot.

Serum Clinical Chemistry. As shown in Table 2, there were no significant differences in the relative liver weights among the treatment groups. However, absolute liver weight was greater ($P = 0.02$) in rats drinking the HFCS-55 solution compared to the ddH_2O control. There were no significant differences in biomarkers of liver function: serum aspartate aminotransferase, alanine aminotransferase or bilirubin, among the different treatment groups. There were no significant differences in serum cholesterol, triglyceride, VLDL, LDL or HDL concentrations among any of the treatment groups. There were also no significant differences in fasting serum glucose, insulin or C-peptide concentrations among the different treatment groups.

Reproductive Function. None of the rats were acyclic (one phase for > 15 d) during the 8 weeks study. Of the $n = 44$ rats only those determined to be in estrus at dissection $n = 31$ were used in the analysis of the estrous cycles and hormones. There were no statistical differences in the number of estrous cycles among the treatment groups. For estrous cycle lengths, there was a treatment ($P = 0.02$) and a treatment × time effect ($P = 0.002$). According to Table 3, estrous cycle 6 was longer ($P = 0.04$) in the rats drinking HFCS-55 solution compared to rats drinking the glucose solution. Estrous cycle 7 was longer ($P = 0.01$) in the rats drinking the HFCS-55 solution compared to rats drinking the different caloric sweetened solutions and the ddH_2O control. Lengthened estrous cycles were due to prolonged time in the estrus phase.

As shown in Table 4, rats drinking the HFCS-55 solution had elevated ($P = 0.01$) serum insulin compared to the ddH_2O control. Serum leptin concentration in rats drinking the HFCS-55 solution was greater ($P = 0.02$) compared to rats drinking the ddH_2O, glucose or fructose solution. There were no significant differences in serum testosterone, estradiol or ovarian and uterine weights among any of the treatment groups.

Discussion

Adding caloric sweeteners to water promoted liquid consumption with the amount that rats drank depending on

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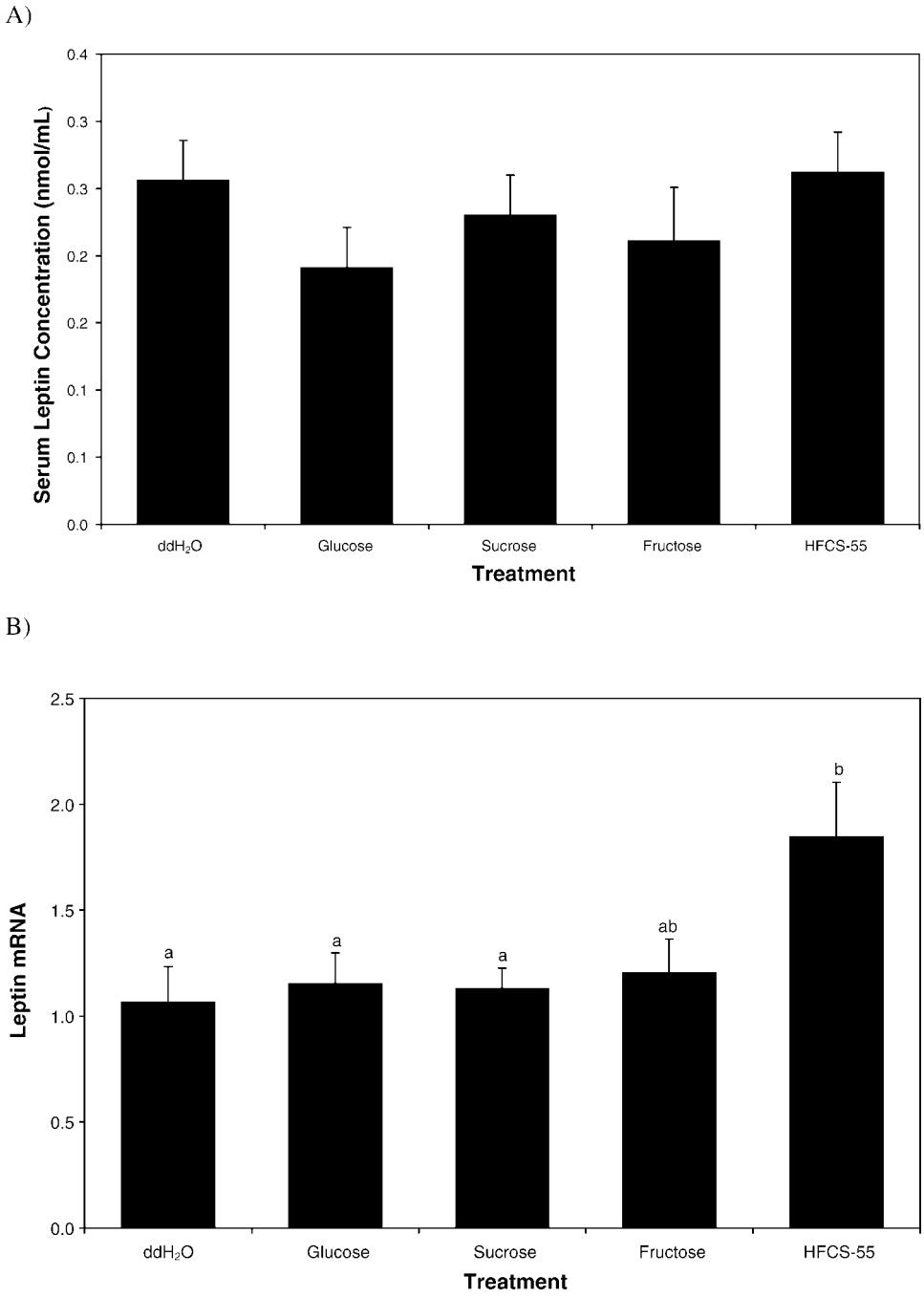

Figure 3. The effects of feeding different caloric sweetened solutions on A) serum leptin concentrations and B) leptin mRNA abundance. Values are the means ± SEM of $n = 8$–9 rats/group. Different superscript letters a, b indicate significant differences at $P < 0.05$ by one-way ANOVA followed by Tukey's test. Same superscript letters ab indicate no significant differences.

the sweetness intensity. Liquid consumption decreased with increasing sweetness intensity, i.e. glucose > sucrose > HFCS-55 = fructose. This resulted in total energy intake during the 8 week study being significantly greater in rats drinking the caloric sweetened water compared to rats drinking ddH_2O. Like humans, rats display hyperphagia when given access to diets containing a greater proportion of sugar than what is found in their typical laboratory rodent diets (24). Whether this preference increased susceptibility to weight gain remains unclear. In our study, rats showed a tendency towards compensation for the kcals ingested from liquid by reducing their caloric intake from the solid diet (Fig. 1). Jurgens *et al.* (12) reported that mice drinking 10% w/v sucrose or 15% w/v fructose solution proportionally reduce their caloric intake from the chow diet to compensate for their total energy intake. In the current study, rats

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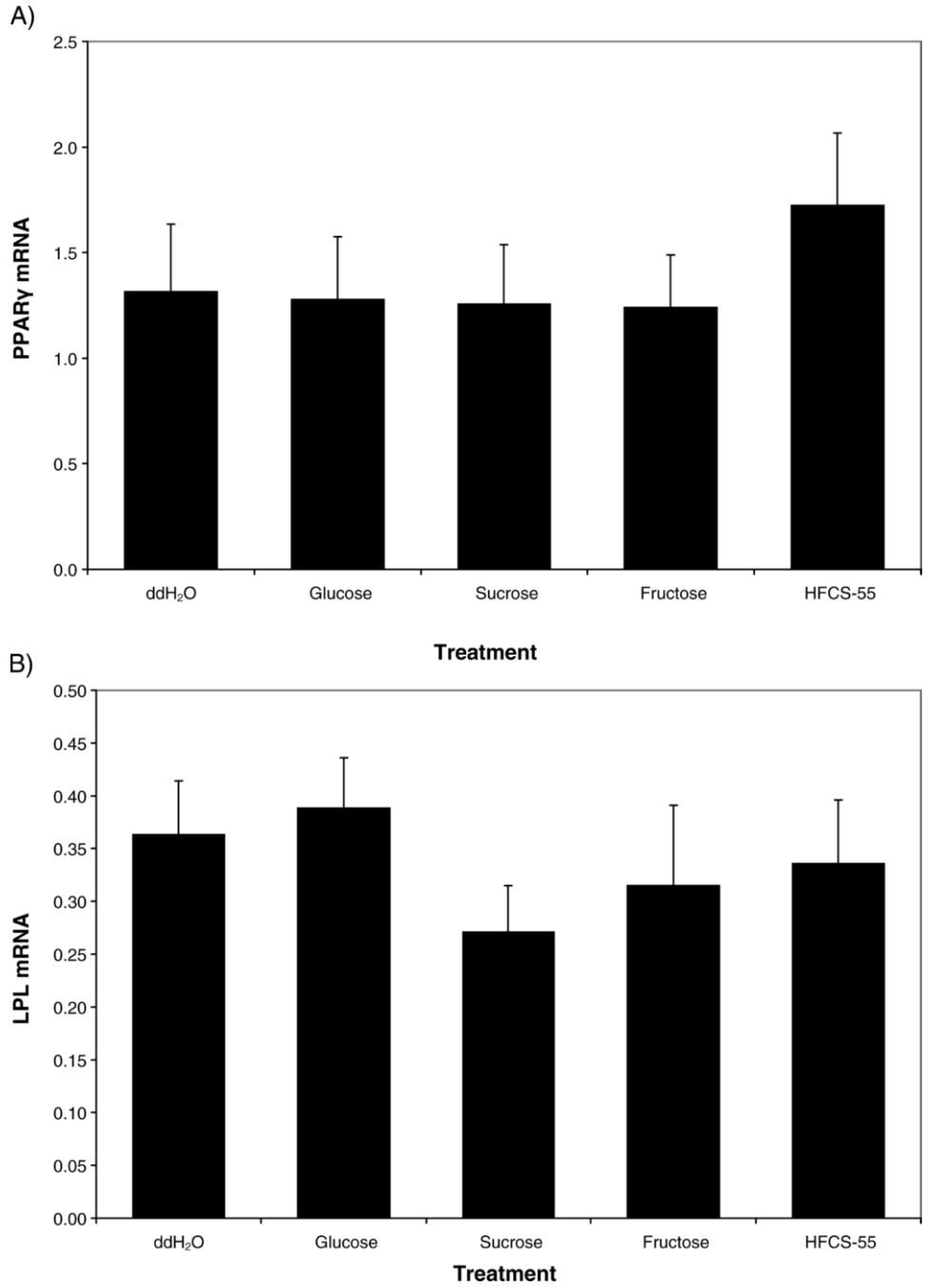

Figure 4. The effects of feeding different caloric sweetened solutions on A) PPARγ mRNA abundance and B) LPL mRNA abundance in the gonadal adipose tissue. Values are means ± SEM of $n = 8$–9 rats/group.

provided caloric sweetened solutions were unable to proportionally reduce their caloric intake from the solid food to completely compensate for their total energy intake.

Rats drinking the glucose solution had the highest liquid kcal intake and the lowest solid food intake, but the highest total energy intake among the treatment groups. Yet, the body weight and fat mass of rats drinking glucose solution were not significantly different than rats drinking ddH_2O, sucrose or fructose solution. Polydipsia (90 mL/d) in rats drinking the glucose solution was accompanied by polyuria (50 mL/d). This resulted in reduced feed efficiency that may explain the absence of significant body weight and fat mass gain in rats drinking glucose solution despite higher total energy intake. Similarly, the body weights of rats drinking the sucrose or fructose solution were not significantly different than rats drinking ddH_2O despite their greater total energy intakes. Kanarek *et al.* (11) reported that weanling male Sprague-Dawley rats consuming a high dose (32% w/v) sucrose solution did not display greater body weights compared to rats drinking water until 8 weeks. A time ×

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Table 2. The Effect of Providing Young Female Rats Different Caloric Sweetened Solutions on Serum Clinical Chemistry

Measurement	Treatments*				
	ddH_2O	Glucose	Sucrose	Fructose	HFCS-55
Liver weight (g)	6.4 ± 0.2[a]	7.0 ± 0.3[ab]	7.4 ± 0.2[ab]	7.6 ± 0.3[ab]	8.1 ± 0.5[b]
Relative liver weight (mg/g)	26.7 ± 0.9	28.8 ± 1.1	28.6 ± 0.7	29.3 ± 1.0	30.0 ± 1.3
Serum aspartate aminotransferase (U/L)	323.7 ± 87.1	279.7 ± 57.2	343.6 ± 35.6	265.1 ± 46.3	282.7 ± 43.4
Serum alanine aminotransferase (U/L)	88.3 ± 12.0	62.0 ± 13.7	71.6 ± 11.2	52.8 ± 9.7	71.0 ± 24.2
Serum bilirubin (μmol/L)	1.9 ± 0.6	1.3 ± 0.4	1.3 ± 0.2	1.5 ± 0.3	1.7 ± 0.1
Serum cholesterol (mmol/L)	1.9 ± 0.1	1.9 ± 0.1	1.8 ± 0.2	1.8 ± 0.1	1.9 ± 0.1
Serum triglyceride (mmol/L)	1.6 ± 0.2	1.4 ± 0.2	1.5 ± 0.1	1.3 ± 0.1	1.5 ± 0.2
VLDL (mmol/L)	0.7 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.7 ± 0.1
LDL (mmol/L)	0.8 ± 0.2	0.6 ± 0.3	0.9 ± 0.2	0.7 ± 0.2	0.7 ± 0.3
HDL (mmol/L)	1.1 ± 0.2	1.1 ± 0.2	1.1 ± 0.1	1.1 ± 0.1	1.3 ± 0.1
Serum fasting glucose (mmol/L)	10 ± 1	13 ± 2	12 ± 1	10 ± 1	13 ± 2
Serum insulin (pmol/L)	322 ± 49	401 ± 73	506 ± 109	384 ± 64	515 ± 124
Serum C-peptide (nmol/L)	0.55 ± 0.05	0.73 ± 0.08	0.79 ± 0.10	0.65 ± 0.08	0.75 ± 0.08

* Values are expressed as the mean ± SEM of $n = 8$–9 rats/group. Different superscript letters a, b within the same rows indicate significant differences at $P < 0.05$ by one-way ANOVA followed by Tukey's test. Same superscript letters ab indicate no significant differences. HFCS-55, high fructose corn syrup 55; ddH_2O, deionized distilled water; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein.

treatment effect on body weight suggested that longer study durations may be needed before obesity develops in rats fed caloric sweeteners at the dose of 13% w/v.

Of the caloric sweeteners only the final body weight and fat mass of rats drinking the HFCS-55 solution were significantly greater than rats drinking ddH_2O or glucose solution at 8 weeks. In a human study, Tordoff *et al*. (25) reported increased *ad libitum* energy intake and body weight in adult subjects consuming HFCS sweetened beverages compared to subjects consuming non-caloric sweetened beverages. Whether HFCS promoted greater increases in energy intake and body weight compared to other caloric sweeteners was not addressed. In our study, there were no significant differences in total energy intake or final body weight in rats drinking HFCS, sucrose or fructose solution. However, linear regression of body weights indicated that

Table 3. The Effect of Providing Young Female Rats Different Caloric Sweetened Solutions on Body Weight, Adiposity, and Estrous Cyclicity*

Treatment	ddH_2O	Glucose	Sucrose	Fructose	HFCS-55
	$n = 6$	$n = 6$	$n = 7$	$n = 6$	$n = 6$
Final body weight (g)	246 ± 3[a]	246 ± 3[a]	260 ± 2[ab]	257 ± 3[ab]	268 ± 3[b]
Gonadal adipose (g)	6.38 ± 0.64[a]	7.82 ± 0.52[a]	9.65 ± 0.57[ab]	9.99 ± 0.59[ab]	11.75 ± 1.65[b]
Relative gonadal adipose (mg/g bw)	25.79 ± 2.33[a]	31.86 ± 2.10[a]	37.64 ± 2.13[ab]	38.36 ± 1.89[ab]	42.14 ± 6.11[b]
Retroperitoneal adipose (g)	2.96 ± 0.25	3.27 ± 0.26	4.11 ± 0.33	4.25 ± 0.22	4.91 ± 0.79
Relative retroperitoneal adipose (mg/g bw)	12.05 ± 1.00	13.32 ± 1.14	16.02 ± 1.20	16.29 ± 0.72	17.46 ± 2.96
Number of cycles	6.67 ± 0.21	7.00 ± 0.10	6.75 ± 0.16	6.83 ± 0.17	6.17 ± 0.40
Cycle length (d)	5.14 ± 0.14	5.00 ± 0.10	5.21 ± 0.14	5.14 ± 0.14	5.81 ± 0.40
Cycle 1 length (d)	5.83 ± 0.31	5.50 ± 0.34	5.00 ± 0.19	5.00 ± 0.26	5.33 ± 0.61
Cycle 2 length (d)	4.67 ± 0.21	5.17 ± 0.31	5.37 ± 0.18	5.00 ± 0.26	5.17 ± 0.31
Cycle 3 length (d)	5.50 ± 0.22	5.50 ± 0.22	4.75 ± 0.25	4.67 ± 0.21	5.50 ± 0.43
Cycle 4 length (d)	5.33 ± 0.21	5.00 ± 0.10	5.00 ± 0.10	5.50 ± 0.56	5.50 ± 0.50
Cycle 5 length (d)	4.80 ± 0.37	4.83 ± 0.17	4.75 ± 0.16	4.80 ± 0.37	5.67 ± 0.33
Cycle 6 length (d)	5.17 ± 0.31[ab]	4.50 ± 0.22[a]	5.13 ± 0.13[ab]	6.00 ± 0.37[ab]	6.17 ± 0.60[b]
Cycle 7 length (d)	5.00 ± 0.26[a]	4.50 ± 0.22[a]	5.00 ± 0.10[a]	4.83 ± 0.40[a]	6.33 ± 0.42[b]
Proestrus (d)	0.96 ± 0.06	1.14 ± 0.11	0.81 ± 0.08	1.06 ± 0.12	0.81 ± 0.08
Estrus (d)	2.10 ± 0.08[a]	2.09 ± 0.08[a]	2.09 ± 0.12[a]	2.09 ± 0.11[a]	2.56 ± 0.10[b]
Metestrus (d)	0.99 ± 0.06	0.91 ± 0.10	1.16 ± 0.07	0.94 ± 0.09	1.10 ± 0.08
Diestrus (d)	1.09 ± 0.08	1.04 ± 0.07	1.04 ± 0.03	1.07 ± 0.06	1.19 ± 0.10

* Values are expressed as the mean ± SEM of $n = 6$–7 rats/group. Only rats confirmed to be in the estrus phase on the morning of dissection were used in the analysis. Of the $n = 44$ rats in the study only $n = 31$ rats were used in these analyses. Different superscript letters a, b within the same rows indicate significant differences at $P < 0.05$ by one-way ANOVA followed by Tukey's test. Same superscript letters ab indicate no significant differences. HFCS-55, high fructose corn syrup 55; ddH_2O, deionized distilled water.

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Table 4. The Effect of Providing Different Caloric Sweetened Solutions to Female on Hormones, Organ Weight, and Serum Clinical Chemistry Measurements*

Treatment	ddH_2O $n = 6$	Glucose $n = 6$	Sucrose $n = 7$	Fructose $n = 6$	HFCS-55 $n = 6$
Hormones					
Serum insulin (pmol/ml)	402 ± 36[a]	574 ± 36[ab]	566 ± 31[ab]	516 ± 36[ab]	688 ± 57[b]
Leptin (nmol/L)	0.20 ± 0.02[a]	0.18 ± 0.02[a]	0.23 ± 0.04[ab]	0.19 ± 0.04[a]	0.34 ± 0.02[b]
Testosterone (nmol/L)	0.29 ± 0.01	0.26 ± 0.01	0.29 ± 0.01	0.26 ± 0.01	0.26 ± 0.01
Estradiol (pmol/L)	117.3 ± 19.4	85.8 ± 8.9	109.6 ± 20.5	114.5 ± 26.7	105.9 ± 14.7
Organ weights					
Ovarian weight (g)	0.12 ± 0.01	0.12 ± 0.01	0.11 ± 0.01	0.12 ± 0.01	0.11 ± 0.01
Relative ovarian weight (mg/g bw)	0.51 ± 0.06	0.46 ± 0.03	0.44 ± 0.02	0.44 ± 0.02	0.41 ± 0.02
Uterine weight (g)	0.34 ± 0.04	0.36 ± 0.03	0.31 ± 0.03	0.37 ± 0.03	0.35 ± 0.04
Relative uterine weight (mg/g bw)	1.39 ± 0.15	1.49 ± 0.15	1.42 ± 0.12	1.21 ± 0.09	1.34 ± 0.16

* Values are expressed as the mean ± SEM of $n = 6$–7 rats/group. Only rats confirmed to be in the estrus phase on the morning of dissection were used in the analysis. Of the $n = 44$ rats in the study only $n = 31$ rats were used in these analyses. Different letters within the same rows indicate significant differences at $P < 0.05$ by one-way ANOVA followed by Tukey's test. HFCS-55, high fructose corn syrup 55; ddH_2O, deionized distilled water.

body weights increased in the order of HFCS-55 > fructose > sucrose > glucose suggesting more rapid weight gain in rats drinking HFCS-55. Therefore, weight gain may be a more complex issue than the overconsumption of calories.

The shift from use of sucrose to HFCS-55 as a sweetener in soft drinks by manufacturers is often blamed for the obesity epidemic (26–27). There is considerable evidence showing fructose metabolism favors de novo lipogenesis (8, 28); however, in our study, fat mass was not greater in rats drinking pure fructose solution. This may be because fructose absorption has been reported to be enhanced in the presence of glucose (29). HFCS is comprised of fructose and glucose and therefore, compositionally, HFCS is more similar to sucrose than fructose (5). In our study, rats drinking HFCS-55, but not sucrose solution, had a greater fat mass compared to rats drinking ddH_2O and glucose. HFCS-55 consists of 55% fructose (6) and this slightly higher ratio of fructose may promote adiposity compared to sucrose which consists of 50% fructose. Metabolically, the carbons from fructose enter glycolysis downstream from the rate-limiting enzyme phosphofructokinase. This enables fructose to provide an unregulated source of three-carbon molecules for the synthesis of glycerol and fatty acids favoring triglyceride production. Triglycerides are packaged in VLDLs that in the circulation can be hydrolyzed by LPL and taken up by adipose tissue, thereby promoting adiposity (30). To determine whether uptake of triglycerides by the adipose tissue contributed to fat mass, we measured circulating triglyceride and gene expression of PPARγ and LPL in the fat pads. PPARγ is highly expressed by adipose tissue and participates in the transcriptional activation of numerous lipogenic genes, including LPL (31). LPL is a key enzyme in lipoprotein clearance and tissue uptake of fatty acids (32). In our study, consumption of HFCS-55 sweetened solution by rats did not promote higher circulating levels of triglycerides or upregulation of adipose PPARγ and LPL mRNA expression. The mechanism is likely complex as there are numerous mechanisms capable of promoting adiposity such as increased glucose flux and triglyceride biosynthesis within the adipose tissue (33). Future studies using pair-feeding of animals to provide isocaloric intakes are required to address whether certain sugar sweeteners independent of energy intake are more capable of promoting lipogenesis.

Adipose tissue is now recognized as an endocrine organ capable of secreting numerous adipokines that influence energy homeostasis. This includes regulation of energy intake as well as energy expenditure. In our study, differences in the rates of weight gain in the absence of differences in energy intake suggest that the type of sweeteners may influence energy expenditure. However, the present study focused on energy intake and did not measure energy expenditure. Of the various adipokines, we focused on leptin due to its role in adiposity as well as reproduction. Fructose has been suggested to promote the development of adiposity due to the absence of leptin stimulation (28). In our study, there were no significant differences in circulating leptin concentrations among the treatment groups; however, leptin expression was greater in rats drinking HFCS-55 solution compared to rats drinking ddH_2O, glucose or sucrose. Leptin expression correlates to the amount of stored triglyceride in the adipose tissue (34) and the increased leptin mRNA abundance in gonadal fat pads of rats drinking HFCS-55 solution may simply reflect a greater fat mass.

Numerous adverse health consequences have been attributed to increased fat mass associated with consuming high sugar diets. Raising the sugar content of the diet has been reported to result in dyslipidemia indicated by elevated serum triglyceride, cholesterol, and LDL (35). In our study, there were no significant differences in serum triglyceride,

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cholesterol, VLDL, LDL or HDL concentrations among any of the treatment groups. Ingestion of sucrose sweetened water has also been observed to increase body weight and to induce glucose intolerance in rats that were not genetically susceptible to obesity or diabetes (36). Studies showed that high fructose diets promoted insulin resistance (37) and elevated C-peptide concentrations (38). In our study, there were no significant differences in fasting glucose, insulin, or C-peptide among the rats drinking the different caloric sweetened solutions. Most studies reporting metabolic disorders have used male rats. Horton *et al*. (39) reported that young male but not female rats fed high sucrose developed insulin resistance. At 8 weeks, the ability of insulin to suppress hepatic glucose in female rats was reduced although this was not statistically significant (39). Therefore, metabolic disorders may be evident only after over a longer duration since metabolic changes become more pronounced over time.

Increased fat mass may also increase reproductive disorders. In a human study, Lake *et al*. (15) reported higher body mass index was associated with increased menstrual problems in adolescent girls. In our animal study, rats drinking caloric sweetened solutions had no effect on the number of estrous cycles. However, estrous cycles 6 and 7 were lengthened in rats drinking the HFCS-55 solution. Lengthened estrous cycles were due to prolonged time in the estrus phase suggesting delayed ovulation. Inhibition of ovulation in gonadotropin primed immature animals by systemic administration of leptin suggests that the primary effect of leptin is on the ovaries (40). In our study, rats euthanized in the estrus phase showed elevated serum leptin in animals drinking HFCS-55 solution, but there was no significant effect on ovarian weight or circulating estradiol concentrations. Duggal *et al*. (40) reported that leptin induced fewer ovulations without changing ovarian weight or steroid concentrations. Therefore, a direct effect of leptin on the ovaries in our study could not be completely ruled out. Hyperleptinemia has also been shown to interfere with gonadotrophin releasing hormone (GnRH), which blunts the feedback mechanism that triggers ovulation, resulting in prolonged estrus (41). The mechanism responsible for altered reproduction in rats drinking HFCS-55 is an area requiring further studies.

In summary, adding caloric sweeteners to water at the dose in soft drinks resulted in higher caloric consumption by rats that were not fully compensated by reducing solid food intake. There were no differences in total energy intake or feed efficiency among rats drinking fructose, sucrose or HFCS-55 solution. Yet, the type of caloric sweetener added to beverages influenced body weight and fat mass. Although compositionally similar to sucrose, only HFCS-55 promoted adiposity in rats compared to the ddH_2O and glucose solution. This may have occurred because drinking HFCS-55 solution comprised of 55% fructose compared to the 50% in sucrose promoted faster body weight gain. Adiposity was not accompanied by metabolic disorders, but reproductive cycles were lengthened. Based on this study, the type of added caloric sweetener should be considered when making dietary recommendation. However, additional mechanistic studies and longer studies are needed to determine if faster body weight gain observed in the HFCS-55 group promotes the development of obesity and whether altered reproductive cycles result in reproductive dysfunction.

1. Bessesen DH. Update on obesity. J Clin Endocrinol Metab 93:2027–2034, 2008.
2. Pereira MA. The possible role of sugar-sweetened beverages in obesity etiology: a review of the evidence. Int J Obes 30 Suppl:28S–36S, 2006.
3. Harrington S. The role of sugar-sweetened beverage consumption in adolescent obesity: a review of the literature. J Sch Nurs 24:3–12, 2008.
4. Malik VS, Schulze MB, Hu FB. Intake of sugar-sweetened beverages and weight gain: a systematic review. Am J Clin Nutr 84:274–288, 2006.
5. Melanson KJ, Angelopoulos TJ, Nguyen V, Zukley L, Lowndes J, Rippe JM. High-fructose corn syrup, energy intake, and appetite regulation. Am J Clin Nutr 88 Suppl:1738S–1744S, 2008.
6. White JS. Straight talk about high-fructose corn syrup: what it is and what it ain't. Am J Clin Nutr 88 Suppl:1716S–1721S, 2008.
7. Drewnowski A, Bellisle F. Liquid calories, sugar, and body weight. Am J Clin Nutr 85:651–661, 2007.
8. Havel PJ. Dietary fructose: implications for dysregulation of energy homeostasis and lipid/carbohydrate metabolism. Nutr Rev 63:133–157, 2005.
9. Toida S, Takahashi M, Shimizu H, Sato N, Shimomura Y, Kobayashi I. Effect of high sucrose feeding on fat accumulation in the male Wistar rat. Obes Res 4:561–568, 1996.
10. Kanarek RB, Marks-Kaufman R. Developmental aspects of sucrose-induced obesity in rats. Physiol Behav 23:881–885, 1979.
11. Kanarek RB, Orthen-Gambill N. Differential effects of sucrose, fructose and glucose on carbohydrate-induced obesity in rats. J Nutr 112:1546–1554, 1982.
12. Jurgens H, Haass W, Castaneda TR, Schurmann A, Koebnick C, Dombrowski F, Otto B, Nawrocki AR, Scherer PE, Spranger J, Ristow M, Joost HG, Havel PJ, Tschöp MH. Consuming fructose-sweetened beverages increases body adiposity in mice. Obes Res 13:1146–1156, 2005.
13. DiMeglio DP, Mattes RD. Liquid versus solid carbohydrate: effects on food intake and body weight. Int J Obes Relat Metab Disord 24:794–800, 2000.
14. Diamanti-Kandarakis E, Kandaraskis HA. Obesity and gonadal function of women. Ped Endocrinol Rev 1 Suppl 3:465S–470S, 2004.
15. Lake JK, Power C, Cole TJ. Women's reproductive health—the role of body mass index in early and adult life. Int J Obes 21:432–438, 1997.
16. Storey ML, Forshee RA, Anderson PA. Beverage consumption in the US population. J Am Diet Assoc 106:1992–2000, 2006.
17. National Research Council. Guide for the care and use of laboratory animals. Washington, DC: National Research Council, pp8–65, 1996.
18. Reeves PG. Components of the AIN-93 diets as improvements in the AIN-76A diet. J Nutr 127 Suppl:838S–841S, 1997.
19. National Research Council. Nutrient requirements of laboratory animals, 3rd rev. Washington, DC: National Research Council, pp3–79, 1995.
20. Tou JC, Grindeland RE, Wade, CE. Effects of diet and exposure to hindlimb suspension on estrous cycling in Sprague-Dawley rats. Am J Physiol Endocrinol Metab 286:E425–E433, 2004.
21. Felmlee MA, Woo G, Simko E, Krol ES, Muir AD, Alcorn J. Effects of the flaxseed lignans secoisolariciresinol diglucoside and its aglycone on

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serum and hepatic lipids in hyperlipidaemic rats. Br J Nutr 16:1–9, 2009.
22. de Moura RF, Ribeiro C, deOliveira JA, Stevanato E, de Mello MA. Metabolic syndrome signs in Wistar rats submitted to different high-fructose ingestion protocols. Br J Nutr 14:1–7, 2008.
23. Guevara R, Valle A, Gianotti M, Roca P, Oliver J. Gender-dependent differences in serum profiles of insulin and leptin in caloric restricted rats. Horm Metab Res 40:38–43, 2008.
24. Eckel LA, Moore SR. Diet-induced hyperphagia in the rat is influenced by sex and exercise. Am J Physiol Regul Integr Comp Physiol 287: R1080–R1085, 2004.
25. Tordoff MG, Alleva AM. Effect of drinking soda sweetened with aspartame or high-fructose corn syrup on food intake and body weight. Am J Clin Nutr 51:963–969, 1990.
26. Bray GA, Nielsen SJ, Popkin BM. Consumption of high-fructose corn syrup in beverages may play a role in the epidemic of obesity. Am J Clin Nutr 79:537–543, 2004.
27. Brown CM, Dulloo AG, Montani JP. Sugary drinks in the pathogenesis of obesity and cardiovascular diseases. Int J Obes (Lond) 32 Suppl 6: S28–S34, 2008.
28. Lê KA, Tappy L. Metabolic effects of fructose. Curr Opin Clin Nutr Metab Care 9:469–475, 2006.
29. Riby JE, Fujisawa T, Kretchmer N. Fructose absorption. Am J Clin Nutr 58 Suppl 5:748S–753S, 1993.
30. Rutledge AC, Adeli K. Fructose and the metabolic syndrome: pathophysiology and molecular mechanisms. Nutr Rev 65:S13–S23, 2007.
31. Ferre P. The biology of peroxisome proliferator-activated receptors: relationship with lipid metabolism and insulin sensitivity. Diabetes 53 Suppl 1:43S–50S, 2004.
32. Merkel M, Eckel RH, Goldberg IJ. Lipoprotein lipase: genetics, lipid uptake, and regulation. J Lipid Res 43:1997–2006, 2002.
33. Wagner EM, Kratky D, Haemmerle G, Hrzenjak A, Kostner GM, Steyrer E, Zechner R. Defective uptake of triglyceride-associated fatty acids in adipose tissue causes the SREBP-1c-mediated induction of lipogenesis. J Lipid Res 45:356–365, 2004.
34. Maffei M, Halaas J, Ravussin E, Pratley RE, Lee GH, Zhang Y, Fei H, Kim S, Lallone R, Ranganathan S, Kern PA, Friedman JM. Leptin levels in human and rodent: measurement of plasma leptin and ob mRNA in obese and weight-reduced subjects. Nat Med 1:1155–1161, 1995.
35. Fried SK, Rao SP. Sugars, hypertriglyceridemia, and cardiovascular disease. Am J Clin Nutr 78:873–880, 2003.
36. Kawasaki T, Kashiwabara A, Sakai T, Igarashi K, Ogata N, Watanabe H, Ichiyanagi K, Yamanouchi T. Long-term sucrose drinking causes increased body weight and glucose intolerance in normal male rats. Br J Nutr 93:613–618, 2005.
37. Stanhope K, Havel P. Endocrine and metabolic effects of consuming beverages sweetened with fructose, glucose, sucrose, or high-fructose corn syrup. Am J Clin Nutr 88 Suppl:1733S–1737S, 2008.
38. Wu T, Giovannucci E, Pischon T, Hankinson SE, Ma J, Rifai N, Rimm EB. Fructose, glycemic load, and quantity and quality of carbohydrate in relation to plasma C-peptide concentration in US women. Am J Clin Nutr 80:43–49, 2004.
39. Horton TJ, Gayles EC, Prach PA, Koppenhafer TA, Pagliassotti MJ. Female rats do not develop sucrose-induced insulin resistance. Am J Physiol Regul Integr Comp Physiol 272:1571–1576, 1997.
40. Duggal PS, Van Der Hoek KH, Milner CR, Ryan NK, Armstrong DI, Magoffin DA, Norman RJ. The in vivo and in vitro effects of exogenous leptin on ovulation in the rat. Endocrinology 141:1971–1976, 2000.
41. Tortoriello DV, McMinn J, Streamson CC. Dietary-induced obesity and hypothalamic infertility in female DBA/2J mice. Endocrinology 145: 1238–1247, 2004.

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