THE EFFECTS OF DIFFERENT DIETARY SUGARS ON MEASURES OF BONE FORMATION,

QUALITY, AND STRENGTH IN GROWING MALE RATS

by

ERICA FRANCES BASS

(Under the Direction of Silvia Giraudo)

ABSTRACT

This study investigated the influence of the consumption of the monosaccharides glucose and fructose on measures of cancellous bone formation and quality as well as bone strength in male Sprague-Dawley rats (60 days old; 225 g) over the course of twelve weeks. Mineral apposition rates (p=0.005), osteoblast surface (p=0.002), and osteoblast number (p=0.005) were higher as a result of glucose and fructose intakes. Despite similar effects on bone formation, trabecular thickness was significantly higher in the fructose group compared to the glucose group (p=0.0016). We did not observe differences in bone strength between groups. The effects of sugar intake on bone were independent of differences in energy intake or bodyweight but heavier epididymal fat pads (glucose vs. chow; p=0.003) and livers (fructose vs. glucose; p=0.000) suggests that disturbances in energy metabolism as a consequence of the consumption of different sugars should be explored as a potential explanation for our findings.

INDEX WORDS: fructose, glucose, bone formation, cancellous bone

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Over 10 million Americans suffer from osteoporosis with another 34 million at risk

of developing the disease due to low bone density [1]. As the population ages, these figures

are expected to increase substantially [2]. The financial burden and poor health outcomes

associated with osteoporotic fractures underscore the importance of understanding more

about osteoporosis prevention [3, 4]. One target for prevention is the optimization of peak

bone mass. According to a consensus statement on osteoporosis prevention, diagnosis, and

therapy by the National Institutes of Health (2001)[5], failure to reach peak bone mass

during adolescence is as important as bone loss to the development of osteoporosis.

Determinants of peak bone mass include genetics and lifestyle factors such as physical

activity and nutrition [6, 7]. Because diet is modifiable, it is important to understand which

dietary factors influence bone development in order to maximize peak bone mass early in

life to protect against the onset of osteoporosis with age.

One dietary factor that may influence the maximization of peak bone mass is sugar

consumption. Over the past three decades, total sweetener availability in the U.S. food

supply has been on the rise [8]. Coincidentally, the age group that consumes the greatest

amount of added sweeteners as a percentage of energy intake is the group most vulnerable

to the effects of diet on bone formation as it relates to peak bone mass—adolescents. The

primary source of added sweeteners in the diets of adolescents is sugar-sweetened

beverages such as soft drinks [9]. It is important to note that as sweetener availability has

1

risen, there has been a shift in the types of sweeteners being used in industry—high fructose corn syrup (HFCS) availability has increased and sucrose availability has decreased [8]. The form of HFCS primarily used to sweetened beverages is HFCS-55. Unlike sucrose, which is composed of 50% glucose and 50% fructose, HFCS-55 is 55% fructose, 42-44% glucose, and 4-6% polysaccharides[10]. Therefore, in order to predict how this shift in the food supply will affect osteoporosis prevalence, it is important to determine the specific effects of glucose and fructose on bone.

Research conducted in both rodents and humans has demonstrated a relationship between fructose and glucose intake and disruptions in bone mineral homeostasis [11-15]. However, few of these studies attempted to quantify the effect of changes in mineral homeostasis in response to sugar consumption on specific parameters of bone health. In humans, observational studies have demonstrated an inverse relationship between soft drink intake and bone health. However, from these data it is difficult to isolate the effect of sugar because of the possibility that other soft drink ingredients such as phosphoric acid and caffeine might adversely affect bone[16]. Multiple animal studies have demonstrated deleterious effects of sugar consumption on bone morphology and strength [17-21]. However, the precise influence of the monosaccharides fructose and glucose remains unclear.

This thesis aims to determine the effects of high glucose and high fructose intakes in the rat diet on measures of bone formation, quality, and strength. Additionally, liver weights and visceral adiposity will also be assessed in order to determine whether there is an association between sugar intake, energy metabolism, and bone. The outcomes of this study will contribute to the growing body of knowledge related to sugar intake and bone

and will hopefully help guide the development of future studies investigating this relationship. Furthermore, the knowledge of how glucose and fructose intake affects the attainment of peak bone mass could potentially help future generations make important dietary choices to minimize the risk of developing osteoporosis in the future.

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CHAPTER 2

LITERATURE REVIEW

Osteoporosis is a condition affecting over 10 million people in the United States. In addition to those diagnosed with osteoporosis, many more are at risk due to low bone density [1]. The financial burden and poor health outcomes associated with osteoporotic fractures underline the importance of understanding more about the prevention of osteoporosis with age [2, 3]. The optimization of peak bone mass during childhood and adolescence has been shown to be a strong indicator of bone health later in life [4]. In order to maximize peak bone mass in children and adolescents, it is important to understand the dietary factors that contribute to bone health [5]. Over the past three decades, total sweetener availability in the U.S. food supply has increased. Concurrently, there has been a shift in the types of sweeteners being consumed—high fructose corn syrup availability has risen and sucrose availability has decreased [6] The age group that consumes the greatest amount of added sweeteners is the group most vulnerable to the effects of diet on bone— adolescents [7].

Multiple animal studies have demonstrated a relationship between sucrose intake and deleterious effects on bone morphology and strength. However, the precise influence of the monosaccharides fructose and glucose are unclear [8]. The original purpose of this study was to investigate the relationship between dietary fructose intake in comparison to glucose intake on bone formation rates, strength, and quality in rats. We hypothesized that fructose intake would adversely influence these parameters in comparison to glucose

based on data from animal and human trials demonstrating disturbances in mineral homeostasis following fructose consumption [9-11]. In light of new information related to the link between energy metabolism and bone [12], the purpose of this study changed. Rather than limit the investigation to the influence of fructose in comparison to glucose alone, we decided to investigate the influences of both monosaccharides in comparison to a chow diet high in starch on bone formation rates, strength and quality in rats.

Osteoporosis

Osteoporosis is a condition characterized by an increased risk of fractures due to diminished bone strength. Bone strength is a combination of both bone density and bone quality. For an individual, bone density is determined by the degree of peak bone mass achieved during adolescence and the amount of bone lost over time. Bone quality is determined by a number of characteristics including bone geometry, architecture, turnover, damage accumulation and mineralization [13]. An estimated 10 million people suffer from osteoporosis in the United States [1]. In addition to those diagnosed with osteoporosis, 34 million Americans are at risk due to low bone density. As the American population ages, these figures are expected to increase substantially. By the year 2020 the number of cases of osteoporosis and low bone density are expected to rise to 14 million and 47 million respectively [14]. The growing prevalence of osteoporosis is a public health concern because fractures are associated with increased medical costs and premature mortality in older adults [2, 3].

It is important to note that peak bone mass attained during adolescence is an important determinant of osteoporosis as an adult [15]. In a review of peak bone mass,

Heaney et al (2000) [4] summarizes multiple studies examining the relationship between peak bone mass and osteoporosis by stating that individuals on the high end of the population distribution for bone mass at age 30 will likely be on the high end at age 70. Although it is difficult to pinpoint the age at which peak bone mass is reached, studies suggest that 85-95% of adult bone mass is attained by the age of 18 in girls and 20 in boys [1]. According to a consensus statement on osteoporosis prevention, diagnosis, and therapy by the National Institutes of Health (2001) [13], failure to reach peak bone mass during adolescence is as important as bone loss to the development of osteoporosis later in life. Therefore, factors that interfere with bone development during adolescence will most likely adversely affect future bone health. Determinants of peak bone mass include genetics and lifestyle factors such as physical activity and nutrition [4, 5] . In this study, the effects of sugar consumption on the growing skeleton were explored in young male rats with the purpose of identifying dietary factors that could potentially influence the attainment of peak bone mass.

Features of skeletal growth in rats

The appropriateness of the rat model for this investigation is evident in the similarities between the rat and human skeleton. The long bones of both species elongate and increase in cross-sectional area through the same mechanisms. These mechanisms are epiphyseal growth and periostial growth, respectively. Furthermore, the mechanism of cancellous bone remodeling between rats and humans is similar [16]. One difference seen in the rat skeleton is the lack of Haversian remodeling in the cortical bone. In humans, Haversian remodeling is associated with increasing cortical bone porosity with age.

To overcome this limitation, researchers often use the ovariectomized rat as a model for osteoporosis.

Like humans, rats also transition from modeling to remodeling as the primary activity in both cortical and cancellous bone with age. This transition is accompanied by greatly reduced longitudinal bone growth. Modeling differs from remodeling in that bone formation and resorption occur independently as opposed to being coupled. The age of transition from modeling to remodeling in rats is site and bone type specific. In the lumbar vertebrae, the transition begins at 3 months in cancellous bone and between 3 and 9 months in cortical bone. In the proximal tibial metaphysis, the transition occurs between 6 and 9 months in cancellous bone and between 9 and 12 months in cortical bone [17]. Additionally, the age of epiphyseal growth plate fusion in the proximal tibia occurs at 8 months in female rats and 10 months in male rats [16]. The age of epiphyseal growth plate fusion differs by site. Some long bones in rats retain their capacity for longitudinal growth throughout life. The age of peak bone mass attainment in rats is approximately 10 months [17]. Therefore, in order to use rats as a model for changes in bone that occur during growth, the age of the rat and bone site of interest must be considered.

Trends in sweetener intake

It is important to investigate the effects of dietary sugars on bone due to the continued rise of sweetener availability and intake in the United States. From 1978-2003, per capita total sweetener availability in the food supply increased by 16.6%. It is important to note, that as sweetener availability increased, there was a shift in the types of sweeteners being used in industry. Over the same time period, sucrose availability

decreased by 32.7% (76.5 g/d) while the availability of high fructose corn syrup (HFCS) increased 60.8% (to 74.2 g/d) [6].

Although both sucrose and HFCS supply glucose and fructose in the diet, there are differences in their compositions. Sucrose is a crystalline disaccharide produced from the processing of sugarcane and sugar beets that is made up of 50% glucose and 50% fructose. HFCS is a liquid composed of glucose and fructose monosaccharides. Glucose from milled corn is converted to syrup and then a percentage is enzymatically isomerized to produce a mixture of glucose and fructose [18]. The two main types of HFCS used commercially are HFCS-55 and HFCS-42. HFCS-55 is 55% fructose, 42-44% glucose and 4-6% polysaccharides and is primarily used by the beverage industry. HFCS-42 is 42% fructose, 53-55% glucose, and 4-6% polysaccharides. HFCS-42 is used commercially in baked goods, dairy products and sweetened sports drinks [19]. The shift in industry usage of sweeteners from sucrose to HFCS and the overall increase in sweetener consumption in the U.S. necessitated exploration of how changes in the ratio glucose to fructose in the diet may affect bone.

Children and adolescents consume an average of 365 kcal/day from added sugars, mostly in the form of sugar-sweetened beverages [20]. Males and females age 12-17 have the highest mean intake of total added sweeteners as a percentage of total energy [7]. Males 15-22 are also the greatest consumers of fructose (75 g/d). Females age 15-18 and 19-22 consume 55 and 61 g/d of fructose respectively [6]. Because children and adolescents consume more sugar and fructose than any other age group, it is especially important to understand the relationship between sugar intake and bone. The primary

dietary sources of added sweeteners include sugar-sweetened beverages, sugars/sweets, milk/milk products, sweetened grains and breakfast cereals [6, 7, 20].

Dietary sugar consumption and mineral homeostasis

Multiple animal studies have demonstrated a relationship between dietary sugar consumption and changes in mineral homeostasis. Koh et al (1989) [21] found that fructose consumption, when coupled with diets low in magnesium, led to greater calcium deposition in the kidneys and hearts of weanling rats when compared to glucose and starch consumption. Theses findings suggest a possible interaction between fructose consumption, magnesium status, and the calcification of soft tissues. In another study examining this relationship, Bergstra et al (1993) [11] compared the effects of diets high in fructose and glucose (77% of kcals) on kidney calcification in female rats. The effects of dietary fructose and glucose were compared when all nutrient requirements were met and also in diets high in phosphorus and diets low in magnesium. Researchers found that regardless of the micronutrient composition of the diets, the diets with added fructose resulted in higher kidney calcium concentrations than diets with added glucose. Apparent calcium absorption was not different between the rats fed the different sugars, however, higher urinary concentrations of phosphorus and magnesium were found in the fructose fed rats. The authors of the study suggested higher urinary concentrations off these minerals was due to increased absorption in the small intestine induced by fructose. The specific relationship between sugar intake and mineral homeostasis could not be determined from the data collected in these studies.

Multiple studies examining the relationship between dietary sugar and mineral homeostasis have also been conducted in humans. Holbrook et al (1989) [22] found that healthy male subjects (aged 21-57 years) fed low-copper fructose diets (20% of calories) for 7 weeks had positive balances for calcium, magnesium, copper, iron, manganese, and zinc when compared to low-copper starch diets. Fecal excretion of minerals was lower and urinary excretion was higher in the fructose group suggesting greater absorption of minerals in the intestine. The authors hypothesized that fructose may form complexes with minerals in the gut to enhance absorption. The effect of positive mineral balances on bone could not be determined due to a lack of bone specific measurements.

Milne and Nielsen (2000) [9] demonstrated that fructose intake disrupts mineral balance in men (aged 22-40 years) when compared to starch. Changes in mineral homeostasis were explored in four diet conditions: fructose or starch was 20% of energy intake and dietary magnesium was either high or low. Participants were exposed to each diet for a period of 42 days. Researchers found that fructose consumption resulted in decreased phosphorous ($p<0.005$) and calcium balance ($p<0.007$) when compared to starch consumption. The effect was stronger when fructose intake was coupled with low magnesium intake. Serum osteocalcin, a marker of bone formation and osteoblast activity, was not different between the different treatments. One limitation of this study is that fructose was provided as HFCS which is a combination of glucose and fructose. Therefore, it is difficult to determine whether fructose alone is responsible for the changes in mineral balance.

In another human study, Ivaturi and Kies (1992) [10] compared the effects of sucrose, fructose and HFCS on mineral balance in adult men and women. When fructose

was consumed, participants had negative calcium, magnesium and phosphorus balance compared to sucrose. Further, HFCS had no effect on mineral homeostasis. There is concern that the amount of fructose supplied (60g/d) caused intestinal disturbances in study participants, which may have adversely affected mineral homeostasis as fecal excretion of minerals was higher in this group. Intestinal disturbances were not observed when HFCS was consumed. This is likely explained by the enhanced absorption of fructose when consumed with glucose [23].

Studies in humans related to the effects of dietary glucose on calcium absorption have also been conducted. In 8 males and females (aged 21-51 years), Wood et al (1987) [24] demonstrated that calcium absorption was enhanced when administered with 50 g of free glucose or glucose polymers. Absorption was enhanced 20% when calcium was taken with free glucose and 27% when taken with glucose polymers compared to calcium taken with water ($p<0.01$). In contrast, Francis et al (1986) [25] saw no change in calcium absorption when calcium was administered with 10 g of glucose in postmenopausal women. The differences in the findings of the two studies may be explained by discrepancies in the doses tested. Wood et al (1987) used doses of 50 g while Francis et al (1986) used a dose of 10 g.

Although these findings suggest a relationship between sugar and changes in mineral homeostasis that could potentially affect bone, a causal relationship cannot be determined based on these data alone. One reason is that the findings are inconsistent. Some studies showed that fructose consumption leads to positive mineral balance [22] while others lead to negative mineral balance [9, 10]. Another factor that makes it difficult to compare results from these studies is differences in the amount and source of fructose

(fructose vs HFCS) supplied to study participants. Similar inconsistencies are seen in studies examining the effects of glucose on calcium absorption. Further, there is a lack of specific bone measurements to determine if changes in mineral homeostasis, whether positive or negative, induced by sugar consumption affected parameters of bone such as bone formation and bone strength.

Dietary sugar consumption and bone

In a review by Tsanzi et al (2008), it was concluded that there is an inverse relationship between soft drink intake and bone health. However, it is difficult to implicate the sugar in soft drinks due to the observational design of the studies because of the possibility that other ingredients in soft drinks adversely affect bone. For example, some studies suggest that phosphoric acid binds calcium, preventing calcium absorption. However, studies exploring the relationship between phosphoric acid and bone have shown little to no effects. Caffeine is another ingredient found in soft drinks that may have an effect on bone health. However, like phosphoric acid, studies on caffeine and bone have not supported such a relationship. Another possible explanation for the relationship between soft drink intake and bone is the displacement of calcium rich foods due to increased soft drink consumption [26]. Clearly, low intakes of calcium rich foods would have an independent effect on bone despite possible effects from ingredients in soft drinks.

In an in vitro study, Terada et al (1998) exposed human osteoblast like cells to varying concentrations of glucose. The authors found that cells exposed to the highest concentrations of glucose had reduced growth indicated by reduced cell number and DNA synthesis. These findings suggest that osteoblasts may be adversely affected by exposure to

high glucose levels [27]. However, similar effects must be demonstrated in vivo before the precise relationship between glucose exposure and osteoblasts can be determined.

Li et al (1990) compared the effects of feeding young female rats a high-fat sucrose (HFS) diet and a low-fat complex carbohydrate (LFCC) diet for 10 weeks on bone morphology and mechanical properties. The tibias of the rats fed the HFS diets had a lower maximum load, failure energy, elastic modulus, and density than the tibias of rats fed the LFCC diet as determined by three point bending. Increased cortical cross-sectional area of the HFS metatarsals was also observed despite no differences in the cross sectional area of the tibial mid-diaphysial cross sectional area between the groups. The authors proposed hypercalciuria as a result of hyperinsulinemia and reduced calcium absorption as possible mechanisms for the observed changes in bone in the HFS group[28]. In a similar study, Zernicke et al (1995) examined the long-term effects of the HFS diet on bone. Weanling female rats were fed either the HFS diet or LFCC diet for two years. The findings of the long-term study were compared to the findings of previously conducted short-term studies [29]. The effects of the HFS diet on the mechanical properties of bone were the same regardless of study length. However, the degree of the effects was significantly greater in the rats in the long-term study. These findings suggest that the deleterious effects of the HFS diet on bone worsen as a function of time of exposure.

In a later study conducted by Lorincz et al (2010), exposure to the HFS diet for 10 weeks resulted in higher serum tartrate resistant acid phosphatase (TRAP), but no differences in serum osteocalcin levels in mice. Serum TRAP is a marker of bone resorption while serum osteocalcin is a marker of bone formation. These findings coupled with reduced cross sectional area and cortical thickness in the tibias of HFS mice suggest diet

related changes in the balance between bone formation and resorption. In addition, receptor activator of nuclear factor κβ ligand (RANKL) mRNA was upregulated in the tibias of HFS mice [30]. RANKL is expressed on the surface of osteoblast precursors and stimulates osteoclast differentiation by binding to receptor activator of nuclear factor κβ (RANK) on osteoclast precursor cells. Osteoprotegerin (OPG) regulates this process by blocking the action of RANKL [31]. Increased osteoclast activity could not be associated with changes to the OPG/RANKL system because the ratio of OPG/RANKL mRNA was not significantly different between groups. Instead, the authors suggested that the chronic inflammatory state induced by obesity (HFS mice were 40% heavier with 14.9% more body fat than LFCC mice) was to blame for the changes in bone [30].

The previously mentioned studies suggest that diets high in sucrose and fat adversely affect bone. In order to understand the specific relationship between sugar and bone, studies exploring the effects of diets high in sugar and low in fat are reviewed. Tjaderhane and Larmas (1998) investigated the effects of a high sucrose diet on bone growth, mineral composition and strength in weanling male and female rats over a 5-week period. The widths of the tibias in the female rats fed sucrose were smaller compared to controls ($p<0.05$). However, this difference was not seen in male rats. Differences in tibia densities compared to controls were evident for both sexes exposed to the high sucrose diets ($p<0.001$ for females and $p<0.01$ for males). Similarly, the bending strength of femurs ($p<0.001$ for females and $p<0.05$ for males) and tibias ($p<0.001$ for females and $p<0.01$ for males) was lower in the sucrose group compared to rats in the control group. Researchers also found differences in bone mineral content between the groups. Tibia bone calcium ($p<0.001$) and phosphorus ($p<0.001$) as well as femur bone calcium ($p<0.05$) was lower in

female rats in the sucrose group. Bone mineral content was not measured in male rats. The authors of this study concluded that although sucrose induced changes in bone for both sexes, differences in growing female rats were greater than those observed in males [32]. Conclusions could not be made regarding the specific effects of fructose and glucose as sucrose is composed of both sugars in a 50:50 ratio.

Tsanzi et al (2008) supplied female adolescent rats (aged 35 days) with water sweetened with glucose, sucrose, fructose or HFCS-55 at levels found in commercial soft drinks (13% weight/volume) in combination with a regular diet for 8 weeks. Rats consuming the glucose-sweetened beverage had the highest beverage and caloric intake but the lowest intake of food. This finding suggests that beverage consumption displaced dietary intake and thus resulted in decreased mineral intake. The biggest differences were observed between the glucose- and fructose-sweetened beverage groups. Rats consuming the glucose-sweetened beverage had reduced tibia and femur bone mineral density (BMD) and bone mineral content (BMC) when compared to the fructose group. However, there was no difference in bone mass, bone strength, or serum bone turnover markers between the groups. The authors suggest that changes in bone mass and strength as the result of sweetened-beverage consumption during growth may be evident in rats with mature skeletons and therefore the study should be conducted for a longer period of time [8].

Studies have demonstrated that sugar consumption has a deleterious affect on bone morphology and strength. However, theses studies have not determined the relationship between sugar consumption and bone formation. Further, the independent effects of glucose and fructose on bone has not been established. Tsanzi et al (2008) [8] showed that the consumption of glucose-sweetened water resulted in reduced BMD and BMC when

compared to the consumption of fructose-sweetened water. However, it is difficult to determine whether the effects were due to the type of sugar or the reduced intake of minerals as a result of decreased food intake.

Bone and energy metabolism

Recent evidence points to the skeleton as a player in energy metabolism. Specifically, bone is regulated by leptin, a hormone produced by white adipose tissue that helps to regulate appetite and metabolism [12]. The effects of leptin on bone may be site (appendicular vs. axial) and bone type (cortical vs. trabecular) specific. In a study by Hamrick et al (2004) [33], the femurs of leptin deficient (ob/ob) mice were significantly shorter and had lower bone mineral content, bone mineral density, cortical thickness, and trabecular bone volume than lean controls. In contrast, vertebral length, BMC, BMD and trabecular bone volume were increased in ob/ob mice versus controls. However, as seen in the femurs of ob/ob mice, decreased cortical thickness of vertebrae was observed. Leptin also differentially affected the number of marrow adipocytes at the different bone sites. Marrow obtained from the femurs of ob/ob mice showed an increased number of adipocytes when compared to lean controls and the marrow obtained from ob/ob vertebrae. These results suggest leptin influences axial and appendicular skeletal sites differently. However, in another study, Bartell et al (2011) [34] investigated the effects of central vs. peripheral administration of leptin on appendicular and axial skeletal sites in ob/ob mice. Exogenous leptin resulted in increased BMC, BMD, bone area, and mineral apposition rates as well as reduced numbers of marrow adipocytes in ob/ob mice regardless of bone site or route of administration.

The discussion of the influence of leptin on bone is pertinent because fructose and glucose consumption influence circulating leptin levels differently. Leptin production depends on insulin-dependent glucose metabolism in adipocytes [35]. Because fructose does not stimulate the β-cells of the pancreas to secrete insulin, smaller postprandial insulin levels following fructose consumption lead to lower circulating levels of leptin [36]. The reduction of 24-hour serum leptin levels following fructose consumption when compared to glucose consumption has been demonstrated in rhesus macaques and in humans [37, 38]. Therefore, lower circulating leptin levels in response to fructose consumption should be explored as a possible mechanism for differences in bone formation and strength between rats fed high glucose or high fructose diets should they be observed.

Bone has also been shown to influence glucose homeostasis through the production of undercarboxylated osteocalcin in response to insulin binding to its receptor on the surface of osteoblasts [39]. The binding of insulin on osteoblasts decreases the production of OPG. This leads to a decrease in the ratio of OPG to RANKL and stimulates osteoclast activity. The acidic environment associated with bone resorption serves to decarboxylate osteocalcin to the undercarboxylated form. Undercarboxylated osteocalcin stimulates the proliferation of pancreatic β-cells, insulin secretion, and insulin sensitivity [12]. This sequence of events has been shown to influence whole body glucose metabolism in mice lacking the insulin receptor in osteoblasts. These mice developed glucose intolerance and insulin insensitivity when fed a normal diet. Undercarboxylated osteocalcin has also been shown to be associated with glucose homeostasis in humans. In a study by Pollock et al (2011) [40], prepubertal overweight children with prediabetes had lower serum levels of

undercarboxylated osteocalcin than children without prediabetes. There was also an association between impaired β-cell function and undercarboxylated osteocalcin in children in the prediabetes group. In another study using the same cohort, it was shown that children with prediabetes and lower undercarboxylated osteocalcin levels had a 4% lower BMC than children without diabetes [41]. These findings suggest a possible relationship between bone mass, undercarboxylated osteocalcin, and glucose homeostasis in humans. However, due to the cross-sectional design of the study, a causal relationship cannot be determined.

As mentioned previously, fructose consumption does not stimulate insulin secretion. In fact, post-prandial insulin responses are significantly lower following short term fructose consumption than following glucose consumption [38]. Further, fructose consumption over time has been associated with the development of insulin resistance [36]. Huang et al (2010)[42] investigated the effects of insulin resistance on bone in mice to try and understand the relationship between diabetes and bone—greater bone mass and increased fractures. Higher trabecular and cortical bone mass, slower rates of bone formation, and fewer osteoclasts were observed in the tibias and verterbrae of the insulin resistant mice when compared to controls. In addition, serum bone resorption markers and osteoclast progenitors were decreased in the insulin resistant mice. The authors suggest that changes in bone turnover as a result of decreased sensitivity to insulin may explain why diabetics have higher bone masses. Additionally, the authors concluded that the elevated fracture rate seen in diabetics could be a result of decreased bone quality due to decreased bone turnover.

Summary

Over the past 30 years, sweetener intake has been on the rise [6]. Presently, dietary sugar intake remains high and the development of osteoporosis continues to be a concern for older adults [43]. Coincidentally, adolescents consume more sugar than any other segment of the population [6]. There is evidence of a relationship between sugar intake and bone in the existing literature. Glucose may alter osteoblast activity [27] and fructose and glucose may cause disruptions in calcium, magnesium and phosphorus homeostasis [9-11, 21]. Additionally, metabolic changes induced by consumption of high sugar diets have the potential to affect bone mass and quality [12, 36, 38]. The purpose of this study was to investigate the influence of the monosaccharides glucose and fructose on specific bone measures such as bone formation and bone strength. Greater understanding of this relationship will provide insight into the role of dietary sugar consumption in the achievement of peak bone mass and ultimately in the development of osteoporosis later in life.

Hypothesis

Diets high in the monosaccharides glucose and fructose will adversely affect measures of bone formation rates, strength, and quality in rats compared to a chow diet where the main carbohydrate source is supplied in the form of starch.

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CHAPTER 3

THE EFFECTS OF DIFFERENT DIETARY SUGARS ON MEASURES OF BONE FORMATION,

QUALITY, AND STRENGTH IN GROWING MALE RATS

ABSTRACT

This study investigated the influence of the consumption of the monosaccharides glucose and fructose on measures of cancellous bone formation and quality as well as bone strength in male Sprague-Dawley rats (60 days old; 225 g) over the course of twelve weeks. Mineral apposition rates (p=0.005), osteoblast surface (p=0.002), and osteoblast number (p=0.005) were higher as a result of glucose and fructose intakes. Despite similar effects on bone formation, trabecular thickness was significantly higher in the fructose group compared to the glucose group (p=0.0016). We did not observe differences in bone strength between groups. The effects of sugar intake on bone were independent of differences in energy intake or bodyweight but heavier epididymal fat pads (glucose vs. chow; p=0.003) and livers (fructose vs. glucose; p=0.000) suggests that disturbances in energy metabolism as a consequence of the consumption of different sugars should be explored as a potential explanation for our findings.

INTRODUCTION

Osteoporosis is a condition characterized by an increased risk of fractures due to diminished bone strength. Bone strength is a combination of both bone density and bone quality. For an individual, bone density is determined by the degree of peak bone mass achieved during adolescence and the amount of bone lost over time. Bone quality is determined by a number of characteristics including geometry, architecture, turnover, damage accumulation and mineralization [1]. It is important to note that peak bone mass attained during adolescence is an important determinant of osteoporosis as an adult [2]. Therefore, factors that interfere with bone development during adolescence will most likely adversely affect future bone health. Determinants of peak bone mass include genetics and lifestyle factors such as physical activity and nutrition [3, 4].

Studies conducted in both rodents and humans have demonstrated a relationship between fructose and glucose intake and disruptions in bone mineral homeostasis [5-9]. Although inconsistent, the findings of these studies have prompted other researchers to more closely examine the relationship between sugar and bone. Multiple animal studies have demonstrated deleterious effects of sugar consumption on bone morphology and strength [10-14]. This evidence as well as the fact that adolescents are the group that consumes the greatest amount of added sugars as a percentage of energy intake [15], underlines the importance of understanding how dietary sugar influences parameters of bone health. This understanding is necessary in order to maximize peak bone mass and protect against osteoporosis.

Over the past three decades, total sweetener availability in the U.S. food supply has risen 16.6% and with this, there has been a shift in the types of sweeteners being used in

industry—high fructose corn syrup (HFCS) availability has increased and sucrose

availability has decreased [15]. The form of HFCS primarily used to sweeten beverages, the

primary source of sweetener intake in adolescents [16], is HFCS-55. Unlike sucrose, which

is composed of 50% glucose and 50% fructose, HFCS-55 is 55% fructose, 42-44% glucose,

and 4-6% polysaccharides [17]. Therefore, in order to predict how this shift in the food

supply will affect osteoporosis prevalence, it is important to determine the specific effects

of glucose and fructose on parameters of bone health.

This purpose of this study was to determine the effects of high glucose and high

fructose intakes in the rat diet on measures of bone formation, quality, and strength. To do

this, we utilized the methods of histology and histomorphometry to assess differences in

microarchitectural arrangement and bone formation in cancellous bone as a consequence

of high glucose and fructose intakes in 2-month-old male Sprague-Dawley rats. To

determine differences in bone strength, we used a three-point bending test. Additionally,

liver weights and visceral adiposity were assessed in order to determine whether there is

an association between sugar intake, energy metabolism, and bone. Our overall hypothesis

was that diets high in glucose and fructose would adversely affect measures of bone

formation rates, strength, and quality in rats compared to a chow diet where the main

carbohydrate source is starch.

METHODS

Animal model, diets, and study design

The guidelines for animal procedures were approved by the University of Georgia

Institutional Animal Care and Use Committee. Male Sprague-Dawley rats (aged 60 days,

n=24) were obtained from Harlan Laboratories (Pratville, AL). Upon arrival, the animals were housed individually in clear plastic shoebox cages attached to the BioDAQ Food Intake Monitoring System (Research Diets, New Brunswick, NJ) in a room kept at 21°C with a 12 h light/dark cycle. The animals were acclimated to the feeding system with *ad libitum* access to food (LabDiet PicoLab Rodent Diet 20 5053, Brentwood, MO) and water for 7 days before being randomly assigned to receive a high fructose diet (n=9), a high glucose diet (n=9), or to continue to receive the chow diet consumed during the acclimation period (n=6). The animals had *ad libitum* access to their assigned diets and water throughout the 12 week feeding study. The animals were run in two cohorts of 12 animals due to a limited number of cages in the feeding system. In the first cohort, rats were randomly assigned to receive either the fructose diet (n=6) or the glucose diet (n=6). In the second cohort, rats were randomly assigned to receive the fructose diet (n=3), the glucose diet (n=3), or the chow diet (n=6).

The high fructose group received a diet consisting of 20% kcal from protein, 10% kcal from fat, and 70% from carbohydrate: 40% fructose, 10% glucose, 20% corn starch (Research Diets, New Brunswick, NJ #D02022708). The high glucose group received a diet consisting of 20% kcal from protein, 10% kcal from fat, and 70% from carbohydrate: 49% glucose, 20% cornstarch (Research Diets, New Brunswick, NJ #D08082606). Both the high fructose and high glucose diets supplied 3.8 kcal/gram. The remaining rats received a diet consisting of 25% kcal from protein, 13% from fat, and 62% from carbohydrate: 44% cornstarch, 4% sucrose, 2% lactose, and 12% other carbohydrate (<1% fructose or glucose). The third diet supplied 3.07 kcal/gram. The compositions of the diets are

displayed in **Table 1**. Food intake was measured continuously and animal weights were recorded weekly.

Bone labeling and tissue collection

Chlorotetracycline hydrochloride (Sigma-Aldrich, St. Louis, MO), a fluorochrome used to label bone, was dissolved in water at a concentration of 25 mg/kg. The rats were injected with the solution intraperitoneally at 7 and 2 days before sacrifice [18]. The labels allowed for the quantitative measurement of bone formation. After 12 weeks, the animals were sacrificed by decapitation. Epididymal and perirenal fat pads were removed and weighed as a measure of abdominal adiposity. The right hind limb was dissected free, cut at the midshaft of the femur and the tibia, and fixed in 10% neutral buffered formalin for 48 hours. Samples were then stored in 70% ethanol prior to histological and histomorphometric analysis. The left hind limbs were removed, wrapped in saline soaked tissue and stored at -20°C prior to mechanical testing.

Bone histology and histomorphometry

Histology and histomorphometry of the right distal femurs were performed by the University of Alabama at Birmingham Center for Metabolic Bone Disease using the following methods. Longitudinal sections (5 μm thick) were cut at the 50% plane from methyl methacrylate (MMA) embedded blocks using a Leica 2265 microtome. These were stained with Goldner's Trichrome stain for the static measurements, and additional sections were cut at 10 μm, and left unstained for dynamic measurements.

A region of interest was selected that is exactly 250 μm distal to the growth plate, and extending 1 mm downward through the metaphysis of the femur. Standard bone

histomorphometry was performed by the methods of Parfitt et al (1987)[2] using Bioquant Image Analysis software (R & M Biometrics, Nashville, TN). Four types of primary measurements were made—area, length (perimeter), distance, and number. These referents, such as tissue volume, bone volume, bone surface, and osteoid surface were used to derive other indices, such as trabecular number and trabecular separation.

Dynamic measurements were made in the same region of interest using an unstained section. The fluorescent measurements are made of single-labeled surface, double-labeled surface, and interlabel width. The interlabel period will be applied for the calculation of mineral apposition rate as well as formation and resorption rates. All nomenclature for histomorphometry will be used as described by Parfitt et al (1987) [19].

Bone biomechanics

A digital caliber was used to measure the lengths, widths, and thicknesses of the left tibias. Three-point bending was conducted using an Instron 3365 test instrument (Instron, Norwood, MA). Left tibias were thawed in normal saline for 2 hours and then mounted on stainless steel fixtures. Tibias were loaded to failure using a 100 N static load cell (10-kg) at a displacement rate of 2 mm/min. Load-displacement data (50 data points per second) was collected by a PC using Bluehill® 2 Materials Testing Software (Instron, Norwood, MA). A load-displacement curve was generated and used to determine maximum flexure load, maximum stress, energy at maximum flexure extension, and other parameters of bone strength.

Statistical Analysis

Statistical analyses were performed using SPSS statistical software (Version 20.0, Chicago, IL). Differences in bone measures, total energy intake, final bodyweights, fat pads, liver weights, and bone measures among the three treatment groups were determined using a one-way ANOVA. Post-hoc comparisons were conducted using Tukey's test. For measures that violated the assumption of equal variance, differences were determined using the Welch test. When the Welch test was used, a Games-Howell test was used to make post-hoc comparisons between groups. The level of statistical significance for differences between groups was defined at $p < 0.05$. Results are presented as mean ± SEM.

RESULTS

Cohort comparisons

In order to determine whether there were differences between the two cohorts, final body weights and total intakes were compared between rats consuming the same diet from different cohorts. There were no significant differences in final body weights or total energy intakes for either diet group when rats from the two cohorts were compared (data not shown).

Energy intake and body weight

As shown in **Table 2**, there were no significant differences in body weights between groups at baseline. Week 11 body weights are reported because energy intake was diminished following tetracycline injection. This resulted in weight loss for all rats in the final week of the study. The week 11 body weights and total weight gain from baseline to

week 11 were highest in the chow group. However, the differences in week 11 body weights and total weight gain from baseline to week 11 were not significantly different between groups. Additionally, there were no significant differences in the amount of weight lost between weeks 11 and 12. Mean total energy intake over 12 weeks was in the order of fructose> chow> glucose. However, total energy intake was not significantly different between treatment groups.

Fat pads and livers

Epididymal, Perirenal, and total (epididymal + perirenal) fat pad masses were corrected for differences in body weight between groups and are expressed as mg/g BW. The means for each experimental group are shown in **Figure 1**. Rats fed the fructose and glucose diets had heavier relative epididymal fat pads than rats fed the chow diet. However, only the fat pads from the rats in the glucose group were significantly heavier than the fat pads from the chow group (p= 0.003). This same relationship was not observed for the perirenal fat pad or total fat pad masses as neither differed significantly between groups. Corrected liver weights were significantly heavier in the rats fed the chow and fructose diets compared to those fed the glucose diet (p= 0.000; **Figure 2**).

Bone histomorphometry

Static histomorphometry data is displayed in **Table 3**. The type of sugar consumed did influence the microarchitecture of the right distal femurs. Bone volume (BV) and tissue volume (TV) were significantly lower in rats fed the fructose and glucose diets when compared to rats fed the chow diet (BV: p=0.009; TV: p=0.047). These differences were lost once bone volume was normalized to tissue volume (%BV/TV). There were no differences

in bone surface (BS) or trabecular bone surface (BS/TV) between groups. Trabecular thickness (Tb.Th) was highest in the fructose group compared to both the chow and glucose groups. However, only the mean Tb.Th of the glucose group was significantly lower than the fructose group (p =0.016). There were no differences in trabecular number (Tb.N) between groups. Trabecular space (Tb.S) was lower in the chow group compared to both the fructose and glucose groups, however the difference between groups was not statistically significant.

The quiescent perimeter (QS) was significantly lower in both the fructose and glucose groups, but only the fructose group was significantly different from the chow group (p=0.005). Both the osteoblast surface (ObS/BS) and the osteoblast number (N.Ob/BS) were significantly higher in the rats fed the fructose and glucose diets (Obs/BS: p=0.002; N.Ob/BS: p=0.005). There was no significant difference in osteoclast surface (OcS/BS) or number (N.Oc/BS) between groups.

Dynamic histomorphometry data are displayed in **Table 4**. Neither the mineralizing surface (MS) nor the percentage of active, forming bone surface (%MS/BS) was influenced by the consumption of dietary sugar. The mineral apposition rate (MAR) was higher in both the fructose and glucose groups compared to the chow group (p=0.005). The bone formation rate (BFR/BS) was higher in both the fructose and glucose groups but neither was significantly different from the chow group. The bone formation rate per bone volume (BFR/BV per day) was significantly higher in both the fructose and glucose groups compared to the chow group (p=0.001). However, there were no significant differences between treatment groups in the bone formation rate per tissue volume (BFR/TV per day).

Bone biomechanical data are displayed in **Table 5**. There were no significant differences in left tibia thickness or width between treatment groups. The mean maximum flexure load was highest in the tibias from the chow group. This value was higher than the maximum flexure load experienced by the tibias from the fructose and glucose fed rats but the differences were not significant. Similarly, the flexure modulus was highest in the tibias from the chow group and lowest in the glucose group. However, the values for the two groups were not significantly different. Additionally, no significant differences between groups were observed for maximum stress, energy at maximum flexure extension, maximum flexure extension, flexure strain at maximum flexure extension, or flexure stress at maximum flexure extension.

DISCUSSION

The purpose of this study was to investigate the influence of the consumption of the monosacchariades glucose and fructose on measures of bone formation, quality, and strength. Before reviewing our results and the relevant literature, we must acknowledge that a lack of standardization between the chow diet and the experimental diets could have contributed to the differences observed between the groups. In future studies, differences in macronutrient percentages and the amount of micronutrients between diets should be eliminated to ensure that the type of carbohydrate is the only diet related variable between groups.

One of the key findings of this study was that consumption of diets high in the monosaccharides glucose and fructose was associated with increased measures of

cancellous bone formation in the distal femurs of growing male Sprague-Dawley rats. Specifically, the consumption of glucose and fructose resulted in higher mineral apposition rates (MAR, um/day) than the consumption of starch. As to be expected with increased bone deposition, the osteoblast surface (Ob.S/BS, %) and the number of osteoblasts (N.Ob/BS) were also higher in the bones of rats consuming glucose and fructose. These changes occurred without significant changes in osteoclast surface (Oc.S/BS, %) or osteoclast number (N.Oc/BS) suggesting an anabolic effect of sugar consumption on bone. However, despite observing a higher MAR, the consumption of glucose and fructose did not lead to significant differences in the mineralizing surface (MS/BS, %) or the bone formation rate (BFR/BS, um/day) between groups. Additionally, BFR/BV, a measure of bone turnover [19] was significantly higher in the glucose and fructose groups than in the starch group.

Another key finding of this study was that glucose and fructose influenced bone microarchitecure of the distal femurs differently. Trabecular thickness (Tb.Th, mm^{-1}) was greatest in the rats that consumed the fructose diet and least in the rats consuming the glucose diet. This finding is important because the thinning of trabeculae is associated with age related bone loss, especially in men [20]. Therefore, any modifiable factor that thickens trabeculae or protects them from thinning may be beneficial in preventing the development of osteoporosis later in life. In this study we have shown that diets high in fructose and glucose have similar effects on cancellous bone formation. Therefore, it is unclear why there was such a big difference in Tb.Th between the fructose and glucose groups. It is possible that some other effect of either fructose or glucose not explored in this study contributed to this difference. Other measures related to trabecular microarchitecture such as trabecular space (Tb.S) and trabecular number (Tb.N) were not

significantly different between groups. However, Tb.S was greater in both the glucose and fructose groups compared to the chow group. It is possible that with an increased sample size or longer study duration, significant differences in Tb.S would be observable.

To our knowledge, this is the first study that has examined the influence of high sugar diets on bone formation and structure in cancellous bone using methods of histology and histomorphometry. However, many other studies have investigated the effects of dietary sugar consumption on bone morphometry, bone mineral content (BMC), bone mineral density (BMD), and mechanical strength. The consumption of a high-fat sucrose (HFS) diet has been shown to have deleterious effects on bone strength and morphology in growing female rats [10, 11]. Both short-term (10 weeks) and long-term (2 years) exposure resulted in lower measures of mechanical strength in L6 vertebrae and the femoral neck when compared to exposure to a low-fat complex carbohydrate diet (LFCC). In female mice (aged 9 weeks) fed a HFS diet for 10 weeks, tibial cortical thickness, cross-sectional area, and strength were lower than in mice fed a LFCC diet [12]. To investigate possible mechanisms for these changes, researchers also explored differences in molecular and endocrine markers of bone turnover. They concluded that differences in bone between the two groups was likely due to osteoclast activation secondary to a chronic inflammatory state induced by obesity (HFS mice were 40% heavier with 14.9% more body fat than LFCC mice) as indicated by upregulation of cyclo-oxygenase 2 (COX-2) mRNA in the tibias of mice fed the HFS diet

It is difficult to elucidate the specific effects of sugar on bone from these data due to the high fat content of the experimental diets. Tjaderhane and Larmas (1998) [13] demonstrated lower measures of mechanical strength in the tibias and femurs of male and

female weanling rats fed a low-fat high sucrose diet when compared to rats fed diets in which the main source of carbohydrate was starch. Additionally, total tibia and femur calcium were significantly lower in the female rats fed the sucrose diet. The authors cited increased urinary excretion of calcium as a result of hyperinsulinemia following sucrose consumption [21] as a potential mechanism for the observed differences in bone calcium content and ultimately the differences in mechanical strength between groups. The results of this study show that sucrose intake adversely effects bone independent of fat intake. However, it is difficult to directly compare our results because our study investigated the effects of the monosaccharide components of sucrose, glucose and fructose, on bone.

A study by Tsanzi et al (2008) [14] found that rats drinking a glucose-sweetened beverage had lower total femur and tibia BMC and BMD when compared to rats drinking a fructose-sweetened beverage. This finding is of particular interest because, as with our study, it demonstrates a difference in the effects of glucose and fructose on bone. However, It is difficult to directly compare the findings of our study to those of the one performed by Tsanzi et al (2008) because of differences in study design and bone variables tested. The biggest difference is that Tsanzi et al (2008) supplied glucose and fructose in addition to a regular diet as components of sweetened beverages (13% weight/volume) while in our study, they were provided as 40-50% of total energy intake. As a result of their study design, rats in the glucose group consumed significantly more of the beverage and less of the diet compared to rats in the fructose group. Therefore it is difficult to discern whether observed changes were due to the intake of the different sugars or due to the displacement of nutrient-rich foods by beverage consumption in the glucose group.

As with our findings, Tsanzi et al (2008) was unable to demonstrate differences in mechanical strength of tibias between experimental groups. The authors implicated their short study duration (8 weeks) as a possible explanation. In our study, it is more likely that small sample size is to blame as Tjaderhane and Larmas (1998) observed changes in the bending strength of tibias after 5 weeks of exposure to a high sucrose diet. Tsanzi et al (2008) investigated changes in bone turnover as a possible mechanism for the differences between the groups but found no differences in serum osteocalcin (*ocn*), serum alkaline phosphatase (ALP), or urinary deoxypyridinoline (DPD). The authors concluded that changes in bone turnover may be site specific and that these measures were indicative of bone formation and resorption activity occurring throughout the entire skeleton.

Most of the aforementioned feeding studies have explained their findings by referencing studies that demonstrate disturbances in mineral homeostasis with the consumption of different sugars [5-9]. However, the specific relationship between sugar consumption and mineral homeostasis is difficult to define due to inconsistencies between studies and the lack of specific bone measurements.

Therefore, in order to better understand the influence of sugar intake on bone measures, it is important to investigate other potential mechanisms for the observed differences.

The absence of differences in bodyweight and total energy intake between the treatment groups suggests that the observed differences in bone formation and microarchitecture are independent of these factors. Additionally, no significant differences in total relative visceral adiposity were observed between groups. When compared independently, there were no differences in the weights of perirenal fat pads. However, relative epididymal fat pads were significantly heavier in the glucose group compared to

the chow group. Relative epididymal fat pads were also heavier in the fructose group but the difference was not significant. The assessment of differences in body composition would have been helpful in determining changes in metabolism as a consequence of sugar consumption. These data coupled with information about fat pad weights would have provided a clearer picture of how different sugars influence total body fat and the pattern of fat deposition.

Results from recent cross-sectional studies have demonstrated an inverse association between visceral adiposity and measures of bone density and morphology. Gilsanz et al (2009) [22] found that visceral fat was negatively associated with femoral cross-sectional area and cortical bone area in 15-25 year old women. Similarly, in a comparison between overweight children 7-11 years old, Pollock et al (2010) [23] found that visceral adiposity, but not total adiposity, was inversely related to BMD. Due to the cross-sectional nature of these studies, a causal relationship between visceral adiposity and the negative effects on bone cannot be determined. However, along with our findings, these data should compel researchers to further explore the relationship between nutrient intake, the pattern of fat deposition, and the influence on bone.

A new area of research that points to the skeleton as a player in energy metabolism may provide an avenue for trying to understand the influence of sugar on bone. Specifically, bone is regulated by leptin, a hormone produced by white adipose tissue that helps to regulate appetite and metabolism [24]. The effects of leptin on bone may be site (appendicular vs. axial) and bone type (cortical vs. trabecular) specific. A study by Hamrick et al (2004)[25] showed that BMC, BMD, and trabecular bone volume were greater in the axial skeleton and lower in the appendicular skeleton in leptin deficient (ob/ob) mice when

compared to lean controls. These results suggest leptin influences axial and appendicular skeletal sites differently. However, in another study, Bartell et al (2011) [26] investigated the effects of central *vs.* peripheral administration of leptin on appendicular and axial skeletal sites in ob/ob mice. Exogenous leptin resulted in increased BMC, BMD, bone area, and mineral apposition rates as well as reduced numbers of marrow adipocytes in ob/ob mice regardless of bone site or route of administration.

The discussion of the influence of leptin on bone is pertinent because fructose and glucose consumption influence circulating leptin levels differently. Leptin production depends on insulin-dependent glucose metabolism in adipocytes [27]. Because fructose does not stimulate the β-cells of the pancreas to secrete insulin, smaller postprandial insulin levels following fructose consumption lead to lower circulating levels of leptin [28]. Therefore, lower circulating leptin levels in response to fructose consumption should be explored as a possible mechanism for differences in bone measures between rats fed high glucose or high fructose diets.

Bone has also been shown to influence glucose homeostasis through the production of undercarboxylated osteocalcin in response to insulin binding to its receptor on the surface of osteoblasts [29]. The binding of insulin on osteoblasts decreases the production of osteoprotegerin (OPG). This leads to a decrease in the ratio of OPG to receptor activator of nuclear $\kappa\beta$ ligand (RANKL) and stimulates osteoclast activity. The acidic environment associated with bone resorption serves to decarboxylate osteocalcin to produce the undercarboxylated form. Undercarboxylated osteocalcin stimulates the proliferation of pancreatic β-cells, insulin secretion, and insulin sensitivity. This sequence of events has been shown to influence whole body glucose metabolism in mice lacking the insulin

receptor in osteoblasts. These mice developed glucose intolerance and insulin insensitivity when fed a normal diet [24]. In humans, Pollock et al (2011) showed that prepubertal overweight children with prediabetes had lower serum levels of undercarboxylated osteocalcin than children without prediabetes. These findings suggest that insulin resistance may also alter the activities of bone remodeling and in turn alter glucose homeostasis.

As mentioned previously, fructose consumption does not stimulate insulin secretion. In fact, post-prandial insulin responses are significantly lower following short- term fructose consumption when compared to glucose consumption [30]. Further, fructose consumption over time has been associated with the development of insulin resistance [28]. This relationship is related to lipid deposition in the liver in response to long-term fructose consumption [31]. In our study, the livers of fructose fed rats were significantly heavier than the livers of glucose fed rats despite controlling for differences in bodyweight. One possible explanation for this difference is that exposure to fructose over the 12-week feeding study lead to the accumulation of fat in the livers of rats. However, without exploring the composition of the livers, we cannot conclude that the livers of the fructose fed rats were heavier due to fat accumulation.

Although our study is one of the first to show that the consumption of diets high in fructose and glucose influence cancellous bone formation and structure, we were unable to determine the mechanisms through which these changes occurred. In order to make associations between energy metabolism dysregulation and changes in bone, future studies should include biochemical measures of serum leptin, undercarboxylated osteocalcin, and fasting serum glucose and insulin levels in response to sugar consumption over time.

Additionally, although the histological and histomorphometric techniques used in this study provided valuable information about bone formation and structure, they are limited in that they only allow for the measurement of bone in two dimensions. The utilization of imaging techniques such as micro-computed tomography (μCT) would allow for the measurement of both cortical and cancellous bone as well as the visualization of the arrangement of trabecular bone in three dimensions. This would provide information about trabecular bone connectivity and anisotropy—qualities that have been shown to be better predictors of fracture risk than trabecular thickness [20]. Other techniques such as dual energy x-ray absorptiometry densiometry (DXA) could be utilized in order to understand how sugar consumption influences whole bone BMC and BMD.

Another limitation of this study was that animals were run in two cohorts. Although we controlled for factors such as animal age, body weight, and the environment in which they were raised, the only way to completely control potential variables is to test all animals at the same time. It is also likely that differences in variance made it difficult to detect whether measures, especially those related to bone strength, were significantly different between groups. A larger sample size would help to minimize this problem. Because male rats achieve peak bone mass around 10 months of age [32], a longer study duration would be necessary to determine whether changes in bone formation and quality due to sugar consumption limited or enhanced the potential for skeletal growth. An even longer study duration would be necessary to determine whether these changes influenced the development of osteoporosis with aging. Finally, we do not know how reduced intake and weight loss in response to chlorotetracycline injections during the final week of the study may have affected the outcomes of this study. Whatever the effect, it is likely to have

been similar in all groups as there were no significant differences in the amount of weight lost between groups. It would be worthwhile to find another fluorochrome that would not cause decreased intake following administration for use in future studies.

CONCLUSION

The high fructose and glucose diets used in this study were found to enhance parameters of cancellous bone formation in the distal femurs of growing male Sprague-Dawley rats. This suggests that sugar consumption may have an anabolic effect on bone during growth. This effect, as well as differences in properties of cancellous bone structure between groups, may be related to differences in energy metabolism in response to long-term fructose versus glucose consumption. Despite these observations, no differences in bone strength were detected in response to sugar intake. This may indicate that sugar intake influences cortical bone differently than cancellous bone as the tibias were stressed at their midpoint. Further research is still needed to determine the mechanism involved in the alteration of bone formation and structure in response to sugar consumption and whether these changes ultimately lead to differences in bone quality and strength.

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Table 1. Macronutrient compositions and energy densities of the experimental diets.

	Chow		Glucose		Fructose	
Protein	25%		20%		20%	
Carbohydrate	62%		70%		70%	
Glucose		____		50%		10%
Fructose		____		____		40%
Starch		44%		20%		20%
Sucrose		4%		____		____
Lactose		2%		____		____
Fat	13%		10%		10%	
Kcals/gram	3.07		3.8		3.8	

Table 2. Effects of dietary sugar intake over a twelve week period on total energy intake, body weight, and weight gain. Data are means ± SEM. Means with different superscripts (a, b) are significantly different at p<0.05.

	Chow	Glucose	Fructose	P-value
Total Energy Intake (Kcals)	5822 ± 98.0	5683 ± 108	5932 ± 116	NS
Initial body weight (g)	287.5 ± 1.5	289.1 ± 3.2	281.9 ± 3.5	NS
Week 11 body weight (g)	427.6 ± 3.4	412.5 ± 7.1	409.3 ± 8.3	NS
Weight gain-baseline to week 11 (g)	160.6 ± 4.0	140.2 ± 5.4	143.8 ± 6.7	NS
Weight lost-week 12 (g)	20.5 ± 1.9	16.8 ± 5.4	16.4 ± 6.7	NS

Table 3. Effects of dietary sugar intake over a twelve week period on static histomorphometry measures. Data are means ± SEM. Means with different superscripts (a, b) are significantly different at p<0.05.

Parameter	Chow	Glucose	Fructose	P-Value
TV (mm^2)	2.24 ± 0.64^a	1.61 ± 0.15^b	1.60 ± 0.12^b	0.047
BV (mm^2)	5.65 ± 0.75^a	4.03 ± 0.25^b	3.56 ± 0.26^b	0.009
BV/TV (%)	41.4 ± 5.4	39.4 ± 1.5	45.0 ± 1.69	NS
BS (mm)	51.2 ± 6.1	39.3 ± 3.2	33.4 ± 1.9	NS
BS/BV (mm^{-1})	23.0 ± 0.42^{ab}	24.7 ± 1.0^a	21.2 ± 0.81^b	0.025
BS/TV (mm^{-1})	9.45 ± 2.6	9.67 ± 0.25	9.46 ± 0.18	NS
Tb.Th (um)	87.2 ± 1.6^{ab}	81.7 ± 3.0^a	95.3 ± 3.6^b	0.016
Tb.N (mm^{-1})	4.73 ± 0.57	4.84 ± 0.13	4.73 ± 0.23	NS
Tb.S (um)	105.0 ± 12.3	125.9 ± 5.1	116.6 ± 4.6	NS
QS (mm)	47.6 ± 5.1^a	35.7 ± 2.8^{ab}	29.4 ± 2.2^b	0.005
ObS (mm)	5.29 ± 0.56	7.94 ± 1.1	7.12 ± 0.75	NS
OcS (mm)	3.50 ± 1.0	2.70 ± 0.35	2.69 ± 0.47	NS
ObS/BS	10.8 ± 1.3^a	20.1 ± 1.6^b	21.4 ± 2.0^b	0.002
OcS/BS	6.24 ± 1.3	6.77 ± 0.63	8.17 ± 1.38	NS
N.Ob/BS	6.0 ± 0.81^a	12.2 ± 1.2^b	11.6 ± 1.2^b	0.005
N.Oc/BS	1.68 ± 0.67	1.73 ± 0.15	1.94 ± 0.19	NS

Table 4. Effects of dietary sugar intake over a twelve week period on dynamic bone measures. Data are means ± SEM. Means with different superscripts (a, b) are significantly different at p<0.05.

Parameter	Chow	Glucose	Fructose	ANOVA
MS (mm)	27.5 ± 5.5	23.8 ± 3.6	20.1 ± 3.8	NS
MS/BS (%)	56.1 ± 11	56.4 ± 6.1	51.6 ± 8.0	NS
MAR (um/day)	1.23 ±0.046^a	1.53 ± 0.089^b	1.56 ± 0.052^b	0.005
BFR/BS (um/day)	0.70 ± 0.13	0.83 ± 0.064	0.786 ± 0.11	NS
BFR/BV (per day)	0.0078 ± 0.0016^a	0.022 ± 0.0020^b	0.018 ± 0.0024^b	0.001
BFR/TV (per day)	0.0078 ± 0.0016	0.0084 ± 0.0074	0.0075 ± 0.0011	NS

Table 5. Effects of dietary sugar intake over a twelve week period on bone strength. Data are means ± SEM. Means with different superscripts (a, b) are significantly different at p<0.05.

| Parameter	Chow	Glucose	Fructose	P-value
Thickness (mm)	3.43 ± 0.06	3.49 ± 0.04	3.48 ± 0.05	NS
Width (mm)	3.06 ± 0.06	3.00 ± 0.04	2.95 ± 0.04	NS
Max flexure load (kgf)	5.26 ± 0.13	4.82 ± 0.04	5.05 ± 0.08	NS
Max stress (kgf/cm^2)	576.7 ± 47.95	515.1 ± 10.68	555.38 ± 22.9	NS
Flex modulus (kgf/cm^2)	88057.7 ± 16318.7	41697.4 ± 2217.7	73180.8 ± 13754.2	NS
Energy at max flexure extension (kgf-mm)	4.53 ± 0.61	5.43 ± 0.23	4.87 ± 0.67	NS
Max flexure extension	1.23 ± 0.23	1.65 ± 0.08	1.53 ± 0.19	NS
Flexure strain at max flexure extension	0.04 ± 0.01	0.05 ± 0.00	0.05 ± 0.01	NS
Flexure stress at max flexure extension	279.1 ± 26.8	258.1 ± 14.6	264.5 ± 23.7	NS
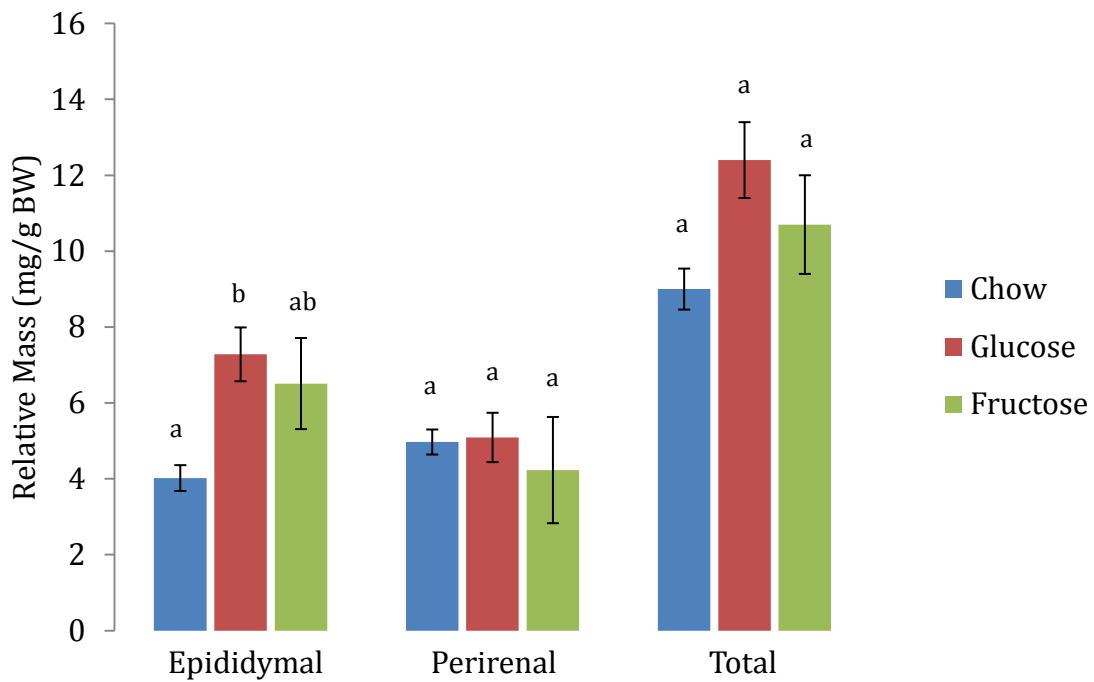
Figure 1. Effects of dietary sugar intake over a twelve week period on fat pad masses. Data are means ± SEM. Means with different superscripts (a, b) are significantly different at p<0.05.
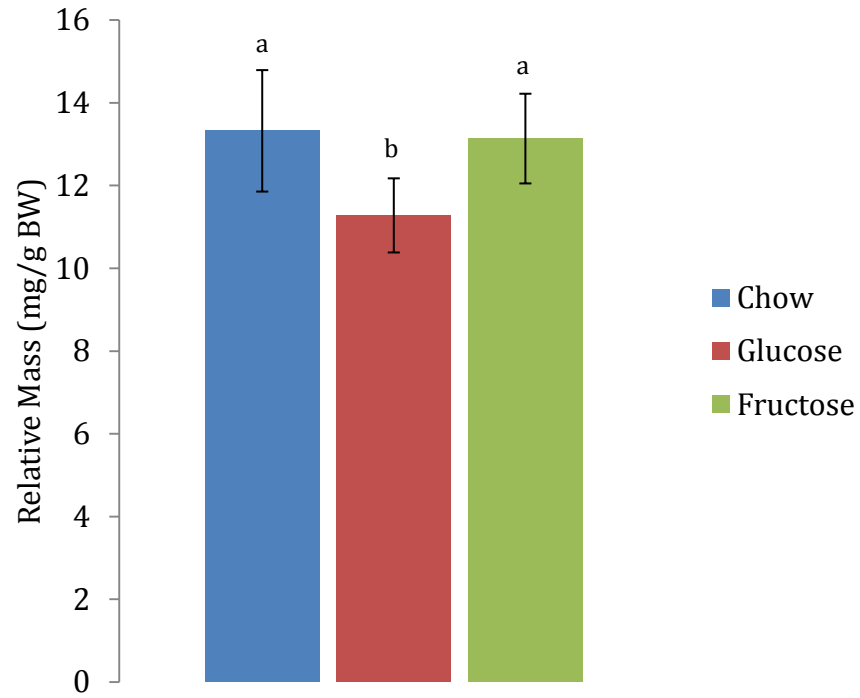
Figure 2. Effects of dietary sugar intake over a twelve week period on liver mass. Data are means ± SEM. Means with different superscripts (a, b) are significantly different at p<0.05.

CHAPTER 4

CONCLUSION

This thesis project examined the effects of high glucose and fructose intakes in the

rat diet on measures of bone formation, quality, and strength. The high glucose and

fructose diets used in this study were found to enhance parameters of cancellous bone

formation in the distal femurs of growing male Sprague-Dawley rats. This suggests that

sugar consumption may have an anabolic effect on bone during growth. This seemingly

contradicts the findings of other studies that have explored the relationship between

dietary sugar and bone[1, 2]. However, those studies looked at the effect on the whole bone

or cortical bone, not cancellous bone. Interestingly, the consumption of glucose and

fructose diets had distinctive effects on trabecular architecture with the consumption of

fructose resulting in thicker trabeculae. Possible explanations for this difference include

sugar specific disruptions of mineral homeostasis and/or differences in energy metabolism

in response to long-term fructose versus glucose consumption. Despite these observations,

no differences in bone strength were detected in response to the consumption of the

different sugars.

This thesis demonstrates that the relationship between dietary sugar intake and bone

is very complex. Unfortunately, because we did not assess parameters of metabolism or

mineral homeostasis, it is difficult to draw conclusions regarding the specific mechanisms

related to our findings. However, our findings related to differences in liver weights and fat

pads between groups may point to disturbances in energy metabolism as a consequence of

the consumption of different sugars. Specifically, the livers of fructose fed rats were significantly heavier than those of glucose fed rats and the epididymal fat pads of glucose fed rats were significantly heavier than those of chow fed rats.

In future projects examining the relationship between dietary sugar, energy metabolism, and bone, our research group will assess the effects of sugar consumption on measures of serum leptin, fasting serum glucose and insulin levels, glucose tolerance, and serum undercarboxylated osteocalcin. This knowledge is relevant in light of recent evidence that the hormones insulin and leptin, regulators of energy metabolism, also play a role in the regulation of bone [3]. Additionally, glucose and fructose have been shown to influence leptin and insulin secretion differently [4, 5]. Therefore, further investigation into the relationships between these factors will help to clarify the effects of glucose and fructose on bone and ultimately the connections between diet, metabolism, and the skeleton.

Additionally, in future studies we would utilize other methods of assessing bone in combination with the histological and histomorphometric techniques employed in this study. The utilization of imaging techniques such as micro-computed tomography (μCT) would allow for the measurement of both cortical and cancellous bone as well as the visualization of the arrangement of trabecular bone in three dimensions. This would provide information about trabecular bone connectivity and anisotropy—qualities that have been shown to be better predictors of fracture risk than trabecular thickness [6]. Other techniques such as dual energy x-ray absorptiometry densiometry (DXA) could be

utilized in order to understand how sugar consumption influences whole bone BMC and BMD. By using a combination of these methods, we can gain a better understanding of how sugar consumption affects both specific areas of bone and the bone as a whole. This will be helpful in guiding future studies seeking to explore this topic.

In summary, the findings of this thesis suggest that high intakes of glucose and fructose in the rat diet influence measures of cancellous bone formation in a similar manner but effect measures of cancellous bone structure differently. The long-term consequences of these effects on bone strength could not be determined in this study. Further research, particularly in the area of how these findings relate to changes in energy metabolism, is necessary to understand the mechanisms related to these findings. Additionally, further research related to the effects of sugar consumption during growth on future skeletal health is necessary to determine whether our findings will be useful in the ongoing search for methods of osteoporosis prevention.

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