Research Article

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Kefir protects the liver against high fructose corn syrup induced phosphodiesterase hyperactivity

Kefir karaciğeri yüksek fruktozlu mısır şurubu kaynaklı fosfodiesteraz hiperaktivitesine karşı korur

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Abstract

Objectives: Phosphodiesterases (PDEs) mediate several physiological activities, and alterations in PDE expressions might cause conflicts between functional and clinical effects. This study clarifies the eventual relationship between the hepatic insulin resistance-associated signaling elements and PDEs together with inflammatory markers and investigates the role of kefir in the treatment.

Methods: Male Wistar rats were grouped as Control, Kefir, HFCS (high-fructose corn syrup), and HFCS + Kefir. Daily HFCS (20% w/v) and kefir (1 mL/100 g weight) were given for 8-weeks. Hepatic expressions of PDE isoforms and insulin signaling elements were determined with qPCR and Western blot. The changes in hepatic phospholipase A2 (cPLA2) and insulin-like growth factor 1 receptor-α (IGF-1-Rα) were investigated histologically.

Results: HFCS upregulated hepatic PDEs while repressed primary insulin signaling elements at gene and protein levels. It also augmented cPLA2 and IGF-1Rα expression. Kefir suppressed the PDEs and normalized the insulin signaling, and down-regulated cPLA2 and IGF-1Rα in the liver of HFCS-fed rats.

Conclusions: The disruption of the insulin signaling pathway and activation of PDEs were negatively correlated in liver tissues of the HFCS-fed rats. Kefir treatment achieved a remarkable improvement in HFCS-dependent modifications, and it could be an excellent functional food against HFCS-induced insulin resistance, PDE hyperactivity, and inflammation.

Keywords: HFCS; inflammation; insulin signaling pathway; kefir; phosphodiesterase.

Özet

Amaç: Fosfodiesterazlar (PDE'ler) çeşitli fizyolojik aktivitelere aracılık eder, bu nedenle PDE ifadelerindeki değişiklikler, fonksiyonel ve klinik etkiler arasında farklılıklara neden olabilir. Bu çalışma, hepatik insülin direnci ile ilişkili sinyal elemanları, PDE'ler ve inflamatuar belirteçler arasındaki ilişkiyi ortaya çıkarmak ve kefirin tedavideki rolünü araştırmak üzere gerçekleştirilmiştir.

Gereç ve Yöntem: Erkek Wistar sıçanları Kontrol, Kefir, YFMS (yüksek fruktozlu mısır şurubu) ve YFMS +Kefir olarak

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gruplandırıldı. 8 hafta boyunca hayvanlara günlük YFMS (%20 a/h) ve kefir (1 mL/100 g ağırlık) verildi. PDE izoformlarının ve insülin sinyal elementlerinin hepatik ekspresyonları, qPCR ve Western blot ile belirlendi. Hepatik fosfolipaz A2 (cPLA2) ve insülin benzeri büyüme faktörü 1 reseptör-α'daki (IGF-1Ra) değişiklikler histolojik olarak araştırıldı.

Bulgular: YFMS, hepatik PDE'leri yukarı yönde regüle ederken, gen ve protein seviyelerinde primer insülin sinyal elementlerini baskıladı. Ayrıca cPLA2 ve IGF-1Ra ifadesini artırdı. Kefir, HFCS ile beslenen sıçanların karaciğerinde PDE'leri baskıladı ve insülin sinyalini normalleştirdi. Buna ilave olarak cPLA2 ve IGF-1Ra'yı aşağı yönde regüle etti.

Sonuç: İnsülin sinyal yolunun bozulması ve PDE'lerin aktivasyonu, YFMS ile beslenen sıçanların karaciğer dokularında negatif korelasyon gösterdi. Kefir tedavisi, YFMS'ye bağlı modifikasyonlarda kayda değer bir iyileşme sağlayarak YFMS'nin neden olduğu insülin direncine, PDE hiperaktivitesine ve inflamasyona karşı mükemmel bir fonksiyonel gıda olabilir.

Anahtar Kelimeler: YFMS; kefir; fosfodiesteraz; insülin sinyal yolu; inflamasyon.

Introduction

The primary physiological stimulator of insulin secretion from the pancreatic β-cells is glucose, and high levels of glucose levels in β-cells close the ATP-sensitive K^+-channels, which results in the depolarization of cells and opening of the voltage-gated Ca^{2+} channels. The influx of calcium ions starts the insulin exocytosis from the β-cells [1]. While it is known that cyclic adenosine monophosphate (cAMP) is commonly believed as a booster of insulin excretion prompted by Ca^{2+} elevation in the β-cells, the role of cyclic guanosine monophosphate (cGMP) has not yet been fully elucidated [2]. Conversion of ATP to cAMP activates the several cAMP-dependent protein kinases, which are highly regulated by phosphodiesterase (PDE) activities [3]. The role of PDEs in carbohydrate metabolism and insulin secretion has been debated for years. Many studies emphasized that inhibition of PDEs may be beneficial in treating diseases such as diabetes and metabolic syndrome [4–7]. PDEs consist of 11 structurally similar isoforms, and the PDE1 family is widely distributed in the body and explicitly suppresses insulin secretion [8]. PDE3B is expressed in tissues generally associated with the regulation of glucose and lipid metabolism [9]. Many animal experiments have demonstrated the essential roles of PDE3B in regulating energy homeostasis and metabolism in the liver, adipocytes, and pancreatic β-cells [10, 11]. Besides, PDE2s are expressed in various tissues, involving the liver, lung, brain, heart, adrenal gland, and adipose tissue, and hydrolyze both cAMP and cGMP nucleotides [12, 13].

Today, the increase in the incidence of metabolic disorders like diabetes, metabolic syndrome, insulin resistance, and obesity are correlated with the increase in refined sugar intake that makes consuming foods containing high fructose suspicious [14]. The main reason for this claim is the inability of insulin to regulate fructose metabolism [15]. In addition to consuming fruits and vegetables as a source of fructose in daily life, high fructose corn syrup [HFCS) sweetened foods and beverages are also consumed [16]. Therefore, the metabolic effects of HFCS and fructose consumption are still under investigation. Recent studies have shown that excess fructose consumption in the diet causes insulin resistance [17], vascular dysfunction [18], fatty liver [19], omental adiposity [20], as well as oxidative stress [21], and inflammation in the kidney tissues [22]. The disruption of the insulin receptor substrate (IRS), phosphoinositide 3-kinases (PI3K), and protein kinase B (AKT) pathway in different tissues could be the basis of these pathologies developed due to fructose consumption [19, 23]. However, there is no evidence of an association between fructose diet-dependent variables, particularly impaired insulin signaling pathway with PDEs.

Probiotics, known as beneficial bacteria, are nutritional supplements that are still being investigated and used in digestive system diseases, allergies, and inflammatory diseases by renewing the intestinal microbiota [24]. These bacteria exert efficacy by improving bowel function and strengthening the lymphoid system [25]. Recent studies have pointed to the anti-inflammatory [19], antiviral [26], and anti-allergic [27] effects of kefir consumption. However, their beneficial effects have also been demonstrated against fructose or HFCS-induced disorders [19, 21, 22, 28]. We have recently investigated the amelioration of tissue inflammation induced by HFCS consumption in the masseter muscle and gingival tissue with kefir as a food supplement [29]. Based on the above findings, we designed this study to evaluate whether dietary HFCS with or without kefir supplementation regulates PDEs in the liver tissues in conjunction with insulin signaling pathway elements; insulin receptor substrate-1 (IRS1), phosphoinositide 3-kinase (PI3K), and protein kinase B (AKT). Besides, the changes in inflammatory markers such as phospholipase A2 (cPLA2) revealed whether HFCS and kefir modulate inflammatory responses.

Materials and methods

Animal protocols

Four-week-old male Wistar rats having approximately 100 g weight were given a standard rodent chow diet and kept at a 12-h night and day cycle in an environment of 24–26 °C and 50–60% humidity before the experiment. A total of 48 animals were used, 12 in each group. The Ethical Animal Research Committee of Afyon Kocatepe University approved this study (AKUHADYEK 49533702–276). The rats were cared for under the Experimental Animal Principles and Guidelines organized by the National Health and Medical Research Council and the Experimental Animal Care and Use Guidelines (NIH issue no. 85–23, 1985 revised) prepared by the National Institute of Health. Before practical applications, they were acclimatized for one week, and then four groups were formed as Control, Kefir, HFCS, and HFCS + Kefir. Kefir was given as 1 mL per 100 g of the animal via gastric gavage daily; however, HFCS (56% fructose and 37% glucose, Cargill F55, Minnesota, USA) was given in diluted form as 20% (w/v) in drinking water *ad libitum* to the rats for eight weeks. After fructose and kefir application, rats were sedated with xylazine (10 mg/kg) and ketamine (100 mg/kg), and their liver tissues were isolated. Physiological saline solution was used to wash samples frozen with liquid nitrogen before storage at –85 °C. Some parts of the tissues were fixed in 10% neutral formalin for the immunohistochemical experiments.

Preparation and composition of kefir

The kefir grains were obtained commercially (Sevdanem, Danem Kefir, Isparta, Turkey) and used (5% w/v) to inoculate the pasteurized cow's whole milk. The fermentation was carried out at 22 °C for 24 h. Kefir was freshly prepared every other day, and the composition of freshly prepared kefir was *Lactobacillus* (10.54 log CFU/mL), *Lactococcus* (10.62 log CFU/mL), *Yeast* (2.89 log CFU/mL), *Lactobacillus acidophilus* (8.25 log CFU/mL) and *Bifidobacterium* (7.78 log CFU/mL). Detailed ingredients were *Lactobacillus reuteri, Lactobacillus fermentum, Lactobacillus parakefiri, Lactobacillus casei, Lactobacillus acidophilus, Lactococcus lactis, Lactobacillus helveticus, Leuconostoc mesentereoides, Lactobacillus bulgaricus, Lactobacillus kefiranofacien, Kluyveromyces marxianus, Acetobacter pasteurianus, Bifidobacterium bifidum, Saccharomyces cerevisiae, Streptococcus thermophilus,* and *Kluyveromyces lactis.*

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

Relative expression levels of genes: *pde1a, pde1b, pde1c, pde2a, pde3b, pi3k, akt,* and *irs-1* with respect to *gadph* (glyceraldehyde-3-phosphate dehydrogenase) were determined with a semiquantitative real-time polymerase chain reaction (*qRT-PCR*). Firstly, we isolated total RNAs from the livers with a commercial RNA isolation kit (GeneJET RNA Purification Kit, Thermo Fisher Scientific, USA) according to manual and then agarose gel electrophoresis and spectrophotometric measurements to confirm their quality and quantity. Then, reverse transcription of one µg RNA was conducted (RevertAid First Strand cDNA Synthesis Kit, Thermo Scientific, USA). Relative gene expression levels of respective genes were measured with qRT-PCR as we described previously [29], and the data were normalized with the expression of the *gadph* housekeeping gene. The sequences of primer pairs used in this study are summarized in Table 1. For all genes, at least triplicate measurements were conducted, and melt analysis confirmed the product specificities. Efficiency corrected advance relative quantification tool of the LightCycler 480 SW 1.5.1 software was utilized for the data analysis.

Table 1: Primer sequences used in gene expression analysis with qRT-PCR.

Gene	Forward primer sequence (5′ → 3′)	Reverse primer sequence (5′ → 3′)
pde1a	CCACTTTGTGATCGGAAGTC	TTCTGCTGAATGATGTCCACC
pde1b	CAGGGTGACAAGGAGGCAGAG	GACATCTGGTTGGTGATGCC
pde1c	TCATCAAGCCTCCTTGCGT	GAATTCGCCCTTGGTCGGT
pde2a	CCTCCTGTGACCTCTCTGACC	TGAACTTGTGGGACACCTTGG
pde3b	ACAAATGCACCTCAGGCAGT	GATCTTTTGCTGGGCCGTTG
irs-1	GCCAATCTTCATCCAGTTGC	CATCGTGAAGAAGGCATAGG
akt	GAAGAGGCTCGCCTCCAT	GAAGGAGAAGGCCACAGGTC
pi3k	ATGCAACTGCCTTGCACATT	CCCTGAAGCTGAGCAACAT
gapdh	TCCTTGGAGGCCATGTGGGCCAT	TGATGACATCAAGAAGGTGG TGAAG

Determination of protein expressions by Western blot

Liver tissue samples were homogenized in fourfold volumes of homogenization medium containing Tris (pH:7.4, 50 mM), sodium chloride (150 mM), phenylmethylsulfonyl fluoride (0.2 mM), EDTA (5 mM), sodium fluoride (50 mM), sodium deoxycholate (0.26% w/v), sodium orthovanadate (0.1 mM) and Triton X-100 (1% w/w) and centrifuged at 1,500*g* for 10 min at +4 °C. Protein concentrations of supernatants were determined [30] and used in Western blot analysis as we described previously [29]. Briefly, 50–100 µg of proteins were separated by SDS-PAGE and then transferred onto the polyvinylidene fluoride membranes. After blocking, primary antibodies were incubated overnight. The used antibodies were PDE1A (anti-PDE1A rabbit IgG, Santa Cruz, CA, USA, 1:500), PDE1C (anti-PDE1C rabbit IgG, Santa Cruz, CA, USA, 1:500), PDE2A (anti-PDE2A rabbit IgG, Santa Cruz, CA, USA, 1:500), PDE3B (anti-PDE3B rabbit IgG, Santa Cruz, CA, USA, 1:1,000), pIRS-1 (anti-pIRS-1 rabbit IgG, St John's Laboratory, London, UK, 1:1,000), pAKT (Phosphorylated Protein kinase B) (anti-pAKT rabbit IgG, Santa Cruz, CA, USA, 1:500), IRS-1 (anti-IRS-1 rabbit IgG, Abcam, France, 1:1,000), AKT (anti-AKT rabbit IgG, Abcam, France, 1:5,000), and PI3K (anti-PI3K rabbit IgG, Santa Cruz, CA, USA, 1:500), GAPDH (anti-GAPDH Rabbit IgG, Santa Cruz, CA, USA, 1:1,000). After washing excess antibodies, secondary antibodies conjugated with horseradish peroxidase (HRP) (Goat Anti-rabbit IgG-HRP conjugate; Santa Cruz, CA, USA, 1:10,000) were applied for 1 h. After exposing to Clarity™ Western ECL (Bio-Rad Laboratories, Hercules, CA, USA) for 5 min, we obtained blots' images with a ChemiDoc™ MP Chemiluminescence detection system (Bio-Rad Laboratories, Hercules, CA, USA). ImageLab 4.1 software (Bio-Rad Laboratories, Hercules, CA, USA) was utilized for data analysis.

Histochemical staining

Tissue samples were fixed in 10% neutral formalin and embedded in paraffin for histological processes. 5 µm thin sections were taken from paraffin blocks and stained with hematoxylin-eosin (H-E) (Sigma). Intralobular microsteatosis was graded as Grade 1 not affected or minimal; Grade 2 slight; Grade 3 moderate; Grade 4 marked or severe Also, Kupffer cells in sinusoids were counted in five different areas under ×40 magnification with a light microscope (Eclipse E-600 Nikon, Tokyo, Japan).

Immunohistochemistry

Deparaffinized and rehydrated liver samples were treated with citrate buffer (pH:6.0) in a microwave for antigen retrieval. Three percent hydrogen peroxide in methanol blocked the endogenous peroxidase activity, and the sections were incubated with cPLA2 (1/200, Abcam, France, ab58375) and IGF-1Rα (1/200, Santa Cruz, USA, sc-712) primary antibodies overnight at 4 °C. HRP secondary antibody kit (Lab Vision UltraVision Detection System: anti-Polyvalent, Thermo Fisher Scientific) was used as a secondary antibody, colored with aminoethyl carbazole (AEC) and counterstained with Mayers hematoxylin. All samples were evaluated under a light microscope (Eclipse E-600 Nikon, Tokyo, Japan), and image analysis was made with Image Analysis Software (NIS Elements Nikon, Tokyo, Japan). H-Score was determined according to the intensity of each antibody.

Statistical analysis

Gene and protein expressions of HFCS, Kefir, and HFCS + kefir groups were normalized to the mean of the control groups, whereas HFCS + Kefir groups were compared with HFCS, and data were also normalized with corresponding housekeeping protein GAPDH. All data is represented as mean ± standard error of the mean (SEM) throughout the study. Statistical comparisons were performed using one-way ANOVA followed by an appropriate post-hoc test (Tukey). For microsteatosis scoring, the Chi-Square test was performed. Pearson Chi-Square and Fisher's Exact Test were used in crosstabs. Comparisons giving p-values less than 0.05 were accepted as statistically significant. All analyses were done using SPSS 21.0 software (IBM Corporation, Armonk, NY, USA).

Results

The data, representing the body & omentum weights, food, liquid, and caloric intake, as well as some metabolic parameters of rats, have been published in our recent study [29]. Briefly, we have shown that intervention with dietary HFCS increased body weight and caused hyperglycemia, hyperinsulinemia, hyperlipidemia, and omental adiposity; however, these disorders were diminished with kefir treatment [29].

The effects of HFCS and kefir consumption on the mRNA expressions of hepatic PDEs and insulin signaling pathway components in rats

Gene expression levels of several PDEs and insulin signaling elements were measured with semiquantitative qRT-PCR with respect to the housekeeping gene; GADPH, and the results were expressed as fold-change values against a control group. Figure 1 summarizes the results, and the data demonstrate the enhanced gene expressions of *pde1a* (a), *pde1b* (b), *pde1c* (c), and *pde3b* (e), but reduced gene expressions of *pi3k* (f), *akt* (g), and *irs-1* (h) in HFCS treated rats. There was also a tendency toward augmentation in *pde2a* (d) mRNAs, but this difference did not achieve a significance level. However, probiotic supplementation with kefir suppressed the mRNA expressions of *pde1a*, *pde1b*, *pde1c*, and *pde3b* in HFCS-fed rats' livers and normalized the *pi3k*, *akt*, and *irs-1* gene expressions in the same group.

The effects of HFCS and kefir consumption on the protein levels of hepatic PDE and insulin signaling elements

The results of protein expression studies carried out with Western blot analysis are shown in Figure 2. According to our findings, HFCS intake triggered the protein levels of PDE isoforms as PDE1A (a), PDE1C (b), PDE2A (c), and PDE3B (d), which were significantly improved by kefir supplementations as in parallel with mRNA expressions. Additionally, hepatic protein expressions of some insulin signaling elements such as pIRS-1 (e), pAKT (f), IRS-1 (g), and PI3K (i) were significantly downregulated in HFCS-fed rats. There was also a tendency toward reducing AKT (h), but it is not significant. The supplementation of kefir normalized the expressions of hepatic proteins as pIRS-1, pAKT, IRS-1, AKT, and PI3K in HFCS-fed rats.

Immunohistochemical changes of PLA2 and IGF-IRα and histopathological modifications in hepatic tissues

Dietary HFCS elevated the hepatic cytosolic phospholipase A2 (cPLA2) (Figure 3A) and insulin-like growth factor 1 receptor-α (IGF-1Rα) (Figure 3B) levels, and supplementation with kefir reduced cPLA2 and IGF-1Rα staining in HFCS-fed rats. Besides, kefir also suppressed cPLA2 in

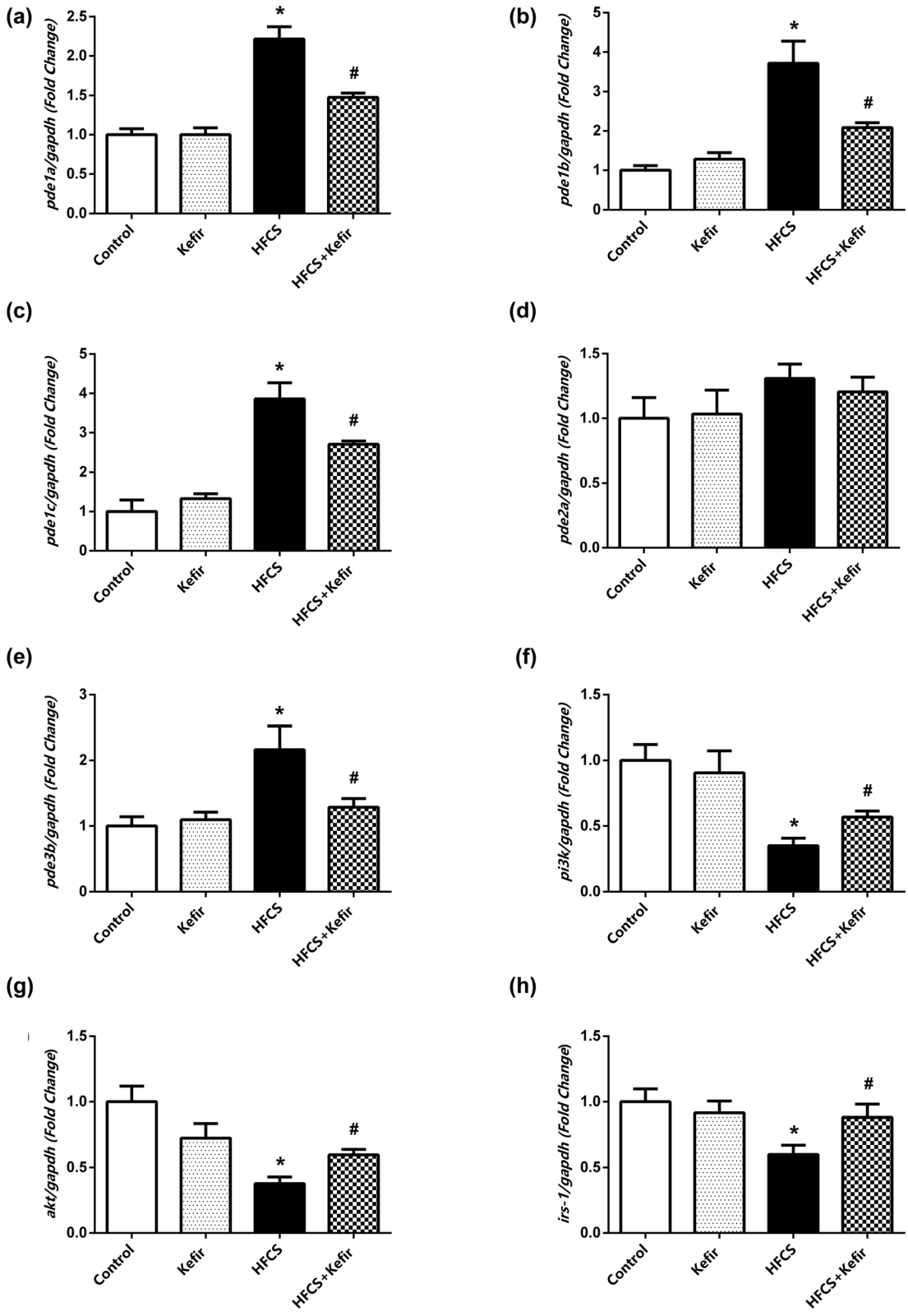

Figure 1: Changes in gene exspression levels of PDEs and insulin signaling elements.
The mRNA expressions of *pde1a* (a), *pde1b* (b), *pde1c* (c), *pde2a* (d), *pde3b* (e), *pi3k* (f), *akt* (g), and *irs-1* (h) in the liver tissues of rats from the Control, Kefir, HFCS, and HFCS + Kefir groups. Data were 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.

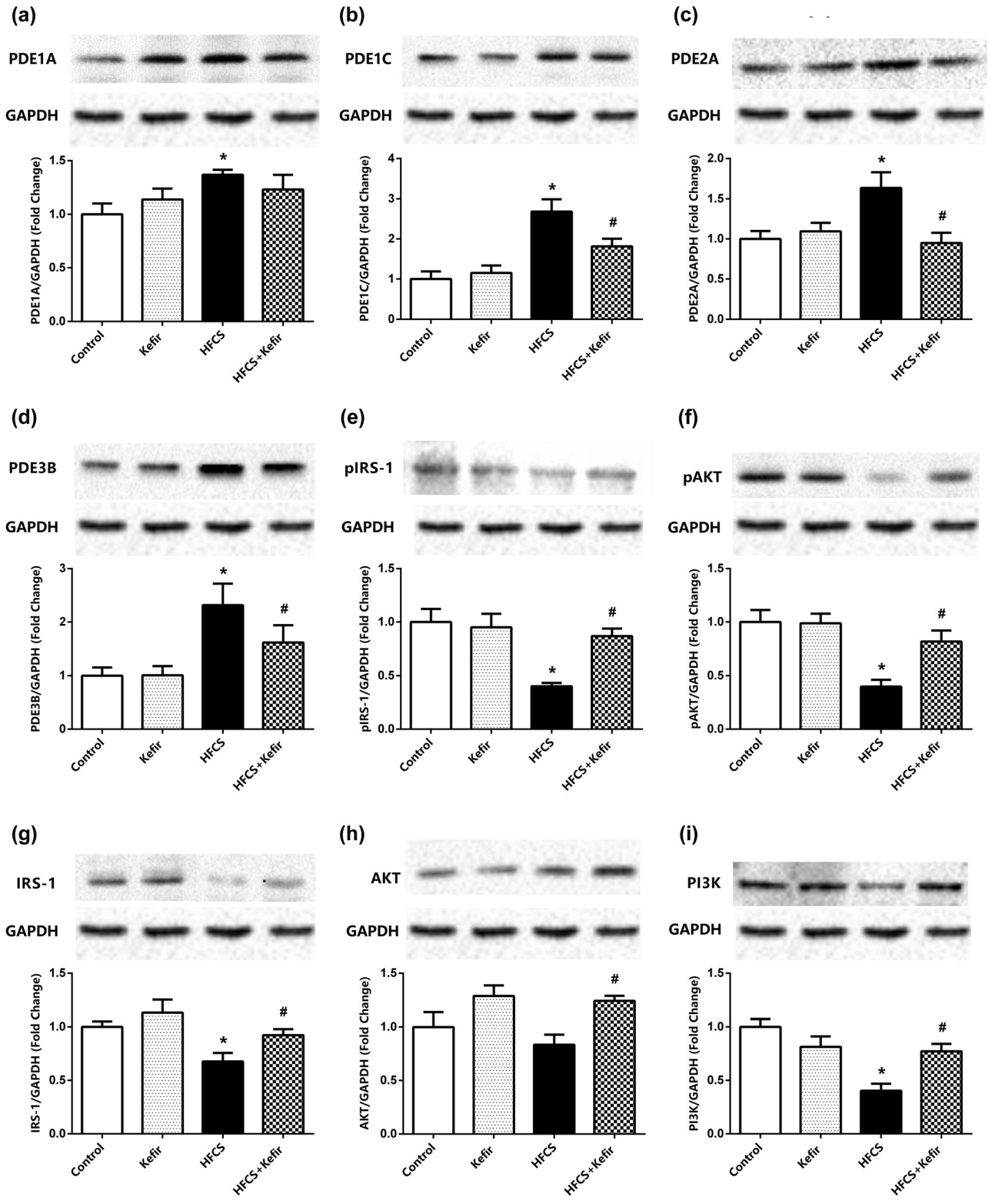

Figure 2: Changes in protein levels of PDEs and insulin signaling elements.
Representative *Western blot* images for PDE1A (a), PDE1C (b), PDE2A (c), PDE3B (d), pIRS-1 (e), pAKT (f), IRS-1 (g), AKT (h), and PI3K (i) in the liver tissues from the Control, Kefir, HFCS, and HFCS + Kefir groups. Each band, presented in a row, derived from the same experiment, and the blots were processed in parallel experiments. Whole data from protein expressions were quantified using densitometry and normalized with corresponding GAPDH. Each bar represents at least six samples. Values are expressed as mean ± SEM, *Significantly different ($p<0.05$) compared to Control group; #significantly different ($p<0.05$) compared to HFCS group.

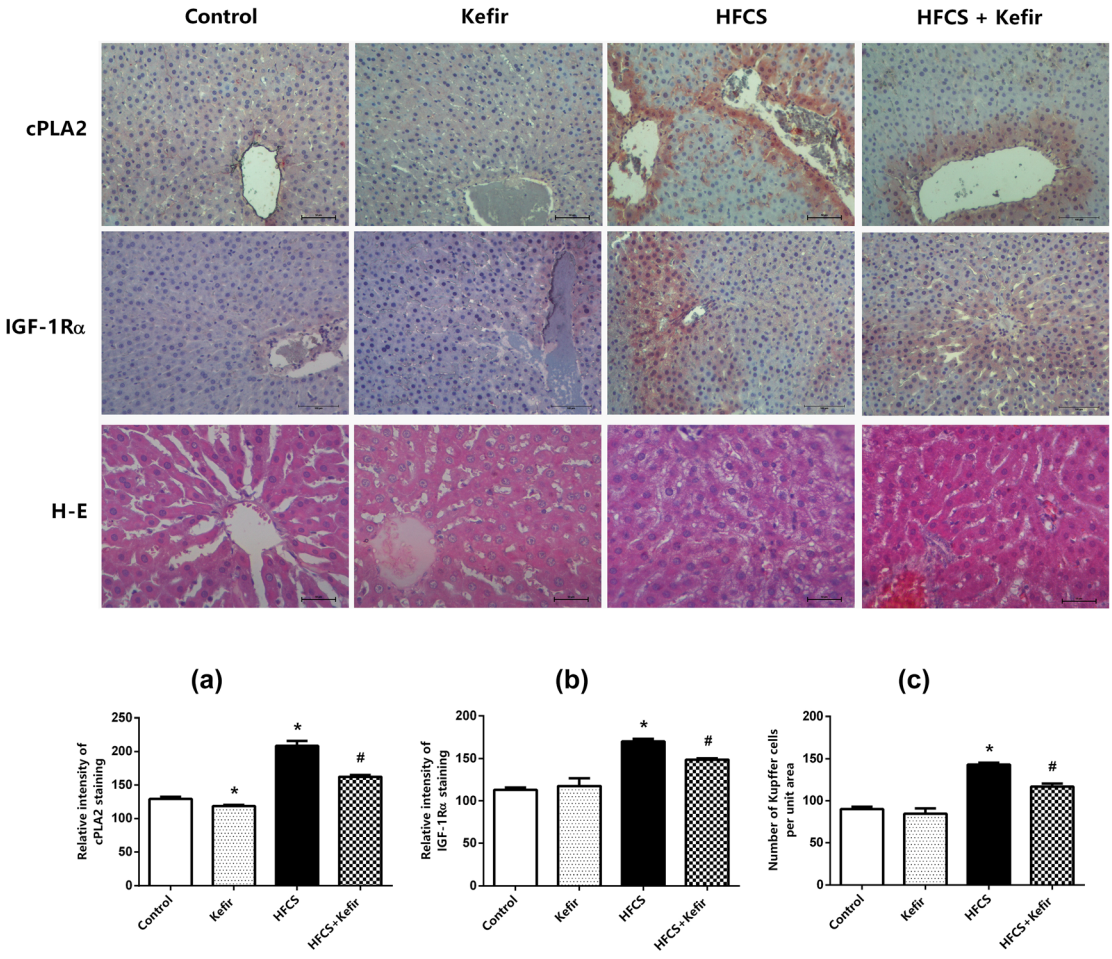

Figure 3: Immunohistochemistry results. Immunohistochemical staining for cPLA2 (a) and IGF-1Rα (b) proteins (×20 magnifications) and the number of Kupffer cells per unit area (c) (×40 magnifications) from control, kefir, HFCS, and HFCS + Kefir groups. Histopathological features of the liver sections with hematoxylin-eosin staining show increased microsteatosis grade in the HFCS-fed rats while reducing with kefir.

healthy rats. However, HFCS enhanced the number of Kupffer cells per unit area (Figure 3C). Kefir alone did not significantly affect the microsteatosis grade levels in control rats. However, there was a massive increment in the microsteatosis grade of the liver of the HFCS group, which was almost normalized after kefir treatment.

Discussion

Increasing consumption of HFCS in foods or beverages is generally considered the leading cause of many metabolic disorders worldwide. PDEs are a family of enzymes that provide hydrolysis of cAMP and cGMP, which serve as secondary messengers in intracellular signaling pathways in the body. Therefore PDEs are also thought to have roles in many metabolic disorders. This study assessed the eventual interactions of HFCS diet-induced impaired insulin signaling pathway-dependent insulin resistance in rats with PDEs explicitly localized in the liver and the potential effects of kefir supplementation on these changes. Herein, we found that disruption of the insulin signaling pathway and activation of PDEs were negatively correlated in the liver tissues of the HFCS-fed rats. Moreover, kefir treatment generally achieved remarkable effects in HFCS-dependent disorders in the liver.

The PDE1, 2, and 3 isoforms, predominantly located in adipocytes, liver, and pancreas [31], have been shown to inhibit insulin secretion by activating IGF-1 and leptin in β-cells [32]. It has been reported that insulin resistance and glucose intolerance developed with PDE3B overexpression in mice with high fat diet-induced metabolic syndrome [33]. In contrast, PDE3B levels were low in the adipose tissues of obese mice [34]. The PDE1 family may play a key role in Ca^{2+} dependent signaling pathways in pancreatic β-cells and the primary catalyst of cAMP and cGMP

hydrolysis; PDE1C was shown to downregulate glucose-induced insulin secretion [34], and its inhibition could increase insulin release. A recent study also demonstrated high expression of PDE1A and PDE1C in the insulinoma cell line [35], and inhibition of these PDEs can have therapeutic benefits. PDE inhibitors can also increase serum glucose levels under pathological circumstances involving the liver since intracellular cAMP levels might modulate the phosphorylation state of crucial gluconeogenesis enzymes [36]. Even though the effects of PDEs in the liver are limited, it was shown that increased hepatocyte cAMP levels suppress the genes involved in *de novo* lipogenesis [37]. Thus, it is conceivable that inhibition of PDEs in the liver might prevent adiposity. Previously, we have shown the development of hyperinsulinemia, hyperlipidemia, and consequently omental adiposity in HFCS-fed rats [29]. In this current study, HFCS has triggered hepatic phosphodiesterase hyperactivity with both increased gene expression and protein levels of PDE1A, PDE1B, PDE1C, PDE2A, and PDE3B. The development of microsteatosis and increased Kupffer cells also support this claim. Moreover, Kupffer cells, the tissue-resident liver macrophages, are enriched with the HFCS diet [38]. Thus, these findings indicate the beginning of inflammatory response with HFCS to emerge with steatosis. In similar other studies, it has been repeatedly shown that the liver tissues of experimental animals develop inflammation with dietary HFCS, which is presented as a carbohydrate source in sugary foods and beverages in daily life. Insulin resistance due to fructose consumption and/or HFCS was also evidenced [19, 39, 40]. The present study determined the suppression of hepatic IRS-1 and PI3K protein and pIRS-1 and pAKT levels, which are insulin signaling pathway elements, by HFCS intake. Similarly, qPCR results revealed that suppression of insulin signaling is controlled at gene expression levels since mRNA levels of those were also downregulated. However, the increase in IGF-1Rα levels detected by the immunohistochemical method and the rise in plasma glucose level despite hyperinsulinemia tendency prove that insulin resistance develops due to HFCS consumption [29]. In similar other studies related to the subject, it has been found that insulin resistance develops due to fructose-feeding, and the IRS/PI3K/AKT pathway is impaired in different tissues [19, 20]. In the light of these findings, HFCS added in drinking water to the diet of rats as a fructose source reduced the levels of IRS-1, AKT, and PI3K and induced PDEs in the liver. Induction of PDEs might reduce the intracellular cAMP and cGMP levels and, therefore, could inhibit insulin activity.

Nowadays, different alternatives for the treatment have emerged as a result of the deterioration of energy metabolism due to changes in diet and lifestyle. The use of nutritional supplements containing probiotics and/or beneficial bacteria is becoming widespread in terms of their more pronounced efficacy and ease of use. Kefir is a fermented food containing many types of bacteria, and it could be an alternative protective agent. Studies examining the effects of kefir indicate its anti-apoptotic, anti-inflammatory, and anti-allergic effects [19, 27, 28]. Similar to this study, metabolic irregularities due to fructose consumption were eliminated using different types of kefir [19, 41]. Also, the metabolic effects of some strains of bacteria in kefir are stunning. It has been shown that by administering *Lactobacillus plantarum* to rats consuming 10% fructose in drinking water, renal antioxidant enzymes were increased [21], and inflammation markers were suppressed [22]. However, it was determined that the IRS1/AKT/eNOS pathway, disrupted by the fructose diet, was improved by administering *L. plantarum* [23]. According to these findings, kefir-treatment suppressed the PDEs; however, it enhanced the insulin signaling pathway via increasing hepatic IRS1, AKT, and PI3K, both protein and mRNA expressions in the rats. Our recent study found that plasma glucose and fructose levels increased in HFCS-fed rats despite decreased daily caloric intake [29]. In the same study, we also demonstrated normalization of plasma glucose and fructose levels of HFCS animals administered with kefir. In this respect, kefir would improve the disorders caused by HFCS over-consumption by enriching the intestinal microbiota.

Conclusions

Dietary HFCS, which is presented as a carbohydrate source in sugary foods and beverages in daily life, might cause insulin resistance by downregulating the signaling elements in the liver tissues of rats. Our study revealed the association of insulin resistance with increased PDEs expression and suppressed insulin signal pathway components; IRS1/PI3K/AKT. Its inflammatory potential is proved with increased immunoreactivity of cPLA2. The induction of IGF-1Rα could indicate cell growth that promotes survival and proliferation. Therefore, this study uncovers some basic molecular mechanisms underlying pathological events induced by HFCS. Kefir treatment altered the disrupted insulin efficiency by increasing insulin signaling elements, suppressing PDEs' expression, and restoring the inflammatory and proliferative markers, cPLA2 and IGF-1Rα, in hepatic tissues. These results point to the existence of kefir's anti-inflammatory and cell growth inhibitory potential. Although the relevance of

these results in humans has not yet been determined, we propose that kefir may be an excellent functional food against HFCS-induced insulin resistance and tissue inflammation in a mechanism involving cellular PDEs.

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Competing interests: The authors of this manuscript report no conflict of interest. All co-authors have seen and agreed with the contents of the manuscript.

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