# High-sucrose effect on bone structure, hardness and biomechanics in an obesity model using Wistar male rats

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#### Abstract:

**Introduction:** Excessive consumption of sugar-sweetened beverage is positively related to overweight. Despite the epidemic of childhood obesity, body mass can have a positive or negative effect on bone health. **Material and methods:** Wistar rats 8 weeks olds were randomly assigned to consume water (Control group, n = 10), sucrose 30% (HS group, n = 10) and water + sucrose 30% (WHS group, n = 14) for 8 weeks. All animals received standard laboratory chow ad libitum. Femur measurements included microhardness, bone mineral density (BMD) by DXA, mechanical compression test and microcomputed tomography (microCT) analysis. **Results:** We observed significant difference in final body weight in HS and WHS groups, significant increase in triacylglycerol/fructosamine in HS and WHS groups, significantly high BMD in WHS group, increased periosteal/endosteal cortical microhardness in WHS group. Compared with control, microCT parameters evidenced lower amount of connected trabecular bone, decreased bone volume, lower trabecular number with high trabecular separation in distal epiphysis in WHS animals. **Conclusion:** High-sucrose consumption causes obesity induced by a liquid diet with negative effects on cancellous bone.

Keywords: cortical bone, obesity, rats, sucrose, trabecular boné.

# 1 Introduction

Obesity is a major public health problem affecting more than 300 million people globally and is associated with many chronic conditions including diabetes and osteoarthritis. There is increasing concern about a possible relationship between high levels of consumption of sugar-sweetened beverages and obesity especially in children, and attention has been focused on both decreased physical activity and increased energy intake. Although all sources of energy contribute to weight gain and the development of obesity if consumed in excess of energy requirement, high fat and carbohydrate consumption, and specifically high sugar consumption, are often considered harmful. Body fat mass is one of the most important obesity indexes, and a substantial body of evidence indicates that fat mass may have beneficial effects on bone. Contrasting studies, however, suggest that excessive fat mass may not protect against osteoporosis or osteoporotic fracture. In diet-induced obesity in animal studies, the structure and mechanical properties of cortical and cancellous bone were negatively affected by diets with high fat content (DIMEGLIO and MATTES, 2000; LEONARD, SHULTS, WILSON et al., 2004; ZAO, LIU, HAMILTON, 2007; WETZSTEON, PETIT, MACDONALD et al., 2008; CAO, GREGOIRE and GAO, 2009; VAN BAAK and ASTRUP, 2009; IONOVA-MARTIN, DO, BARTH et al., 2010).

Diet is certainly of importance in the incidence of obesity. There is increasing documentation of a positive association between beverage consumption and body weight or body mass index (BMI) but the mechanisms by which beverages and solid food forms elicit differential appetitive and dietary responses are not known. A strong body of evidence indicates that energy-yielding beverages are contributing to the positive energy balance and increasing incidence and prevalence of overweight/obesity with attention to the potential role of fluid calories in positive energy balance and weight gain (MATTES, 2006; MOURÃO, BRESSAN, CAMPBELL et al., 2007). As the prevalence of overweight and obesity is increasing drastically worldwide, the role of visceral abdominal fat and insulin resistance have gained recognition, and new heterogeneous clinical disorders strongly associated with abdominal obesity have been identified as major risk factors for cardiovascular disease.

Whereas obesity on a high-fat diet is associated with hyperphagia and elevated lipids, obesity on high-carbohydrate diet with a similar increase in body fat shows none of these characteristics, suggesting the existence of multiple forms of obesity with different underlying mechanisms that are diet dependent (DOURMASHKIN, CHANG, GAYLES et al., 2005; NOVELLI, DINIZ, GALHARDI et al., 2007; DINIZ, BURNEIKO, SEIVA et al., 2008; WETZSTEON, PETIT, MACDONALD et al., 2008).

Despite the epidemic of childhood obesity, the effect of obesity from high-sucrose beverage (HS) consumption on bone mineral accrual during growth is poorly understood. However, in general, a significant decrease in mechanical performance (reduced bone quality), concurrent with an increase in bone size (increased bone quantity) has been reported (LEONARD, SHULTS, WILSON et al., 2004). The need for accurate assessment of the structural effects of obesity on bone mineral accretion is underscored by conflicting reports of the effects of increased fat mass and body mass index (BMI) on fracture risk in childhood.

# 2 Materials and methods

#### 2.1 Animals and diets

All animal procedures were in compliance with the São Paulo State University/Araçatuba Dental School Care Committee rules and regulations on their Ethical Principles in Animal Experiments. After a week of acclimatization, thirty four male Wistar rats, 8-weeks-old, were randomly divided into Control (n = 10, 307.80 g at day 1) group (C), high-sucrose (n = 10, 302.31 g at day 1) group (HS) and water + high-sucrose (n = 14, 284.00 g at day 1) group (WHS). They were housed in plastic cages (three per cage), in a temperature (22 ± 1 °C) and humidity-controlled (54-56%) closed cabinet with ventilation; a 12 hours light/12 hours dark cycle, and were given free access to a standard Purina Laboratory Rodent chow during the 56 days of the experimental period. The C Group had free access to the commercial diet and water only. Rats in the HS Group were given free access to commercial diet and a 30% sucrose solution, ad libitum, in distilled water. The WHS group had free access to commercial diet and water and 30% sucrose, ad libitum, in distilled water. The sucrose solution was composed of commercially available pure cane sugar (Docito, Brazil), prepared at the same time (8:00-9:00 AM) and provided in 500 mL glass bottles with rubber stoppers. Animal body weight was determined once a week (on the same day and time, 8:00-9:00 AM). Water and sucrose consumption was measured every two days (8:00 to 9:00 AM).

#### 2.2 Biometrical and biochemical measurements

At the time of sacrifice, the abdominal circumference (AC, immediately posterior to the forefoot), thoracic circumference (immediately anterior to the foreleg), body length, body mass index (BMI, body weight/length<sup>2</sup>) were determined (NOVELLI, DINIZ, GALHARDI et al., 2007). After 28 and 56 days of treatments, all rats were fasted overnight (12 hours) and glycemia was measured with the use of a blood glucose analyzer and test strips (Optium Xceed monitor and strips, Abbott, Oxfordshire, U.K.), cutting the tip of the tail of each rat.

After fasting overnight, the rats were killed on day 56 under ketamine (0.07 mL.100 g<sup>-1</sup>)- xylazine (0.03 mL.100 g<sup>-1</sup>) anesthesia and exsanguination. After being anesthetized, animal blood was collected from the abdominal aorta artery, placed in heparin-treated tubes and kept on ice until centrifuged. The plasma collected was then frozen (-80 °C) for subsequent calcium, triacylglycerol, total cholesterol, HDL-cholesterol and fructosamine analyses using the manufacturer's instructions provided with the kits (Labtest, São Paulo, Brazil). The right femurs were dissected, cleaned of adherent muscles and other tissues and stored in saline solution at -20 °C.

Each left femur was scanned using dual energy X ray absorptiometry (DXA) to determine bone mineral density (BMD). Bones were thawed at room temperature (23 °C), placed in a plexiglass container filled with deionized water, aligned in an anterior-posterior position, and scanned using a Lunar DPX Alpha (Madison, U.S.A.) with small-animal software coupled to a computer. The region of interest was moved to cover: total bone, mid- diaphysis, and proximal and distal epiphysis.

#### 2.4 Bone microbardness

Fresh undecalcified femoral mid-diaphyseal bone was cut (5 mm) perpendicular to the bone long axis, bone marrow was irrigated and samples were then embedded in a thermoplastic mounting powder (Extec, Enfield, U.S.A.), under pressure (150 kgf.cm<sup>-2</sup>) and temperature for complete penetration of the resin into marrow cavity. The bone surface was carefully polished with special carbide grinding paper of decreasing particle size (400, 600, 800, 1200, Buehler Carbimet Paper Discs, U.S.A.) and finally with a diamond suspension (1/4 micron, Metadi Diamond Suspension, U.S.A.) on special paper. Knoop hardness testing was carried out with use of a Shimadzu Microhardness Tester (model HMV-2000, Japan) with a Knoop diamond indenter, set at a 25 g of load for 10 seconds, and measured at 500× magnification. The microhardness tester was linked to a computer with CAMS-WIN program (NewAge, U.S.A.). Knoop hardness was measured along 2 lines at 100 and 300 µm from the periosteal surface. Twenty measurements, separated by 100-120 µm, were taken along each line on cortical bone. Knoop hardness (KH) was expressed as the mean of these measures.

#### 2.5 Mechanical testing

The structural and material properties of the femoral head were determined by destructive mechanical compression testing. The bones were slowly thawed at room temperature at least 12 hours before testing and kept wrapped in saline-soaked gauzes except during measurements and then placed on a computer-controlled EMIC DL 3000 universal testing machine (São José dos Pinhais, PR, Brazil), with a 2000 N load cell (speed of 5 mm/min). The right femoral epiphysis was placed with the distal portion two-thirds vertically into a specially constructed round metal tube (5.0 cm long, 2.7 cm wide), fixed in the center of the tube with six adjustable screws and the femoral neck was tested with a downward vertical load applied parallel to the bone long axis, on the top of the femoral head, using a small concave steel cup at its end, until fracture.

#### 2.6 Bone microstructure

The influence of dietary sucrose on trabecular bone microstructure was assessed at the femoral distal epiphysis. After mechanical testing, the epiphysis was removed (12 mm from distal end), placed in a holder and scanned using a Skyscan 1172 microCT (Skyscan, Aartselaar, Belgium) with X ray source power of 100 kV and 100  $\mu$ A, with a resolution of 12 × 12 × 12  $\mu$ m (TATSUMI, ITO, ASABA et al., 2008) for nondestructive three dimensional (3D) evaluation of bone architecture. The region of interest (ROI) in the

cancellous bone was manually interpolated, beginning in the thinner slice (above the cartilaginous growth plate). A direct three-dimensional evaluation of trabecular bone parameters was performed in a ROI that consisted of a volume of interest (VOI) with 100 slices. The reconstruction and 3D quantitative analyses were performed with Skyscan software. Cancellous bone was separated and the following 3D parameters in the defined VOI were analyzed (CAO, GREGOIRE and GAO, 2009): tissue volume, bone volume, percent bone volume, trabecular thickness, trabecular number, trabecular separation, bone surface, bone surface/ volume ratio, bone surface density, trabecular pattern factor and structure model index.

# 2.7 Statistics

Data on all parameters were expressed as group means with SD. Statistical differences between groups were assessed by ANOVA (Tukey's post-hoc test).

## 3 Results

### 3.1 Biometrical and biochemical measurements

There was a manifest option for 30% sucrose solution in WHS animals when water and sucrose was given for 8 weeks (Table 1), particularly after the fourth week. At the end of the study (8 weeks on experimental sucrose intake) control animals were 36.2% heavier. Animals from the HS group were 50.5% heavier, and those from the WHS group 77.3% heavier than on the initial day, characteristic of obesity state clearly influenced by high sucrose intake during 8 weeks. In the biochemical determinations (Table 2) high values of triacylglycerol but not cholesterol were observed in the HS and WHS groups. As expected, animals from HS and WHS

Groups presented hyperglycemia and hypertriglyceridemia after 8 weeks on the high sucrose diet, and high fructosamine in the HS and WHS groups compared with the C Group, confirming the sustained high glycemia for approximately 20 days.

# 3.2 Biomechanical, bone mineral density (BMD) and microhardness results

DXA measurements from the femoral BMD from WHS Group were higher in the whole bone, proximal epiphysis and mid-diaphysis, probably due to the size of femur and mineralization of the cortical bone (Table 3). Surprisingly, cortical bone Knoop microhardness was higher in the WHS group at 100 and 300  $\mu$ m from periosteal surface, with higher BMD from DXA in the same region, expressing differences in cortical mineralization between the groups (Table 3).

#### 3.3 Bone structure

Non-destructive microCT was used to evaluate the effect of sucrose intake on cancellous bone structure in the distal epiphysis (Table 4). Tb.Pf is a connectivity index of trabecular bone developed to calculate the index of concavity or convexity of the total bone surface. Concavity indicates connectivity, and convexity indicates isolated disconnected structures. As a result, lower Tb.Pf signifies better connected trabecular lattices while higher Tb.Pf means a more disconnected trabecular structure. The prevalence of enclosed cavities and concave surfaces pushed the Tb.Pf into negative values. It should be noted that the concave surfaces of enclosed cavities represent negative convexity to the SMI parameter. Regions of bone containing a prevalence of enclosed cavities can have negative SMI values. In WHS group a significantly lower trabecular number with high

Table 1. Biometrical parameters from male rats 8-week-old drinking sucrose 30% for 56 days.

Parameters	C group	HS group	WHS group
Initial body weight (g)	307.80 (37.17)	295.80 (37.50)	284.00 (23.87)
Final body weight (g)	419.20 (48.53)	445.20 (56.88)	503.57*+ (32.42)
Water consumption (mL/wk/animal)	111.94 (2.16)		115.37 (46.43)
Sucrose consumption (mL/wk/animal)		$105.22\;(13.30)$	117.83+ (14.19)
Body weight gain (g/day)	1.99 (0.48)	2.67* (0.67)	$3.92^{*+}(0.59)$
Body weight variation (%)	36.52 (9.32)	52.39* (15.45)	78.12*+ (14.33)
Body length (cm)	23.75 (0.57)	24.90* (0.82)	23.85+ (0.51)
Thoracic circumference (cm)	17.69 (0.79)	18.08(1.01)	18.80* (1.07)
Abdominal circumference (cm)	19.17 (1.28)	19.77 (1.39)	20.88* (0.86)
Body mass index (g.cm <sup>-2</sup> )	0.74(0.09)	0.72(0.06)	$1.16^{*+}(0.09)$
Glycemia 4 weeks (mg.dL <sup>-1</sup> )	75.70 (8.34)	102.90* (13.57)	103.71* (10.67)
Glycemia 8 weeks (mg.dL <sup>-1</sup> )	89.80 (14.64)	132.20* (27.62)	123.86* (9.93)

Values are expressed as mean  $\pm$  SD. \* = P < 0.05 versus C Group, + = P < 0.05 versus HS Group. HS, sucrose 30%; WHS, water and sucrose 30%; wk, week.

 Table 2. Plasma parameters from male rats 8-week-old drinking sucrose 30% for 56 days.

Parameters	C group	HS group	WHS group
Calcium (mg.dL <sup>-1</sup> )	11.13 (1.24)	9.66 (1.87)	11.50 (0.99)
Triacylglycerol (mg.dL <sup>-1</sup> )	45.14 (20.92)	125.82* (46.70)	131.08* (66.25)
Total cholesterol (mg.dL <sup>-1</sup> )	99.12 (22.18)	95.39 (18.73)	111.17 (17.08)
HDL cholestrol (mg.dL <sup>-1</sup> )	26.19 (2.48)	29.59 (8.29)	30.53 (8.44)
Fructosamine (µmol.L <sup>-1</sup> )	207.46 (39.41)	281.71* (43.88)	306.64* (37.73)

Values are expressed as mean  $\pm$  SD. \* = P < 0.05 versus C Group. HS, sucrose 30%; WHS, water and sucrose 30%.

for 56 days.			
	C group	HS group	WHS group
Femur ultimate load (N)	173.05 (38.01)	185.46 (33.15)	156.30 (29.45)
Femur stiffness (N.mm <sup>-1</sup> )	151.94(48.41)	166.17 (38.41)	216.72* (66.64)
Femur thoughness (N.mm <sup>-1</sup> )	129.48 (49.48)	138.63 (59.20)	108.42 (43.44)
Area whole femur (mm <sup>2</sup> )	1.57(0.099)	1.53(0.057)	$1.84^{**}(0.178)$
BMD whole femur (g.cm <sup>-2</sup> )	0.224 (0.020)	0.240 (0.015)	0.246* (0.017)
BMD proximal epiphysis (g.cm <sup>-2</sup> )	0.201 (0.019)	0.230* (0.018)	0.255** (0.020)
BMD distal epiphysis (g.cm <sup>-2</sup> )	0.271(0.028)	0.256(0.032)	$0.282\ (0.018)$
BMD diaphysis (g.cm <sup>-2</sup> )	0.216 (0.021)	0.230 (0.018)	0.238* (0.020)
Periosteal cortical hardness (HK)	35.75 (3.79)	40.52 (3.76)	48.18** (7.42)

Table 3. Femoral biomechanical measurements, DXA and microhardness results from male rats 8-week-old drinking sucrose 30% for 56 days.

Values are expressed as mean  $\pm$  SD. \* = P < 0.05 versus C Group, + = P < 0.05 versus HS Group. BMD, bone mineral density; HK, Knoop microhardness; HS, sucrose 30%; WHS, water and sucrose 30%.

42.08 (1.98)

43.41\* (7.13)

37.95 (3.49)

Table 4. 3-D microCT parameters measured in femoral distal epiphysis from male rats 8-week-old drinking sucrose 30% for 56 days.

Parameters	C group	HS group	WHS group
TV (mm <sup>3</sup> )	18.33 (1.96)	17.96 (2.02)	19.03 (2.33)
BV (mm <sup>3</sup> )	3.13 (0.86)	3.42 (0.82)	2.50+ (0.85)
BV/TV (%)	16.89 (3.65)	18.96 (3.13)	12.86** (3.44)
Tb.Th (µm)	55.00 (9.20)	50.92 (2.08)	77.46** (5.78)
Tb.Nb (mm <sup>-1</sup> )	3.19 (0.92)	3.71 (0.54)	$1.64^{*+}(0.36)$
Tb.Sp (µm)	241.71 (44.60)	226.73 (51.39)	317.07*+ (54.56)
$Tb.Pf(mm^{-1})$	-7.84(13.89)	-15.32(2.50)	17.31** (4.82)
SMI	0.44(0.88)	0.015 (0.11)	2.16** (0.21)
BS (mm <sup>2</sup> )	286.60 (103.85)	334.05 (79.29)	140.19** (36.61)
$BS/BV (mm^{-1})$	89.90 (18.20)	98.19 (9.97)	58.32*+ (7.45)
$BS/TV (mm^{-1})$	15.43 (4.93)	18.27 (2.72)	7.28*+ (4.82)

Values are expressed as mean  $\pm$  SD. \* = P < 0.05 versus C Group; + = P < 0.05 versus HS Group. HS, sucrose 30%; WHS, water and sucrose 30%; tissue volume (TV); bone volume (BV); percent bone volume (BV/TV); trabecular thickness (Tb.Th); trabecular number (Tb.N); trabecular separation (Tb.Sp); trabecular pattern factor (Tb.Pf); structure model index (SMI); bone surface (BS); bone surface/volume ratio (BS/BV); bone surface density (BS/TV).

separation was observed reflecting a higher significant trabecular pattern factor and a structure model index (increase in SMI and Tb.Pf suggests a less well connected trabecular network).

Endosteal cortical hardness (HK)

The sucrose solution for the present study was based on data regarding to its ability to induce obesity in rodents (SCAFLANI and XENAKIS, 1984; LONDON, LALA, BERGER et al., 2007; DINIZ, BURNEIKO, SEIVA et al., 2008), and our results of body weight gain, abdominal circumference, BMI, sucrose consumption obtained in 8-week-old rats drinking 30% sucrose solution and water-30% sucrose solution, are in agreement with these studies. They pointed that it is well established that animals offered diets that are tasty and calorically rich in sugar overeat and become obese, as observed in our animals. Beverages rich in sugar can be classified as providing low satiety but with high impact on body weight, revealing that they are less satiating than solid foods (MATTES, 2006). Although a variety of factors may influence the consumption of these foods, palatability is thought to be an especially important one. Beverages have lower expected satiety value, lower demand for oral processing, shorter gastrointestinal transit times and the energy they contain has greater bioaccessibility and bioavailability. Each of these attributes has been associated

with weaker effects on appetite and dietary compensation (MOURÃO, BRESSAN, CAMPBELL et al., 2007). Sweet taste is a stimulus that promote ingestion in animals, and rats offered a sugar solution, in addition to chow and water, increase their caloric intake and body weight compared to controls fed on chow only. Rats given access to palatable sugar solutions typically consume 60% of their daily energy intake from the solution, with the remaining 40% taken from a standard non- purified diet (LONDON, LALA, BERGER et al., 2007).

According the results of the present study, only the WHS Group presented a higher consumption of both free water and high-sucrose. It is obvious that the similar intake of water in animals from C group and high-sucrose in the HS Group promoted the overweight in the latter. Cumulative sucrose intake was higher by the HS and WHS Groups with greater total daily energy intake derived from sucrose, and body fat (especially abdominal fat) was higher than that of the control animals. One supposed that the control group ate more nonpurified diet each day (LONDON, LALA, BERGER et al., 2007). The act of masticating the solid food may provide an internal satiety signal not triggered by simply swallowing the liquid and that viscous or solid stimuli are greater than those of fluids (DIMEGLIO and MATTES, 2000; MATTES, 2006). The physical nature of the food influences food intake, with liquids being less satiating than solids and the possible mechanisms included absence of mastication, faster gastric emptying, osmotic effect and cognitive factors (MATTES, 2006; MOURÃO and BRESSAN, 2009). There is strong evidence that liquid foods elicit weaker appetitive and dietary responses than solid foods and the sensation of satiety occurs more rapidly following solid compared with liquid consumption (TIEKEN, LEIDY, STULL et al., 2007).

As expected, after 8 weeks triacylglycerol, glycemia and fructosamine in the HS and WHS Groups were significantly higher when compared with the C group. Fructosamine determinations confirm the hyperglycemic state during approximately the latter 3 weeks, certainly promoted by persistent high-sucrose intake, contrary to another study (LONDON, LALA, BERGER et al., 2007) when low plasma glucose concentration was observed in animals whether they were deprived of food or not. Moreover, a great deal of fat accumulation was observed in the abdominal region expressed by higher circumference and body mass indexes in the WHS Group. These accumulated abdominal fat (our own observation) were similar to those obtained by other authors (LONDON, LALA, BERGER et al., 2007; NOVELLI, DINIZ, GALHARDI et al., 2007; CAO, GREGOIRE and GAO, 2009).

There was difference in BMD of both cancellous and cortical bone when the WHS Group was compared with the C group. Obese animals had significantly higher BMD in the whole bone, proximal epiphysis and femoral mid-diaphysis. Cortical parameters from obese mice were not affected by a high-fat diet, and a finding of cancellous rather than cortical bone affected by the high-fat diet is not surprising, whereas cancellous bone is more responsive than cortical bone to diet or drug treatments because the cancellous bone is more actively remodeled than cortical bone due to the larger surface to volume ratio (CAO, GREGOIRE and GAO, 2009). Bone strength is influenced by a number of determinants and the intrinsic material properties of the tissue and the degree of mineralization not only influence the mechanical resistance of bone but partly determine the bone mineral density (BOIVIN, DOUBLIER, FARLAY et al., 2008).

Bone quality depends on bone geometry and microarchitecture (distribution of bone tissue in space) and on the intrinsic properties of bone tissue such as the degree of bone mineralization. Primary mineralization on surfaces may alter bone morphology and enhance its strength by the sheer addition of high quality tissue. The mineralization is affected by crystallinity and collagen characteristics, included in secondary mineralization and thereby crystal size, orientation, maturation, and perfection have been suggested as factors that influence mechanical behavior (BUSA, MILLER, RUBIN et al., 2005; WU, BERGOT, JOLIVET et al., 2009). Hardness is a measurement to assess the resistance of material deformation and the level of mineralization is an important determinant of the microhardness of bone tissue (BOIVIN, DOUBLIER, FARLAY et al., 2008). Our results of mid-diaphyseal cortical microhardness evidenced that high-sucrose consumption had a positive effect on the periosteal and endosteal envelopes, evidenced by higher mineralization at these levels in the HS and WHS Groups. These results showed that both cancellous and cortical bone are affected by caloric liquid sucrose, irrespective of their reaction when the animal is submitted to a different diet, such as separated compartments with a diverse response (WANG, BANU, MCMAHAN et al., 2001). Cortical

bone hardness could be improved by mechanical loading provided by body weight as observed in the HS and WHS animals (BUSA, MILLER, RUBIN et al., 2005). On the other hand, previous results (WETZSTEON, PETIT, MACDONALD et al., 2008) affirmed that reports of higher BMD in overweight children are a result of greater bone size and increased cortical thickness rather than differences in bone mineralization per se. But the hardness and BMD results (whole femur and diaphysis) of the our study reinforce their results when they pointed out that overweight children had a greater increase in periosteal apposition over 16 months. They explained these findings from a mechanical perspective, related to bending forces that result in bone apposition on the periosteal surface, along the axis of bending.

As previously suggested (WETZSTEON, PETIT, MACDONALD et al., 2008) a very different picture emerged at the distal femoral epiphysis, which is comprised of a higher proportion of cancellous bone. Femoral microCT results confirmed that high sucrose consumption adversely affected trabecular bone structure in experimentally induced obesity in growing rats when high-sucrose and water was given simultaneously (WHS group). Although Tb.Th was significantly increased in these animals, high Tb.Sp with significantly decreased Tb.N, BV/TV and trabecular pattern factor were observed in distal epiphysis in WHS animals. These results are in agreement with other (CAO, GREGOIRE and GAO, 2009) that found lower tibial trabecular volume, lower trabecular number and greater trabecular separation in diet obese mice. The suggestion of these results is related to increased bone resorption from enhanced osteoclastic activity in cancellous bone in obese animals. Another study (LORINCZ, REIMER, BOYD et al., 2010) showed serum tartrate-resistant acid phosphatase (a marker of bone resorption) levels elevated in mice consuming high fat/sucrose solid (HFS) food, but serum osteocalcin (a marker of bone formation) levels were not different between cohorts, appearing that ingesting an HFS diet may have disrupted the balance between bone formation and resorption, favouring resorption. Increased trabecular thickness but not trabecular number was also observed, and high-fat diet adversely affected trabecular bone structure seemed contrary to reports in humans that body mass index is positively associated with BMD. While mechanical loading provided by body weight has a positive effect on bone formation, it has been questioned whether excessive fat mass is beneficial to bone or is a protective factor for osteoporosis, as has been suggested (CAO, GREGOIRE and GAO, 2009).

The most recent discoveries in bone biology are coming from scientists taking a whole-organism approach and implicate the skeleton as a participant in the regulation of global energy metabolism, describing a new bone-pancreas endocrine loop (CLEMENS and KARSENTY, 2011). Future work is needed to determine why the effect of high-sucrose only was positive on bone and to analyze the effects of insulin, leptin serotonin and other molecules participating in the central control of bone mass, based on the fact that energy metabolism affects bone mass accrual by acting through a neuronal relay on one cell type, the osteoblast, raised the testable hypothesis that, in turn, the osteoblast might secrete one or several hormones affecting energy metabolism (CLEMENS and KARSENTY, 2011; KARSENTY, 2006).

# 4 Conclusion

Micro-CT was essential for study the microarchitecture of femoral cancellous bone and DXA was limited to analyze growing long bone. Obesity induced by high-sucrose is detrimental to bone structure in cancellous bone from the distal femur of young Wistar rat.

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