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The effect of feeding different sugar-sweetened beverages to growing female Sprague–Dawley rats on bone mass and strength ☆

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Abstract

Consumption of sugar beverages has increased among adolescents. Additionally, the replacement of sucrose with high fructose corn syrup (HFCS) as the predominant sweetener has resulted in higher fructose intake. Few studies have investigated the effect of drinking different sugar-sweetened beverages on bone, despite suggestions that sugar consumption negatively impacts mineral balance. The objective of this study was to determine the effect of drinking different sugar-sweetened beverages on bone mass and strength. Adolescent (age 35d) female Sprague–Dawley rats were randomly assigned (n=8–9/group) to consume deionized distilled water (ddH_2O, control) or ddH_2O containing 13% w/v glucose, sucrose, fructose or high fructose corn syrup (HFCS-55) for 8weeks. Tibia and femur measurements included bone morphometry, bone turnover markers, determination of bone mineral density (BMD) and bone mineral content (BMC) by dual energy X-ray absorptiometry (DXA) and bone strength by three-point bending test. The effect of sugar-sweetened beverage consumption on mineral balance, urinary and fecal calcium (Ca) and phosphorus (P) was measured by inductively coupled plasma optical emission spectrometry. The results showed no difference in the bone mass or strength of rats drinking the glucose-sweetened beverage despite their having the lowest food intake, but the highest beverage and caloric consumption. Only in comparisons among the rats provided sugar-sweetened beverage were femur and tibia BMD lower in rats drinking the glucose-sweetened beverage. Differences in bone and mineral measurements appeared most pronounced between rats drinking glucose versus fructose-sweetened beverages. Rats provided the glucose-sweetened beverage had reduced femur and tibia total P, reduced P and Ca intake and increased urinary Ca excretion compared to the rats provided the fructose-sweetened beverage. The results suggested that glucose rather than fructose exerted more deleterious effects on mineral balance and bone.

Keywords: Glucose; Fructose; High fructose corn syrup; Bone mineral density; Calcium

Introduction

In the absence of a cure for osteoporosis, emphasis is placed on prevention. A major preventive measure is to maximize peak bone mass (PBM) during the bone formation stage. During childhood and puberty, bone formation exceeds bone resorption resulting in bone mass acquisition. After this stage, bone resorption exceeds bone formation resulting in steady bone loss [1]. Therefore, to prevent future risk of osteoporosis it is important to identify modifiable factors that may improve PBM during this critical stage of bone acquisition [2].

Nutrition is an important modifiable factor that can influence PBM. Dietary approaches to prevent bone loss have focused primarily on calcium (Ca) intake and factors that affect Ca balance. Several studies have reported that sugar consumption displaces consumption of Ca rich foods or negatively impacts Ca balance [3–5]. It is important to determine the effect of sugar consumption on bone because over the past 20 years the consumption of added sugars has increased [6] with the largest

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source of added sugars being non-diet beverages [7]. Among children and adolescents in the US, sugar-sweetened beverages have assumed an increasingly significant proportion of the total energy intake [7,8].

In addition, we investigated different sugars because the type of sugar added to beverages has changed from sucrose consisting of 50% fructose to high fructose corn syrup (HFCS) [9]. The three major forms of HFCS are HFCS-42, HFCS-55, and HFCS-90. The form primarily utilized in the US beverage industry is HFCS-55 which is comprised of 55% fructose, 42% glucose and 3% higher saccharides [10]. However, few studies have investigated the impact of drinking sugar-sweetened beverages on bone despite continual rising sugar intake and a shift in the type of sweeteners from sucrose to HFCS.

Studies have reported an association of soft drink consumption with low bone mineral density (BMD) and fractures in adolescent girls [11–13]. Despite the high sugar content of soft drinks, attention has mainly been given to other ingredients in soft drinks such as: phosphoric acid and caffeine. Studies that directly determine the effect of sugar consumption on bones and the potential mechanisms whereby sugar may affect bone are needed. A better understanding of the role of sugar consumption on bone will assist in the determination of dietary recommendations to reduce the future risk of osteoporosis. The objective of this study was to determine the effect of providing growing female rats beverages containing different sugars, at the level present in soft drinks, on bone mineralization and bone strength. Providing sugar in the drinking water rather than in the diet provides caloric intake that is devoid of nutrients and matches the situation of increased sugar intake in beverage form by humans.

Materials and methods

Animal model and diets

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 [14] and were approved by the Animal Care and Use Committee at West Virginia University. Immature (age 28d) female Sprague–Dawley rats ($n=44$) were purchased from Taconic Farms (Rockville, MD). Upon arrival, animals were housed in a room kept at 21°C with a 12h light/dark cycle and individually caged in metabolic cages for the mineral balance study.

All animals were given *ad libitum* access to deionized distilled water (ddH_2O, control) and the American Institute of Nutrition 93 G (AIN-93G) diet (Harlan Teklad, WI). The ddH_2O was used because it does not contain minerals. The AIN-93G diet was fed because it is a standard diet that meets the National Research Council [15] nutrient requirements for growing rats and is a purified diet with defined ingredient sources and precise levels of sucrose and minerals.

After a 7 d acclimation, animals were randomly assigned ($n=8$–9 rats/group) to drink ddH_2O (control) or ddH_2O containing 13% w/v sugar. We studied adolescent rats (age 35d), a stage of active skeletal growth to match the situation of increased sugar-sweetened beverage consumption by humans during the bone acquisition stage of childhood and adolescence. The level of 13% was chosen because this is the sugar content of most soft drinks [16]. Sugars were added as glucose, sucrose, fructose or HFCS-55 (Cornsweet®55, Archer Daniels Midland, IL). Rats were given free access to the powdered AIN-93G diet as well as to their assigned sugar-sweetened beverage throughout the 8 week feeding study. Food and beverage intake were measured twice weekly and replaced with fresh diet and their respective assigned beverage. Body weight was measured weekly.

Bone morphometry, bone mineral area, bone mineral content (BMC) and BMD

At the end of the 8week feeding study, rats were euthanized by CO_2 inhalation. The femur and tibia were collected. The bones were defleshed with care being taken not to damage the periosteum. Each bone was individually wrapped in saline soaked gauze and stored at − 20°C. For analysis, each bone was brought to room temperature and the bones weighed. Bone length, width and depth were determined using a vernier caliper.

BMD, BMC and area of the whole bone and the distal diaphysis and proximal region of both the tibia and femur were determined using dual energy X-ray absorptiometry (DXA). The femur and tibia were placed in a dish of deionized water and scanned using DXA equipped with a small animal high resolution collimator (Hologic Delphi A, Bedford, MA). The coefficient of variance for total BMD and BMC was 0.12%. Bone regions were identified according to Gouveia et al. [17]. Proximal and distal regions of the tibia and femur were determined as one-fourth of the total bone length and the diaphysis as half of the total bone length.

Bone biomechanics

Bone strength parameters were assessed using a TA.HDi Texture Analyzer (Texture Technologies Corp, NY) outfitted with a three-point bending apparatus. Femora and tibiae were placed on supports (1mm width at tip) and bent until broken by lowering a centrally placed blade (1mm width) at constant crosshead speed (0.1mm/s). The load-deflection data collected by a PC interfaced with the TA.HDi Texture Analyzer was used to determine bone biomechanical measurements of peak force, bending failure energy, ultimate stiffness, ultimate bending stress (UBS) and Young's modulus as described in Yuan and Kitts [18].

Bone turnover markers

Following euthanasia, the chest cavity was opened and the aorta punctured to collect blood. Blood was centrifuged at 1500×*g* for 10min at 4°C and serum collected. Serum samples were stored at − 80°C until assayed. Serum alkaline phosphatase (ALP) was measured by Vet 16 rotor using a Hemagen Analyst automated spectrophotometer (Hemagen Diagnostics Inc., Columbia, MD). Serum osteocalcin was determined as an indicator of osteoblast activity using a commercially available rat specific enzyme immunoassay (EIA) (Biomedical Technologies, Stoughton, MA). Deoxypyridinoline (DPD) in the urine was determined as an indicator of osteoclast activity using a commercially available rat specific EIA (Quidel Corp, San Diego, CA). Optical density for serum osteocalcin was measured at 450nm and urinary DPD at 405nm using a Spectramax Plus microplate reader (Molecular Devices, Sunnyvale, CA).

Mineral intake and balance

Rats were individually housed in metabolic cages to determine food and water intake and to collect feces and urine. Ca and P intake were calculated from known concentrations provided by the purified AIN-93G diet. Ca intake was calculated as food intake (g)×0.5% Ca. P intake was calculated a food intake (g)×0.3% P. Ca apparent absorption was calculated as (Ca intake − fecal Ca excretion) / Ca intake×100. Calcium retention was determined by calculating Total Ca intake − (fecal Ca excretion+urinary Ca excretion). P absorption and retention was calculated using the same formulas as used for Ca.

Baseline and final fecal and urine samples were collected. Baseline fecal and urine consisted of samples pooled over the 7 d of the acclimation period. Final fecal and urine specimens consisted of samples pooled over the final 7 d of the 8 week feeding study. For urine collection, ascorbic acid (0.1%) was added to the collection tubes as a preservative and 1mL mineral oil to prevent evaporation. Collected urine samples were centrifuged at 1500×*g* for 10min at 4°C. Following centrifugation, urine samples were aliquoted into fresh tubes and stored at − 20°C until assayed. Fecal samples were freeze–dried for 48h then stored at − 20°C until assayed.

For the determination of bone and fecal Ca and P content, feces were freeze–dried and bone fragments were dried at 110°C for 48h. Dried bones and freeze–dried fecal samples were ashed by placing the samples in a muffle furnace at

600°C for 24h. To measure fecal, urine and bone Ca and P content, samples were dissolved in 2mL of 70% nitric acid. The acidified samples were neutralized in 5mL of ddH_2O and filtered through Whatman no. 1 paper. Samples were then diluted to volume with ddH_2O in a 50mL volumetric flask. Ca and P concentrations were measured using an inductively coupled plasma optical emission spectrometry (model P400 Perkin Elmer, Shelton, CT).

Urinary Ca and P values were corrected for creatinine. Kidney function was determined by assessing blood urea nitrogen (BUN), creatinine, BUN/creatinine ratio, total protein, albumin, serum Ca and P by the Vet 16 rotor using the Hemagen Analyst automated spectrophotometer (Hemagen Diagnostics Inc., Columbia, MD).

Statistical analyses

A 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 $P < 0.05$. All statistical analyses were done using the statistical software SigmaStat 3.1 (Systat Software Inc., San Jose, CA).

Results

Energy intake and body weight

As shown in Table 1, rats provided the sugar-sweetened beverages had increased caloric intake compared to the animals drinking ddH_2O. Rats provided glucose or sucrose-sweetened beverages drank more ($P<0.001$), but had reduced food intake compared to the rats drinking ddH_2O. Rats provided the HFCS-55 sweetened beverages had reduced food intake ($P<0.001$), but did not drink significantly more than rats provided ddH_2O. Only the rats drinking the HFCS-55 sweetened beverage had a greater ($P<0.05$) final body weights than the rats drinking ddH_2O.

Among the rats drinking sugar-sweetened beverages, the animals provided the glucose-sweetened beverages had the highest ($P<0.001$) beverage intake and greatest ($P<0.001$) caloric intake. Food intake was lower in rats drinking the glucose compared to rats drinking the fructose and HFCS-55, but not the sucrose-sweetened beverage. Rats provided the sucrose-sweetened beverage consumed less food, but drank more and had higher caloric intakes than the rats provided fructose-sweetened beverage. Rats drinking the HFCS-55 sweetened beverage had lower ($P<0.05$) caloric intake, but greater ($P<0.05$) body weight than the rats drinking the glucose-sweetened beverage.

Bone morphometry

Table 1 shows that relative femur weight was lower ($P=0.04$) in rats provided glucose and the HFCS-55 sweetened beverages compared to animal fed ddH_2O, but there were no significant differences in the other femur morphometry measurements. Among the rats drinking sugar-sweetened beverages, the animals provided the glucose-sweetened beverage had lower ($P=0.03$) femur weights than animals drinking the fructose-sweetened beverage. There were no significant differences in tibia relative weight or other morphometry measurements among the treatment groups (Table 2).

BMC

According to Table 1, there was no significant difference in the BMC of the whole femur in rats drinking sugar-sweetened

Table 1
Body weight, food, beverage and energy intake, femur morphometry, mineral content and mineral density of the whole bone and of the different femur regions in growing female rats provided different sugar-sweetened beverages[§]

Treatment	Control	Glucose	Fructose	Sucrose	HFCS-55
Initial body mass (g)	80.2±2	82.5±4	81.3±3	88.2±3	86.4±4
Final body mass (g)	256±5	256±3	276±5	274±3	283±10[ab]
Beverage intake (mL/d)	29.3±3.1	81.8±5.8[a]	26.6±4.0[b]	54.0±4.9[abc]	44.4±3.7[b]
Food intake (g/d)	15.6±0.5	8.9±0.5[a]	13.9±0.3[b]	10.4±0.6[ac]	12.2±0.6[ab]
Energy intake (kcal/d)	48.1±13.6	346.2±23.7[a]	133.4±17.7[ab]	238.6±20.4[abc]	166.1±12.8[abc]
Femur morphometry					
Length (mm)	28.04±0.23	28.25±0.28	28.34±0.34	28.47±0.18	29.92±0.25
Width (mm)	3.64±0.06	3.59±0.06	3.72±0.07	3.70±0.07	3.64±0.05
Depth (mm)	2.96±0.05	2.93±0.04	3.06±0.07	3.04±0.03	2.98±0.06
Weight (mg)	572±17	539±9	593±16[b]	584±9	565±9
Relative Weight (mg/g bwt)	2.23±0.03	2.10±0.04[a]	2.15±0.02	2.13±0.03	2.00±0.05[a]
BMC (mg)					
Whole	289±14	270±5	308±9[b]	306±6[b]	299±6
Proximal	64±3	51±6	69±3[b]	62±3	63±2
Diaphysis	131±7	131±4	148±5	149±4[ab]	141±5
Distal	103±9	91±3	100±3	100±3	102±3
BMD (mg/cm^2)					
Whole	219±6	209±2	226±2[b]	225±3[b]	227±3[b]
Proximal	204±6	186±4	211±2[b]	211±5[b]	212±5[b]
Diaphysis	216±9	207±2	219±3	217±3	222±3
Distal	239±6	232±2	249±4[b]	248±4[b]	246±3

[§]Values are expressed as mean±SEM of $n=8$–9 rats/group. HFCS-55, high fructose corn syrup 55; BMC, bone mineral content; BMD, bone mineral density. Letters a = $P<0.05$ vs control, b = $P<0.05$ vs glucose, c = $P<0.05$ vs fructose by one-way ANOVA followed by Tukey's test.

Table 2
Tibia morphometry, mineral content and mineral density of the whole bone and of the different tibia regions in growing female rats provided different sugar-sweetened beverages[§]

Treatment	Control	Glucose	Fructose	Sucrose	HFCS-55
Tibia morphometry					
Length (mm)	34.88±0.35	34.88±0.22	35.13±0.24	35.23±0.15	34.98±0.20
Width (mm)	3.80±0.04	3.78±0.04	3.88±0.07	3.91±0.05	3.78±0.07
Depth (mm)	2.93±0.05	2.72±0.07	2.87±0.09	2.83±0.08	2.83±0.09
Weight (mg)	417±13	396±8	439±13	434±7	420±13
Relative weight (mg/g bwt)	1.63±0.02	1.55±0.04	1.59±0.02	1.58±0.03	1.49±0.05
BMC (mg)					
Whole	205±8	194±5	220±7[b]	219±6	211±6
Proximal	49±2	44±2	49±2	45±6	42±5
Diaphysis	94±5	93±4	104±6	103±4	106±5
Distal	71±3	61±4	76±3	71±4	69±3
BMD (mg/cm^2)					
Whole	179±3	171±1	186±3[b]	185±3[b]	185±2[b]
Proximal	185±4	177±2	194±6	191±3	187±5
Diaphysis	165±3	162±1	171±3	172±3[b]	170±2
Distal	196±6	182±3	206±2[b]	204±3[b]	211±2[b]

[§]Values are expressed as mean±SEM of $n=8$–9 bones/group. HFCS-55, high fructose corn syrup 55; BMC, bone mineral content; BMD, bone mineral density. Letters a = $P<0.05$ vs control, b = $P<0.05$ vs glucose by one-way ANOVA followed by Tukey's test.

beverages compared to the ddH_2O control. Among the rats drinking sugar-sweetened beverages, the BMC of the whole femur was lower ($P=0.02$) in rats drinking the glucose compared to the sucrose and fructose-sweetened beverages, but did not differ from animals drinking the HFCS-55 sweetened beverage. There were no significant differences in the area of the whole femur among the treatment groups (data not shown).

As shown in Table 1, there were no significant differences in the distal femoral BMC between any of the treatment groups but the proximal femoral BMC was lower ($P=0.02$) in the rats drinking the glucose compared to the fructose-sweetened beverage. The BMC of the femoral diaphysis was higher ($P=0.03$) in the rats drinking sucrose-sweetened beverage compared to the rats drinking the glucose-sweetened beverage and the ddH_2O control. There were no significant differences in the area of the distal, diaphysis or proximal femur among the treatment groups (data not shown).

Table 2 presents the results for the tibia. There were no significant differences in the BMC of the whole tibia between rats drinking sugar-sweetened beverages compared to the ddH_2O control. Among the rats provided sugar-sweetened beverages, BMC of the whole tibia was lower ($P=0.04$) in rats drinking the glucose compared to the fructose-sweetened beverage. There were no significant differences in the tibia proximal, diaphysis and distal BMC among the treatment groups. There were no significant differences in area of the whole, distal, diaphysis or proximal tibia among the treatment groups (data not shown).

BMD

Table 1 shows that in the femur, there were no significant differences in the BMD of the whole femur between rats drinking sugar-sweetened beverages compared to the ddH_2O control. Among the rats drinking sugar-sweetened beverages, the BMD of the whole femur was lower ($P=0.005$) in the rats drinking the glucose-sweetened beverage compared to all the other sugar-sweetened beverage groups.

There were no significant differences in the femur proximal, diaphysis or distal BMD between rats drinking sugar-sweetened beverages and the ddH_2O control. Among the rats provided sugar-sweetened beverages, the rats drinking the glucose-sweetened beverage had lower ($P=0.02$) proximal femur BMD compared to rats drinking the sucrose, fructose or HFCS-55 sweetened beverages. BMD in the distal femur was lower ($P=0.01$) for rats drinking the glucose compared to the sucrose and fructose-sweetened beverages. There were no significant differences in femur diaphysis BMD among any of the treatment groups.

Table 2 shows that in the tibia, there were no significant differences in the BMD of the whole tibia between rats drinking sugar-sweetened beverages compared to the ddH_2O control. Among the rats provided sugar-sweetened beverages, rats drinking the glucose-sweetened beverage had lower ($P<0.001$) BMD of the whole tibia compared to the rats drinking the other sugar-sweetened beverages.

There was no significant difference in the BMD of the proximal, diaphysis and distal tibia between rats drinking sugar beverages compared to the ddH_2O control. Among rats provided sugar-sweetened beverages, rats drinking the glucose-sweetened beverage had lower ($P=0.001$) BMD in the distal tibia compared to the other sugar-sweetened beverage groups. BMD in the tibia diaphysis was lower ($P=0.01$) in the rats drinking the glucose compared to the sucrose-sweetened beverage. No differences were detected in the BMD of the proximal tibia among any of the treatment groups.

Bone biomechanical strength

Table 3 shows that there were no significant differences in bone strength as determined by peak force, ultimate stiffness,

Table 3
Femur and tibia biomechanical strength measurements in growing female rats provided different sugar-sweetened beverages[§]

Treatment	Control	Glucose	Fructose	Sucrose	HFCS-55
Femur					
Peak force (N)	161.0±16.4	155.3±9.5	155.1±10.5	177.0±6.9	155.2±10.2
Ultimate stiffness (N/s)	1630.5±113.2	1313.9±167.3	1227.5±176.5	1447.1±135.5	1321.5±183.9
Bending failure energy (N·s)	9.4±1.6	9.4±1.2	9.3±1.3	12.2±1.1	8.3±1.1
Ultimate bending stress (N/mm^2)	197.8±20.9	215.8±12.9	197.1±15.4	226.2±18.3	203.7±15.8
Young's modulus (N/mm^2)	2266.5±266.2	2900.8±208.9	2304.1±230.1	2762.5±203.4	2535.3±268.5
Tibia					
Peak force (N)	78.2±2.9	68.7±2.6	82.2±5.2	76.4±2.3	76.8±2.5
Ultimate stiffness (N/s)	506.5±34.2	462.2±17.5	516.1±47.7	470.9±21.7	527.8±21.6
Bending failure energy (N·s)	7.5±0.8	5.6±0.6	7.9±0.7	7.6±0.8	6.5±0.4
Ultimate bending stress (N/mm^2)	131.3±8.1	143.0±11.0	138.0±7.3	134.4±5.9	131.7±5.2
Young's modulus (N/mm^2)	3771.6±542.6	5909.4±865.1	3968.8±432.5	4106.5±383.4	3877.0±447.0

[§]Values are expressed as the mean±SEM of $n=8$–9 bones/group. HFCS-55, high fructose corn syrup 55.

bending failure energy, ultimate bending stress and Young's modulus in the femur or tibia among any of the treatment groups.

Ca intake and balance

Table 4 shows that there was no difference in fecal and urinary output or Ca homeostasis between the treatment groups at baseline. During the final week, the rats drinking sugar-sweetened beverages had lower ($P<0.001$) Ca intake than the rats drinking ddH_2O. The rats drinking glucose or sucrose-sweetened beverages had significantly higher apparent Ca absorption compared to the rats drinking ddH_2O. There was no statistical difference in final fecal output among the treatment groups. However, fecal Ca excretion was lower ($P=0.01$) in the rats drinking sugar-sweetened beverages compared to the rats drinking ddH_2O. Rats provided the glucose, sucrose and HFCS-55 sweetened beverages had higher ($P<0.05$) urinary output compared to the ddH_2O control. Urinary Ca excretion was higher in the rats drinking the glucose, sucrose and HFCS-55 sweetened beverages compared to the rats provided ddH_2O. Retention of Ca was lower ($P<0.01$) in the rats drinking sugar-sweetened beverages compared to the ddH_2O control.

Among the rats provided sugar-sweetened beverages, rats drinking the glucose-sweetened beverage had lower ($P=0.001$) Ca intake and fecal Ca output compared to rats drinking the

Table 4
Urinary and fecal output, sugar, calcium intake, excretion, absorption, retention and bone calcium in growing female rats provided different sugar-sweetened beverages[§]

Treatment	Control	Glucose	Fructose	Sucrose	HFCS-55
Baseline					
Urinary output (mL/d)	3.34±0.59	5.00±0.74	2.99±0.49	3.73±0.32	3.93±0.52
Fecal output (mg/d)	1.53±0.11	1.73±0.18	1.49±0.11	1.79±0.21	1.60±0.11
Ca intake (mg/d)	70.49±2.81	70.98±3.13	70.34±3.06	72.02±3.01	73.49±3.46
Ca apparent absorption* (%)	86.61±0.65	81.14±1.66	83.58±0.93	84.55±1.36	82.33±0.98
Fecal Ca excretion (mg/d)	12.23±0.57	13.08±0.94	11.50±0.72	10.89±0.73	13.13±1.15
Urinary Ca excretion (mg/d)	0.07±0.03	0.08±0.02	0.03±0.01	0.06±0.02	0.09±0.04
Ca retention[†] (mg/d)	58.19±2.46	57.84±3.67	58.81±2.86	61.06±3.37	58.63±2.72
Final week					
Urinary output (mL/d)	11.24±1.76	49.90±4.41^{a}	9.46±2.81^{b}	22.59±2.14^{abc}	29.31±3.81^{abc}
Fecal output (mg/d)	1.99±0.13	2.54±0.16	1.91±0.10	2.46±0.18	2.47±0.21
Sugar intake (g/d)	7.21±0.11	13.20±0.18^{ab}	6.66±0.11^{b}	9.95±0.21^{abc}	8. 85±0.5^{b}
Ca intake (mg/d)	77. 87±2.40	44.59±2.46^{a}	69.66±1.34^{ab}	51. 83±3.09^{ac}	61.11±2.85^{ab}
Ca apparent absorption* (%)	69.58±1.41	80.44±2.17^{a}	72.39±1.36^{b}	77.89±2.76^{ab}	72.96±1.81^{b}
Fecal Ca excretion (mg/d)	24.12±1.64	7.77±1.54^{a}	17.55±1.36^{ab}	11.06±1.85^{ac}	14.90±1.26^{ab}
Urinary Ca excretion (mg/d)	0.28±0.09	0.98±0.43^{a}	0.22±0.12^{b}	1.42±0.54^{ac}	0.99±0.22^{ac}
Ca retention[†] (mg/d)	66.56±2.48	23.28±3.76^{a}	50.78±2.58^{ab}	37.16±3.01^{abc}	40.92±2.81^{ab}
Serum Ca (mg/dL)	9.25±0.58	9.12±0.69	8.32±0.66	8.39±0.72	8.54±0.52
Femur total Ca (mg)	24.85±0.95	25.46±1.43	25.17±0.80	24.11±0.65	24.79±0.72
Tibia total Ca (mg)	24.52±1.56	21.85±1.43	23.35±0.56	22.49±0.76	22.88±0.53

[§]Values are expressed as the mean±SEM of $n=8$–9 rats/group. Ca, calcium; HFCS-55, high fructose corn syrup 55.
Letter a = $P<0.05$ vs control, b = $P<0.05$ vs glucose, c = $P<0.05$ vs fructose by one-way ANOVA followed by Tukey's test. *Calcium apparent absorption (%)= (Ca intake − fecal Ca excretion)/Ca intake × 100. [†]Calcium retention = Total Ca intake − (fecal Ca excretion + urinary Ca excretion).

Table 5
Phosphorus intake, excretion, absorption, retention and bone phosphorus in growing female rats provided different sugar-sweetened beverages[§]

Treatment	Control	Glucose	Fructose	Sucrose	HFCS-55
Baseline					
P intake (mg/d)	44.50±1.78	44. 80±1.98	44.42±1.92	45.48±1.92	46.40±2.18
P apparent absorption* (%)	62.49±3.13	56.39±6.35	65.33±2.72	66.86±2.48	60.92±2.81
Fecal P excretion (mg/d)	8.29±0.67	9.48±1.04	7.78±0.72	7.44±0.43	9.21±1.00
Urinary P excretion (mg/d)	0.01±0.002	0.02±0.003	0.01±0.003	0.01±0.003	0.01±0.004
P retention[†] (mg/d)	13.95±0.98	12.91±1.77	14.42±0.81	15.23±1.04	13.91±0.70
Final week					
P intake (mg/d)	46.72±1.44	26.75±1.47[a]	41. 8±0.80[b]	31.10±1.85[ac]	36.67±1.71[ab]
P apparent absorption* (%)	28.91±1.53	66.84±4.39[a]	50.34±4.31	64.59±7.21[a]	49.58±5.17
Fecal P excretion (mg/d)	17.84±1.47	4.22±0.94[a]	10.07±1.16[ab]	5.81±1.54[a]	8.71±0.90[ab]
Urinary P excretion (mg/d)	1.78±0.36	1.61±0.57	0.84±0.28	2.02±0.71	3.08±0.80[c]
P retention[†] (mg/d)	5.35±1.12	6.42±0.89	9.93±1.06[a]	7.82±1.23	5.48±1.43
Serum P (mg/dL)	7.43±0.60	8.23±0.62	7.43±0.50	7.04±0.56	7.20±0.54
Femur total P (mg)	38.46±1.07	35.09±0.84	40.62±1.02[b]	39.59±0.56[b]	38.81±1.13[b]
Tibia total P (mg)	29.04±0.94	26.24±0.81	30.02±0.64[b]	29.16±0.67	29.66±0.58[b]

[§]Values are expressed as the mean±SEM of $n=8$–9 rats/group. P, phosphorus; HFC-55, high fructose corn syrup 55.
Letters a = $P<0.05$ vs control, b = $P<0.05$ vs glucose, c = $P<0.05$ vs fructose by one-way ANOVA followed by Tukey's test.
*Phosphorus apparent absorption (%)=(P intake − fecal P excretion)/P intake × 100.
[†]Phosphorus retention=Total P intake − (fecal P excretion + urinary P excretion).

fructose and HFCS-55 sweetened beverages, but did not differ from the rats consuming the sucrose-sweetened beverage. Urinary Ca excretion was higher in rats provided the glucose compared to the fructose-sweetened beverage. However, rats provided the glucose-sweetened beverage had higher apparent Ca absorption than all the other treatment groups. Ca retention was lower ($P<0.001$) in rats drinking the glucose-sweetened beverage compared to all the other treatment groups. There were no significant differences in femur or tibia total Ca content (Table 4).

P intake and balance

Table 5 shows that there was no difference in P intake and P homeostasis between the treatment groups at baseline. During the final week, the rats provided the glucose, sucrose or HFCS-55 sweetened beverages had lower ($P=0.001$) P intakes compared to the ddH_2O control. Rats drinking the glucose and sucrose-sweetened beverages had higher ($P<0.001$) apparent P absorption than the rats drinking ddH_2O. Fecal P excretion was lower ($P<0.001$) but not urinary P excretion, in rats drinking sugar-sweetened beverages compared to rats drinking ddH_2O. Retention of P was higher ($P<0.05$) in the rats drinking the fructose-sweetened beverage compared to the rats drinking ddH_2O.

Among the animals consuming sugar-sweetened beverages, rats drinking the fructose-sweetened beverage had higher P intake compared to the rats drinking glucose ($P<0.001$) and sucrose ($P=0.01$) but not HFCS-55 sweetened beverages (Table 5). There were no differences in apparent P absorption among the animals drinking sugar-sweetened beverages. Rats drinking the glucose-sweetened beverage had lower ($P=0.02$) fecal P excretion compared to rats drinking the fructose or the HFCS-55 sweetened beverage. Rats drinking the HFCS-55 sweetened beverage had higher ($P<0.05$) urinary P excretion compared to rats provided the fructose, but not the glucose or sucrose-sweetened beverages. No differences were detected in P retention among the rats drinking sugar-sweetened beverages. Femur total P content was lower in rats drinking the glucose-sweetened beverage compared to the other sugar-sweetened

Table 6
Serum biochemistry for kidney function and bone turnover markers in growing female rats provided different sugar-sweetened beverages[§]

Variable	Control	Glucose	Fructose	Sucrose	HFCS-55
Serum BUN (mg/dL)	15.49±1.39	11.79±1.48	13.12±1.95	11.27±1.23	12.71±1.10
Serum creatinine (mg/dL)	0.50±0.07	0.78±0.09	0.56±0.09	0.54±0.08	0.61±0.06
Serum BUN/creatinine	36±6	17±3	24±4	27±8	22±2
Serum total protein (g/dL)	5.71±0.33	5.41±0.45	5.26±0.46	5.70±0.47	5.18±0.36
Serum albumin (g/dL)	4.04±0.25	3.89±0.26	3.57±0.29	3.64±0.31	3.71±0.21
Bone turnover markers					
Alkaline phosphatase (U/L)	66.63±7.45	79.22±7.95	68.78±8.98	86.89±12.81	59.22±6.38
Osteocalcin (ng/mL)	1.88±0.29	1.52±0.20	2.09±0.41	1.81±0.33	1.39±0.12
Urinary DPD (nmol/mmol creatinine)	166.69±53.6	80.37±25.2	107.56±36.7	105.8±46.3	197.61±66.7

[§]Values are expressed as mean±SEM of $n=8$–9 rats group. HFCS-55, high fructose corn syrup 55; BUN, blood urea nitrogen; DPD, deoxypyridinoline.

beverages. Tibia total P content was lower in rats drinking the glucose compared to the fructose and HFCS-55 sweetened beverages (Table 5).

Serum biochemistry and bone turnover markers

Serum biochemistry for kidney function showed no significant differences in blood urea nitrogen (BUN), creatinine, BUN/creatinine ratio, total protein, or albumin concentrations among the treatment groups (Table 6).

For bone turnover markers, there were no significant differences in the urinary DPD, serum osteocalcin and ALP among any of the treatment groups (Table 6).

Discussion

In the present study, rats drinking the glucose-sweetened beverage showed no difference in bone mass or strength despite having the lowest food intake, but the highest beverage and caloric consumption. Only in comparisons among the rats provided sugar-sweetened beverage were femur and tibia BMD lower in the rats drinking the glucose-sweetened beverage. The type of sugar appears to be a factor by influencing the amount of sugar-sweetened beverage consumed. Polydipsia in the rats drinking the glucose-sweetened beverage and the order of highest beverage intake of glucose > sucrose > HFCS-55 > fructose, suggests that glucose content influences the amount of beverage consumed. In turn, beverage consumption influenced mineral homeostasis by altering mineral intake and excretion. Rats provided the beverages sweetened with pure glucose or sucrose (i.e. 50% glucose) had reduced Ca and P intake and increased Ca excretion compared to the rats provided the fructose-sweetened beverage. Rats provided HFCS-55 containing 42% glucose had no effect on mineral intake but urinary Ca and P excretion was increased compared to rats drinking the fructose beverage. Despite increased urinary mineral loss there were no differences in femur or tibia BMD suggests that the mechanism responsible for the reduced BMD associated with sugar-sweetened beverage intake was predominantly due to the displacement of mineral rich foods.

According to Fitzpatrick and Heaney [19], low BMD and increased bone fractures with soft drink consumption is due to the displacement of milk consumption rather than a direct negative effect of any soft drink component. In our study, polydipsia in rats drinking the glucose-sweetened beverage promoted increased caloric intake from the beverage which the rats compensated for by decreasing their caloric intake from the diet. Decreased food intake resulted in lower Ca intake because the AIN-93G diet but not the water used in our study provided an adequate mineral and Ca:P ratio for bone mineralization [20]. Although, rats drinking the glucose-sweetened beverage had reduced Ca intake this was accompanied by increased Ca absorption. Similarly, other studies observed that feeding glucose increased intestinal Ca absorption in rats [21,22]. In human subjects, Norman et al. [23] observed that glucose consumption resulted in a two-fold increase in intestinal Ca absorption. Despite the increased Ca absorption in the current study, the greater urinary output that accompanied polydipsia decreased Ca retention in the rats provided glucose-sweetened beverage. Negative Ca homeostasis had no significant effect on femoral or tibial total Ca, but lower P intake decreased total P in the femur and tibia.

Another possible mechanism for reduced BMD may be a direct effect of glucose on bone. Terada et al. [24] observed *in vitro* that high glucose concentrations inhibited osteoblast proliferation and in turn, this can impair bone formation resulting in bone loss. In the current study, there was no impairment of osteoblast activity or bone formation as indicated by the absence of a reduction in serum osteocalcin and ALP in the rats drinking the glucose-sweetened beverage compared to the ddH_2O and the other sugar-sweetened beverages. The absence of changes in bone turnover markers despite changes in femoral and tibial BMD may have occurred because bone turnover markers are an indicator of whole body skeletal activity.

It is important to examine the effect of different added sugars because the type of sugars used to sweeten beverages has changed, resulting in altered glucose to fructose intakes [25]. Among the rats drinking sugar-sweetened beverages, bone differences appeared most pronounced between rats drinking glucose and fructose-sweetened beverages. The rats drinking the glucose-sweetened beverages had reduced BMD and BMC of the whole femur and tibia compared to animals drinking the fructose-sweetened beverages. In addition, rats provided the glucose-sweetened beverages had reduced proximal femur BMD and BMC. BMC and BMD loss in the proximal region is important because the proximal end of the femur, near the hip joint, is prone to fragility fractures. Despite the reduced femoral and tibial total BMD and BMC in rats drinking the glucose-sweetened beverage, no differences in bone strength were detected. Reduced bone mass in the young rats may manifest as decreased bone strength later with aging. At the end of our study, rats were still young (age 3 months old), a model of skeletal maturity would require rats at least 6 months old, an age when bone growth slows [26]. Therefore, a study conducted over a long period of time may be required. Other researchers have observed decreased bone strength at a similar or younger age than the rats in our study [27–29]. However, these studies used either high fat diets or higher sugar concentrations than our study.

Increased glucose intake may accompany higher sugar consumption because HFCS-42, a form of HFCS having a higher glucose to fructose ratio, is commonly used in baked goods, dairy products and sport drinks [9]. However, a higher fructose to glucose ratio has been the focus of public attention because the predominant sugar used to sweeten beverages has changed from sucrose consisting of 50% fructose to HFCS-55 consisting of 55% fructose [30]. In a human study, Milne and Nielsen [4] observed elevated ALP with high fructose intake. In our study, fructose in the drinking water had no effect on serum ALP or osteocalcin. However, Ca intake was higher and urinary Ca excretion was lower resulting in higher Ca retention in rats drinking the fructose compared to the glucose-sweetened beverage. Contrary to our findings, Milne and Nielsen [4] observed negative Ca balance with high fructose intake and this effect

was more marked when dietary magnesium was low. The authors [4] reported that high dietary fructose promoted mineral deficiency and kidney calcification. The AIN-93G diet used in our study provided adequate levels of magnesium and minerals to meet requirements in the rat [31]. Future research should include use of low mineral diets to determine whether added sugars promote mineral deficiencies.

It is important to specifically compare the effect of HFCS-55, rather than pure fructose, to sucrose. In our study, there were no differences in femoral or tibial morphometry, BMC, BMD, Ca and P content or bone strength between rats drinking the HFCS-55 sweetened beverage and the rats drinking the sucrose-sweetened beverage. We also observed no significant differences in Ca intake, absorption and retention between rats drinking the HFCS-55 sweetened beverage and the rats drinking the sucrose-sweetened beverage. Our observations are in agreement with the Ivaturi and Kies [5] study findings in human subjects that HFCS did not negatively affect mineral balance compared to sucrose consumption. However, rats provided the HFCS-55 sweetened beverage had higher body weights than rats drinking glucose-sweetened beverage or ddH_2O. Greater body weight has been reported to exert bone protective effect due to increased mechanical loading [32].

In our study, BMD and bone strength of the rats drinking HFCS-55 was not significantly different from the rats drinking the other sugar-sweetened beverages or the ddH_2O. The only bone difference between rats drinking sugar-sweetened and non-sweetened beverages was that rats provided the glucose-sweetened beverage had lower relative femur weights than the ddH_2O control, despite their higher caloric intake. The absence of a similar effect in the tibia may be due to the changes being more readily detected in the slower maturing femur [33]. In contrast, other studies have reported that rats fed diets high in added sugars had reduced bone mineralization and strength compared to animals fed diets low in added sugars [27–29]. The absence of a significant reduction in bone mineralization and strength in rats drinking sugar-sweetened beverages may be due our shorter study duration (8 weeks) compared to the Li et al. [27] and the Zernicke et al. [28] studies of 10 weeks and 2 years, respectively. In addition, Li et al. [27] and the Zernicke et al. [28] provided rats with a high fat diet that may negatively affect bone. In a shorter study, Tjaderhane and Larmas [29] reported reduced bone mass and strength after 5 weeks. The amount of sucrose used in their study of ~43% was ~23% higher than the combination of sugar in the solid diet and beverage used in our study. At this level of sugar in the diet, gastrointestinal problems that contribute to increased Ca losses may occur. In our study, we did not observe gastrointestinal problems in the animals or differences in fecal weight among any of the treatment groups. The amount of sweetener used in our study of 13% is comparable to that typically found in soft drinks [16] and thus, more physiologically relevant to human consumption. Although the level of sweetener added to the beverage in our study was similar to that in soft drinks there was no significant difference in the BMD of rats drinking sugar-sweetened beverages compared to the ddH_2O control. In contrast, Garcia-Contreras et al. [34] observed decreased BMD in rats consuming soft drinks compared to those consuming tap water. This suggested that other ingredients in soft drinks such as phosphoric acid and caffeine may be responsible for the bone loss.

In summary, there were no statistical differences in bone mass and bone strength of animals consuming sugar added beverages compared to the non-sugar ddH_2O control. Of the different added sugars examined, glucose promoted beverage consumption and this in turn, affects mineral homeostasis by altered mineral intake and excretion. Therefore, in terms of bone health, high sugar consumption that leads to a glucose: fructose ratio that increases glucose may need to be the focus of research rather than the current attention being given to the increased fructose intake associated with HFCS-55 beverage consumption.

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