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ORIGINAL ARTICLE

Kefir alters craniomandibular bone development in rats fed excess dose of high fructose corn syrup

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Abstract

Introduction Dietary high fructose corn syrup (HFCS) is involved in the pathogenesis of oral diseases as well as metabolic diseases. The aim of this study was to investigate the effects of HFCS-feeding on the craniomandibular bone development at an early age and also the potential of milk kefir for preventive treatment.

Materials and methods In this study, Control, Kefir, HFCS, and HFCS plus Kefir groups were formed; kefir was given by gastric gavage, while HFCS (20% beverages) was given in drinking water; for 8 weeks.

Results Based on morphological evaluations, immunohistochemical, and gene expression results, it was clearly determined that excess dose of HFCS consumption decreased osteoblastic activity in craniomandibular bones while increasing osteoclastic activity. However, it has been determined that the intake of kefir with the HFCS-feeding greatly suppresses the effects of HFCS on bone tissues.

Conclusion In conclusion, dietary the excess dose of HFCS at an early age has been observed to pose a risk for cranial and mandible bone development. The healing effects of kefir may be a new approach to the treatment via kefir consumption in young's.

Keywords HFCS · Kefir · Craniomandibular bone · RANKL · RUNX2

Abbreviations

BMP 2–7	Bone morphogenetic protein 2–7
DKK	Dickkopf WNT signaling pathway inhibitor 1
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
HE	Hematoxylin–eosin
HFCS	High fructose corn syrup
IBSP	Integrin-binding sialoprotein
LRP5	LDL receptor related protein 5
NF-κB	Nuclear factor-kappa B
Ob.S/BS	Osteoblast surface
OS/BS	Osteoid surface
OCN	Osteocalcin
OPN	Osteopontin
OSN	Osteonectin
OSP	Osteoprotegerin
qRT-PCR	Quantitative real-time PCR
RANKL	Receptor activator of nuclear factor kappa-B ligand
RUNX2	Runt-related transcription factor 2
TNF-α	Tumor necrosis factor-α

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Introduction

The consumption of calorie sweeteners in the daily diet is common in developed and developing countries. Among sweeteners, there has been a transition towards high fructose corn syrup (HFCS), especially since the 1980s [1]. It is known that adversely affects the mineral balance of sugar intake in the diet; therefore, it could have important implications for bones [2]. In a recent study, we demonstrated the disorders of the HFCS diet on masseter muscle and gingival tissue [3]. In this study, we aimed to investigate the possible effects and potential mechanisms of action of HFCS-feeding on cranial bone and mandible development at an early age.

Recent studies show that diabetes, metabolic syndrome, insulin resistance, and cardiovascular diseases are associated with carbohydrate consumption [4]. It is claimed in many studies that the main cause of this situation is fructose monosaccharide, which is included in sugar derivatives. Fructose is a sugar consumed in HFCS as well as organic foods, such as fruits and vegetables [5]. According to studies examining the effects of HFCS or fructose using alone, the detrimental effects of both carbohydrate sources are parallel. For instance, it has been shown that rats fed with 10% fructose [6, 7] or 20% HFCS [8, 9] developed endothelial and liver dysfunction. Similarly, HFCS consumption may cause bone formation or resorption along with these metabolic disorders. Namely, it has been shown that increased osteoclastic activity and occurred bone resorption with dietary high fructose for 12 and/or 15 weeks in animals [10, 11]. Bone morphogenetic proteins (BMPs) are involved in the formation and development of bone structure and belonging to the transforming growth factor (TGFβ) superfamily [12]. Experimental studies have shown that mutations in BMP genes play critical roles in the musculoskeletal system and also in infantile bone formation [13, 14]. Currently, two specific BMP types (BMP-2 and BMP-7) are known to be used in the clinic for tibial open fractures and setting of long bone nonunions [15]. The main purpose of treatment is to increase osteoblastic activity. Osteoblasts are derived from mesenchymal stromal cells (MSCs) and called as cells that build bone. The formation of osteoblasts begins with the transformation of MSCs into osteoprogenitor cells by the activated runt-related transcription factor 2 (RUNX2) initially. These middle form cells transform into osteoblasts under the influence of BMPs. Subsequently, mature osteoblasts that perform their function produce receptor activator of nuclear factor-κB (NfκB), receptor activator of NF-κB ligand (RANKL), and osteoprotegerin (OPG) which modulate the differentiation of osteoblasts into osteoclasts [16]. However, in this process, the proteins such as osteopontin (OPN) [17], osteonectin (OSN or SPARC) [18], Dickkopf WNT signaling pathway inhibitor 1 (DKK1) [19], low-density lipoprotein receptor-related protein 5 (LRP5) [20], and integrin-binding sialoprotein (IBSP) [21] responsible for the development of osteoblasts and osteocytes and also ensure bone mineralization.

Probiotics are dietary supplements consumed for the prevention and treatment of gastrointestinal diseases, allergies, and inflammation-dependent diseases. Microorganisms known as probiotics are naturally found in kefir, yogurt, cheese and other fermented dairy products. The main mechanisms underlying its effects can be listed as improving intestinal barrier function, microbiota modification in mucosa/epithelium, and enhancement of the lymphoid-dependent immune system [22]. In addition to the anti-inflammatory and anti-allergic properties [23] of daily kefir consumption in childhood, anti-viral effects [24] are also known. However, it has also been shown in many studies that kefir consumption improves the impaired insulin, glucose and lipid metabolism due to fructose or HFCS consumption [25, 26]. Interestingly, recent studies suggest that consumption of milk-kefir may prevent osteonecrotic bone loss [27, 28]. In previous study, we showed the favorable effects of kefir on mouth evolution by preventing inflammation of masseter muscle and gingival tissue in the young rats [3].

The aim of this study is to investigate the effects of possible detrimental effects of HFCS-feeding on the craniomandibular bone tissue at a molecular grade and to determine to what extent milk-kefir may straighten back these effects related to HFCS.

Results

The data representing body & omentum weights, food, liquid, and caloric intake as well as some metabolic parameters of rats, have been published in our recent study (Table 1) [3].

The influences of HFCS and kefir on the mRNA expressions of bone markers

As shown in Fig. 1, the gene expression levels of BMP-2 (a), BMP-7 (b), DKK1 (c), IBSP (d), LRP5 (e), OCN (f), OPN (g), OSN (h), OSP (i), RANKL (j), and RUNX2 (k) in the craniomandibular bones (A refers to the cranial bone; B refers to the mandible) from rats were established by real-time PCR analysis. HFCS-feeding decreased IBSP, LRP5, OCN OPN, OSN, OSP, and RUNX2 and increased RANKL mRNA expressions in the craniomandibular tissue samples from rats, whereas no changes were observed in BMP-2, BMP-7, and DKK1 mRNA expressions ($P < 0.05$ compared to control). Kefir consumption enhanced IBSP, LRP5, OCN OPN, OSN, OSP, and RUNX2 mRNA expressions in rats

Table 1 Primer sequences of BMP-2, BMP-7, DKK1, IBSP, LRP5, OCN, OPN, OSN, OSP, RANKL, RUNX2 and internal standard GAPDH used for the mRNA expression determination of qRT-PCR

Gene	Forward primer sequence (5′→3′)	Reverse primer sequence (3′→5′)	Product lenght (bp)
BMP-2	CACGAGAATGGACGTGCCC	GCTTCAGGCCAAACATGCTG	249
BMP-7	CATGGTCATGAGCTTCGTCA	TGTGATCTGGAAGGTCTCGT	187
DKK1	CTCAGTGTGGCACTTACCTG	TCTGCACCCTAGAGACAAAAG	200
IBSP	AGGCAACGAGTACAACACTG	CATAGCCATGCCCCTTGTAG	116
LRP5	GAGATTGACTGCATCCCTGG	GAGACAGACAGCATCGCAG	159
OCN	CGCTACCTCAACAATGGACT	AACACATGCCCTAAACGGTG	166
OPN	CGATCGATAGTGCCGAGAAG	TGAAACTCGTGGCTCTGATG	112
OSN	GGAAGCTGCAGAAGAGATGG	GGTCTTGTTGTCATTGCTGC	286
OSP	CTAGACAAGCAGGGGTAGGT	AACGTTGGGGGCAATATCAA	222
RANKL	CCCATCGGGTTCCCATAAAG	CAGGTTATGCGAACTTGGGA	241
RUNX2	CGCCTCACAAACAACCACAG	TCACTGCACTGAAGAGGCTG	225
GAPDH	TGATGACATCAAGAAGGTGGTGAAG	TCCTTGGAGGCCATGTGGGCCAT	249

upon HFCS-feeding, but RUNX2 mRNAs only in the cranial bone is not significant. There was a blunted decrease with kefir in BMP-2, BMP-7, and RANKL mRNAs in the craniomandibular bones of both healthy rats and in response to HFCS-feeding, but RANKL mRNAs of the Kefir group only in the cranial bone was not changed.

The influences of HFCS and kefir on the bone markers immunohistochemically

The expression level of OSN (a), OPN (b), and OCN (c) proteins measured by immunohistochemical staining was decreased in the craniomandibular tissues of HFCS-fed rats, whereas TNFα (d) and NfκB (e) were increased compared to controls (Fig. 2; A refers to the cranial bone; B refers to the mandible). Moreover, all these changes were normalized by kefir treatment in both cranial bone and mandibles of HFCS-fed rats. There was no change in the expression levels of OSN, OPN, OCN, TNFα, and NfκB with kefir treatment in the craniomandibular bones of healthy rats, but OSN level only in the cranial bone was increased.

Dietary kefir avoids bone degeneration in the HFCS-attenuated craniomandibular tissues of rats

Histological examination with HE staining showed that in Fig. 3, osteoblast surface (Ob.S/BS) (a) and osteoid surface (OS/BS) (d) were shrunken in the craniomandibular tissues (A refers to the cranial bone; B refers to the mandible) of HFCS-fed rats compared to controls, while osteoclast surface (Oc.S/BS) (b) and eroded surface (ES/BS) (c) were widened. However, kefir supplementation in the craniomandibular tissues of both healthy ($P < 0.05$ compared to control) and HFCS-fed rats ($P < 0.05$ compared to HFCS) increased OS/BS and Ob.S/BS. Whereas, kefir-treatment reduced Oc.S/BS and ES/BS in the mandibles; moreover, it decreased ES/BS in the cranial bones, significantly, but the reduction of Oc.S/BS only in the cranial bone was not significant.

Discussion

The prevalence of metabolic syndrome, visceral obesity, dyslipidemia, insulin resistance, and cardiovascular diseases are rapidly increased worldwide the last 40 years approximately. Studies conducted in this process claim that changes in lifestyle and diet are largely involved in the pathogenesis of the diseases [4]. The positive synchronization in the prevalence of metabolic syndrome and obesity with the increase in refinery sugar consumption made HFCS consumption controversial [1]. Although the cardiovascular and metabolic effects of HFCS consumption have been largely elucidated recently [8, 9], its effects on early age and oral development are unknown. In the first stage of our study, we demonstrated the detrimental effects of the excess dose of HFCS on masseter muscle and gingival tissue [3]. In this study, we showed its effects on cranial bone and mandible formation. In addition, the therapeutic effects of kefir consumption against the effects of the excess dose of HFCS in our previous study make our current study even more important.

Bone formation and modelling are directly related to calcium and phosphate homeostasis. This balance is due to the action of several systemic factors, especially multiple cytokines and hormones [29]. Osteoclast formation, named osteoclastogenesis, begins with mononuclear cell proliferation and results in the formation of circulating preosteoclasts. At this stage, in the presence of RANKL, preosteoclasts fuse with each other to forming immature osteoblasts and its re-evolution into osteoclasts occurs in

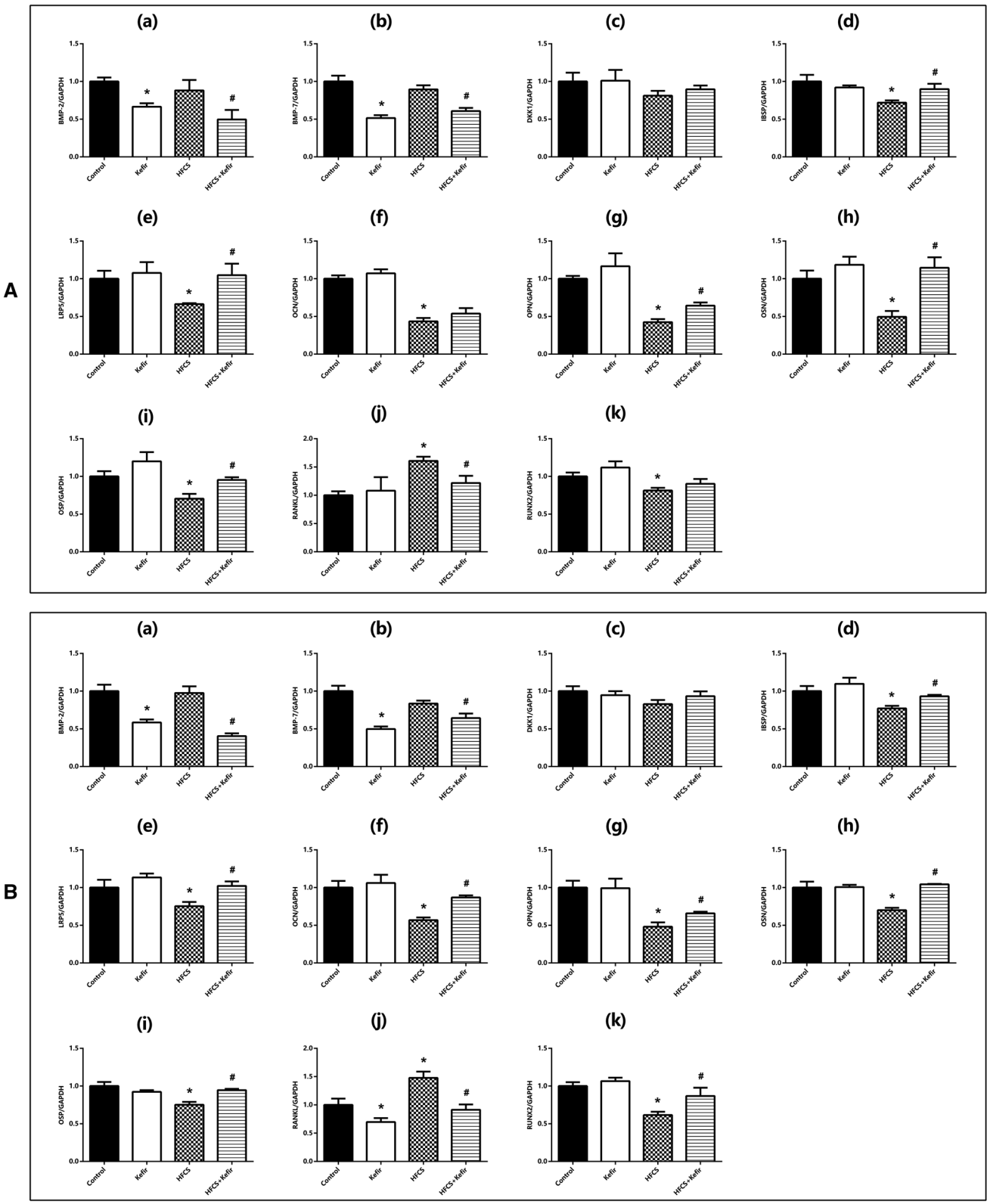

Fig. 1 mRNA expression levels of *BMP-2* (**a**), BMP-7 (**b**), DKK1 (**c**), IBSP (**d**), LRP5 (**e**), OCN (**f**), OPN (**g**), OSN (**h**), OSP (**i**), RANKL (**j**), and RUNX2 (**k**) in the cranial bones (**A**) and mandibles (**B**) of male rats from the Control, Kefir, HFCS, and HFCS + Kefir groups. Data was normalized by GAPDH. Each bar represents the means of at least six rats. Values are expressed as mean ± SEM, $n = 6–12$. ∗Significantly different ($P < 0.05$) compared to Control group; #significantly different ($P < 0.05$) compared to HFCS group

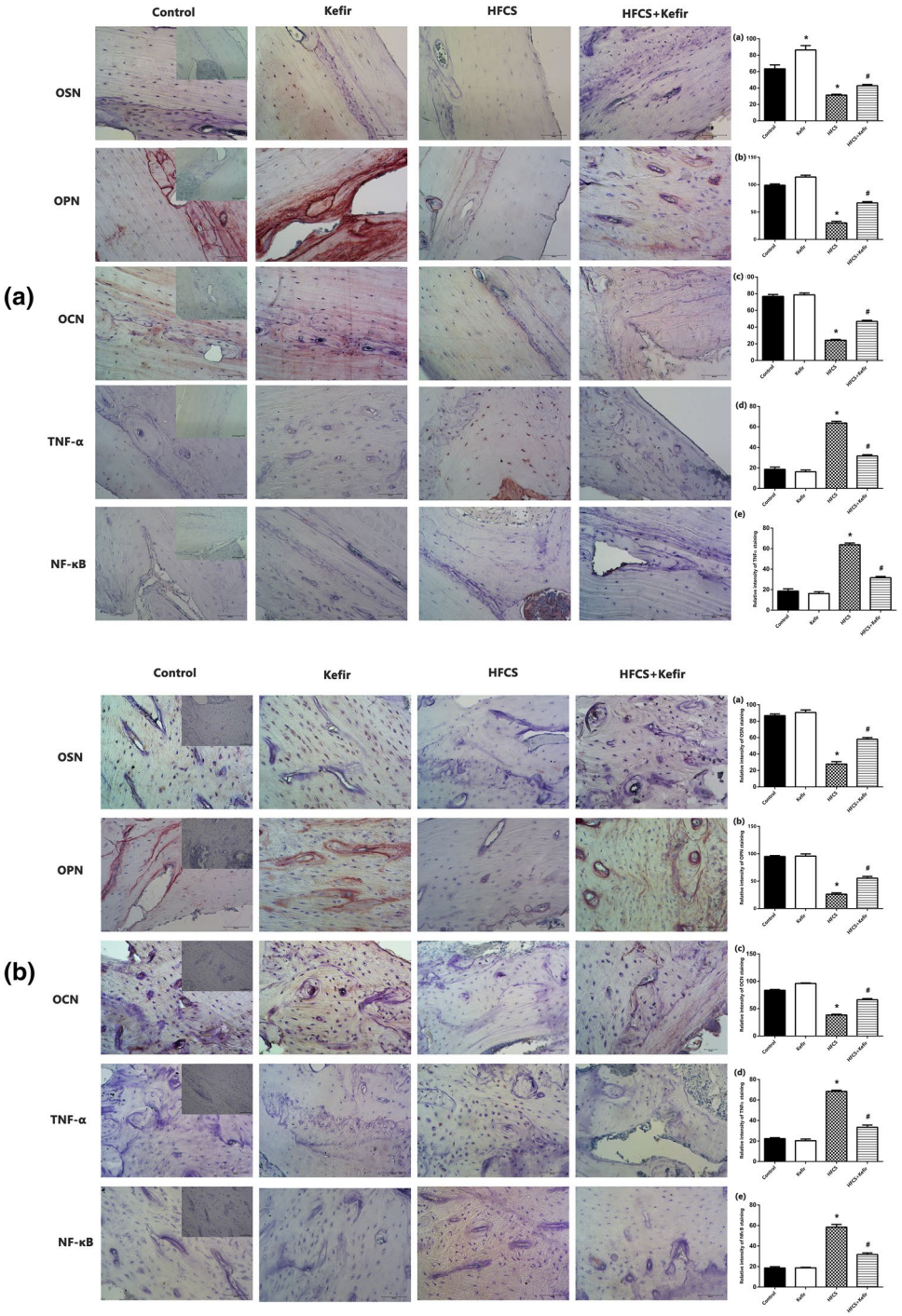

Fig. 2 Relative immunostaining of OSN (**a**), OPN (**b**), OCN (**c**), TNFα (**d**), and NfκB (**e**) protein expressions in the cranial bones (**A**) and mandibles (**B**) of male rats from the Control, Kefir, HFCS, and HFCS + Kefir groups (× 100 magnifications). Values are expressed as mean ± SEM, $n = 6–12$. ∗ Significantly different ($P < 0.05$) compared to Control group; #significantly different ($P < 0.05$) compared to HFCS group

the presence of RANKL. In the continuation, OSP which produced by osteoblasts and bone marrow stromal cells inhibits this mechanism by binding RANKL. In contrast, TNFα, NfκB, and many cytokines synthesized from calvarial cells inducing RANKL, suppressing OSP and provide osteoclastic activity [16]. Moreover, osteoblasts regulate overexpression of several transcription factors, such as RUNX2. The newly formed preosteoblasts that are recognized by the synthesis of IBSP create mature osteoblasts through the expression of RUNX2 and several fragments of the Wnt signaling pathway, such as BMP-2, BMP-7, DKK1, LRP5, OCN, OPN, and OSN [17–21]. However, reproductive hormones as well as parathormone and anabolic hormones also have a bioactive role in this mechanism. Although estrogen and testosterone derivatives have bone-building effects, disruption of the balance between them may also disrupt this mechanism [30]. Therefore, both androgens and estrogens would be needed for bone

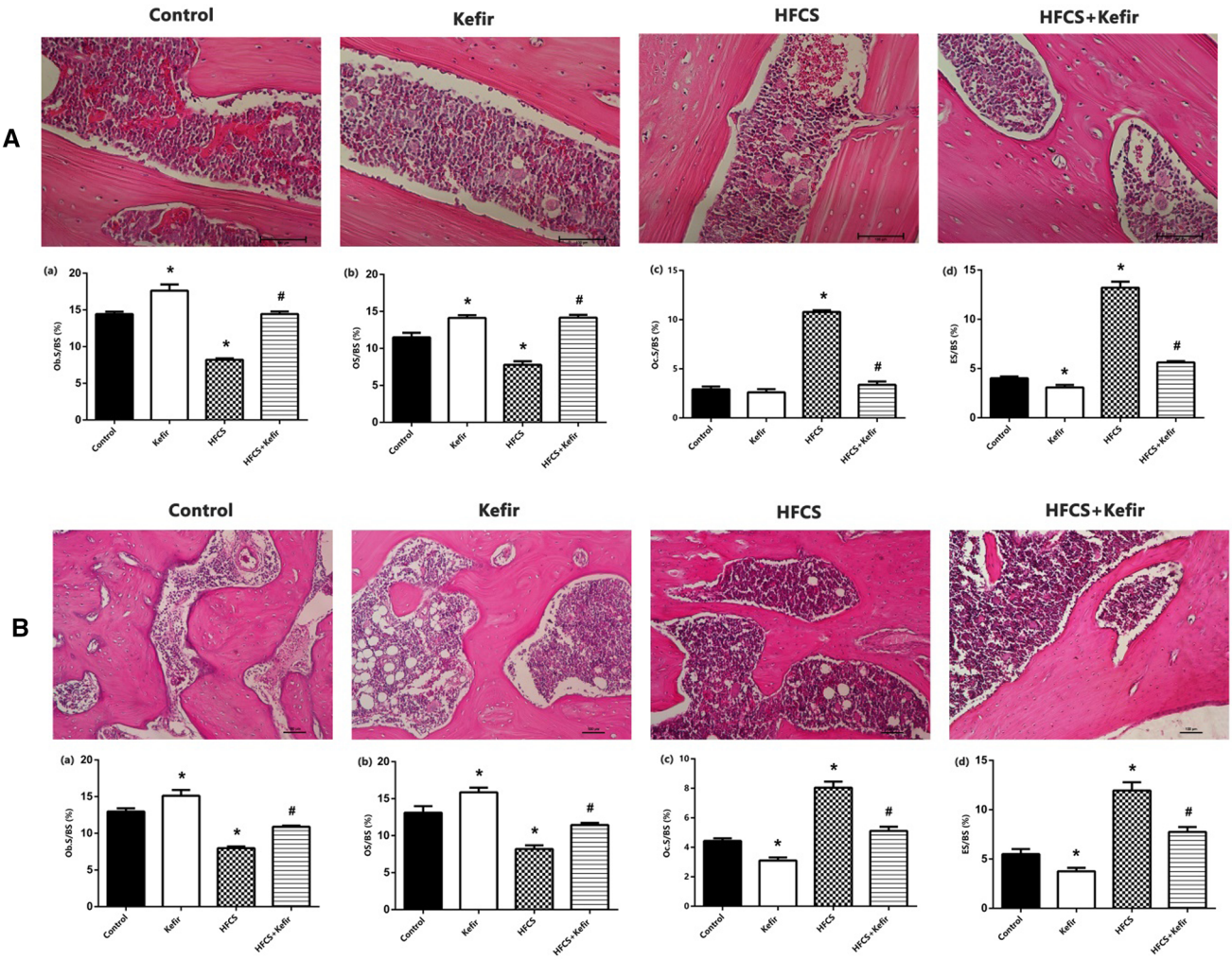

Fig. 3 Representative micrographic photos of decalcified cranial bones (**A**) and mandibles (**B**) with performed histomorphometric analyses from the Control, Kefir, HFCS, and HFCS + Kefir groups (Hematoxylin–eosin; × 200 magnifications). Osteoblast surface (Ob.S/BS) (**a**); osteoclast surface (Oc.S/BS) (**b**); eroded surface (ES/BS) (**c**); osteoid surface (OS/BS) (d). Values are expressed as mean ± SEM, $n = 6$–12. * Significantly different ($P < 0.05$) compared to Control group; #significantly different ($P < 0.05$) compared to HFCS group

reconstruction by inducing differentiation of osteoblasts and increasing their life span [29].

Experimental and retrospective studies point to the osteonecrotic effects of a high-fat or carbohydrate diet on bone formation, as well as diseases, such as metabolic syndrome and diabetes. It has been shown that femoral bone mineral and surface density reduced, OCN, OPN, and OSP levels decreased, and RANKL levels increased after the administration of 10% fructose to adult rats for 12 weeks [31]. In a similar study, it was stated that cancellous bone density and surface decreased and serum OCN levels increased in high-fat-fed obese rats [32]. In contrast, decreased serum OCN levels have also been shown in HFCS-fed rats [33]. Moreover, it was found that TNFα and NfκB increased, whereas BMP-2 and OCN levels decreased in bone tissue samples of streptozotocin-induced diabetic rats [34]. In another study, according to the static histomorphometric analysis results of the distal femoral metaphysis of rats fed with 10% fructose in the diet for 14 days, it was found that the osteoblast surface (Ob.S/BS) and osteoid surface (OS/BS) tended to increase, while the osteoclast surface (Oc.S/BS) and eroded surface (ES/BS) did not change [35]. According to the results of retrospective studies conducted in US and Korea, it was stated that the bone mass and mineral density of adult individuals diagnosed with metabolic syndrome were low [36, 37]. In our previous study [3], we stated that due to the excess dose of HFCS intake, plasma calcium level did not change, whereas estradiol-testosterone balance was impaired. According to our current study results, BMP-2, BMP-7, and DKK1 mRNA expressions did not change with the excess dose of HFCS consumption, IBSP, LRP5, OCN, OPN, OSN, OSP, and RUNX2 levels

were decreased, and RANKL, which provides osteoclastic activity, and the TNFα and NfκB that induce it appear to be increased. These findings show that the dietary excess dose of HFCS reduced bone formation by inhibiting the WNT signaling pathway without changing BMP-2, BMP-7, and DKK1 levels. In a similar study, decreased levels of relevant protein were associated with higher glucose levels and impaired serum calcium homeostasis [34], whereas, in our study, tolerable plasma glucose level and unchanged calcium level were determined. Hence, unchanged mRNA levels of the respective proteins may be attributed to this difference. Supportively, both the cranial bone and mandible results are clearly similar, and the relative densities of OSN, OPN, and OCN and mRNA expressions are in one-to-one synchronization, making our findings acceptable. In contrast, a study suggests that serum OCN levels increased with a diet high in fructose or high fat [32]. In our study, it indicates that craniomandibular OCN levels decreased. This difference may be related to the increase in the density of OCN in the blood due to the disruption of androgen–estrogen balance in our study. Similarly, a study indicate that OS/BS, Ob.S/BS, Oc.S/BS, and ES/BS did not change significantly with fructose-feeding [35]; but our findings showed that enhanced OS/BS and Ob.S/BS, however, reduced Oc.S/BS and ES/BS. It can be state that these variations are caused by differences, such as diet duration and food source.

There are many studies of the use of the milk-kefir preventing dyslipidemia, digestion, hyperglycemia, and its anti-inflammatory, antioxidant, and antiviral effects are also widely known [23–26, 38]. Kim et al., showed that, kefir treatment (0.2 ml/day) reduced *Firmicutes*, *Proteobacteria*, *Enterobacteriaceae,* and *Firmicutes/Bacteroidetes* ratio, whereas enriched intestinal microbiota with *Bacteroidetes*, *Lactobacillus*, and *Lactococcus* bacteria for 3 weeks [39]. Similarly, in the clinical trial, kefir 180 (ml/day) increased *Actinobacteria* and suppressed inflammation in patients with metabolic syndrome [40]. However, it has been shown that milk-kefir treatment in rats with periodontitis decreased inflammation via suppressing TNFα and IL-1β, and reduced alveolar bone loss [27]. In the current study, it is evident that the mRNA expressions of IBSP, LRP5, OPN, OSN, OSP, and RANKL were normalized by kefir-treatment in the craniomandibular bones. However, in the mandibles, kefir supplementation increased OCN and RUNX2, but it caused a tendency towards an elevation in the cranial bones. According to the immunohistochemical results, OSN, OPN, and OCN findings are consistent with gene results. It is understood that kefir consumption provides benefits in bone formation by suppressing TNFα and NfκB, which are inflammatory structures that cause bone loss. These results also supported by the histological examinations that OS/BS and Ob.S/BS increase with intake of kefir, and reduction of the Oc.S/BS and ES/BS. Interestingly, kefir consumption decreased BMP-2 and BMP-7 levels in the craniomandibular tissues of both HFCS-fed and healthy rats. In a study conducted to elucidate the mechanism of action of BMPs, it was revealed that BMPs are induced by limiting bone formation and if an erosive source is in question [41]. The acceleration of bone formation with kefir consumption may suppress BMPs as a compensatory.

In conclusion, feeding for 8 weeks with the excess dose of HFCS-diet at an early age impairs the craniomandibular bone-building mechanism and thus bone formation in rats. In this process, it is seen that daily kefir consumption highly prevents HFCS-dependent damage. These results indicate that intake of kefir could have beneficial effects on mouth formation in childhood, but further studies are needed to clarify the mechanism of action of kefir on these bones.

Materials and methods

Animals and protocols

The Ethical Animal Research Committee of Afyon Kocatepe University (AKUHADYEK 49533702-93) approved the study. Four-week-old male *Wistar* rats having approximately 100 g weight were given a standard rodent chow (Korkutelim Yem Sanayi A.Ş., Antalya/TURKEY) diet that composed of 62% starch, 23% protein, 4% fat, 7% cellulose, standard vitamins and salt mixture, ad libitum. Before practical applications, they were acclimatized for 1 week, and then four groups were formed as: Control, Kefir, HFCS, and HFCS + Kefir. According to the manufacturer's reports, kefir yeast contained *Lactobacillus helveticus, Lactobacillus parakefiri, Lactobacillus casei, Lactobacillus reuteri, Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus fermentum, Leuconostoc mesentereoides, Lactococcus lactis, Lactobacillus kefiranofacien, Acetobacter pasteurianus, Streptococcus thermophilus, Bifidobacterium bifidum, Kluyveromyces marxianus, Saccharomyces cerevisiae,* and *Kluyveromyces lactis*. It was obtained commercially (DanemKefir, Isparta, Turkey). Kefir was given as 1 ml/100 g weight/day in the Kefir and HFCS + Kefir groups, while saline (1 ml/100 g weight/day) was used as a vehicle in Control and HFCS group via gastric gavage; however, HFCS (Cargill F55, Minnesota, USA) was given in diluted form as 20% (w/v) in drinking water ad libitum to rats for 8 weeks. After fructose and kefir application, rats were anesthetized with ketamine (100 mg/kg), and xylazine (10 mg/kg), and cranial bones and mandibles were isolated. Physiological saline solution washed the bone tissues, which were frozen with liquid nitrogen before storage at − 85 °C. Some parts of the tissues were fixed in 10% neutral formalin for the immunohistochemical experiments.

Determination of the gene expressions with real-time polymerase chain reaction

Relative expression levels of genes: BMP-2, BMP-7, DKK1, IBSP, LRP5, OCN, OPN, OSN, OSP, RANKL, and RUNX2 with respect to GAPDH were determined with semi-quantitative real-time polymerase chain reaction (qRT-PCR). Accordingly, bone tissues were pulverized with liquid nitrogen, and total RNAs were isolated with a commercial kit (GeneJET RNA Purification Kit, Thermo Fisher Scientific, USA). Isolated RNAs were subjected to agarose gel electrophoresis and spectrophotometer at 260/280 for quality control and quantification. One microgram of total RNA was reverse transcribed to cDNA with RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, Waltham, MA, USA). Relative expression levels were measured by mixing 5-µl 2X SYBR Green Master mix (Fast Start Universal SYBR Green Master Mix, Roche, Basel, Switzerland), 4 µl of primer mixture of 400 nM each and 1 µl of cDNA. qRT-PCR (LightCycler480 II, Roche, Basel, Switzerland) was performed in which samples were initially denatured 95 °C for 10 min. Then 40 repeated cycles of 95 °C for 10 s, 58 °C for 15 s, and 72 °C for 15 s were conducted with the measurement of green fluorescence at the end of each extension step. The primer pairs (Table 2) were designed with the NCBI primer blast tool for rat databases, and their specificities were double-checked with the other species on the databases. All the PCR reactions were performed in triplicates, and to confirm the specificity of PCR products, a melt analysis was carried out. GAPDH was the internal control, and the relative expressions of the genes were calculated with the efficiency-corrected advance relative quantification tool of the LightCyclerVR 480 SW 1.5.1 software.

Immunohistochemistry

Deparaffinized and rehydrated tissue Sects. (5 µm) were subjected to antigen retrieval process using citrate buffer (0.1 M, pH: 6.0) in a microwave. Peroxidase activity was inhibited with 3% hydrogen peroxide. OSN (1/100, sc 73472, Santa Cruz Biotechnology), OPN (1/100, sc 21742, Santa Cruz Biotechnology, OCN (1/100, sc-365797, Santa Cruz Biotechnology, TNF-α (1/100, ab220210, Abcam), and NfκB (1/100, sc 8008, Santa Cruz Biotechnology primary antibodies were used for antigen detection in fixed tissues. Primed samples were probed with a secondary antibody conjugated with HRP (LabVision). 3-Amino-9-ethylcarbazole (AEC) (LabVision) was used as a chromogen. Mayers hematoxylin counterstained all the slides, which were mounted with a water-based mounting medium. The previously described histological scoring method was conducted to evaluate immunoreactivity38 under a light microscope (Nikon, Eclipse E600, Tokyo, Japan). Image analysis was conducted with Image Analysis Software (NIS Elements Nikon, Japan).

Histochemical staining

The craniomandibular samples were fixed in 10% neutral formalin, demineralized and processed histologically. Then samples were embedded in paraffin blocks. Five µm thick samples were taken on classic and poly-l-lysine slides and then deparaffinized. After standard Hematoxylin–Eosin (HE) staining, Histomorphometric analyzes of cranial bone and mandible slices were performed separately by light microscopy (Nikon, Eclipse E600, Tokyo, Japan) with the image analysis system (NIS Elements Nikon, Japan). In histomorphometric analysis osteoblast surface (Ob.S/BS, %),

Table 2 Effects of kefir, high-fructose corn syrup (HFCS), and their combinations (HFCS + kefir) on body and omentum weights, food, liquid, and caloric intake with some metabolic parameters of rats

Groups	Control	Kefir	HFCS	HFCS + Kefir
Terminal body weight (g)	291 ± 5	322 ± 3*	365 ± 9*	335 ± 2#
Omentum weight/body weight (%)	0.66 ± 0.1	0.75 ± 0.05	1.72 ± 0.12*	1.14 ± 0.09#
Food intake (g/day)	25.5 ± 1.2	26.1 ± 1.8	14.4 ± 0.9*	20.6 ± 2.7#
Liquid intake (ml/100 g bw)	16.1 ± 2.1	15 ± 2.3	13.2 ± 1.8	13.7 ± 1.6
Total caloric intake (kcal)	89.4 ± 1.2	92.4 ± 3.5	77.5 ± 3.4*	99.1 ± 2.6#
Glucose (mg/dL)	73 ± 2.9	79 ± 1.6	106 ± 4*	87 ± 0.7#
Insulin (ng/mL)	0.62 ± 0.05	0.7 ± 0.07	195 ± 0.05*	0.91 ± 0.06#
Triglyceride (mg/dL)	109 ± 2.8	146 ± 2.1*	179 ± 1*	160 ± 1.5#
VLDL (mg/dl)	21.8 ± 0.6	29.3 ± 0.4*	36 ± 0.2*	32 ± 0.3#
Cholesterol (mg/dl)	58.5 ± 3.1	59.6 ± 3.8	67.1 ± 3.1*	55.3 ± 2.4#
Fructose (µmol/L)	144 ± 3	146 ± 5	159 ± 7*	142 ± 3#
Calcium (mg/dL)	0.22 ± 0.01	0.25 ± 0.02	0.21 ± 0.03	0.24 ± 0.01
Estradiol (pg/ml)	10.7 ± 0.9	9.1 ± 0.9	12.9 ± 0.9*	9.8 ± 1.6
Total testosterone (ng/ml)	2.82 ± 0.29	2.91 ± 0.16	2.81 ± 0.19	2.92 ± 0.18

*Significantly different ($P < 0.05$) compared to Control group; #significantly different ($P < 0.05$) compared to HFCS group

osteoclast surface (Oc/BS, %), eroded surface (ES/BS, %) and osteoid surface (OS/BS), %) was measured.

Statistical analysis

Gene expressions of Kefir and HFCS plus Kefir groups were normalized to the mean of the Control groups, whereas HFCS plus Kefir groups was compared to HFCS, and data was also normalized with corresponding GAPDH. All data is represented as mean ± standard error of the mean (SEM) through the study. Statistical comparisons were performed using one-way ANOVA followed by appropriate post-hoc test (Tukey). Comparisons giving *P* values less than 0.05 were accepted as statistically significant.

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Author's contributions O.E., A.M.G., and M.B.P. contributed equally by conceiving and designing the study. M.G.B., E.A., H.G., O.A.K., G.S., O.E., and M.B.P. performed the tissue collection, experiments, and analyzed the data. M.B.P., and A.M.G. wrote the paper.

Declarations

Conflict of interest The authors declare no competing interests.

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