Lactobacillus helveticus and *Lactobacillus plantarum* modulate renal antioxidant status in a rat model of fructose-induced metabolic syndrome

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Abstract: High dietary fructose intake causes a metabolic disorder and augments the risk of chronic kidney disease most likely due to oxidative stress. Probiotics could have antioxidant, antiinflammatory and immunoregulatory properties. The present study examined the influence of *Lactobacillus helveticus* and *Lactobacillus plantarum* supplementation on dietary fructose-induced metabolic changes and renal antioxidant/oxidant status of rats. Male Wistar rats were divided into four groups as follows: control; fructose; fructose plus *L. helveticus*; fructose plus *L. plantarum*. Fructose was given to the rats as a 20% solution in drinking water for 15 weeks. The probiotic supplementation was applied by gastric gavage once a day for six weeks. Several metabolic parameters in the plasma, gene and protein expressions of the main antioxidant enzymes in renal tissues of rats were measured. Dietary fructose-induced elevations in plasma insulin, triglyceride, VLDL, creatinine as well as renal urea levels were alleviated after treatment with *L. helveticus* and *L. plantarum*. Moreover, *L. helveticus* and *L. plantarum* supplementation recovered the changes in renal protein expression level of SOD1, SOD2 and CAT. In conclusion, supplementation with *L. helveticus* and *L. plantarum* has an improving effect on specific metabolic parameters and renal antioxidative enzymes in a fructose-induced metabolic disorder.

Key words: dietary fructose; renal antioxidant status; antioxidant enzymes; *Lactobacillus helveticus; Lactobacillus plantarum.*

Abbreviations: CAT (catalase); Fruc (fructose); FAS (fatty acid synthase); GAPDH (glyceraldehyde 3-phosphate dehydrogenase); GPx (glutathione peroxidase); HDL (high density lipoprotein); IL-1β; 6 (interleukin-1β; 6); iNOS (inducible nitric oxide synthase); MDA (malondialdehyde); NF-κB (nuclear factor-kappa B); PAGE (polyacrylamide gel electrophoresis); PVDF (polyvinylidene floride); SOD (superoxide dismutase); qRT-PCR (quantitative real-time PCR); SDS (sodium dodecyl sulfate); SREBP1 (sterol regulatory element-binding transcription factor 1); TBARS (thiobarbituric acid reactive substances); TNF-α (tumor necrosis factor-α); VLDL (very low-density lipoprotein).

INTRODUCTION

Excess sugar intake, especially of fructose, has been shown to induce metabolic syndrome and kidney diseases via several molecular mechanisms, including activation of inflammatory

processes and generation of oxidative stress [1,2]. We previously described the upregulation of the inflammatory pathway and induction of the superoxide-producing system in various tissues with high dietary fructose intake [3-9]. Development of a proinflammatory condition, as manifested by nuclear factor-kappa B (NF-κB) activation, high expression of tumor necrosis factor-α (TNF-α), inducible nitric oxide synthase (iNOS) and interleukin-6 (IL-6), was demonstrated in the renal tissue of high-fructose-fed rats [10]. Furthermore, oxidative stress, as evidenced by decreased antioxidant enzyme activities and glutathione levels and increased malondialdehyde (MDA) contents in kidney tissues was shown in high-fructose-fed rats [11-14]. Fructose-induced uric acid generation is proposed to lead to increased mitochondrial oxidative stress, endothelial dysfunction and maladaptive immune and inflammatory responses [15,16].

Probiotics are currently defined as nonpathogenic microorganisms and have been reported to improve metabolic diseases such as obesity and diabetes through modulation of intestinal microorganisms [17,18]. Their beneficial health effects could be attributed to their antiinflammatory and immunoregulatory properties [19]. Some probiotic strains were shown to induce significant antioxidant activity through action on superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) [20]. Lactic acid bacterial strains, which are the principal representatives of probiotics in both food and pharmaceutical forms, have been shown to ameliorate metabolic diseases by increasing insulin sensitivity, reducing inflammatory reactions and oxidative stress [18,21]. Supplementation with *Lactobacillus plantarum* along with a high-fat fructose diet in rats was shown to improve metabolic dysfunction by modulating blood proinflammatory cytokines, IL-1β, IL-6 and TNF-α, as well as the antioxidant enzymes, SOD, CAT and GPx [22]. The combination of *L. curvatus* and *L. plantarum* in high-fructose-fed rats lowered plasma glucose, insulin, triglyceride and oxidative stress, and also suppressed hepatic lipogenesis via downregulation of sterol regulatory element-binding transcription factor 1 (SREBP1) and fatty acid synthase (FAS) mRNA levels [23]. Knowledge of the influence of probiotic treatment on high-fructose-induced metabolic syndrome is limited. Therefore, in the present study, an attempt was made to investigate whether supplementation with *L. helveticus* and *L. plantarum* improves metabolic parameters and the renal antioxidant/oxidant status of fructose-fed rats.

MATERIALS AND METHODS

Chemicals

Chemicals were purchased from the Sigma Chemical Co. (St. Louis, MO) unless otherwise stated. Fructose was obtained from Danisco Sweeteners OY (Finland). *L. helveticus* and *L. plantarum* strains were obtained from Chr. Hansen (Denmark; ATCC: 15009 and ATCC: 14917 respectively) and grown in our laboratory.

Animals and diets

All protocols for animal usage were approved by the Ethical Animal Research Committee of Afyon Kocatepe University (Akuhadyek-49533702). 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 animals were fed with a standard rodent chow diet composed of 62% starch, 23% protein, 4% fat, 7% cellulose, standard vitamins and salt. At the end of the acclimation for one week, the rats were randomly divided into four groups, designated as Control; Fructose (Fruc); Fructose+*L. helveticus* (Fruc+LH) and Fructose+*L. plantarum* (Fruc+LP). Fructose was given to the rats as a 20% solution (w/v) in drinking water for 15 weeks. *L. helveticus* and *L. plantarum* ($1x10^9$ CFU per 100 g of body weight of animal) in 2 mL of saline were given by gastric gavage once a day during the final six weeks. The control and fructose groups were administered the same volume of saline by gavage for the same period. Body weights, food, and fluid intake were recorded weekly during the follow-up period.

At the end of the follow-up period, the rats were anesthetized with a mixture of ketamine-xylazine (100 and 10 mg/kg, respectively, i.p.) and blood samples were rapidly collected via cardiac puncture. The kidneys of rats were blotted dry, weighed, frozen in liquid nitrogen and stored at -85°C.

Preparation of *L. helveticus* and *L. plantarum*

L. helveticus and *L. plantarum* were cultured in de Man, Rogosa and Sharpe broth (MRS; Oxoid; Unipath Ltd., Basingstoke, Hampshire, England) at 30°C in a rotary shaker at 150 rpm. Stock cultures were stored at -80°C in MRS broth containing 20% (v/v) glycerol. Erlenmeyer flasks containing 20 mL of MRS were inoculated with 1.5 mL of glycerol stock culture and the cultures were incubated at 35°C±1°C in a rotary shaker at 150 rpm and grown to an optical density of 1.0 at 600 nm (cell density corresponding to $1x10^8$ CFU/mL). The culture was divided into 10 mL tubes ($1x10^9$ CFU) and the cells were harvested at 5000 x *g* for 5 min at 4°C. The cell pellets were washed with isotonic saline solution and lyophilized under a freeze drier.

Measurement of metabolic parameters in plasma and renal tissue

Cardiac blood samples of non-fasted rats were immediately centrifuged at 10000 x *g* and 4°C for 30 min. Kidney samples were homogenized in 0.1 M phosphate buffer 1:10 (w/v), pH 7.4, and 24000 cycles/min (Ultra Turrax, IKA Works Inc., USA), and ultrasonicated at 20000 cycles/s for 1 min (Dr. Hielscher, Germany). Homogenates were centrifuged at 10000 x *g* and 4°C for 15 min, and the supernatants were collected. All the samples were stored at -85°C until analysis. Plasma triglyceride, very low-density lipoprotein (VLDL), high-density lipoprotein (HDL) and total cholesterol levels were determined by standard enzymatic techniques. Insulin (Elabscience, USA), creatinine, urea, uric acid, albumin and total protein levels (Biolabo, France) were assessed using enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturers' instructions. Glucose levels were measured using a glucometer (Roche Diagnostics, USA) in blood collected from the tail veins of rats. MDA levels were measured with a TBARS assay kit (Cayman Chemical, USA).

Determination of *sod1, sod2, cat,* and *gpx* gene expressions by real-time polymerase chain reaction (qRT-PCR)

Total RNAs were isolated from kidney tissue using RNeasy total RNA isolation kit (Qiagen, Venlo, Netherlands) as described in the manufacturer's protocol. After isolation, the amount and the quality of the total RNA was determined by spectrophotometry and agarose gel electrophoresis. One µg of total RNA was reverse-transcribed to cDNA using a commercial first strand cDNA synthesis kit (Thermo Scientific, USA). Expression levels of *sod1, sod2, cat,* and *gpx* were determined by qRT-PCR (LightCycler480 II, Roche, Basel, Switzerland). One µL of cDNA, 5 µL 2X SYBR Green Master mix (Roche FastStart Universal SYBR Green Master Mix) and 2 µL primer pairs (*sod1*-F: GCAGAAGGCAAGCGGTGAAC; *sod1*-R: TAGCAGGACAGCAGATGAGT; *sod2*-F: GCACATTAACGCGCAGATCA; *sod2*-R: AGCCTCCAGCAACTCTCCTT; *cat*-F: GCGAATGGAGAGGCAGTGTAC; *cat*-R: GAGTGACGTTGTCTTCATTAGCACTG; *gpx*-F: CTCTCCGCGGTGGCACAGT; *gpx*-R: CCACCACCGGGTCGGACATAC; *gapdh*-F: TGATGACATCAAGAAGGTGGTGAAG; *gapdh*-R: TCCTTGGAGGCCATGTGGGCCAT) were mixed and 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, 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.

Immunoblot analysis of antioxidant enzymes, SOD1, SOD2, CAT and GPx

For determination of SOD1, SOD2, CAT and GPx protein contents, kidney tissue was homogenized in 2-fold volumes of homogenization medium (50 mM Tris, 150 mM NaCl, 5 mM EDTA, 1 % (w/w) Triton X-100, 0.26% (w/v) sodium deoxycholate, 50 mM sodium fluoride, 0.1 mM sodium orthovanadate and 0.2 mM phenylmethylsulfonyl fluoride (PMSF)) with a Tissue Ruptor^{TM} (Qiagen, Netherlands) homogenizer. The homogenates were centrifuged at 1500 x *g* for 10 min at 4ºC. After the removal of the supernatants, the protein concentrations were determined by the Lowry method [24]. Ten to 50 µg of total proteins were separated by SDS-PAGE and transferred on to PVDF membranes using a semi-dry electroblotting apparatus (TransBlot Turbo, BioRad, Germany). Blotted membranes were blocked with 5% (w/v) nonfat dried milk and incubated with primary antibodies for SOD1 (Anti-SOD1 Sheep IgG, Calbiochem-574597, 1:5,000), SOD2 (Anti-SOD2 Rabbit IgG, Santa Cruz; sc:30080, 1:100), CAT (Anti-CAT Rabbit IgG, Abcam, ab:6731,1:6,000), GPx (Anti-GPx Rabbit IgG, Santa Cruz, sc:30147,1:100) for 2 h at room temperature or overnight at 4ºC. As an internal control, GAPDH proteins were also labeled with anti-GAPDH Rabbit IgG (Santa Cruz, sc:25778, 1:2,000). Horseradish peroxidase-conjugated secondary antibody (Santa Cruz, sc:2030 or sc:2770, 1:10,000) was incubated for 1 h, and the blots were incubated in Clarity^{TM} Western ECL (Bio-Rad Laboratories, Hercules CA, USA) substrate solution. Images of the blots were obtained using the ChemiDoc^{TM} MP Chemiluminescence detection system (Bio-Rad Laboratories, Hercules CA, USA) equipped with a CCD camera. The relative expression of proteins with respect to GAPDH was calculated using ImageLab5.2 software.

Statistical analysis

All data are presented as the mean±standard error of the mean; n is the number of rats. Statistical comparisons were performed using unpaired Student's t-test or one-way ANOVA followed by the Tukey *post hoc* test. *P* values smaller than 0.05 were considered as statistically significant.

RESULTS

The effects of dietary fructose and the supplementation of *L. helveticus* and *L. plantarum* on body weight, caloric intake and kidney weight

As shown in Table 1, the high-dietary-fructose intervention did not change the final body weights of rats despite the increase in total caloric intake. There was a trend toward an increase in final body weight in Fruc+LH group when compared to the fructose group, but the differences were not significant. Besides, no significant change was found in the final body weights of rats between Fruc and Fruc+LP groups. The total caloric intakes were not changed after supplementation with *L. helveticus* and *L. plantarum* when compared to the fructose group. Moreover, the right and left kidney absolute weights, as well as the ratio of right or left kidney weights to the body weight were not significantly altered between the groups (Table 1).

The effects of dietary fructose and the supplementation with *L. helveticus* and *L. plantarum* on metabolic and biochemical parameters in plasma and kidney

As shown in Table 2, high dietary fructose increased the plasma glucose and insulin levels as compared to control group. The probiotic supplementation did not alter the plasma glucose levels. However, *L. plantarum* supplementation significantly reduced the plasma insulin levels. The dietary fructose-induced increase in plasma triglyceride and VLDL levels was markedly reduced with *L. helveticus* and *L. plantarum*. Fructose feeding and *L. helveticus* supplementation did not affect the HDL and total cholesterol levels, but *L. plantarum* supplementation significantly enhanced plasma HDL contents.

Plasma MDA levels, but not the renal concentration, increased in rats that were fed with fructose. Moreover, supplementation with *L. helveticus* and *L. plantarum* did not modulate the MDA levels. Dietary fructose also increased the plasma creatinine levels, and conversely, *L. helveticus* and *L. plantarum* supplementation reduced the creatinine levels to the control value.

Significant changes in plasma urea, uric acid, albumin and total protein contents were determined between groups: plasma uric acid levels in the *L. helveticus*-treated group were decreased. High-fructose intake increased renal urea concentration, which was decreased by *L. helveticus* and *L. plantarum* supplementation. Additionally, *L. plantarum* treatment significantly decreased the renal uric acid levels as compared to the fructose group.

The effects of dietary fructose and supplementation with *L. helveticus* and *L. plantarum* on SOD1, SOD2, CAT and GPx gene and protein expression

Relative changes in antioxidant enzyme gene and protein expression levels in kidney tissue were measured by qRT-PCR and Western blot analysis, respectively. The results show the suppression of *sod1* and *sod2* mRNA expression in fructose-fed rats. However, treatments with *L. helveticus* and *L. plantarum* did not alter *sod1* expression, but *L. plantarum* significantly increased *sod2* mRNA levels (Fig. 1A and 1B). Moreover, high-fructose intake significantly increased *cat* mRNA expressions but not *gpx* levels. Supplementation of *L. helveticus* and *L. plantarum* did not modulate the *cat* and *gpx* gene expressions significantly (Fig. 1C and D).

Fig. 2 summarizes the changes in renal protein expression of the main antioxidant enzymes, SOD1, SOD2, CAT and GPx, by dietary fructose and probiotic bacteria. As shown in Fig. 2A and B, SOD1 and SOD2 protein expression was decreased in fructose-fed rats. Supplementation with *L. helveticus* and *L. plantarum* increased both SOD1 and SOD2 protein expression (Fig. 2A and B). Parallel with the gene expression pattern, CAT protein expression was increased in the fructose group (Fig. 2C) but significantly decreased after treatments with *L. helveticus* and *L. plantarum*. No change was observed in GPx levels among all examined groups (Fig. 2D).

DISCUSSION

Fructose is commonly used as an industrial sweetener and is excessively consumed in the regular human diet. Overconsumption of fructose has been proposed as a risk factor for the metabolic syndrome, manifesting in dysfunctional adipose, liver, kidney, intestine and cardiovascular tissues [25]. One of the leading driving forces for the fructose-induced disturbances is the oxidative stress arising from an imbalance between the prooxidant and antioxidant systems [26]. This study demonstrated some favorable influences of probiotic supplementation with *L. helveticus* and *L. plantarum* on the metabolic parameters and renal antioxidant/oxidant status of rats maintained on a high-fructose diet.

Probiotics have emerged as a therapeutic potential for metabolic syndrome, especially in the high-fat diet model [27-29]. Data describing the influence of probiotic treatment on high-fructose-induced metabolic syndrome is limited [22,30]. Therefore, we evaluated the effects of the supplementation of two commonly used probiotic microorganisms, *L. helveticus* and *L. plantarum*, on metabolic, plasma and renal antioxidant/oxidant markers. The findings presented herein demonstrated hyperinsulinemia and hypertriglyceridemia with high-fructose intake, which is in accordance with our previous studies [3,4,6,9]. There were no significant alteration in the body weights and the ratio of renal weights to body weights in rats fed with high-fructose diet. *L. helveticus* and *L. plantarum* supplementation diminished the increase in plasma triglyceride. Moreover, *L. plantarum* treatment restored the elevated plasma insulin levels. The plasma glucose- and lipid-lowering effects of *L. plantarum* were previously reported in high-fat- or high-fructose-induced metabolic disorders [22,27,28,30]. Moreover, the consumption of a combination of *L. curvatus* and *L. plantarum* probiotic strains was shown to reduce plasma triglyceride levels in hypertriglyceridemic subjects [31]. Administration of the probiotic *L. kefiri* to mice fed with a fructose-rich diet prevented the increase in plasma triglycerides and leptin [32]. An early sign of nephropathy is an increase in serum creatinine, which is associated with the progression of renal damage [33]. Serum urea and uric acid levels are also essential parameters of renal functions. Fructose-induced hyperglycemia may impair renal functions and

increase plasma creatinine, urea and uric acid levels as well as oxidative stress [15,34]. In this study, a significant increase in the levels of plasma creatinine and renal urea could be taken as a reflection of a fructose-induced renal disorder. The correction of these abnormalities by *L. helveticus* and *L. plantarum* supplementation could be valuable in prevention of the disease. Previously, it has been demonstrated that probiotic therapy with *L. plantarum* strains improves urinary oxalate, calcium, uric acid, creatinine and serum uric acid levels in rats with increased renal calcium oxalate deposition [35].

A diet rich in fructose can lead to the induction of marked oxidative stress and mitochondrial dysfunction [36]. Oxidative stress arising from a high-fructose diet has been shown to suppress antioxidant enzymes in different tissues [37-41]. Renal SOD, CAT and GPx levels were reduced in rats with fructose-induced metabolic syndrome [12,42,43]. However, contradictory results have demonstrated the upregulation of catalase in hepatic tissues [44]. Besides, the enzymes in the liver or kidney tissues were also reported to be unchanged in similar animal models [45]. Recently, we demonstrated significant suppression of both CAT, GPx and SOD gene and protein expression in the renal tissues of diabetic rats [46]. The suppression was attributed to a moderate increase in tissue oxidative biomarkers. In this study, we demonstrated alterations of oxidative stress markers in plasma and reduction in renal SOD1 and SOD2 gene and protein expressions. These results indicate the presence of oxidative stress in fructose-fed rats. On the contrary, both CAT gene and protein expression were upregulated in renal tissue of fructose-fed rats. Such discrepancy might result from differences in intracellular locations since most of the CAT is present in peroxisomes which could prevent it from participating in primary oxidation reactions arising from excessive mitochondrial input through the electron transport systems [47]. Upregulation of CAT could be seen as an adaptation process to the moderate increase in oxidative stress biomarkers in peroxisomes.

Recent studies proposed that probiotics can stimulate the antioxidant systems of the host and augment the activities of antioxidant enzymes [20]. Supplementation with probiotics and vitamin C increased the activity of antioxidant enzymes in the serum, brain and kidneys in type 1 diabetic rats [48]. Administration of *L. casei* with inulin to healthy volunteers resulted in a significant increase in plasma CAT activity [49] and in a significant decrease in plasma MDA and oxidized glutathione concentrations and increased free thiol and glutathione contents [50]. Moreover, probiotics provided significant protection against mercury-induced toxicity by preventing alterations in the levels of GPx and SOD [51]. In another study, upregulation of serum SOD, GPx and hepatic CAT activities with dietary *L. fermentum* was demonstrated [52]. Moreover, yeast probiotic supplementation increased the body weights and serum antioxidant enzyme activities in chicks [53]. In line with these findings, a study conducted with humans demonstrated increased SOD and GPx activities in erythrocytes of diabetic patients supplemented with *L. acidophilus* and *Bifidobacterium lactis* [54]. In our study, treatment with *L. helveticus* and *L. plantarum* strains did not affect the gene expression patterns of *sod1* and *cat*, however, *L. plantarum* upregulated *sod2*. In contrast to gene expression levels, the changes in protein expression levels of these enzymes were normalized with both probiotics. These results suggested that *L. helveticus* and *L. plantarum* might have a positive influence on renal antioxidant capacity in the dietary fructose-induced metabolic disorder.

In conclusion, supplementation of *L. helveticus* and *L. plantarum* has an improving effect on specific metabolic parameters and renal antioxidative enzymes in a fructose-induced metabolic disorder.

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REFERENCES

1. Prasad K, Dhar I. Oxidative stress as a mechanism of added sugar-induced cardiovascular disease. Int J Angiol. 2014;23:217-26.
2. Hannou SA, Haslam DE, McKeown NM, Herman MA. Fructose metabolism and metabolic disease. J Clin Invest. 2018;128:545-55.
3. Akar F, Uludağ O, Aydın A, Aytekin YA, Elbeg S, Tuzcu M, Sahin K. High-fructose corn syrup causes vascular dysfunction associated with metabolic disturbance in rats: protective effect of resveratrol. Food Chem Toxicol. 2012;50:2135-41.
4. Babacanoglu C, Yildirim N, Sadi G, Pektas MB, Akar F. Resveratrol prevents high-fructose corn syrup-induced vascular insulin resistance and dysfunction in rats. Food Chem Toxicol. 2013;60:160-7.
5. Sadi G, Ergin V, Yilmaz G, Pektas MB, Yildirim OG, Menevse A, Akar F. High-fructose corn syrup-induced hepatic dysfunction in rats: improving effect of resveratrol. Eur J Nutr. 2015;54(6):895-904.
6. Pektas MB, Yücel G, Koca H, Sadi G, Yıldırım O, Öztürk G, Akar F. Dietary fructose-induced hepatic injury in male and female rats: influence of resveratrol. Drug Res. (Stuttg). 2017;67:103-10.
7. Pektas MB, Koca HB, Sadi G, Akar F. Dietary fructose activates insulin signaling and inflammation in adipose tissue: modulatory role of resveratrol. Biomed Res Int. 2016;2016:8014252.
8. Pektas MB, Sadi G, Akar F.Long-term dietary fructose causes gender-different metabolic and vascular dysfunction in rats: modulatory effects of resveratrol. Cell Physiol Biochem. 2015;37:1407-20.
9. Yildirim OG, Sumlu E, Aslan E, Koca HB, Pektas MB, Sadi G, Akar F. High-fructose in drinking water initiates activation of inflammatory cytokines and testicular degeneration in rat. Toxicol Mech Methods. 2018;DOI: 10.1080/15376516.2018.1543745.
10. Prince PD, Lanzi CR, Toblli JE, Elesgaray R, Oteiza PI, Fraga CG, Galleano M. Dietary (–)-epicatechin mitigates oxidative stress, NO metabolism alterations, and inflammation in renal cortex from fructose-fed rats. Free Radic Biol Med. 2016;90:35-46.
11. Palanisamy N, Viswanathan P, Anuradha CV. Effect of Genistein, a soy isoflavone, on whole body insulin sensitivity and renal damage induced by a high-fructose diet. Ren Fail. 2008;30:645-54.
12. Nasri R, Abdelhedi O, Jemil I, Daoued I, Hamden K, Kallel C, Elfeki A, Lamri-Senhadji M, Boualga A, Nasri M, Karra-Châabouni M. Ameliorating effects of goby fish protein hydrolysates on high-fat-high-fructose diet-induced hyperglycemia, oxidative stress and deterioration of kidney function in rats. Chem Biol Interact. 2015;242:71-80.
13. Wang W, Ding X-Q, Gu T-T, Song L, Li J-M, Xue Q-C, Kong L-D. Pterostilbene and allopurinol reduce fructose-induced podocyte oxidative stress and inflammation via microRNA-377. Free Radic Biol Med. 2015;83:214-26.
14. Qiao Y, Xu L, Tao X, Yin L, QiY, Xu Y, Han X, Tang Z, Ma X, Liu K, Peng J. Protective effects of dioscin against fructose-induced renal damage via adjusting Sirt3-mediated oxidative stress, fibrosis, lipid metabolism and inflammation. Toxicol Lett. 2018;284:37-45.
15. Chaudhary K, Malhotra K, Sowers J, Aroor A. Uric Acid - key ingredient in the recipe for cardiorenal metabolic syndrome. Cardiorenal Med. 2013;3:208-20.
16. Johnson RJ, Nakagawa T, Sanchez-Lozada LG, Shafiu M, Sundaram S, Le M, Ishimoto T, Sautin YY, Lanaspa MA. Sugar, uric acid, and the etiology of diabetes and obesity. Diabetes. 2013;62:3307-15.
17. Wang J, Tang H, Zhang C, Zhao Y, Derrien M, Rocher E, van-Hylckama Vlieg JE, Strissel K, Zhao L, Obin M, Shen J. Modulation of gut microbiota during probiotic-mediated attenuation of metabolic syndrome in high fat diet-fed mice. ISME J. 2015;9:1-15.
18. Rad AH, Abbasalizadeh S, Vazifekhah S, Abbasalizadeh F, Hassanalilou T, Bastani P, Ejtahed H-S, Soroush A-R, Javadi M, Mortazavian AM, Khalili L. The future of diabetes management by healthy probiotic microorganisms. Curr Diabetes Rev. 2017;13:582-9.
19. Plaza-Díaz J, Ruiz-Ojeda FJ, Vilchez-Padial LM, Gil A. Evidence of the anti-inflammatory effects of probiotics and synbiotics in intestinal chronic diseases. Nutrients. 2017;9(6):555.
20. Wang Y, Wu Y, Wang Y, XuH, Mei X, Yu D, Wang Y, Li W. Antioxidant properties of probiotic bacteria. Nutrients. 2017;9(5):521.
21. Zielińska D, Kołożyn-Krajewska D. Food-origin lactic acid bacteria may exhibit probiotic properties: review. Biomed Res Int. 2018;2018:5063185.
22. Huang H-Y, Korivi M, Tsai C-H, Yang J-H, Tsai Y-C. Supplementation of Lactobacillus plantarum K68 and fruit-vegetable ferment along with high fat-fructose diet attenuates metabolic syndrome in rats with

insulin resistance. Evid Based Complement Alternat Med. 2013;2013:943020.
23. Park D-Y, Ahn Y-T, Huh C-S, McGregor RA, Choi M-S. Dual probiotic strains suppress high fructose-induced metabolic syndrome. World J Gastroenterol. 2013;19(2):274-83.
24. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193:265-75.
25. Zhang D-M, Jiao R-Q, Kong L-D. High dietary fructose: direct or indirect dangerous factors disturbing tissue and organ functions. Nutrients. 2017;9(4):335.
26. Della Corte KW, Perrar I, Penczynski KJ, Schwingshackl L, Herder C, Buyken AE. Effect of dietary sugar intake on biomarkers of subclinical inflammation: a systematic review and meta-analysis of intervention studies. Nutrients. 2018;10(5):606.
27. Andersson U, Bränning C, Ahrné S, Molin G, Alenfall J, Önning G, Nyman M, Holm C. Probiotics lower plasma glucose in the high-fat fed C57BL/6J mouse. Benef Microbes. 2010;1:189-96.
28. Choi I-D, Kim S-H, Jeong J-W, Lee DE, Huh C-S, Hong SS, Sim J-H, Ahn Y-T. Triglyceride-lowering effects of two probiotics, Lactobacillus plantarum KY1032 and Lactobacillus curvatus HY7601, in a rat model of high-fat diet-induced hypertriglyceridemia. J Microbiol Biotechnol. 2016;26:483-7.
29. Martinic A, Barouei J, Bendiks Z, Mishchuk D, Heeney DD, Martin R, Marco ML, Slupsky CM. Supplementation of Lactobacillus plantarum improves markers of metabolic dysfunction induced by a high fat diet. J Proteome Res. 2018;17:2790-802.
30. Yakovlieva M, Tacheva T, Mihaylova S, Tropcheva R, Trifonova K, Tolєkova A, Danova S, Vlaykova T. Influence of Lactobacillus brevis 15 and Lactobacillus plantarum 13 on blood glucose and body weight in rats after high-fructose diet. Benef Microbes. 2015;6:505-12.
31. Ahn HY, Kim M, Chae JS, Ahn Y-T, Sim J-H, Choi I-D, Lee S-H, Lee JH. Supplementation with two probiotic strains, Lactobacillus curvatus HY7601 and Lactobacillus plantarum KY1032, reduces fasting triglycerides and enhances apolipoprotein A-V levels in non-diabetic subjects with hypertriglyceridemia. Atherosclerosis. 2015;241:649-56.
32. Zubiría MG, Gambaro SE, Rey MA, Carasi P, Serradell M de L.Á, Giovambattista A. Deleterious metabolic effects of high fructose intake: the preventive effect of Lactobacillus kefiri administration. Nutrients. 2017;9:470.
33. Dabla PK. Renal function in diabetic nephropathy. World J Diabetes. 2010;1:48-56.
34. Aoyama M, Isshiki K, Kume S, Chin-Kanasaki M, Araki H, Araki S-I, Koya D, Haneda M, Kashiwagi A, Maegawa H, Uzu T. Fructose induces tubulointerstitial injury in the kidney of mice. Biochem Biophy Res Commun. 2012;419:244-9.
35. Sasikumar P, Gomathi S, Anbazhagan K, Abhishek A, Paul E, Vasudevan V, Sasikumar S, Selvam GS. Recombinant Lactobacillus plantarum expressing and secreting heterologous oxalate decarboxylase prevents renal calcium oxalate stone deposition in experimental rats. J Biomed Sci. 2014;21:86.
36. Cioffi F, Senese R, Lasala P, Ziello A, Mazzoli A, Crescenzo R, Liverini G, Lanni A, Goglia F, Iossa S. Fructose-rich diet affects mitochondrial DNA damage and repair in rats. Nutrients. 2017;9(4)323.
37. Girard A, Madani S, Boukortt F, Cherkaoui-Malki M, Belleville J, Prost J. Fructose-enriched diet modifies antioxidant status and lipid metabolism in spontaneously hypertensive rats. Nutrition. 2006;22:758-66.
38. Francini F, Castro MC, Schinella G, García ME, Maiztegui B, Raschia MA, Gagliardino JJ, Massa ML. Changes induced by a fructose-rich diet on hepatic metabolism and the antioxidant system. Life Sci. 2010;86:965-71.
39. Taleb-Dida N, Krouf D, Bouchenak M. Globularia alypum aqueous extract decreases hypertriglyceridemia and ameliorates oxidative status of the muscle, kidney, and heart in rats fed a high-fructose diet. Nutr Res. 2011;31:488-95.
40. Ali Hussei S, M Abd El-O, S Hemdan H. Protective effect of L-carnitine on metabolic disorders, oxidative stress, antioxidant status and inflammation in a rat model of insulin resistance. Int J Biol Chem. 2014;8:21-36.
41. Abdel-Kawi SH, Hassanin KMA, Hashem KS. The effect of high dietary fructose on the kidney of adult albino rats and the role of curcumin supplementation: A biochemical and histological study. Beni-Suef Univ J Basic Appl Sci. 2016;5:52-60.
42. Rajasekar P, Viswanathan P, Anuradha CV. Renoprotective action of L-carnitine in fructose-induced metabolic syndrome. Diabetes Obes Metab. 2008;10:171-80.
43. Sivakumar AS, Viswanathan P, Anuradha CV. Dose-dependent effect of galangin on fructose-mediated insulin resistance and oxidative events in rat kidney. Redox Rep. 2010;15:224-32.
44. Nogales F, Ojeda ML, del Valle PM, Serrano A, Murillo ML, Carreras Sánchez, O. Metabolic syndrome and selenium during gestation and lactation. Eur J Nutr. 2017;56:819-30.
45. Demirtas CY, Pasaoglu OT, Bircan FS, Kantar S, Turkozkan N. The investigation of melatonin effect on liver antioxidant and oxidant levels in fructose-mediated metabolic syndrome model. Eur Rev Med Pharmacol Sci. 2015;19:1915-21.

46. Sadi G, Şahin G, Bostancı A. Modulation of renal insulin signaling pathway and antioxidant enzymes with streptozotocin-induced diabetes: effects of resveratrol. Medicina (B. Aires). 2019;55(1):3.
47. Sadi G, Sadi Ö.Antioxidants and regulation of antioxidant enzymes by cellular redox status. Turkish J Sci Rev. 2010;3:95-107.
48. Aluwong T, Ayo J, Kpukple A, Oladipo O. Amelioration of hyperglycaemia, oxidative stress and dyslipidaemia in alloxan-induced diabetic wistar rats treated with probiotic and vitamin C. Nutrients. 2016;8(5):151.
49. Kleniewska P, Hoffmann A, Pniewska E, Pawliczak R. The influence of probiotic Lactobacillus casei in combination with prebiotic inulin on the antioxidant capacity of human plasma. Oxid Med Cell Longev. 2016;2016:1340903.
50. Kleniewska P, Pawliczak R. Influence of synbiotics on selected oxidative stress parameters. Oxid Med Cell Longev. 2017;2017:9315375.
51. Majlesi M, Shekarforoush SS, Ghaisari HR, Nazifi S, Sajedianfard J, Eskandari MH. Effect of probiotic Bacillus coagulans and Lactobacillus plantarum on alleviation of mercury toxicity in rat. Probiotics Antimicrob Proteins. 2017;9:300-9.
52. Wang AN, Yi XW, Yu HF, Dong B, Qiao SY. Free radical scavenging activity of Lactobacillus fermentum in vitro and its antioxidative effect on growing-finishing pigs. J Appl Microbiol. 2009;107:1140-8.
53. Aluwong T, Kawu M, Raji M, Dzenda T, Govwang F, Sinkalu V, Ayo J. Effect of yeast probiotic on growth, antioxidant enzyme activities and malondialdehyde concentration of broiler chickens. Antioxidants (Basel). 2013;2(4):326-39.
54. Ejtahed HS, Mohtadi-Nia J, Homayouni-Rad A, Niafar M, Asghari-Jafarabadi M, Mofid V. Probiotic yogurt improves antioxidant status in type 2 diabetic patients. Nutrition. 2012;28(5):539-43.

Table 1. The effects of dietary fructose, *L. helveticus* and *L. plantarum* supplementation on body weight, kidney weight, food, liquid and caloric intake.

Groups	**Control**	**Fruc**	**Fruc+LH**	**Fruc+LP**
Initial body weight (g)	90.7±5.2	92.1±3.7	93.1±3.4	92.3±1.8
Final body weight (g)	354.6±5.2	359.3±12.7	373.4±17.7	354.2±19.2
Food intake (g/day)	21.4±0.2	14.7±0.4*	15.6±1.6	16.9±1.5
Liquid intake (mL/day)	48.3±2	40.1±2.8*	37.5±1.2	38.1±0.7
Total caloric intake (kcal)	74.9±3.1	84.3±2.1*	85.4±2.1	90.4±4.2
Left kidney absolute weight (g)	1.5±0.1	1.4±0.05	1.5±0.1	1.4±0.1
Ratio of left kidney weight to body weight (%)	0.4±0.01	0.4±0.02	0.4±0.01	0.4±0.01
Right kidney absolute weight (g)	1.5±0.1	1.4±0.05	1.5±0.1	1.4±0.1
Ratio of right kidney weight to body weight (%)	0.4±0.01	0.4±0.02	0.4±0.01	0.4±0.01

Values are expressed as the mean±SEM, n = 6-8; *P<0.05, significantly different from the control.

Table 2. The effects of dietary fructose, *L. helveticus* and *L. plantarum* supplementation on metabolic parameters in the plasma and kidney tissue.

Groups	**Control**	**Fruc**	**Fruc+LH**	**Fruc+LP**
Glucose (mmol/L)	6.1±0.3	8.5±0.3*	8.7±0.9	8.2±0.5
Insulin (pmol/L)	2.77±0.7	5.9±1.4*	5.5±1.6	3.62±0.5$^{\#}$
Triglyceride (mmol/L)	1.39±0.1	3.75±0.1*	2.11±0.1$^{\#}$	1.73±0.1$^{\#}$
VLDL (mmol/L)	0.58±0.05	1.63±0.04*	0.97±0.03 $^{\#}$	0.79±0.02 $^{\#}$
HDL (mmol/L)	0.61±0.03	0.61±0.05	0.66±0.04	0.76±0.03 $^{\#}$
Total Cholesterol (mmol/L)	1.14±0.1	1.42±0.1	1.29±0.1	1.53±0.1
MDA (µmol/L)	21.5±1.1	27.1±2*	29.1±3.2	23.6±0.9
Creatinine (µmol/L)	15±1.8	21.2±2.7*	14.1±2.6$^{\#}$	15±2.7$^{\#}$
Urea (mmol/L)	11.6±0.9	12.6±1	10.9±0.4	11.5±0.7
Uric Acid (µmol/L)	142.7±11.9	127.9±11.8	107.1±5.9$^{\#}$	136.8±11.9
Albumin (g/L)	44±3	44±3	44±4	42±4
Total Protein (g/L)	74±1	79±1	79±2	81±1
Renal MDA (µmol/g protein)	1.47±0.1	1.6±0.2	1.32±0.2	1.27±0.2
Renal Urea (mg/g protein)	14.7±1.2	21.6±1.3*	16.3±2.1$^{\#}$	14.1±1.2$^{\#}$
Renal Uric Acid (mg/g protein)	8.6±0.6	10.2±0.9	8.8±0.6	7.2±0.8$^{\#}$

Values are expressed as the mean±SEM, n = 6-8; *P<0.05, significantly different from the control; $^{\#}P$<0.05, significantly different from the fructose-treated rats.

Figure Legends

Fig. 1. Changes in expression levels of *sod1* (**A**), *sod2* (B), *cat* (**C**) and *gpx* (**D**) mRNAs in kidney tissues of Control, Fruc, Fruc+LH, and Fruc+LP 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 fructose. Fruc – Fructose, LH – *L. helveticus*, LP – *L. plantarum.*

Fig. 2. Changes in protein expression of the antioxidant enzymes in kidney tissues of Control, Fruc, Fruc+LH, and Fruc+LP groups. SOD1 (**A**), SOD2 (**B**), CAT (**C**) and GPx (**D**) protein levels were quantified using densitometry and normalized with GAPDH. Representative Western blot images are included above the corresponding figures. Each bar represents at least six rats. *P<0.05, significantly different from control; $^{\#}P$<0.05, significantly different from fructose. Fruc – Fructose, LH – *L. helveticus*, LP – *L. plantarum.*

Figure 1.

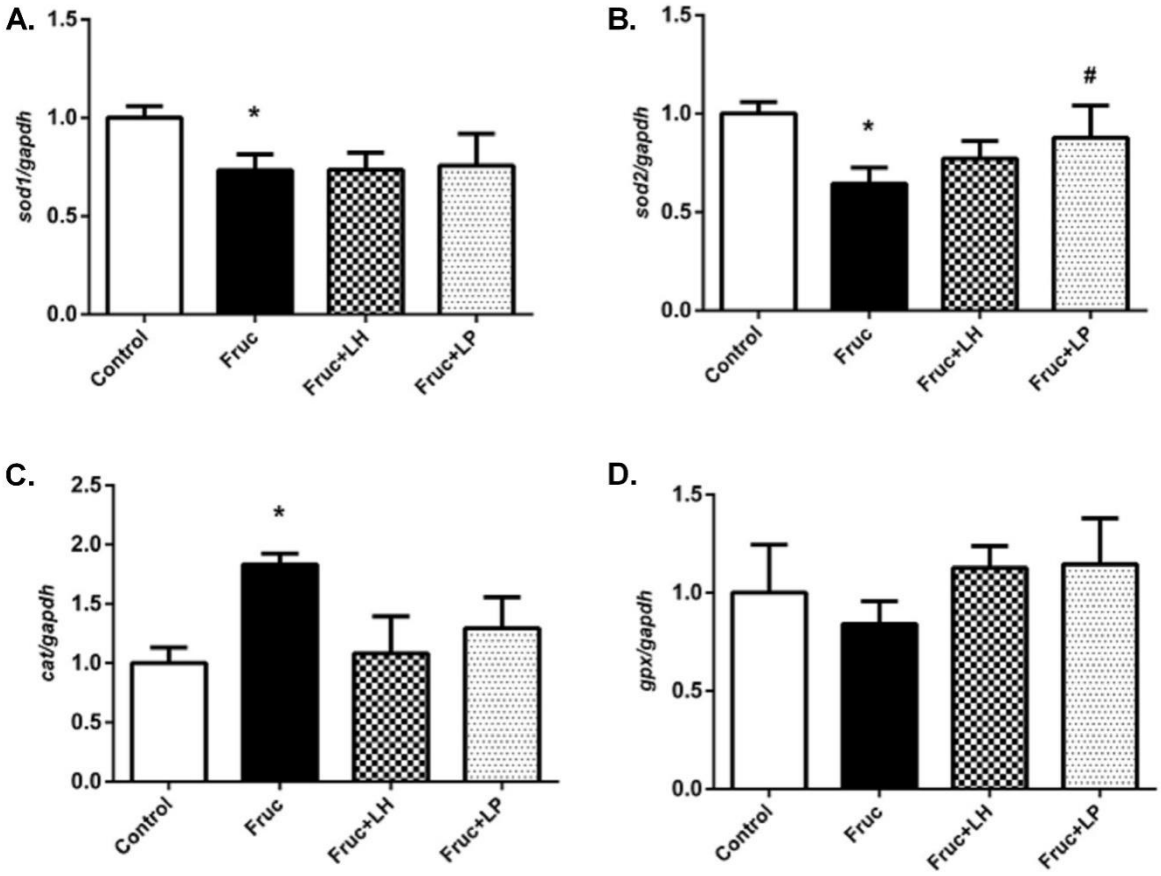
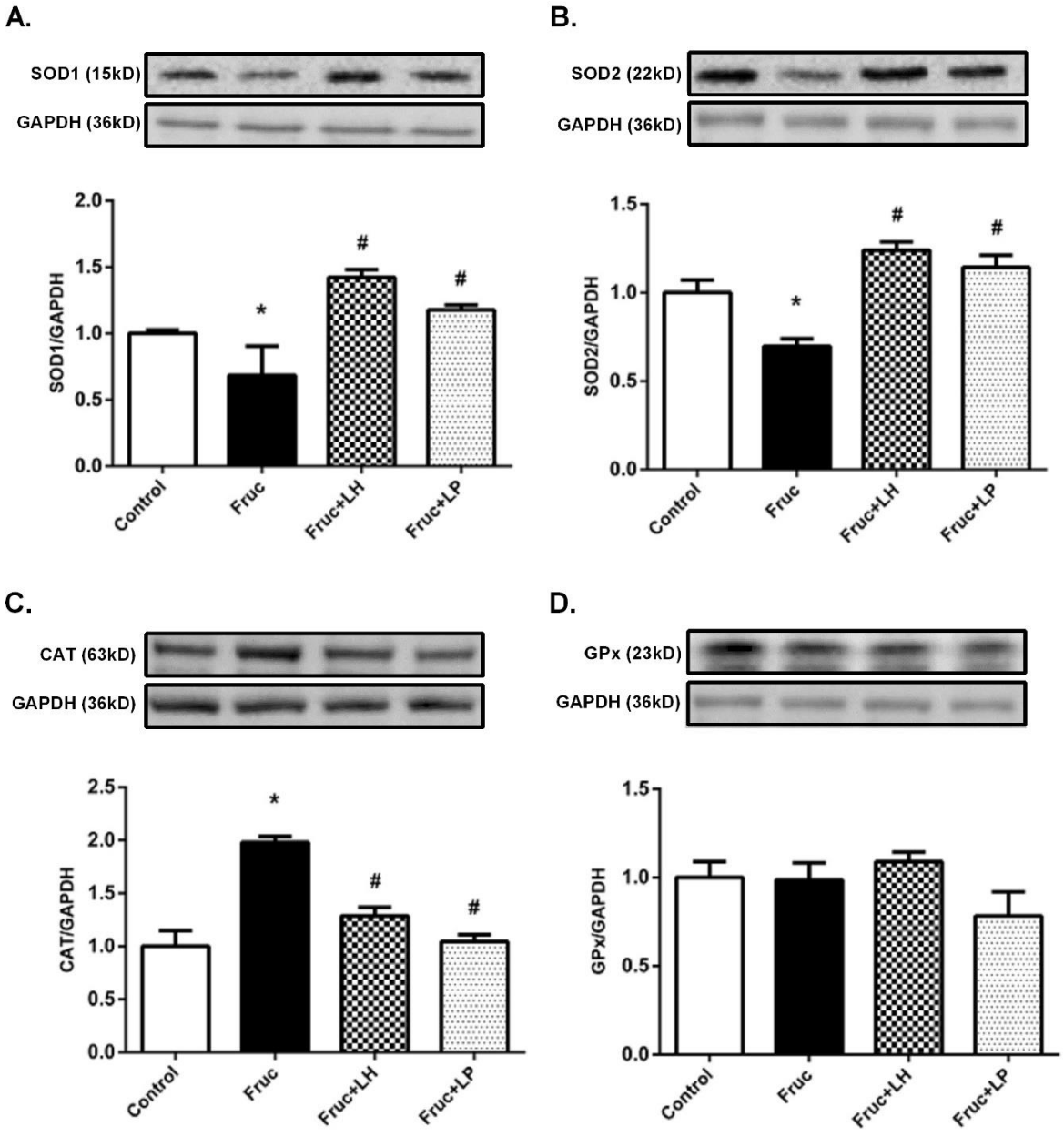

A.
sod1/gapdh
1.5
1.0
0.5
0.0
*
Control
Fruc
Fruc+LH
Fruc+LP
B.
sod2/gapdh
1.5
1.0
0.5
0.0
*
#
Control
Fruc
Fruc+LH
Fruc+LP
C.
cat/gapdh
2.5
2.0
1.5
1.0
0.5
0.0
*
Control
Fruc
Fruc+LH
Fruc+LP
D.
gpx/gapdh
1.5
1.0
0.5
0.0
Control
Fruc
Fruc+LH
Fruc+LP

Figure 2.


A.
SOD1 (15kD)
GAPDH (36kD)
SOD1/GAPDH
2.0
1.5
1.0
0.5
0.0
*
#
#
Control
Fruc
Fruc+LH
Fruc+LP
B.
SOD2 (22kD)
GAPDH (36kD)
SOD2/GAPDH
1.5
1.0
0.5
0.0
*
#
#
Control
Fruc
Fruc+LH
Fruc+LP
C.
CAT (63kD)
GAPDH (36kD)
CAT/GAPDH
2.5
2.0
1.5
1.0
0.5
0.0
*
#
#
Control
Fruc
Fruc+LH
Fruc+LP
D.
GPx (23kD)
GAPDH (36kD)
GPx/GAPDH
1.5
1.0
0.5
0.0
Control
Fruc
Fruc+LH
Fruc+LP