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Histopathological evaluation of effects of high fructose diet on bone healing in tibial defects: An Experimental study

Evaluación histopatológica de los efectos de una dieta alta en fructosa sobre la curación ósea en defectos tibiales: un estudio experimental

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ABSTRACT

The purpose of this study was to examine the impact of a high-fructose diet on bone regeneration in defects created in rat tibias. The experimental setup was performed with 24 female Sprague-Dawley rats in the same estrus period; the rats were divided into two groups as control and experimental groups. In the control defect group (n=12), a cylindrical defect of 4 mm in diameter and 4 mm in depth was surgically created in the corticocancellous bone of the metaphyseal part of the right tibia of each rat. No other application was made in this group during the experimental setup. For each rat in the high-fructose-fed defect group (n=12), cylindrical defects of 4 mm in diameter and 4 mm in depth were surgically created in the corticocancellous bone of the metaphyseal part of the right tibia. Fructose supplements of the groups were added to the drinking water at a rate of 20% (w/v). All rats were sacrificed at the end of the 12th week of the surgical application. The histological samples were evaluated under a light microscope. There was no significant differences in the bone regeneration between control animals and high fructose diet consumption group.52.2 ± 9 % for Controls, versus 49.8 ± 7.67 % for HFD (P>0,05). Further research is needed to determine the mechanisms responsible for these changes in bone structure and how these changes affect bone quality and strength with age.

Key words: Fructose; high fructose diet; bone healing; bone metabolism; bone formation

RESUMEN

El objetivo de este estudio fue investigar el efecto de la alimentación con dieta alta en fructosa sobre la regeneración ósea en defectos creados en tibias de ratas. El experimento se realizó con 24 ratas Sprague-Dawley hembras en el mismo período de celo; las ratas se dividieron en dos grupos: grupo control y grupo experimental. En el grupo control con defecto (n=12), se creó quirúrgicamente un defecto cilíndrico de 4 mm de diámetro y 4 mm de profundidad en el hueso corticoesponjoso de la parte metafisaria de la tibia derecha de cada rata. No se realizó ninguna otra aplicación en este grupo durante el experimento. Para cada rata del grupo con defecto alimentado con alta fructosa (n=12), se crearon quirúrgicamente defectos cilíndricos de 4 mm de diámetro y 4 mm de profundidad en el hueso corticoesponjoso de la parte metafisaria de la tibia derecha. Los suplementos de fructosa de los grupos se agregaron al agua potable a una tasa del 20% (p/v). Todas las ratas fueron sacrificadas al final de la semana 12 de la aplicación quirúrgica. Las muestras histológicas fueron evaluadas bajo un microscopio óptico. No hubo diferencias significativas en el caso de la regeneración ósea entre los grupos control y dieta alta en fructosa 52,2 ± 9 % para animales en la dieta control, versus 49,8 ± 7,67 % para HFD. (P>0,05). Se requieren más investigaciones para identificar los mecanismos responsables de estas alteraciones en la estructura ósea y determinar si los cambios afectan en última instancia la calidad y la resistencia ósea con la edad.

Palabras clave: Fructosa; dieta alta en fructosa; curación ósea; metabolismo óseo; formación ósea

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INTRODUCTION

Recently, many changes were detected in the human diet. Commonly consumed foods and beverages such as beverages, cookies, bread, processed snacks, fermented milk products and chocolate products contain large amounts of sweeteners. Sucrose, high fructose syrups, glucose syrup, fruit juices, honey and molasses, which are frequently used in the chocolate, cake and biscuit industry, can be included in these sweeteners [1].

In humans it was reported that fructose consumption significantly increases de novo lipogenesis, whereas eucaloric glucose intake does not [2 , 3]. It can be said that fructose has a more lipogenic property compared to glucose. Additonally, it can be said that this lipogenic effect is more pronounced in individuals with insulin resistance or type 2 diabetes [4 , 5]. Fructose does not induce the production of insulin or leptin, hormones. These hormones are important and necessary for long-term energy regulation. Long-term fructose consumption, which reduces insulin responsiveness and leptin production, may have harmful effects on energy regulation and body fat metabolism [6].

Recently, excessive fructose consumption has been associated with obesity and metabolic syndrome [7]. And high fructose consumption in rats has been reported to cause hypertension, and increasing blood triglyceride, insulin, and insulin resistance [8]. Fructose is more lipogenic when compared with glucose and leads to higher triglyceride levels [9]. The increase in triglycerides increases the intramyocellular triglyceride content in skeletal muscle, due to this mechanisms insulin resistance develops in the individual [10 , 11].

High-fructose diets contribute both directly and indirectly to metabolic syndrome, insulin resistance, and cardiovascular disease. The direct effect occurs when fructose consumption disrupts lipid and carbohydrate metabolism, and the indirect effect occurs when sugar creates a positive energy balance that leads to fat accumulation and weight gain [12 , 13 , 14 , 15]. Studies in rats fed 10% fructose and 20% high-fructose syrup have also seen endothelial and liver dysfunction [12 , 13].

In the bone, osteoblasts and fat cells originate from the same mesenchymal stem cells. Local and systemic conditions can affect osteoblasts and fat cells [16]. Notably, diet-related negative impacts on trabecular bone structure may change by sex, with male mice showing greater changes compared to female mice [17].

Studies examining the relationship between bone microarchitecture and nutrition in female rodent models may help to exhibit the relationship between nutrition and bone metabolism in humans. In addition, studies on rodents regarding effects of the diets on bone metabolism in long-term may also be valuable in revealing the long-term relationship between nutrition and bone tissue in humans. Histomorphometric examinations have shown that bone microarchitecture deteriorates in animals fed a fatty diet. It may also be of interest to examine diet-induced changes in osteoblast function [18].

Dietary habits can affect bone metabolism and bone remodeling processes. It was stated that high-saturated fat diet increases osteoclast activity, which is the main cells of trabecular bone destruction, and increases the level of fat in the bone marrow in rodent models [19 , 20 , 21 , 22]. Ex vivo examination of fructose-rich diets, which are converted to triglycerides in the liver and are directly linked to insulin resistance, has shown that their osteogenic properties are reduced and the adipogenic properties of bone marrow stromal cells are increased [6 , 23]. It has been reported that the combination of high-fat and high-fructose diets disrupts the physiological balance between bone formation and bone destruction, called remodeling, and leads to a decrease in trabecular bone volume [24]. In addition, it has been reported in studies conducted on both minipigs and mice that a high-fat diet also negatively affects the osseointegration process, where bone metabolism is extremely important [25 , 26].

The areas of interest of reconstructive bone surgery include congenital bone disorders, bone losses due to trauma, tumoral formations and bone losses due to severe infections. Bone defects in a small size may heal spontaneously, while bone defects in a large size may require various materials. The aim of bone defect treatment in surgical applications is to repair and renew bone tissue [27 , 28]. The aim of this study is to examined the healing levels of bone defects created in the tibias of rats (*Rattus norvegicus*) fed a high-fructose diet for 12 weeks using histopathological methods.

MATERIALS AND METHODS

Animals and study design

All experimental processes of this study were performed at Fırat University Experimental Research Center (Elazig, Turkiye) after receiving approval from Fırat University Animal Experiments Local Ethics Committee (Elazig, Turkiye) (Protocol No: 30 December 2022-13363). All rats included in this study were obtained from rats produced at Fırat University Experimental Research Center. During all experimental phases, the Declaration of Helsinki for the care and welfare of animals was strictly followed.

The experimental setup was performed with 24 female Sprague-Dawley rats in the same estrus period by using vaginal smear method; the rats were divided into two groups as control and experimental groups. A cylindrical defect of 4 mm in diameter and 4 mm in depth was surgically created in the corticocancellous bone of the metaphyseal part of the right tibia of each rat in both of the groups [27 , 28]. No other application was made in the control group during the experimental protocol. The high-fructose-fed defect group was supplemented with 20% fructose in their drinking water (w/v) [12]. All rats were euthanized at the end of the 12th week after surgery [22].

Surgical procedure

All rats were left without food for 8 hours (h) before the creation of the defects. All surgical procedures performed on the subjects were developed under general anesthesia. Xylazine hydrochloride (Rompun®, Bayer, Germany) (10 mg/kg) and Ketamine hydrochloride (Ketasol®, Richter Pharma, Austria (40 mg/kg) were used to provide anesthesia. After the operation area was shaved, antisepsis was provided using povidone iodine solution. A 1.5 cm surgical incision was made on the tibial crest in the surgical area and a periosteal elevator was used to scrape the soft tissue in the corticocancellous bone area where the defect would be created.

In all rats included in the study, monocortical bone defects of 4 mm in diameter and 4 mm in depth were created in the corticocancellous bone layer in the metaphyseal parts of the right tibia bones using a surgical drill (WH Physiodispenser, Austria) under sterile saline cooling. After surgery, all soft tissues and skin were sutured to their original positions (5/0 vicryl, Ethican, Inc. USA). Intramuscularly antibiotic (Penicillin, 50 mg/kg) and analgesic (Tramadol hydrochloride, 0.1 mg/kg) were administrated for infection and pain control for 3 day (d) after surgery. All rats in the control group fed with a normal diet and in the experimental group fed with a high-fructose diet were euthanized in the 12th week of the study. Bone blocks containing defects in the right tibia bones of the rats were removed, decalcified by using %10 formic acid solution and subjected to histopathological analysis.

Application of fructose diet

Fructose supplements of the groups was added to the drinking water at a rate of 20% (w/v) [12]. The rats access to water and food was (will be) provided with ad *ad libitum.*

Histopathological analyses

Histopathological analyses were performed at the Department of Pathology, Faculty of Medicine, Fırat University (Elazig, Turkiye). After hematoxylin and eosin staining, histological samples were evaluated under a light microscope (Olympus BX43, Tokyo, Japan).

Histopathological analysis was performed by measuring the ratio (%) of new bone formation in the healing bone defect area to the defect area [27 , 28] Images of all histological sections were captured with a digital camera (Olympus Bx51; Olympus Corporation, Tokyo, Japan) connected to a light microscope and recorded on a computer. Imaging software (Olympus DP71; Olympus Corporation, Tokyo, Japan) was used to perform histomorphometric analyses [27 , 28].

Statistical analysis

IBM SPSS Statistics version 22 was used to evaluate the data obtained in this study. The Kolmogorov-Smirnov test was used to evaluate whether the data were normally distributed. Student's T test was used to compare between groups in the evaluation of normally distributed data. Data are given using mean and standard deviation. Significance was assessed at P<0.05.

RESULTS AND DISCUSSION

As seen in the TABLE I, no significant difference was detected between control rats and high fructose diet rats in histological newly regenerated bone ratios (%) (P>0,05 P:0,529) (52,2± 9 % for Controls, versus 49,8 ± 7,67 % for HFD) (FIG. 1 A,B).

TABLE I. Newly regenerated bone (NRB) of the groups. *Student T Test.

GROUPS	MEAN (%) (NRB)	ST. DEV.	P*
CONTROL (n=12)	52.2	9.00	0.529
HIG FRUCTOSE DIET (n=12)	49.8	7.67	

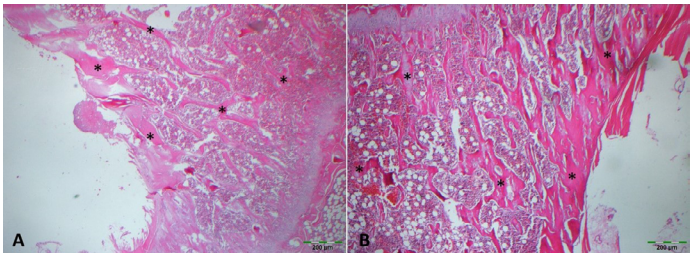

FIGURE 1. Decalcified histologic images of the groups; A: Control Group, B: High Fructose Diet Group. Newly reegenerated bone (NRB) tissues in defect site and are surrounded by fatty bone marrow. Bone filling and maturation of the defects in the experimental group; high fructose diet, were not statistically significantly different compared with controls (P>0.05). (Hematoxylin-Eosin). *Newly regenerated bone

Diet can directly affect the bone remodeling process and bone metabolism, which is an important parameter of healing in bone defects. It has been reported that saturated high-fat diets negatively affect the trabecular bone microstructure in rodents, disrupt the microstructure, increase osteoclast activity and function, and increase the fatty part in the bone marrow. Fructose-rich diets are directly metabolized to triglycerides in the liver, which directly causes insulin resistance. In ex vivo studies examining the relationships between fructose diets and bone tissue, it has been shown that fructose diets reduce the osteogenic potential in stromal cells in the bone marrow and suppress osteogenic properties by increasing the adipogenic potential. In addition to their use alone, it has been stated that high-fat and high-fructose diet combinations also disrupt bone metabolism and lead to a decrease in trabecular bone volume [12]. When examining the link between diet and bone metabolism, it is seen that both osteoblasts and adipocytes originate from common mesenchymal stem cells in the bone marrow. Both adipocytes and osteoblasts can be affected by local and systemic conditions and changes [29 , 30]. Alterations in bone microstructure may lead to functional alterations, which can be assessed using dynamic histomorphometry-a quantitative method for evaluating bone formation over time. Studies have shown reduced bone formation in animals fed a high-fat diet [18]. Preclinical animal models offer valuable insights into how modifiable factors, such as diet, may impact bone metabolism.

When the literature is examined, it is seen that there are studies examining the effects of sugar diet on bone tissue parameters; bone morphometry-microstructure, bone mineral content-density and biyomechanical strength. It was stated that consumption of high-fat sucrose (HFS) diet has negative effects on bone strength and morphological structure in female rats during growth and development [20 , 31]. According to the data obtained from the Lorincz *et al*. study [32], mice fed high fructose syrup were 40% heavier than mice on the low-fat complex carbohydrate diet and when assessed for body fat, mice fed high fructose syrup had 14.9% more body fat. Additionally, these study when examined at the molecular level, mice fed high fat sucrose diet had increased expression of cyclooxygenase-2 mRNA in the tibia bones compared to control subjects [32]. To uncover the mechanism behind these situation, changes in molecular and endocrine markers of bone turnover were examined; serum tartrate-resistant acid phosphatase, osteocalcin, Receptor activator of nuclear factor kappabeta ligand (RANKL), osteoprotegerin/RANKL and cyclooxygenase-2. Lorincz *et al*. [32] suggested that the difference between the two groups may be due to the triggering of osteoclast cells by chronic inflammation caused by obesity. According to the

data obtained from the Lorincz *et al.* [32] study, HFS mice were 40% heavier and had 14.9% more body fat than low-fat complex carbonhydrate mice. Additionally, increased expression of cyclooxygenase-2 mRNA was detected in the tibias of HFS-fed mice compared to controls [32]. Histopathological data obtained from this study revealed that there was no difference in bone healing in subjects fed a high fructose diet for 12 weeks compared to controls. Within the limitations of this study it can be stated that this result may also be due to the difference in method.

In another study, Douard *et al*. [33] reported that fructose consumption reduces calcium transport in both the intestines and the kidneys. Pregnant and virgin female rats were each randomly assigned to 3 groups fed a 63% glucose, a 63% fructose, or a 63% starch diet modified from a standard, published American Instituteof Nutrition (AIN)-93G formula containing normal Ca^{2+} and P_i levels.They stated that the relationship between fructose consumption and decreased calcium transport is an effect related to the decrease in 1,25-dihydroxyvitamin D3 (1,25(OH)2D3) levels. It is thought that the increased expression of 24-hydroxylase (CYP24A1), which promotes renal catabolism of 1,25-(OH)2D3, and the decreased expression of 1α-hydroxylase, which impairs 1,25-(OH)2D3 synthesis, are related to this mechanism. As a result of the above mentioned mechanism it was suggested that fructose negatively affects calcium metabolism by altering vitamin D regulation [33]. Tsanzi *et al*. [34], in their experimental animal studies examining the effects of different types of sugary drinks on bone mass and biomechanical strength, could not detect any difference in the biomechanical strength of the tibia between the experimental groups. The researchers suggested that this result may have been due to the short duration of the experimental setup. In contrast, Tjäderhane and Larmas [35] stated that they found changes in the bending strength of the tibia after only 5 weeks of feeding in their studies on subjects fed a high sucrose diet [35]. Tsanzi *et al*. [34] also reported that serum osteocalcin, serum alkaline phosphatase and urine deoxypyridinoline, which are considered to be the main markers of bone metabolism, did not create a significant difference between the groups. The authors reported that these results obtained may be due to the fact that the changes in bone turnover are region-specific. In addition, they stated that their measurements may have shown the bone formation and resorption activity of the entire skeletal system rather than regional changes [34]. This study was conducted on the tibial bones of rats. The data obtained from this study may not be valid for the entire skeletal system of the rats included in the study.

Nuche-Berenguer *et al*. [36] studied the changes in bone structure associated with insulin resistance induced by fructose consumption in male rats by feeding them 20% fructose containing drinking water for 8 weeks. The researchers reported that bone trabeculation was higher and bone filling was lower in fructose-fed rats compared to normal water-drinking controls. The researchers also suggested that a higher degree of structural irregularities in trabecular bone was detected in subjects consuming fructose containing water [36]. In nutritional studies, the deterioration in mineral balance caused by sugar consumption has been explained by referring to previous studies [37 , 38]. However, the negative relationship between sugar consumption and mineral balance has not been fully clarified due to uncertainties in the studies, inconsistencies between studies, and lack of bone measurements. More advanced studies are needed on other potential mechanisms to better understand the negative relationship between sugar consumption and bone health. In a study conducted by Tjaderhane and Larmas in male and female weaned rats they noted that the strength in the tibia and femurs of rats fed a low-fat diet was lower, high sucrose diet when compared to those fed a starch-based diet [35]. In addition, the tibia and femur calcium levels of the subjects were significantly lower in female rats fed a sucrose diet. The researchers reported that possible mechanism could be due to increased urinary calcium excretion resulting from hyperinsulinemia following sucrose consumption, and that this mechanism may account for the differences in bone calcium content and mechanical strength observed between the groups. It was stated that sucrose consumption negatively affects bone health independently of fat consumption [36]. Felice *et al*. [23] reported in their studies on rats that fructose diet causes metabolic syndrome, fructose feeding has negative effects on bone microarchitecture and this harmful effect is related to the disruption of bone formation mechanism. They also reported that these changes may be due to deflection in adipogenic/osteogenic potential of mesenchymal stem cells affected by modulation in Runx2/PPARγ ratio [23].

In another study, Yarrow *et al*. [22] investigated the effects of 12 weeks of standard diet (Control), 30% moderate high fat/no sugar diet, or 30%/40% high fat/no sugar diet/high fructose diet on trabecular cancellous and cortical bone development in 8-week-old male Sprague-Dawley rats. Yarrow *et al*. reported that feeding "western" high-fat diet in skeletally immature male rats disrupted the architecture of cancellous bone tissue but not cortical bone, and bone loss was not exacerbated when fructose was consumed along with the high-fat diet [22]. In another study, Bass *et al*. [39] investigated the effects of glucose and fructose diets on bone formation, bone microstructure and architecture, and biomechanical strength of bone tissue. The researchers emphasized that glucose and fructose may also have different effects on bone tissue due to their different metabolic and structural properties. The researchers first randomly divided the two-month-old rats they included in their study into two groups: one group was given a high-fructose diet for 12 weeks, while the other group was given a high-glucose diet for 12 weeks.

According to the data obtained at the end of the study, the researchers determined that the subjects who were given a high-fructose diet had better bone microstructure and architecture than the subjects who were given a high-glucose diet. In another study Khan *et al*. studied the effects of moderate fat/high sugar and high fat/high fructose diets on femur bones in weaned male Wistar rats [40]. The researchers evaluated the mineral content and density of the rats right femur bones, cortical and cancellous bone architecture, and cell populations within the bone. The experimental phase was completed by dividing the rats into three feeding groups: control, high fat/high fructose, and moderate fat/high sugar during the 17-week experimental setup. At the end of the study, bone mineral content and bone mineral density in the rats' femur bones were examined by densitometric method.

Microcomputed tomography was used to evaluate the microstructural features of cortical and cancellous bone. Osteoblast, osteoclast, fat cell, and chondrocyte numbers were evaluated using histomorphometric methods. At the end of the study, the researchers reported that the moderate fat/high sugar diet was largely beneficial for bone, while the high fat/high fructose diet had negative effects on bone mineral content, bone mineral density, bone microstructure and bone cell populations [40]. In this study, no difference was found in bone healing in the rats given a high-fructose diet compared to the control group rats fed a normal diet. These results do not contradict the

study of Bass *et al*., who reported that the application of a high-fructose diet did not worsen bone microstructure [39].

CONCLUSION

Within the limitations of this study, it can be stated that high fructose diet application does not have a suppressive effect on the healing of bone defects. Further research is required to determine the mechanisms responsible for the changes in bone structure caused by diet and to determine the methods of protection against these changes caused by diet.

Conflicts of interest

The author declares that I have no conflict of interest.

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