doi: 10.4149/gpb_2024003

Quercetin decreases fructose drinking in model of fructose-induced insulin resistance. Unexpected uric acid and TBARS lowering effect of methyl cellulose vehicle and fructose combination

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Abstract. The aim of this study was to improve insulin sensitivity in fructose-treated animals by ingestion of flavonoid quercetin. Several signs of insulin resistance have been developed in rats by drinking 10% fructose solution for 9 weeks. The effect of 6-week-gavage-administrated quercetin (20 mg/kg/day in 1% methyl cellulose solution) was monitored. Rats of the control groups received methyl cellulose vehicle as well. The most striking result of the quercetin treatment was the normalization of the fructose solution drinking to the level of drinking water intake. In addition, quercetin supplementation considerably decreased the plasma glucose and Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) index in rats consuming fructose. Surprisingly, fructose ingestion did not elevate plasma uric acid, thiobarbituric acid reactive substances, nitrotyrosine, or advanced glycation end products fluorescence. Instead, a reduction of the above parameters was observed. In summary, these results indicate that quercetin supplementation reduces fructose drinking and decreases plasma glucose and the HOMA-IR index. Furthermore, methyl cellulose, in combination with fructose, causes uric acid – lowering, antioxidant and anti-glycation effects. Thus, methyl cellulose possibly shifts fructose metabolism in favor of the utilization of antioxidant features of fructose. Our results call for using methyl cellulose in sweetened beverages and other sweetened food.

Key words: Quercetin — Fructose-rich diet — Methyl cellulose — Uric acid — TBARS

Introduction

Excessive fructose consumption is linked with metabolic changes and severe health problems (Tappy 2018). Available data suggest that consuming sugar-sweetened beverages and other fructose-sweetened food products is associated with body-weight gain, obesity, inflammation, and cardiometabolic disturbances (Caliceti et al. 2017; Zhang et al. 2017; Andres-Hernando et al. 2020; Wang et al. 2020; Chan et al. 2021; Muriel et al. 2021). In addition, high fructose intake increases oxidation stress (Hokayem et al. 2013; Muriel et al. 2021; Chenni et al. 2022). The majority of fructose is metabolized in the liver (Muriel et al. 2021), and an excess of fructose is converted to fat. Indeed, fructose consumption increases blood triacylglycerols (TAG) (Chan et al. 2021). The TAGs are taken up by adipose tissue for storage – consequently, the

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fructose-fed rats display augmentation of adipose tissue mass (Chan et al. 2021). Besides TAG and very-low-density lipoprotein (VLDL) production, fructose can stimulate adipogenesis by glucocorticoid activation, reactive oxygen species (ROS) generation, renin-angiotensin (RAS) activation, and inhibition of thermogenesis (Hernández-Díazcouder et al. 2019).

Another significant feature of fructose-fed animals and humans is elevated blood uric acid (UA) (Caliceti et al. 2017; Chan et al. 2021). UA originates from ATP breakdown through ADP and AMP to inosine monophosphate, which is further metabolized to xanthine and hypoxanthine, the substrates of xanthine oxidase (Caliceti et al. 2017). The ATP is needed for the initial phosphorylation of fructose by fructokinase, an enzyme lacking feedback regulation, unlike glucose phosphorylation by hexokinase (Hernández-Díazcouder et al. 2019). Thus, the fructose is phosphorylated until ATP exhaustion leading to the enormous production of intracellular and extracellular UA. Hyperuricemia is considered as an indicator of metabolic syndrome, chronic renal disease, cardiovascular disease, and diabetes (Zhang et al. 2020). Hyperuricemia may increase the likelihood of gout. Moreover, it seems that fructose and UA have a beneficial effect on cancer cell growth (Nakagawa et al. 2020; Fini et al. 2021). This fact deserves special attention because cancer cells have an increased expression of the fructose transporter GLUT 5, and fructose can also be formed endogenously from glucose *via* sorbitol employing the so-called polyol pathway (Andres-Hernando et al. 2019; Krause and Wegner 2020).

The UA-producing purine degradation is accompanied by mitochondrial oxidative stress with stimulation of ATP citrate lyase and fatty acid synthetase (Caliceti et al. 2017). Moreover, fructose stimulates NADPH oxidase (NOX). Collectively, high fructose intake causes an increase in oxidative stress leading by such a way to the development of insulin resistance, endothelial cell dysfunction, atherosclerosis, vascular calcification, impaired myocardial energetics, and stimulating the production of pro-inflammatory interleukins and tumor necrosis factor alpha (Caliceti et al. 2017). Accordingly, in rats and humans, fructose consumption results in thiobarbituric acid reactive substances (TBARS) and carbonyl elevation (Hokayem et al. 2013; Chenni et al. 2022). This is accompanied by an increase in lipid hydroperoxide and F2-isoprostane and a decrease in NO.

Quercetin is one of the most commonly occurring bioactive flavonoids in human foods of plant origin (Dobrocsyova et al. 2019). It is present in tea, apples, broccoli, onion berries, and red grapes (Yao et al. 2004). Quercetin is known for its beneficial health effects, mainly anti-inflammatory and antioxidant properties (Li et al. 2013; Vazquez Prieto et al. 2015). Recently, it was confirmed that quercetin could alleviate hepatic fat accumulation resulting in improved dyslipidemia, hypertension, and hyperinsulinemia (Bischoff 2008; Rivera et al. 2008; Kim et al. 2011; Panchal et al. 2012). The effect of quercetin on adipocyte metabolism *in vitro* seems to be concentration dependent: with stimulation of pro-adipogenic genes at 2 μM and inhibition at higher concentrations (Dobrocsyova et al. 2019). *In vivo*, relatively high concentrations of quercetin (50 and 100 mg/kg body weight/day) were used when model 4 weeks 10% fructose in drinking water plus additional 4 weeks combining fructose drinking and quercetin treatment was employed (Hu et al. 2009, 2012; Li et al. 2013). In these experiments, quercetin attenuated fructose-induced hyperuricemia and renal dysfunction and preserved beta cell mass. On the other hand, much lower concentrations of quercetin (2, 10, and 20 mg/kg/day) were effective in ameliorating metabolic syndrome, inflammatory status, hypertension, and vascular function of obese Zucker rats (Rivera et al. 2008; Ferenczyova et al. 2020).

In the presented study, rats with fully developed fructose-induced insulin resistance (9 weeks of 10% fructose treatment) were prepared first and then continuously treated with the combination of fructose and 20 mg/kg/day quercetin for additional 6 weeks. It is worth mentioning that the quercetin was delivered by gavage as solubilized in 1% methyl cellulose.

As mentioned above, quercetin was administered to animals with already developed metabolic syndrome, so we investigated whether the antioxidant quercetin can at least partially improve the developed metabolic disorders. An additional novelty of our study is the monitoring of liquid intake in the fructose model of insulin resistance, which has not been done yet. Last but not least, we also highlight that all experimental groups received methyl cellulose as a vehicle. This experimental design also allowed us to investigate the co-administration of methyl cellulose with fructose which has never been studied before.

Materials and Methods

Animals and treatments

All animal study procedures and protocols were conducted in accordance with the EU Directive (2010/63/EU) on the protection of animals used for experimental and other scientific purposes and were approved by the official Vinča Institute's Ethical Committee for Experimental Animals (323-07-07800/2020-05).

Wistar rats (36 males), 21-day-old, were separated from their mothers and placed three *per* cage, avoiding animals from one litter in the same cage. The rats were initially divided into two groups, 18 rats each, one drank water and the other drank 10% fructose solution. After 9 weeks of such a regime, both groups were divided into two subgroups, each containing 9 rats, as listed below:

C: control-standard food and tap water throughout both periods of the experiment (9+6 weeks), and administra-

tion of vehicle – 1% methyl cellulose, by gavage for the last 6 weeks.

Q: quercetin-treated-standard food and tap water throughout both periods of the experiment (9+6 weeks), and administration of quercetin in 1% methyl cellulose by gavage for the last 6 weeks.

F: standard food and 10% fructose in tap water throughout both periods of the experiment (9+6 weeks), and administration of vehicle – 1% methyl cellulose, by gavage for the last 6 weeks.

FQ: standard food and 10% fructose in tap water throughout both periods of the experiment (9+6 weeks), and administration of quercetin in 1% methyl cellulose by gavage for the last 6 weeks.

A schematic illustration of the experimental design is shown in Figure 1.

A daily dose of quercetin (20 mg/kg) was dissolved just before administration in 1% methyl cellulose. It is essentially insoluble in methyl cellulose aqueous solution, so a homogeneous suspension was obtained by stirring on a magnetic stirrer for 5 minutes and was kept homogeneously suspended until application. The application of the methyl cellulose and quercetin was performed by the gavage technique using metal probes (7.5–16 cm long, depending on the animal's size). Administered dose of quercetin (20 mg/kg/day) was chosen based on literary data (Rivera et al. 2008) and our preliminary data. In the preliminary experiment, the dose of 20 mg of quercetin/kg/day taken for 6 weeks significantly decreased the size of adipocytes in subcutaneous adipose tissue of obese diabetic Zucker rats.

The animals were kept under standard conditions (light regime, room temperature, and air humidity). At the end of the treatment period, animals were sacrificed by decapitation without anesthesia, which could affect the measured parameters. The animals were fasted the night before sacrifice to measure biochemical parameters. Blood samples were collected in commercially available heparinized tubes and plasma was isolated by centrifugation of uncoagulated blood for 10 min at 3000 rpm in a clinical centrifuge.

Food and liquid intake and body weight gain

Body weight was measured weekly, and fluid and food intake were recorded daily. Body weight gain was calculated as the difference between the rat body weight at the end of the experiment and the body weight at the beginning of the treatment.

Glucose and insulin measurement, HOMA-IR index calculation, and advanced glycation end products (AGEs)

Glucose concentration in plasma was measured by using Vitros 250 autoanalyzer (Johnson&Johnson, Rochester, NY, USA). Insulin concentrations were measured in a commercial laboratory (Institute for the application of nuclear energy, Zemun, Serbia). The RIA human insulin kit combined with rat insulin standards was used. The insulin resistance was estimated by the Homeostasis model assessment of insulin resistance (HOMA-IR) index, which was calculated as: fasting insulin (mIU/l) × fasting glucose (mmol/l)/22.5. AGEs fluorescence (excitation 360 nm/emission 450 nm) was measured according to Almenglo et al. (2022) in duplicates of 100 ul plasma in 96 flat bottom solid black polystyrene microplates (Greiner BIO-ONE International GmbH, Kremsmunster, Austria). The results were expressed in arbitrary units (AU/mg protein). AU represents the fluorescence at 360/450 nm.

Plasma lipid profile

Triglyceride and total cholesterol concentration were obtained by using Vitros 250 autoanalyzer. Determination of high-density lipoprotein-cholesterol (HDL-C) is done by the method of spectrophotometry at the Biosystems A25 apparatus. The manufacturer of the reagent is also Biosystems (Spain).

Uric acid, TBARS, and 3-nitrotyrosine (3-NT)

UA concentrations were obtained by using Vitros 250 autoanalyzer. TBARS content in plasma was determined by using a Lipid peroxidation (MDA) assay kit (ab118970, Cambridge,

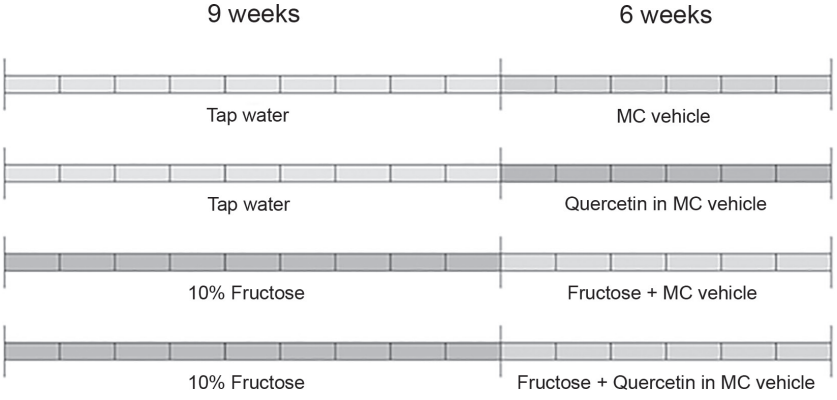

Figure 1. Illustration of experimental design. MC, methyl cellulose.

UK). Oxidative damage of plasma proteins was studied by assessing 3-NT modification. 3-NT content was measured using a Nitrotyrosine ELISA kit (ab 113848, Cambridge, UK).

Total proteins and albumin

Total proteins and albumin concentrations were obtained by using Vitros 250 autoanalyzer.

Statistical analysis

All data are presented as means ± SD (n = 9). Statistical comparisons were performed by two-way ANOVA with a Tukey's test for *post-hoc* comparison using Statistica software (StatSoft Inc., Tulsa, OK, USA). A value of $p < 0.05$ was considered statistically significant.

Two-way ANOVA test is used when the treatment contains two factors, such as in this case fructose and quercetin. As a result of using this test in the first step, information is obtained whether each of the factors individually (fructose and quercetin) influenced the measured parameter and whether there is a significant interaction between the two factors in achieving the effect – denoted as F×Q. Only if there is an interaction between the factors a *post-hoc* test has to be done. Accordingly, asterisk(s) are present only in graphs where interaction e.g. between fructose and quercetin is shown. If there is only a significant effect of individual factors, *post-hoc* test was not performed, but it is considered that the effect is significant regardless of the action of another factor.

The graphs contain the results of multiple comparisons *post-hoc* test for cases where there are interactions between quercetins and fructose.

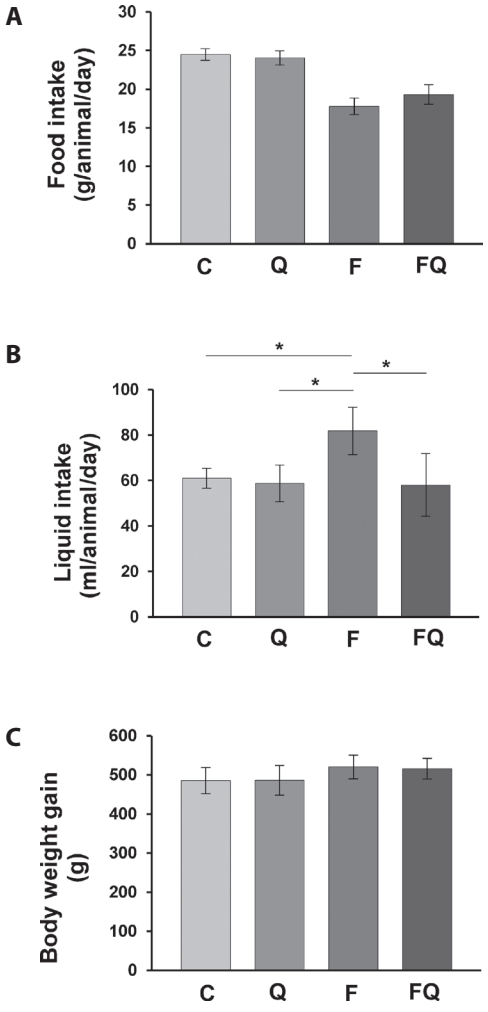

Figure 2. Diet regime and body weight. Food intake (**A**) and liquid intake (**B**) were recorded daily. **C.** Body weight was measured weekly and body weight gain was calculated as the difference between the rat body weight at the end of the experiment and the body weight at the beginning of the treatment. C, control group; Q, quercetin-treated rats; F, fructose diet group; FQ, rats exposed to fructose diet and treated with quercetin. * $p < 0.05$.

Results

Diet regime and body weight

Fructose had a significant effect on food intake ($p < 0.001$) in terms of reduction by 23% and 20% in control rats and those treated with quercetin, respectively (Fig. 2A). On the other hand, when the liquid intake was analyzed (Fig. 2B), the effect of quercetin ($p < 0.05$) and the interaction between fructose and quercetin were observed ($p < 0.05$). Animals that drank fructose ingested significantly more liquid than controls and quercetin-treated rats by 34% and 39%, respectively (F *vs.* C and F *vs.* Q, $p < 0.05$ for both), while rats that underwent combined treatment consumed less fluid by 29% than those only drank fructose (FQ *vs.* F, $p < 0.05$) (Fig. 2B). Regarding body weight gain, the treatments were reflected in such a way that fructose had a significant effect ($p < 0.01$) in terms of a mild increase of 7% in control and 6% in quercetin-treated rats (Fig. 2C).

Glucose homeostasis markers

A diet rich in fructose, unlike quercetin, significantly increased the concentration of insulin in the circulation of rats ($p < 0.01$). The fructose-induced increase was 38% in control and 36% in quercetin-treated rats (Fig. 3A). In the regulation of glucose concentration, there were effects of fructose ($p < 0.05$) and quercetin ($p < 0.001$), and also fructose and quercetin achieved interaction ($p < 0.01$). Rats who drank fructose had elevated glucose levels by 11% in relation to the control group and 17% compared to rats treated with quercetin (F *vs.* C, $p < 0.01$; F *vs.* Q, $p < 0.001$)

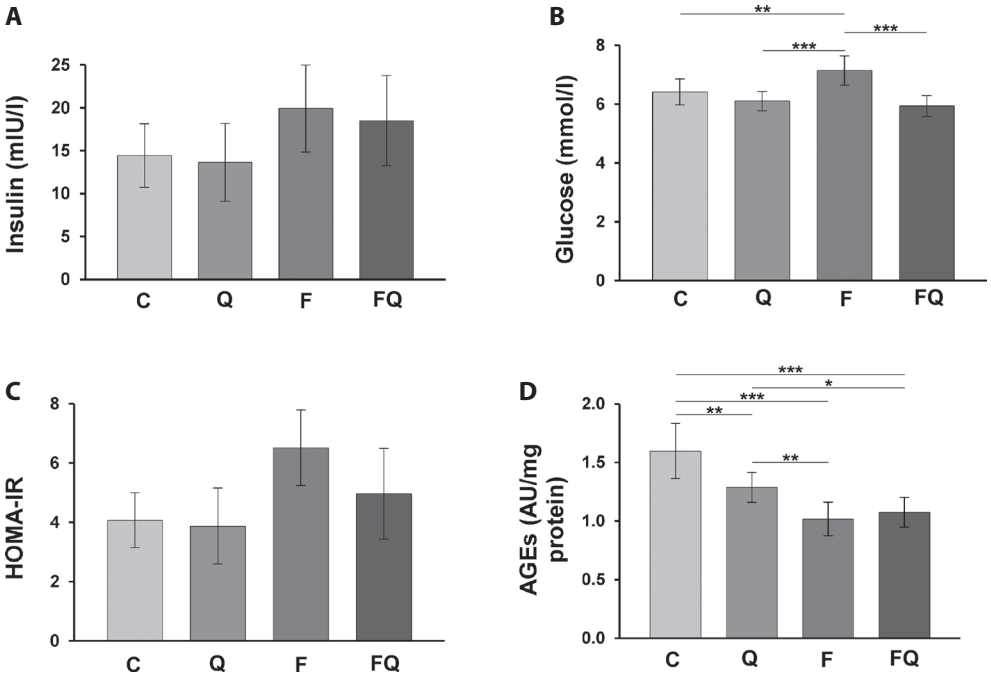

Figure 3. Glucose homeostasis markers: plasma insulin (**A**), glucose (**B**), HOMA-IR index (**C**), and AGEs (**D**). Glucose concentration in plasma was measured by using Vitros 250 autoanalyzer. The RIA method was used to measure insulin. The insulin resistance was estimated by the Homeostasis model assessment – insulin resistance (HOMA-IR) index. AGEs fluorescence was measured according to Almenglo et al. method (Almengló et al. 2022). AGEs, advanced glycation end products. Other abbreviations are explained in Figure 2. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

(Fig. 3B). However, a considerable decrease (17%) in glucose concentration was observed in rats given both fructose and quercetin compared to those consumed only fructose (FQ *vs*. F, $p < 0.001$). Interestingly, when the HOMA-IR index was calculated as a marker of insulin resistance, both factors had a significant effect, fructose diet in terms of increasing ($p < 0.001$) and quercetin in the direction of decreasing HOMA-IR index ($p < 0.05$). The fructose diet elevated HOMA-IR by 60% in control and 28% in quercetin-treated rats. In contrast, quercetin decreased HOMA-IR by 5% and 24% in control and fructose-treated rats, respectively (Fig. 3C). Two-way ANOVA revealed the effects of quercetin ($p < 0.05$) and fructose diet ($p < 0.001$) on AGEs in circulation. Also, the obtained results (Fig. 3D) indicate the interactions of fructose and quercetin in the regulation of AGEs levels ($p < 0.01$). In contrast to starting expectations, rats drinking fructose had reduced levels of AGEs by 36% compared to control animals (F *vs*. C, $p < 0.001$) and by 21% compared to those treated with quercetin (F *vs*. Q, $p < 0.01$). Also, rats exposed to the combined treatment had reduced levels of AGEs by 33% compared to rats from the control group (FQ *vs*. C, $p < 0.001$) and by 17% compared to those from the quercetin-treated group (FQ *vs*. Q, $p < 0.05$).

Blood lipid profile

Concerning plasma lipid profile, a fructose-rich diet significantly elevated TAG level ($p < 0.001$) by 53% in control and by 43% in quercetin-treated rats (Fig. 4A). Quercetin administration raised total cholesterol ($p < 0.001$) by 20% in both control and fructose-consumed rats (Fig. 4B). This increase was associated with an increase in HDL-C levels in both groups of quercetin-treated rats ($p < 0.01$), by 21% in fructose-naive animals and by 16% in fructose-fed animals (Fig. 4C). Neither fructose-rich diet nor quercetin treatment, individually or in combination, had a significant effect on the level of LDL cholesterol (Fig. 4D).

Uric acid and oxidative stress biomarkers

Interactions of fructose with quercetin ($p < 0.01$) were observed in the regulation of UA levels, as well as the effect of fructose ($p < 0.001$). Rats that drank fructose had reduced UA level by 26% in the circulation compared to controls (F *vs*. C, $p < 0.01$) and by 40% compared to those treated with quercetin (F *vs*. Q, $p < 0.001$) (Fig. 5A). Animals subjected to the combined treatment with fructose and quercetin also had lower UA levels compared to control (by 34%) and quercetin-treated rats (by 46%) (FQ *vs*. C and FQ *vs*. Q, $p < 0.001$ for both comparisons). Also, quite surprisingly, animals treated with quercetin had elevated UA level by 23% compared to controls (Q *vs*. C, $p < 0.05$). Completely unexpected, a diet enriched with fructose led to a highly significant decrease in the concentration of malondialdehyde (MDA) in plasma ($p < 0.001$). In both control and quercetin-treated rats, the decrease was 25% (Fig. 5B). The same effect was observed regarding fructose and 3-NT levels ($p < 0.01$). The fructose-rich diet decreased 3-NT level by 55% and 13% in control and quercetin-treated rats, respectively (Fig. 5C).

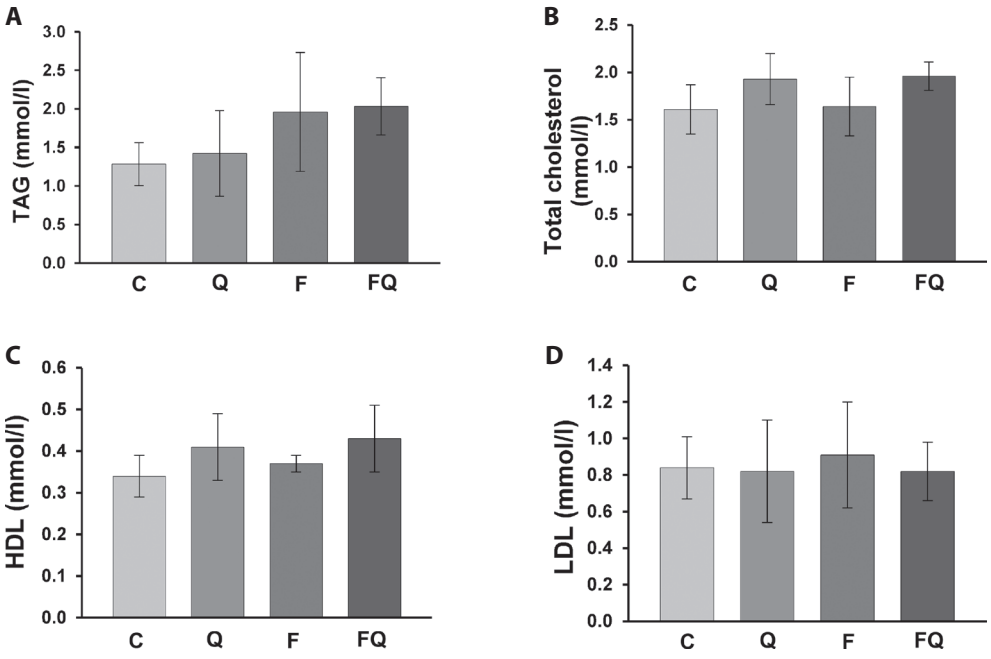

Figure 4. Blood lipid profile: triacylglycerols (**A**), total cholesterol (**B**), HDL cholesterol (**C**) and LDL cholesterol (**D**). Triglyceride and total cholesterol concentration were obtained by using Vitros 250 autoanalyzer. Determination of HDL-cholesterol is done by the method of spectrophotometry at the Biosystems A25 apparatus. LDL was calculated as: total cholesterol – HDL – (TAG/5). TAG, triacylglycerols; HDL, high-density lipoprotein; LDL, low-density lipoprotein. Other abbreviations are given in Figure 2.

Markers of liver protein production

Both treatments, fructose and quercetin, significantly affected the level of total plasma proteins in terms of increase ($p < 0.001$ for F and $p < 0.01$ for Q). The fructose-induced increase was relatively moderate, with 10% in control and 6% in quercetin-treated rats (Fig. 6A). The same can be said about the increase caused by quercetin, which was by 6% in control and only 3% in fructose-drank rats. Regarding plasma albumin, both fructose-consumed and quercetin-treated rats had higher plasma levels of this protein ($p < 0.001$ and $p < 0.01$, respectively). The observed increases were also moderate in this case: fructose raised the albumin level by 13% and 6% in control and quercetin-treated rats, respectively, whereas the increase caused by quercetin treatment was by 10% in control and by 3% in fructose-treated rats (Fig. 6B).

Discussion

Quercetin is one of the most commonly occurring bioactive flavonoids found in various fruits, vegetables, and products of plant origin (Bischoff 2008). Quercetin has several pharmacological effects, including cleavage of oxygen radicals, prevention of lipid peroxidation, and anti-adipogenic and anti-inflammatory effects (Vicentini et al. 2010; Wagner et al. 2010). In addition, quercetin decreases systolic blood pressure and improves vascular relaxation in 6-month-old Zucker diabetic rats without beneficial effects in 1-year-old animals (Ferenczyova et al. 2020). Regarding metabolic effects, long-term treatment with quercetin reduces blood pressure, plasma concentrations of insulin, TAGs, free fatty acids, nitric oxide and increases adiponectin in obese Zucker rats (Rivera et al. 2008). In the above rat model of obesity, quercetin was employed as the solution of suspension in 1% methyl cellulose.

Until now, the effect of quercetin on fructose-induced metabolic syndrome was studied in the following rat model: the treatment with 10% fructose in drinking water was used for 4 weeks with a subsequent combination of fructose and quercetin for additional 4 weeks (Hu et al. 2009, 2012; Li et al. 2013). The concentrations of quercetin were relatively high 50 and 100 mg/kg/day (dissolved in water). Alternatively, simultaneous treatment with fructose and quercetin (15 mg/kg/day in 0.2 % DMSO) (Er et al. 2022) for 10 weeks was applied as well.

In the presented experiment, it was addressed the question of whether quercetin treatment can attenuate already developed fructose-induced metabolic syndrome. Our previous studies corroborate that drinking 10% fructose for 9 weeks develops insulin resistance in rats (Stanišić et al. 2016). It was assumed that additional 6 weeks of simultaneous 10% fructose drinking with quercetin treatment would attenuate at least some of the features of metabolic syndrome.

As expected, fructose alone decreased the food intake. The same happened when a combination of fructose and quercetin treatment was utilized. On the contrary, fructose alone increased the liquid intake. Surprisingly, a combination of fructose and quercetin led to the normalization of the liquid intake. To the best of our knowledge, this is the

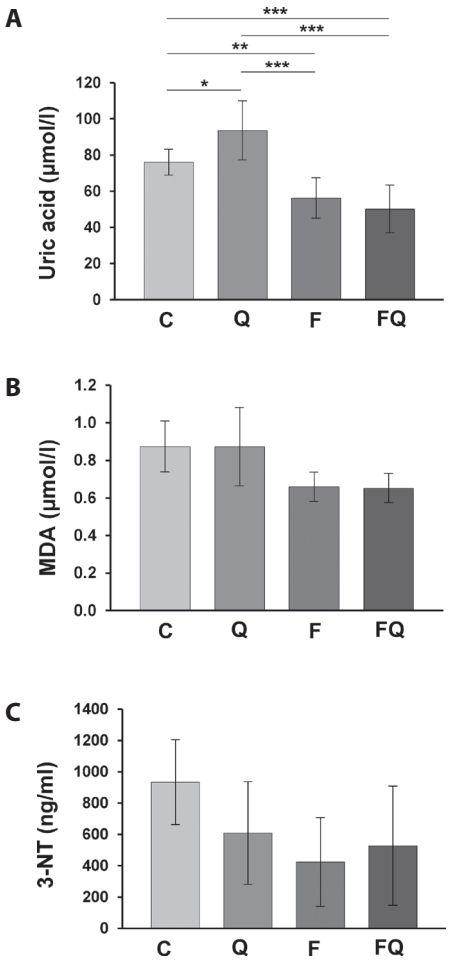

Figure 5. Uric acid and oxidative stress biomarkers: UA (**A**), MDA (**B**), and 3-NT (**C**). UA concentrations were obtained by using Vitros 250 autoanalyzer. TBARS content in plasma was determined by using a Lipid peroxidation (MDA) assay kit. 3-NT content was measured by using a Nitrotyrosine ELISA kit. UA, uric acid; MDA, malondialdehyde; 3-NT, 3-nitrotyrosine. Other abbreviations are given in Figure 2. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

first report describing the inhibition of fructose drinking by quercetin. Previous studies (Hu et al. 2009, 2012) showed rather a tendency towards increasing sweet fluid intake due to quercetin.

Fructose itself and its combination with quercetin have increased body weight gain. However, this body weight gain was very mild and resembled a plateau at 15 weeks since the relative increase in the fructose controls and the fructose and quercetin-treated animals was 7% and 6%, respectively. These increases are close to the value of fructose-treated control (7.5%) animals after 9 weeks of fructose treatment (unpublished results).

Fructose caused systemic insulin resistance after 15 weeks of treatment, as evidenced from glucose, insulin, and HOMA-IR data. The same effect was found in our previous experiments employing 9-week- and 15-week-fructose treatment (Stanišić et al. 2016; Stanisic et al. 2021). Quercetin alone and in combination with fructose significantly attenuated plasma glucose levels. This is in accordance with data published by other authors (Hu et al. 2009; Li et al. 2013; Lu et al. 2017) using much higher concentrations of quercetin (50 and 100 mg/kg/day). In our experiment, 20 mg/kg/day of quercetin did not affect plasma insulin, unlike 50 and 100 mg/kg/day (Hu et al. 2009, 2012). However, 20 mg/kg/day of quercetin significantly decreased the HOMA-IR index in fructose-treated animals. In summary, a significant improvement in glucose metabolism was achieved when quercetin was administrated in a methyl cellulose solution at a dose of 20 mg/kg/day.

On the contrary, quercetin did not exert any lipid-lowering effect at this dose, unlike 50 and 100 mg/kg/day (Hu et al. 2009; Li et al. 2013; Lu et al. 2017). In addition, in our experiment, quercetin elevated total cholesterol in both control and fructose-treated rats. This is in accordance with the data of Er et al. (2022) when using 15 mg/kg/day dose of quercetin in 0.2% of DMSO. However, our study showed that the increase in total cholesterol is at the expense of an increase in HDL-C.

One of the most studied effects of the fructose diet is an increase in plasma UA (Caliceti et al. 2017; Zhang et al. 2020). Therefore, our data on plasma UA greatly surprised us. Quercetin increased the UA concentration, in contrast to pure fructose or a combination of fructose with quercetin, showing the opposite effect. Data on UA were compared with

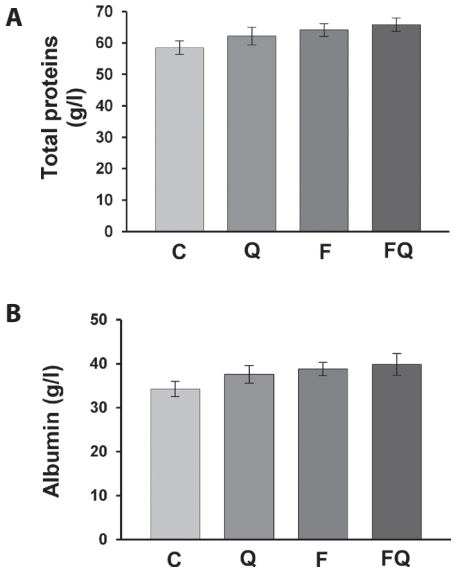

Figure 6. Markers of liver protein production. Total plasma proteins (**A**) and plasma albumin concentration (**B**) were obtained by using Vitros 250 autoanalyzer. Abbreviations are explained in Figure 2.

those of MDA in plasma, a factor also usually elevated after fructose consumption (Hokayem et al. 2013; Chenni et al. 2022). In the case of MDA, the pattern was very similar to the results of UA, and the only difference was in the effect of quercetin.

The above facts have led us to reconsider the entirely accepted axiom of methyl cellulose inertness (Kalasz and Antal 2006; Deng et al. 2020; Sultan et al. 2022). Only sparse information is available on the protective action of methyl cellulose during sperm storage, including a decrease in reactive oxygen species formation (González-Abreu et al. 2017). Therefore, 3-NT was also measured as an indicator of oxidative protein damage. The obtained results confirmed those of UA and MDA with the protective effect of fructose/methyl cellulose combination on oxidative damage of proteins.

In addition, we looked at how changed plasma glucose influences the glycation of plasma proteins. Again**,** it was found that quercetin alone and a combination of fructose with methyl cellulose with or without quercetin decreased AGEs fluorescence. Indeed, it was described that quercetin reduces AGEs (Nayak et al. 2014), but the effect of fructose in combination with methyl cellulose seems to be completely new.

Increased total plasma proteins and albumin in fructose-drank and quercetin-treated animals might suggest a liver-protective effect of both treatments (Ahmed et al. 2022).

We are aware of the missing absolute control group of animals without methyl cellulose vehicle treatment in the presented study. Despite that, the obtained data on fructose/methyl cellulose action is very convincing, and therefore they deserve further investigation. It especially refers to the possibility of adding methyl cellulose to sweetened drinks to reduce their harmful effects on health.

In conclusion, quercetin in 20 mg/kg/day given in methyl cellulose solution partially attenuated the signs of fructose-induced insulin resistance. It mainly reduced glycemia and the HOMA-IR index. In addition, quercetin normalized liquid intake, thus decreasing fructose ingestion.

Another outcome of this study is that combining fructose with methyl cellulose has several positive impacts. This unique combination decreases plasma UA, oxidative stress biomarkers and AGEs fluorescence. Our results support divergent actions of fructose, which might be detrimental as well as protective depending on the conditions, such as dosing, short-term vs. long-term fructose treatment, or the presence of specific substances (Spasojević et al. 2009; Semchyshyn 2013). Here we show that methyl cellulose might be a new substance converting at least a part of the harmful effects of fructose to protective ones. We might speculate that methyl cellulose can shift the fructose metabolism from the ketohexokinase pathway towards the glycolytic one through the hexokinase and/or polyol pathway (Krause and Wegner 2020).

Conflict of interest. The authors declare there are no competing interests.

Acknowledgements. This research was co-funded by Slovak Research and Development Agency (grant APVV-20-0421) and Ministry of Education, Science, Research and Sport of the Slovak Republic (grant VEGA 2/0105/24). This study was also supported by The Ministry of Science, Technological Development and Innovation of the Republic of Serbia (grant No: 451-03-66/2024-03/200017).

Author contribution. GK: Conceptualization, formal analysis, investigation, writing – original draft. JH: Formal analysis, visualization, writing – review and editing. SR: Investigation, formal analysis, writing – review and editing. VD, MS, KK, TI: Investigation. SZ: Conceptualization (lead), writing – original draft (lead), formal analysis. All authors approved the submitted version of the manuscript.

Data availability. The data that support the findings of this study are available on request from the corresponding author.

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Received: January 26, 2024
Final version accepted: February 23, 2024