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Original Article

The inverse association between ANGPTL8 and PI3K-mTOR-PPARγ expressions in adipose tissue of high-fructose-fed rats: The modulatory effect of kefir

Fatma Akar[1], Ceren Güney[1], Hamdi Barbaros Özer[2], Mehmet Bilgehan Pektaş[3], Halit Buğra Koca[4], Aytaç Kocabaş[5], Gökhan Sadi[5]

[1]Gazi University, Faculty of Pharmacy, Department of Pharmacology, Ankara, Turkey
[2]Ankara University, Faculty of Agriculture, Department of Dairy Technology, Ankara, Turkey
[3]Afyonkarahisar Health Sciences University, Faculty of Medicine, Department of Medical Pharmacology, Afyonkarahisar, Turkey
[4]Afyonkarahisar Health Sciences University, Faculty of Medicine, Department of Medical Biochemistry, Afyonkarahisar, Turkey
[5]Karamanoğlu Mehmetbey University, K.Ö. Science Faculty, Department of Biology, Karaman, Turkey

ORCID IDs of the authors: F.A. 0000-0002-5432-0304; C.G. 0000-0002-3267-2886; H.B.Ö. 0000-0001-6669-0444; M.B.P. 0000-0003-0055-7688; H.B.K. 0000-0002-5353-3228; A.K. 0000-0001-7622-1932; G.S. 0000-0002-6422-1203

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ABSTRACT

Background and Aims: The dietary high-fructose intake might be a risk factor for several metabolic diseases. Kefir, a fermented milk product, has been proposed to have beneficial health effects. In this study, we aimed to investigate the effects of fructose consumption and kefir supplementation on the lipogenesis-related genes including angiopoietin-like protein 8 (*angptl8*), phosphoinositide 3-kinase (*pi3k*), mammalian target of rapamycin (*mtor*), and peroxisome proliferator-activated receptor γ (*pparγ*) as well as inflammatory factors in the adipose tissue to provide new mechanistic insights into lipogenesis.
Methods: Fructose was given to the rats as a 20% solution in drinking water for 15 weeks. Kefir was administered by gastric gavage once a day during the final six weeks.
Results: There was an upregulation of *angptl8* mRNA expression in adipose tissue of rats given fructose. However, expressions of *pi3k, mtor,* and *pparγ* mRNAs were impaired in the adipose tissue. The increased interleukin (IL)-1β levels, but decreased IL-10, were also measured. There was no change in expressions of sirtuin1 (*sirt1*) and nuclear factor erythroid 2-related factor 2 (*nrf2*). Kefir supplementation suppressed expression of *angptl8*, but increased *pi3k* and *mtor* in the adipose tissue of high-fructose-fed rats.
Conclusion: Activation of gene expression of *angptl8*, together with the suppression of *pi3k*, *mtor*, and *pparγ*, showed that there was an inverse association between these lipogenic genes in the adipose tissue of rats fed with high-fructose. Kefir supplementation has modulatory effects on fructose-induced changes except for *pparγ* expression. These findings showed that dietary fructose and kefir might reciprocally affect the lipogenesis-related genes in the adipose tissue.

Keywords: Dietary fructose, Kefir, ANGPTL8, PI3K-mTOR-PPARγ pathway, Lipogenesis

Address for Correspondence:
Fatma AKAR, e-mail: fakar@gazi.edu.tr

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INTRODUCTION

The metabolic syndrome is a cluster of conditions including hyperlipidemia, hyperinsulinemia, hypertension, central adiposity, fatty liver disease, and chronic low-grade inflammation. The increased prevalence of metabolic syndrome in the world might be due to high consumption of fructose, particularly in the form of soft drinks, in the current human diet (Hannou, Haslam, McKeown, & Herman, 2018; Jensen et al., 2018). We have previously shown that dietary high-fructose leads to a change in adipose tissue function with an alteration in insulin signaling, activation in inflammatory cytokines, and upregulation in lipogenic genes (Pektas, Koca, Sadi, & Akar, 2016; Akar, Sumlu, Alçığır, Bostancı, & Sadi, 2021). Besides, we have determined that high-fructose-induced metabolic disturbance is more likely related to abdominal fat accumulation, but independent from general obesity (Pektaş, Sadi, & Akar, 2015; Akar et al., 2021). Previously, it was shown that the expansion of white adipose tissue was related to insulin resistance and low-grade inflammation (Bastard et al., 2006). Adipose tissue has endocrine and metabolic functions by secreting several hormones and factors that influence lipid and glucose metabolism as well as the effectiveness of insulin (Scherer, 2006). Therefore, dysfunction of adipose tissue might be associated with metabolic diseases.

Angiopoietin-like proteins (ANGPTLs) have been proposed to play essential roles in lipoprotein metabolism, adipogenesis, and inflammation (Carbone et al., 2018). ANGPTL8, also known as lipasin, refeeding induced fat and liver (RIFL) and betatrophin, is mainly expressed in the liver and adipose tissue (Ren, Kim, & Smas, 2012; Zhang, 2012; Wang et al., 2013). Previously, ANGPTL8 was found to regulate plasma triglyceride levels by inhibiting lipoprotein lipase, a key enzyme in the lipoprotein lipolysis pathway (Quagliarini et al., 2012). Deletion of the *angptl8* gene in mice was shown to reduce plasma triglyceride levels (Ren et al., 2012; Quagliarini et al., 2012). On the contrary, overexpression of ANGPTL8 causes an increase in plasma triglyceride levels in mice (Quagliarini et al., 2012; Zhang, 2012). Moreover, in type 2 diabetic patients with insulin resistance, ANGPTL8 levels were positively correlated with triglyceride levels (Chen, Susanto, Chuang, Liu, & Wang, 2016). Metabolic effects of insulin including adipogenesis and lipid accumulation are mediated by phosphoinositide 3-kinase (PI3K)- protein kinase B (Akt) pathway (Wang & Sul 1998; Sakaue et al., 1998). PI3K-Akt pathway was damaged in obesity and type 2 diabetes resulting in insulin resistance (Huang, Liu, Guo, & Su, 2018). Additionally, the stimulatory effect of insulin on lipid metabolism and deposition was mediated by PI3K-Akt-mammalian target of rapamycin (mTOR) (Han et al., 2015). Indeed, mTOR is involved in the regulation of adipogenesis, lipid, and glucose metabolism, as well as insulin resistance (Laplante & Sabatini, 2012). The mTOR complex, mTORC1, increases the expression of peroxisome proliferator-activated receptor γ (PPARγ), which is the master transcriptional regulator of adipocyte differentiation and lipid storage (Zhang et al., 2009). Moreover, mTOR deficiency was found to cause insulin resistance and downregulation of PPARγ expression in white adipose tissue of mice (Shan et al., 2016). The relationship between ANGPTL8 and PI3K-mTOR-PPARγ pathway in adipose tissue is not understood. Therefore, in this study, the association between these lipogenic genes was examined for the first time in the fat tissue of high-fructose-fed rats.

Kefir, a fermented milk product, contains several lactic acid and acetic acid bacteria in a polysaccharide matrix (Rosa et al., 2017). Kefir consumption was reported to have beneficial effects in several disease models (Kim, Jeong, Kim, & Seo, 2019). Kefir peptides were shown to reduce hepatic lipid accumulation and inflammation in high-fructose-fed mice (Chen et al., 2016). Besides, *Lactobacillus kefiri (L. kefiri)*, a kefir bacterium, reduced epididymal adipose tissue expansion and inflammatory factors in fructose-rich diet-fed mice (Zubiría et al., 2017). In another study, kefir grain powder was shown to suppress the lipid synthesis and inflammatory cytokines in adipose tissue and liver of diet-induced obese mice (Choi et al., 2017). In a very recent study, we showed that kefir supplementation improved the level of plasma triglyceride, hepatic weight, triglyceride content, and fatty degeneration as well as omental fat accumulation in high-fructose-fed rats. Moreover, kefir supplementation reduced expression of lipogenic genes, sterol regulatory element-binding protein (*srebp*)*-1c* and fatty acid synthase (*fasn*), as well as produced a marked downregulation in tumor necrosis factor-alpha (TNF-α) and nuclear factor-kappa B (NF-κB) expressions in the liver, but not in adipose tissue of high-fructose-fed rats (Akar et al., 2021). Tibet kefir milk administration reduced serum triglyceride and abdominal fat mass together with an upregulation of the *angptl4* gene in fat tissue of high-fat diet-fed rats (Gao et al., 2019). Modulation of ANGPTL8 and PI3K-mTOR-PPARγ pathway in adipose tissue by kefir remains to be investigated. In the present study, we have examined the impacts of dietary fructose and kefir supplementation on gene expressions of *angptl8* and PI3K-mTOR-PPARγ pathway elements, as well as the levels of inflammatory cytokines in the adipose tissue of rats in order to provide new mechanistic insights into lipogenesis.

MATERIALS AND METHODS

Animals and diets

The Ethical Animal Research Committee of Gazi University (G.Ü.ET-18.018) approved the protocol for animal usage. Three-week-old male *Wistar* rats were housed in temperature- and humidity-controlled rooms (at 20-22°C and 40-60% humidity), with a 12-h light-dark cycle. The rats were fed with a standard rodent chow diet composed of 62% starch, 23% protein, 4% fat, 7% cellulose, standard vitamins, and salt mixture. At the end of the acclimation of one week, the rats were randomly divided into three groups: as control, fructose, and fructose+kefir. Fructose (Danisco Sweeteners OY, Kotka, Finland) was given to the rats as a 20% solution (w/v) in drinking water ad libitum for 15 weeks. Kefir, which was fermented in our laboratory, was given to the rats once a day as 1 ml per 100 g of body weight of animal by gastric gavage for the final 6 weeks. The same volume of water was also given to the control and fructose groups by gavage in the same period for sham operation. At the end of the feeding periods, the rats were anesthetized with a mixture of ketamine-xylazine (100 and 10 mg/kg, respectively, i.p.). The

omental adipose tissues were immediately dissected, blotted dry, and frozen in liquid nitrogen and stored at -85°C for the measurements.

Preparation of kefir

The kefir grains were obtained from Ankara University, Faculty of Agriculture and used (5% w/v) to inoculate the pasteurized cow's whole milk. The fermentation was carried out at 22°C for 24 h. Afterward, the mixture of kefir grain and fermented milk were separated by filtering through a sieve. Kefir was freshly prepared every other day. Kefir grains were stored at 4°C until further use. It contained 8.74±0.46 log CFU/ml of lactic acid bacteria and 4.12±0.78 log CFU/ml of yeast, not containing acetic acid bacteria. Microbial combination of kefir was determined with a metagenomic approach based on next-generation sequencing technology, as we stated in a very recent study (Akar et al., 2021).

Determination of gene expressions with quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNAs were isolated from adipose tissue using RNeasy total RNA isolation kit (Qiagen, Venlo, Netherlands), as described according to the manufacturer protocol. After isolation, the amount and the quality of the total RNAs were determined by spectrophotometry and agarose gel electrophoresis. Then, one µg of total RNA was reverse transcribed to cDNA using a commercial first-strand cDNA synthesis kit (Thermo Fisher Scientific, Waltham, MA, USA). Expression levels of *angptl8*, *pi3k, mtor, ppary,* sirtuin1 (*sirt1*) and nuclear factor erythroid 2-related factor 2 (*nrf2*) were determined with a real-time quantitative polymerase chain reaction (qRT-PCR, LightCycler480 II, Roche, Basel, Switzerland). To do this, 1 µl cDNA, 5 µl 2X SYBR Green Master mix (Roche FastStart Universal SYBR Green Master Mix, Roche, Basel, Switzerland), and 2 µl primer pairs of each (Table 1) at 0.5 µM concentrations in a final volume of 10 µl were mixed. qRT-PCR was performed as follows: initial denaturation at 95°C for 10 min, denaturation at 95°C for 10 s, annealing at 58°C for 15 s and extension at 72°C for 15 s with 40 repeated thermal cycles measuring the green fluorescence at the end of each extension step. All reactions were performed in triplicate and the specificity of PCR products was confirmed by melt analysis. The relative expression of genes to internal control glyceraldehyde 3-phosphate dehydrogenase (*gapdh*) was calculated with the quantification tool provided by LightCycler® 480 SW 1.5.1 software.

Measurement of inflammatory parameters in the adipose tissue

The adipose tissue samples were homogenized in 0.1 M phosphate buffer 1:10 (w/v), pH 7.4 and 24,000 cycles/min (Ultra Turrax, IKA Works Inc., USA), and then ultrasonicated at 20,000 cycles/s for 1 min (Dr. Hielscher, Germany). Homogenates were centrifuged at 10,000 x g and 4°C for 15 min, and the supernatants were collected. All the samples were stored at -85°C until analysis. Interleukin (IL)-1β, IL-6, and IL-10 levels in the adipose tissue of rats were measured by using ELISA kits (Cusabio, Houston, TX, USA) according to the manufacturer's instructions.

Statistical analysis

The results are given as the mean ± standard error of the mean (SEM); n is the number of rats. Statistical analyses were performed using one-way ANOVA followed by the Bonferroni *post hoc* test. Data were evaluated with GraphPad Prism (version 6.0, GraphPad Software, La Jolla, CA, USA). *P* values smaller than 0.05 were considered as statistically significant.

RESULTS

Metabolic parameters

Firstly, we used adipose tissue of the rats from our very recent study (Akar et al., 2021), where we have presented the data with metabolic parameters, including body weight, omental fat mass, plasma levels of glucose, insulin, and triglyceride in rats subjected to high-fructose diet as well as kefir supplementation. Briefly, in that paper (Akar et al., 2021), we reported that the major bacteria strains found in the kefir were *L. kefiranofaciens* (85.5%) and *L. helveticus* (12.5%), while the most abundant yeast species were *Kluyveromyces marxianus* (70.4%) and *Saccharomyces mikatae* (29.2%). Regarding metabolic parameters, dietary high-fructose or kefir supplementation did not change the body weight of rats. Importantly, high-fructose intake increased omental fat mass of rats which was markedly reduced by kefir supplementation. High-fructose-induced elevation in plasma glucose was not changed by kefir, but the increase in the insulin level was diminished with kefir. However, dietary fructose-induced augmentation in plasma triglyc-

Table 1. Primer sequences *angptl8*, *pi3k, mtor, ppary, sirt1, nrf2* and internal standard *gapdh* used for the mRNA expression determination of qRT-PCR.

Gene	Forward primer sequence (5'→3')	Reverse primer sequence (3'→5')	Genbank accession number
angptl8	CCTTTTTGACCAAGCACTGG	AAGTGTCCTCTTCTGCCTGA	NM_001271710.1
pi3k	ATGCAACTGCCTTGCACATT	CGCCTGAAGCTGAGCAACAT	NM_053481.2
mtor	GCAATGGGCACGAGTTTGTT	AGTGTGTTCACCAGGCCAAA	NM_019906.1
ppary	CTCAGGTCAGAGTCGCCCC	GAGAGAGACCTCGTCAGGCT	NM_001145367.1
sirt1	CGGTCTGTCAGCATCATCTTCC	CGCCTTATCCTCTAGTTCCTGTG	XM_008772947.1
nrf2	GATTCGTGCACAGCAGCA	GCCAGCTGAACTCCTTAGAC	XM_006234397.2
gapdh	TCCTTGGAGGCCATGTGGGCCAT	TGATGACATCAAGAAGGTGGTGAAG	NM_017008.4

eride was reduced by supplementation of kefir. To investigate possible mechanisms of omental fat accumulation due to the dietary high-fructose and modulatory effect of kefir, we tested the adipose tissue of the same animals in the current study.

High-intake of fructose augmented the gene expression level of *angptl8* in the adipose tissue (2.68 fold, p<0.05 versus control group). The supplementation of kefir significantly decreased the expression of *angptl8* in the adipose tissue of rats (2.29 fold, p<0.05 versus fructose group; Figure 1a). However, high-fructose intake reduced the expression level of *pi3k* (5.49 fold, p<0.05 versus control group), which was significantly increased by kefir supplementation (2.40 fold, p<0.05 versus fructose group), in the adipose tissue (Figure 1b). In the adipose tissue, high-fructose intake decreased *mtor* mRNA expression (2.75 fold, p<0.05 versus control group), while kefir treatment improved the expression of this gene (1.43 fold, p<0.05 versus fructose group; Figure 1c). Excess fructose intake suppressed *pparγ* gene expression (1.55 fold, p<0.05 versus control group), and kefir supplementation did not show any marked change of *pparγ* expression in the adipose tissue of rats (Figure 1d). On the other hand, mRNA expression levels of *sirt1* and *nrf2,* which are cytoprotective factors, were not altered by fructose or kefir treatment in the adipose tissue of rats (Figure 2a, b).

High-fructose intake increased the level of proinflammatory cytokine IL-1β (1.66 fold, p<0.05 versus control group), which was not improved by kefir supplementation in the adipose tissue of rats (Figure 3a). Also, dietary high-fructose reduced the level of antiinflammatory cytokine IL-10 (1.64 fold, p<0.05

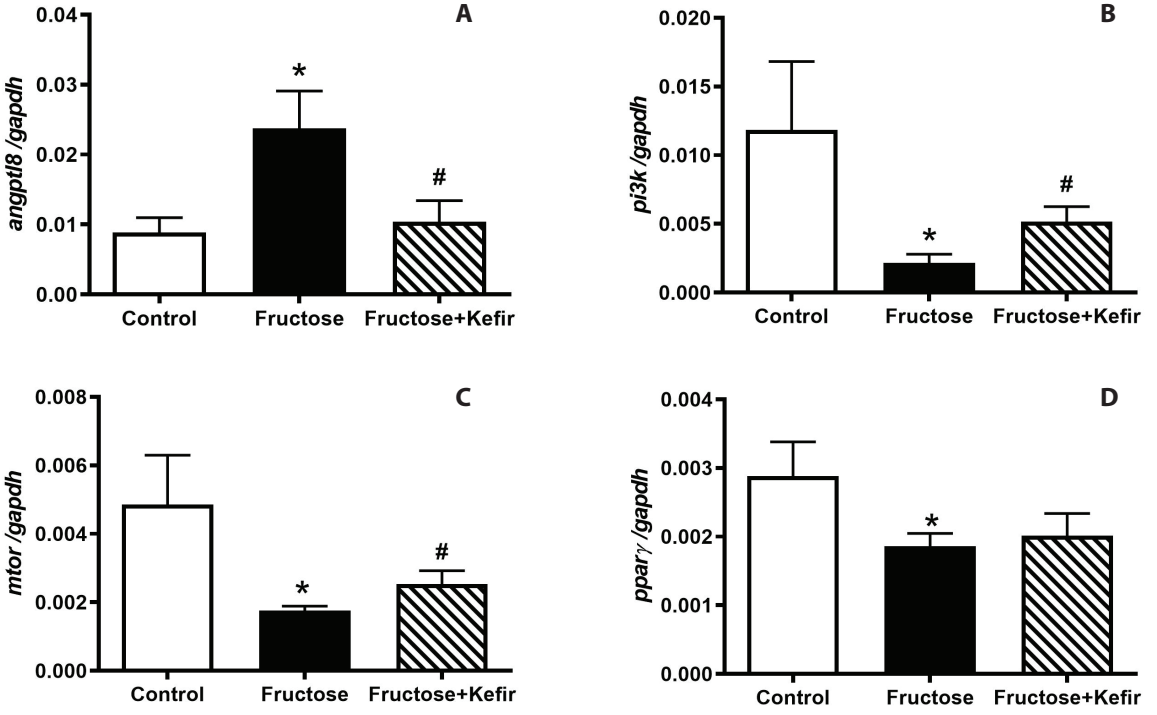

Figure 1. The mRNA expression levels of *angptl8* (A), *pi3k* (B), *mtor* (C), and *ppar*γ (D) in the adipose tissue of control, fructose, and fructose+kefir groups. Data were normalized using *gapdh*. Each bar represents the means from at least six rats. *P<0.05, significantly different from the control; #p<0.05, significantly different from the fructose group.

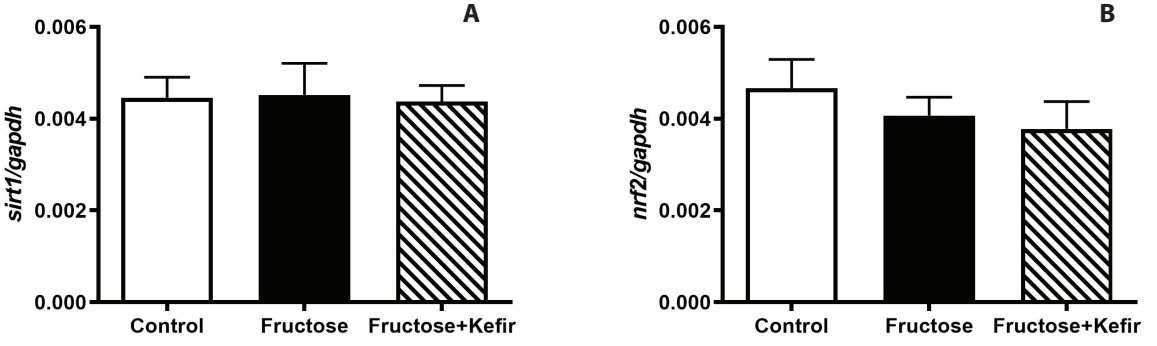

Figure 2. The mRNA expression levels of *sirt1* (A) and *nrf2* (B) in the adipose tissue of control, fructose, and fructose+kefir groups. Data were normalized using *gapdh*. Each bar represents the means from at least six rats.

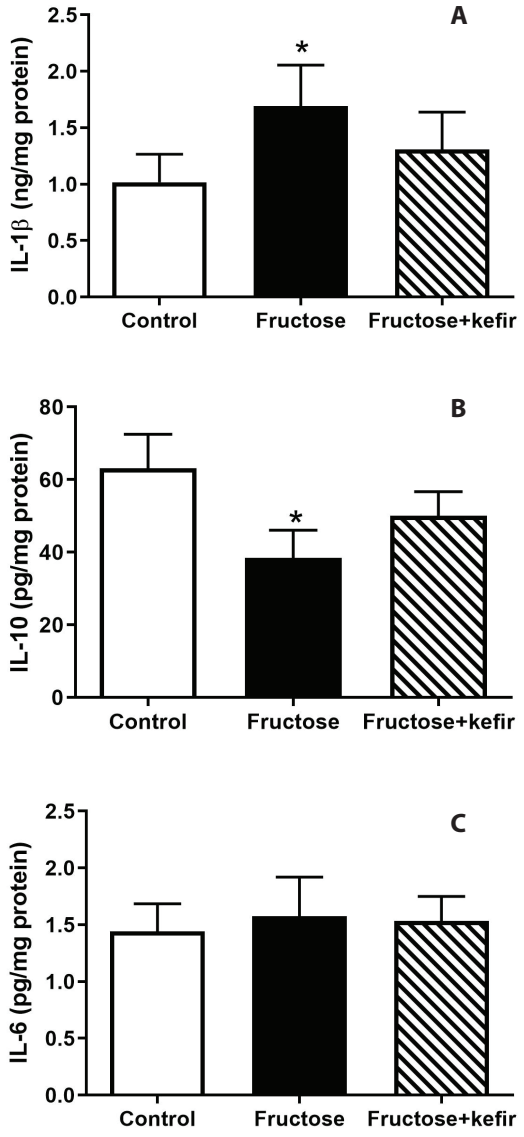

Figure 3. The levels of IL-1β (A), IL-10 (B), and IL-6 (C) in the adipose tissue of control, fructose, and fructose+kefir groups. Each bar represents the means from at least six rats. $^{*}p<0.05$, significantly different from the control.

versus control group) in the adipose tissue, but kefir treatment did not show any regulatory effect on this factor (Figure 3b). Another cytokine IL-6 was not affected by fructose or kefir treatment in the adipose tissue of rats (Figure 3c).

DISCUSSION

The worldwide high frequency of metabolic syndrome is generally accepted due to excess consumption of sugar and fat in the human diet. Accumulating evidence indicates that high intake of fructose provokes metabolic syndrome manifesting dysfunction of the liver, adipose, and vascular tissue (Hannou et al., 2018; Jensen et al., 2018). In our previous studies, we have reported that oral administration of 20% (w/v) fructose causes metabolic syndrome characterized by hypertriglyceridemia, hyperglycemia, hyperinsulinemia, and abdominal fat accumulation leading to dysregulation in vascular, hepatic, renal, intestinal, and testicular functions (Akar et al., 2012, Yildirim et al., 2019; Korkmaz et al., 2019a; Korkmaz et al., 2019b; Sumlu, Bostancı, Sadi, Alçığır, & Akar, 2020; Akar et al., 2021). In the current study, we have shown that dietary fructose increased expression of *angptl8*, but suppressed *pi3k*, *mtor*, and *pparγ* mRNAs together with a change in the levels of inflammatory cytokines in the adipose tissue of rats. Kefir supplementation exerted an improving effect on the expression of these genes, except *pparγ*. Thus, we propose that dietary fructose and kefir may reciprocally affect the lipogenesis-related genes in the adipose tissue.

Insulin plays a vital role in the regulation of energy metabolism by inhibiting gluconeogenesis and promoting lipogenesis (Brown & Goldstein, 2008). In the insulin-resistant state, the hormone loses its ability to reduce glucose production, but paradoxically maintains the lipid synthesis (Brown & Goldstein, 2008; Li, Brown, & Goldstein, 2010). In this context, we showed that fructose intake in the long-term period increased abdominal fat mass, expression of genes involved in insulin signaling, despite induction of proinflammatory markers in the adipose tissues from rats (Pektas et al., 2015; Pektas et al., 2016). Therefore, we propose that the upregulation of insulin signaling pathway in adipose tissue leads to increased visceral adiposity together with an inflammatory status (Pektas et al., 2016). We have also demonstrated that abdominal fat accumulation is accompanied by upregulation of lipogenic genes such as *srebp-1c* and *fasn* expression in the adipose tissue of high-fructose-fed rats (Akar et al., 2021). Indeed, fructose-induced metabolic disturbance is more likely related to increase in abdominal fat mass, independent of general obesity (Pektas et al., 2015; Akar et al., 2021). The contribution of the other lipogenesis-related genes such as *angptl8*, *pi3k*, *mtor* and *pparγ* to abdominal fat accumulation in high-fructose-fed rats was not identified.

The studies showed that overexpression of ANGPTL8 in the liver increases circulating triglyceride concentration and causes activation of insulin secretion (Quagliarini et al., 2012; Zhang, 2012; Wang et al., 2013). However, lacking hepatic ANGPTL8 causes low plasma triglyceride levels and decreases delivery of dietary lipids to adipose tissue (Oldoni et al., 2020; Izumi et al., 2018). Basically, insulin decreases circulating triglycerides by also inhibiting adipose tissue lipolysis (Nidhina Haridas et al., 2015). It was also reported that insulin significantly increases ANGPTL8 expression in the 3T3-L1 adipocytes cell line (Ren et al., 2012). Besides, the circulating ANGPTL8 levels were found to be increased in certain metabolic diseases, including type 2 diabetes mellitus (Abu-Farha et al., 2015; Hu et al., 2014; Chen et al., 2016), non-alcoholic fatty liver disease (Lee et al., 2016; García-Monzón et al., 2018), obesity (Fu et al., 2014), and metabolic syndrome (Abu-Farha et al., 2016; Wang et al., 2016). Herein, we demonstrated an upregulation of *angptl8* mRNA expression in samples of abdominal adipose tissue in high-fructose-fed rats. In the same protocol, we have very recently reported an increase in plasma insulin and triglyceride levels also in abdominal fat mass alongside upregulation of lipogenic genes *srebp-1c* and *fasn* in the adipose tissue (Akar et al., 2021). Overall, it can be evident that abdominal fat accumulation together with hyperinsulinemia and hypertriglyceridemia due to

dietary high-fructose is associated with increased expression of *angptl8* mRNA and other lipogenic genes, namely *srebp-1c* and *fasn* in the adipose tissue.

The gene and protein expression of ANGPTL8 is stimulated by different proinflammatory cytokines such as TNFα and IL-1β in various cell lines. Also, it was observed that there was a correlation between ANGPTL8 level and lipopolysaccharide-induced acute inflammatory response in the different tissues of mice (Zhang et al., 2017). In this study, proinflammatory cytokine IL-1β was increased in the adipose tissues of rats fed with high-fructose consistent with the results of previous studies (Ma et al., 2013; Pektas et al., 2016). Besides, we also showed that dietary high-fructose leads to low level of IL-10, which is known as an antiinflammatory cytokine, in fat tissue, in agreement with an earlier observation (Barroso et al., 2015). Herein, for the first time, we established that upregulation of *angptl8* is associated with an increase in inflammatory factor, but a decrease in counter regulatory response in adipose tissue. Thus, it can be suggested that there was a correlation between ANGPTL8 and inflammatory factors in adipose tissue in dietary intervention with high-fructose. These findings may be valuable to better understand the role of ANGPTL8 in metabolic syndrome.

In adipose tissue, insulin suppresses lipolysis activating PI3K formation (Okada, Kawano, Sakakibara, Hazeki, & Ui, 1994). Adipocytes obtained from type 2 diabetic patients showed diminished PI3K activation (Rondinone et al., 1997). Also, expression levels of *pi3k* and *pparγ* mRNAs were reduced in adipose tissue of mice with high-fat diet-induced insulin resistance (Li, Yu, & Zhao, 2019). PPARγ activation improves hyperglycemia by increasing sensitivity to peripheral insulin, also decreases triglyceride and adipocyte hypertrophy (Yamauchi et al., 2001). Deletion of PPARγ in the adipose tissue of mice resulted in marked adipocyte hypertrophy and elevation in plasma triglyceride together with insulin resistance (He et al., 2003). It has been shown that the PPARγ signaling through mTOR regulates the differentiation of pre-adipocytes. mTOR deficiency was reported to cause insulin resistance and downregulation of PPARγ expression in white adipose tissue of mice (Shan et al., 2016). All these indicated that PI3K, PPARγ, and mTOR work together as insulin downstream effectors in the regulation of adipogenesis. In a previous study, we surprisingly detected an upregulation in insulin downstream effectors, including *pi3k*, *mtor*, and *pparγ* mRNAs in adipose tissue of rats subjected to fructose intake (10% fructose in drinking water) in the long-term period of 24 weeks (Pektas et al., 2016). In the present study, expressions of *pi3k*, *mtor*, and *pparγ* mRNAs were impaired in the adipose tissue of rats given 20% fructose solution for 15 weeks. These discrepancies can be attributed to the differences in concentration and duration of fructose given in diet reflecting adaptive and compensatory changes. Taken all together, the increases in *angptl8* expression and inflammatory factor IL-1β level were associated with the impairment in *pi3k*, *mtor*, and *pparγ* mRNAs, also anti inflammatory factor IL-10 levels, in the adipose tissue of rats fed with high-fructose. Regarding cytoprotective factors such as *sirt1* and *nrf2*, which could be activated as a compensatory mechanism to counteract inflammation in adipose tissues, we did not measure any change in their genes expression by dietary fructose indicating these factors are not closely related to the process in adipocytes during fructose feeding (Yoshizaki et al., 2009; Schneider & Chan, 2013).

Recently we and others showed that, kefir supplementation improved the metabolic parameters including plasma triglyceride, and insulin levels as well as omental fat mass in high-fructose-fed rats and metabolic disorders induced by monosodium glutamate (Akar et al., 2021; Rosa et al., 2016). Furthermore, supplementation with *L. kefiri*, which is one of the probiotic bacteria of kefir, suppressed body weight gain and epididymal adipose tissue expansion in high-fructose-fed mice (Zubiría et al., 2017). Herein, kefir supplementation suppressed the increased *angptl8* mRNA expression in adipose tissue of high-fructose-fed rats. In this line, Tibet kefir intake was shown to reduce plasma triglyceride levels and abdominal fat mass as well as to normalize gene expression of *angptl4* which is an activator of triacylglycerol metabolism (Janssen et al., 2018) in the adipose tissue of high-fat diet-fed rats (Gao et al., 2019). In our previous study, kefir has not normalized the upregulation of lipogenic genes *srebp-1c* and *fasn* in the adipose tissue but reduced abdominal fat mass (Akar et al., 2021). Given, improving effect of kefir on abdominal fat accumulation may depend on downregulation of *angptl8* mRNA in adipose tissue of high-fructose-fed rats. Additionally, for the first time, we have found a decrease in *pi3k* and *mtor* mRNAs, which are downstream effectors of insulin, in the adipose tissue after kefir supplementation to fructose feeding rats. However, kefir supplementation did not change the decreased *pparγ* mRNA expression in this tissue. Treatment with kefir powder obtained from households in Russia reduced epididymal fat pad weight and PPARγ in high-fat diet-induced obese mice (Choi et al., 2017). Notably, lactic acid bacteria strains possessed PPARα/γ agonist activity and ameliorated dyslipidemia in obese mice (Nakamura et al., 2016). More studies are required to elucidate the modulatory effect of kefir on lipogenesis-related genes, including *pi3k*, *mtor*, and *pparγ* in adipose tissue.

In conclusion, dietary fructose-activated gene expression of *angptl8*, together with the suppression of *pi3k*, *mtor*, and *pparγ* mRNAs, showed that there was an inverse association between these two classes of lipogenic genes in adipose tissue of rats fed with high-fructose. These were accompanied by changed inflammatory factors. Kefir supplementation has modulatory effects on fructose-induced changes except for the levels of inflammatory factors and *pparγ* expression. Our findings revealed that dietary fructose and kefir might reciprocally affect the lipogenesis-related genes in the adipose tissue. Further studies are necessary to clarify dietary regulation of lipogenesis in adipose tissue.

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Ethics Committee Approval: This study was approved by the Ethical Animal Research Committee of Gazi University (G.Ü.ET-18.018).

Author contributions: Conception/Design of Study- F.A.; Data Acquisition- F.A., C.G., H.B.Ö., M.B.P., H.B.K., A.K., G.S.; Data Analysis/Interpretation-F.A., G.S., C.G., H.B.K.; Drafting Manuscript- F.A., C.G.; Critical Revision of Manuscript-F.A., G.S.; Final Approval and Accountability- F.A., C.G., H.B.Ö., M.B.P., H.B.K., A.K., G.S.

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