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Dietary Intake of Fructooligosaccharides Protects against Metabolic Derangements Evoked by Chronic Exposure to Fructose or Galactose in Rats

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Scope: Diets rich in fat and sugars evoke chronic low-grade inflammation, leading to metabolic derangements. This study investigates the impact of fructose and galactose, two commonly consumed simple sugars, on exacerbation of the harmful effects caused by high fat intake. Additionally, the potential efficacy of fructooligosaccharides (FOS), a fermentable dietary fiber, in counteracting these effects is examined.

Methods and results: Male Sprague-Dawley rats (six/group) are fed 8 weeks as follows: control 5% fat diet (CNT), 20% fat diet (FAT), FAT+10% FOS diet (FAT+FOS), FAT+25% galactose diet (FAT+GAL), FAT+GAL+10% FOS diet (FAT+GAL+FOS), FAT+25% fructose diet (FAT+FRU), FAT+FRU+10% FOS diet (FAT+FRU+FOS). The dietary manipulations tested do not affect body weight gain, blood glucose, or markers of systemic inflammation whereas significant increases in plasma concentrations of triacylglycerols, cholesterol, aspartate aminotransferase, and alanine aminotrasferase are detected in both FAT+FRU and FAT+GAL compared to CNT. In the liver and skeletal muscle, both sugars induce significant accumulation of lipids and advanced glycation end-products (AGEs). FOS supplementation prevents these impairments.

Conclusion: This study extends the understanding of the deleterious effects of a chronic intake of simple sugars and demonstrates the beneficial role of the prebiotic FOS in dampening the sugar-induced metabolic impairments by prevention of lipid and AGEs accumulation.

1. Introduction

The consumption of a Western-type diet rich in simple sugars, refined grains, and saturated fatty acids has increased steadily over the last decades and may have contributed to an increase in the occurrence of obesity, type 2 diabetes mellitus, and cardiovascular diseases.[1,2] The high intakes of the sugars sucrose and fructose, not the least from sugar sweetened beverages, have been suggested as contributors to the development of obesity, fatty liver disease, and chronic inflammation,[3] particularly when consumed as part of an energy-dense Western dietary pattern. The daily intake of total sugars in adults, given as percentage of the total daily energy intake, ranges from about 14% in Italian males to 25% in 20–29 year old US American females.[1] The consumption of fructose alone in a US American population was estimated at approximately 10% of total daily energy intake.[4]

Fructose may bypass the rate-limiting step that controls the conversion of monosaccharides into fatty acids and

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thereby result in uncontrolled lipid accumulation and derangement of lipid homeostasis.[5] In addition, fructose is one of the most reactive sugars resulting in the formation of advanced glycation end-products (AGEs), a heterogeneous group of sugar-modified proteins whose accumulation is associated with impaired cellular metabolism and may increase the progression of several age-related chronic inflammatory diseases, such as diabetes mellitus, dyslipidemia, neurodegenerative, and cardiovascular diseases[2,6]

Galactose, a constituent of the disaccharide lactose, is one of the most commonly consumed monosaccharides present in dairy products and the most rapidly absorbed simple sugar. Limited data are available on the mean intake of galactose in human populations. The daily consumption of galactose has been estimated at 1.5–3.6 g d^{-1} in a female population in Iran[7] and below 0.5 g d^{-1} in 75% of women in a Japanese cohort.[8] The metabolism of galactose results in intermediate products that accumulate and may evoke long-term metabolic complications and degenerative changes that also occur in aging.[9] Therefore, galactose injection has been widely used to induce and study the effects of accelerated aging in animal models.[9] However, so far, little is known about the metabolic consequences of high intake of galactose, specifically on lipid metabolism and liver function, as the liver is the main organ metabolizing galactose. In addition, the molecular mechanisms underlying the toxicity of AGEs following galactose intake are not fully clear. Although both of these sugars may have adverse metabolic effects at high intakes, to the best of our knowledge, no studies have been reported where galactose and fructose have been directly compared.

On the other hand, frequent consumption of complex carbohydrates, such as prebiotic fructooligosaccharides (FOS), may preserve intestinal barrier function,[10] improve glycemic control prevent or reduce chronic inflammation, enhance immune function, and thus mitigate the risk of chronic metabolic and inflammatory diseases.[11] These effects may be indirectly mediated by increasing the abundance of Bifidobacterium and Lactic Acid bacteria in the large intestine, which has been consistently shown in many studies, or by suppressing the growth of pathogenic bacteria that may result in endotoxemia, which could thereby provide a positive effect on gut barrier integrity and lower sub-clinical systemic inflammatory responses.[12,13] Thus, FOS may counteract the adverse metabolic effects evoked by fructose and galactose.

We recently conducted a rat trial to compare the metabolic effects of the two simple sugars, fructose and galactose, as well as to highlight potential beneficial effects of FOS.[14] However, the high intake of sugars we tested (50% diet content) and the chronic feeding (12 weeks) evoked severe toxic symptoms, which prevented us from studying the nutritional impact of these sugars on chronic inflammation and dyslipidemia and the potential beneficial effects of FOS. A high-fat diet has been implicated in increasing intestinal inflammation, which could contribute to endotoxemia and tissue inflammation.[15,16] However, so far, the mechanisms by which a high-fat diet increases intestinal inflammation, as well as the dietary components mediating these changes, have not been identified. Interestingly, as recently documented,[17] elimination of sugar from a high-fat diet protected mice from developing metabolic derangements by preserving the protective function of intestinal immune cells, thus suggesting interesting cross-talk mechanisms linking simultaneous exposure to fat and sugars, which deserve further investigation.

Hence, the present study was designed to investigate the effects of a chronic high intake of fructose or galactose, when ingested as part of a Westernized high-fat diet, and to test the potential beneficial effects of the simultaneous consumption of FOS to counteract sugar-induced metabolic derangements.

2. Experimental Section

2.1. Animal Experiment

Forty-eight male Sprague-Dawley rats (250–274 g) were purchased from Janvier Labs (Le Genest-Saint-Isle, France) at 7 weeks of age. After a 12 days familiarization period, the rats were randomly divided into the following groups (n = 6 per group): 1) control group fed a standard rodent diet (CNT: 5% fat, 35% starch), and further six groups receiving an isocaloric Westernized diet as follows: 2) positive control (FAT: 20% fat, 35% starch). 3) FAT+10% FOS (FAT+FOS), 4) 20% FAT+25% galactose (FAT+GAL), 5) 25% GAL+10% FOS (FAT+GAL+FOS), 6) FAT+25% fructose (FAT+FRU), 7) FRU+10% FOS (FAT+FRU+FOS). Sunflower oil was rich in pro-inflammatory polyunsaturated fatty acids and was used to facilitate inflammatory processes. Further details on the diet composition were listed in **Table 1**. Rats were housed in groups of three per cage and kept in a controlled environment (20 ± 2 °C, 55 ± 5% relative humidity, 12 h light/12 h dark cycle) with free access to feed and water. Feed intake and body weight were recorded weekly.

After 8 weeks of intervention, rats were fasted overnight, anesthetized with CO_2, and sacrificed by decapitation. Blood was collected into heparinized monovettes (Monovette, Sarstedt, Germany). Tissues (liver, gastrocnemius, spleen, brain, kidneys, ileum, and colon and their contents) were excised, placed in cryotubes, and snap-frozen in liquid nitrogen. All samples were immediately frozen at −80 °C until further analysis. Skeletal muscle and liver tissues were also embedded and preserved in optimal cutting temperature compound (OCT). All animal procedures were carried out in accordance with the Federation of European Laboratory Animal Science Association (FELASA) guidelines for care and use of laboratory animals and approved by the Regional Council (RP S35-9185-99/383, Stuttgart, Baden-Württemberg, Germany).

2.2. Biochemical Analyses

Fasting blood glucose was measured in a drop of whole blood with a glucometer (TESTAmed, Diabetes care, Germany). Plasma concentrations of aspartate aminotransferase (AST), alanine aminotransferase (ALT), triglycerides, and total cholesterol were measured using commercially available clinical assay kits (FAR Diagnostics, Verona, Italy) following the manufacturer's instructions. Plasma concentrations of C-reactive protein (CRP) and tumor necrosis factor α (TNF-α) were quantified using enzyme-linked immunosorbent assays (ELISA kits, Abcam, Boston, MA, USA) according to the manufacturer's instructions.

Table 1. Dietary composition of the experimental diets.

	CNT	FAT (20%)	FAT+FOS	FAT+GAL	FAT+GAL +FOS	FAT+FRU	FAT+FRU + FOS
Total carbohydrates [g kg^{-1}]	400	400	450	400	450	400	450
Starch [g kg^{-1}]	350	350	300	100	50	100	50
Sugar [g kg^{-1}]	0	0	0	250	250	250	250
FOS [g kg^{-1}]	0	0	100	0	100	0	100
Cellulose [g kg^{-1}]	50	50	50	50	50	50	50
Fat [g kg^{-1}]	50	200	200	200	200	200	200
Protein [g kg^{-1}]	250	250	250	250	250	250	250
Minerals [g kg^{-1}]	62	62	62	62	62	62	62
Vitamins [g kg^{-1}]	1	1	1	1	1	1	1
Total energy [kcal kg^{-1}]	**2950**	**4300**	**4300**	**4300**	**4300**	**4300**	**4300**

FOS, fructooligosaccharides; FRU, fructose; GAL, galactose.

2.3. Myeloperoxidase Activity

Myeloperoxidase (MPO) activity was assayed as described previously.[18] In brief, 100 mg of liver or gastrocnemius tissues was homogenized, centrifuged (13 000 × *g* at 4 °C for 10 min) and assayed for MPO activity by measuring the H_2O_2-dependent oxidation of 3,3′,5,5′-tetramethylbenzidine (TMB). MPO activity was expressed as optical density (O.D.) at 650 nm per mg of protein.

2.4. Liver Lipids

Lipid accumulation in the liver was evaluated by Oil Red O staining on 14 μm liver cryostatic sections. Stained tissues were viewed under an Olympus Bx4I microscope (400× magnification) with an AxioCamMR5 photographic attachment (Zeiss, Gottingen, Germany).

2.5. Western Blot Analyses

Liver and gastrocnemius protein extracts were prepared as previously described.[19] Briefly, about 50 mg of liver or gastrocnemius was homogenized at 10% w/v in a Potter Elvehjem homogenizer (Wheaton, Millville, NJ, USA) using a homogenization buffer containing 20 mM HEPES, pH 7.9, 1 mM MgCl2, 0.5 mM EDTA, 1% NP–40, 1 mM EGTA, 1 mM Dithiothreitol (DTT), 0.5 mM phenylmethyl sulphonyl fluoride, and centrifuged at 13 000 × *g* for 10 min, 4 °C. Supernatants were collected and the protein content was determined using a BCA protein assay (Pierce Biotechnology Inc., Rockford, IL, USA) following the manufacturer's instructions. 50 μg of total proteins were loaded for immunoblot experiments. Proteins were separated by either 8% or 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to a polyvinylidene difluoride membrane, which was then incubated with primary antibodies (dilution 1:1000). The antibodies used were: mouse anti-CEL (Transgenic, KAL-KH025); mouse anti-CML (R&D Systems, MAB3247); rabbit anti-RAGE (Santa Cruz, sc-3651); rabbit anti-β-actin (Cell signaling 4970); blots were then incubated with a secondary antibody (Cell Signaling - anti-mouse #7076; anti-rabbit #7074) conjugated with HRP (dilution 1:10 000) and developed using the ECL detection system. The immunoreactive bands were analyzed by the Bio-Rad Image Lab Software 6.0.1 and results were normalized to the corresponding β-actin band and expressed as fold of expression compared to control.

2.6. Quantification of AGEs by Ultra-High-Performance Liquid Chromatography (UHPLC)-Tandem Mass Spectrometry

Tissue homogenates (50 μL) were hydrolysed with 500 μL of 0.6 M trichloroacetic acid and 50 μL of 6 M hydrochloric acid for 2 h at 60 °C. The analyses were performed on a UHPLC coupled to a triple quadrupole mass spectrometer (AB-Sciex Triple Quad 5500, Milan, Italy), equipped with a Turbo ion ESI source. Analytes were separated on a reversed-phase C18 column (Kinetex 100 × 2.1 mm, 1.7 μm particle size, Phenomenex) at a flow rate of 0.35 μL min^{-1}. A gradient mobile phase composition of 95/5–40/60 over 25 min in 5 nM heptafluorobutanoic acid/acetonitrile was adopted. The liquid chromatograph column eluent was delivered to the Turbo ion source using nitrogen as a sheath (GS1) and curtain (CUR) gas, and air as reagent gas (GS2).

The source voltage was set at 4.5 kV in positive mode, CUR 26 arbitrary units (arb), GS1 45 arb, and GS2 50 arb. The heated capillary was maintained at 275 °C. The MRM transitions and parameters were as follows: Nε-(carboxymethyl)-lysine (*m*/*z*) 205@84, declustering potential (DP) 100 V, entrance potential (EP) 7 V, collision energy (CE) 32 V. Quantification of analytes was performed using standard calibration curves for carboxymethyllysine (CML), methylglyoxal-hydroimidazolone-1 (MGH1) at concentrations of 10, 50, 100, 250, 300, and 500 μg L^{-1}. Measured concentration of analytes in samples was always within the linear range of calibration. Coefficient of variation for the above analyses ranged from 8% to 14%.

2.7. qRT-PCR

Total RNA was extracted from liver (10 mg) using Total Tissue RNA Purification Kit (#3700Norgen Biotek Corporation, Thorold,

Table 2. Metabolic parameters at 8 weeks of dietary manipulation.

	CNT	FAT	FAT+FOS	FAT+GAL	FAT+GAL+FOS	FAT+FRU	FAT+FRU+FOS
Body weight [g]	592.7 ± 40.07	618.6 ± 21.1	618.3 ± 28.3	586.8 ± 36.4	588.8 ± 40.4	605.0 ± 54.02	612.5 ± 53.2
Fasting blood glucose [mg dL^{-1}]	83.5 ± 21.9	82.0 ± 14.8	83.5 ± 4.04	82.8 ± 15.8	80.2 ± 8.3	83.7 ± 9.3	77.8 ± 7.7
Plasma triglycerides [mg dL^{-1}]	23.8 ± 6.2	42.5 ± 15.1*	40.4 ± 5.1	40.6 ± 8.1*	28.0 ± 5.0°	51.1 ± 7.9*	37.0 ± 8.1°
Plasma cholesterol [mg dL^{-1}]	37.5 ± 5.4	45.9 ± 9.7	34.3 ± 8.9	63.5 ± 9.9 §*	47.1 ± 7.9°	62.3 ± 3.9*	46.1 ± 5.4§°
AST [U L^{-1}]	36.3 ± 5.8	43.1 ± 1.4	29.9 ± 5.2	63.7 ± 8.8§*	47.9 ± 0.5°	58.3 ± 16.2	34.1 ± 12.0§°
ALT [U L^{-1}]	13.7 ± 4.5	20.2 ± 1.3	22.6 ± 4.6	47.8 ± 17.1§*	25.7 ± 1.1°	41.9* ± 3.5*	23.6 ± 5.4 §°

Data are means ± SD ($n = 6$). * versus CNT. § versus FAT. °versus FAT or FAT+GAL or FAT+FRU, respectively.

ON, Canada) according to manufacturer's instructions. Instead, approximately 30 mg of gastrocnemius muscle were lysed and processed to isolate total RNA using the standard phenol-chloroform method (TRIzol Reagent, #15596026, Thermo Fischer, Waltham, MA, USA). The RNA concentration and purity were measured using a nanodrop (Titertek-Berthold) machine. Total RNA was retro-transcribed using the SensiFAST cDNA SynthesisKit (#BIO-65054 Meridian Bioscience, USA) according to manufacturer's instructions. Transcript levels were determined by Real time qPCR using the SensiFAST SYBR No-ROX Kit (#BIO-98005 Meridian Bioscience, USA) with 10 ng of cDNA per well. PCR reaction was carried out on the CFX Connect Real-Time PCR Detection System (Bio-Rad). Relative gene expression was obtained after normalization to housekeeping genes (18s) using the formula $2-\Delta\Delta CT$ as previously described[20] and folds changes were determined by comparison to CNT group. The GAPDH, CCL2, and CXCL10 primers used in this study were purchased from Eurofins Scientific (Nantes, France).

2.8. Statistical Analyses

Statistical analyses were carried out using GraphPad Prism 9 (San Diego, CA, USA). Changes in body weight were analyzed by repeated-measures analysis of variance (ANOVA). Differences between diets were evaluated by one-way ANOVA with Bonferroni correction. Differences were considered significant at $p < 0.05$ and all reported values were least-square means ± standard deviation (SD).

3. Results

3.1. Effects of Fructose, Galactose, and FOS on Body Weight and Blood Biochemistry

Rats fed the different diets did not differ in body weight gain over the 8-week intervention or in fasting blood glucose at the end of the trial. In contrast, slight differences were found when the lipid profile was analyzed (**Table 2**). The fat-enriched diet led to significantly increased plasma triglyceride concentrations compared to control diet, but intake of fructose or galactose did not further exacerbate this effect (Table 2). The consumption of FOS alone did not modify plasma triglycerides when compared to the Westernized diet (positive control), but when added to the sugar-enriched diets it significantly reduced their levels compared to the respective sugar-fed group. No differences in plasma cholesterol were observed between the control and the Westernized diet. Interestingly, both fructose and galactose, ingested as part of a Westernized diet, increased plasma cholesterol and this effect was prevented by the simultaneous feeding with FOS.

The effects of diet on the systemic lipid profile were paralleled by changes in markers of hepatic dysfunction (Table 2). Plasma concentrations of ALT and AST were not significantly altered by consumption of the Western-type diet with or without FOS in comparison to control. Rats fed fructose or galactose as part of the Westernized diet showed significantly increased plasma concentrations of AST and ALT compared to either the control or the fat-enriched diet and simultaneous consumption of FOS again prevented these increases.

3.2. Effects of Fructose, Galactose, and FOS on Lipid Accumulation in Liver and Gastrocnemius

Liver tissue of rats fed a Westernized diet showed significantly increased triglyceride concentrations compared to control and feeding of both sugars further increased their concentrations (**Figure 1A**). FOS feeding alone did not alter liver triglycerides, whereas simultaneous intake of FOS and sugars dampened the sugar-induced lipid accumulation. Similarly, the consumption of the Westernized diet increased liver cholesterol compared to control, which was further exacerbated by galactose exposure, whereas FOS exposure counteracted the sugar-induced cholesterol local accumulation (Figure 1C).

On the contrary, when lipid accumulation was detected in the skeletal muscle, both fructose and galactose did not further exacerbate the accumulation of both triglycerides and cholesterol evoked by the fat-enriched diet. Interestingly, FOS exerted protective effects in the skeletal muscle when added to either fat- and sugar-enriched diets (Figure 1B,D).

The impact of the diets on hepatic steatosis was confirmed by Oil Red O staining (**Figure 2**), showing cytoplasmic micro- and macro-vacuolar lipid droplet deposition in the liver of rats fed the Westernized diet alone or in combination with fructose or galactose. Intake of FOS significantly reduced lipid accumulation compared to positive control as well as compared to the respective sugar-fed group.

3.3. Effects of Fructose, Galactose, and FOS on AGEs Accumulation

As shown in **Figure 3**, the AGEs carboxymethyl lysine (CML) and carboxyethyl lysine (CEL) were detected by Western blot analysis.

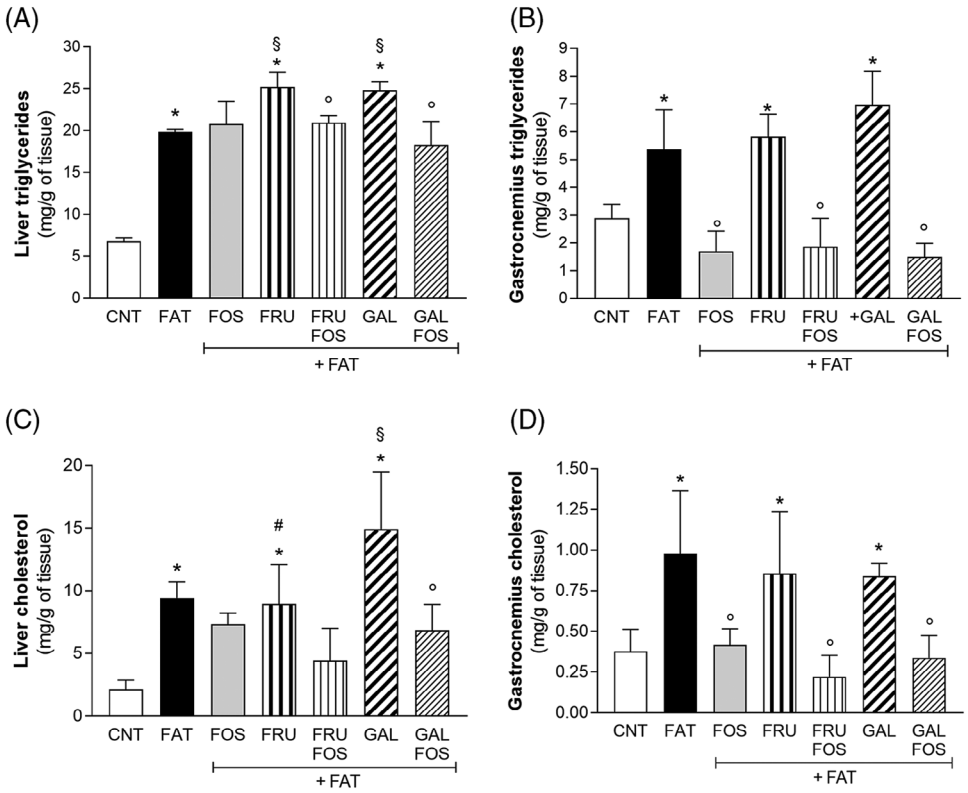

Figure 1. Effect of diets on (A, B) triacylglycerols and (C, D) cholesterol in liver and gastrocnemius. Data are expressed as mean ± SD for $n = 6$ rat per group. * versus CNT. § versus FAT. ° versus FAT or FAT+GAL or FAT+FRU respectively. # versus GAL.

AGEs concentrations were not altered by the consumption of the fat-enriched diet in the liver and skeletal muscle when compared to the control diet. Both fructose and galactose intake induced a significant increase in tissue AGEs accumulation. Most notably, FOS reduced sugar-induced AGEs accumulation, although statistical significance was not reached for all comparisons. As a consequence of AGEs accumulation, we detected a large increase in the expression of the AGE-receptor RAGE in both

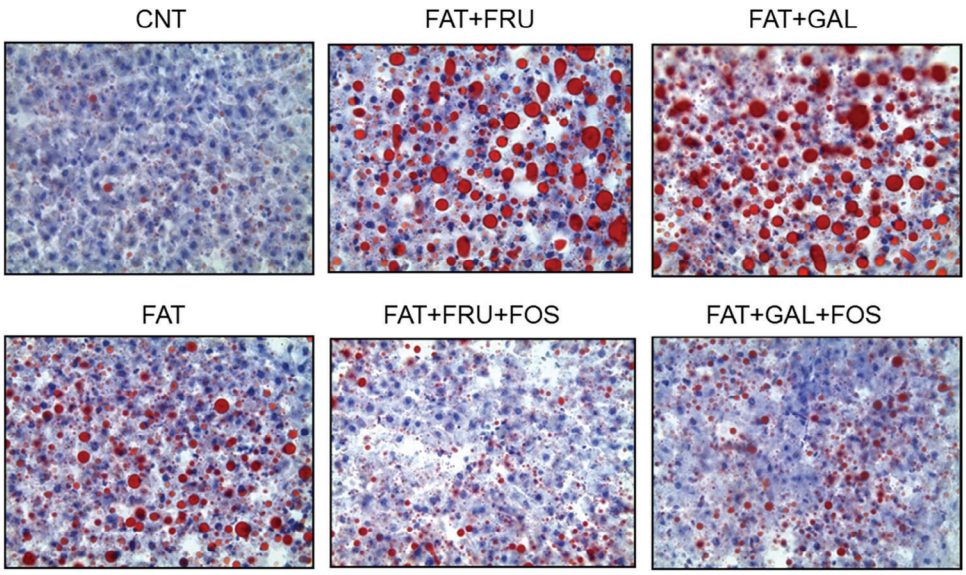

Figure 2. Oil red O staining on liver sections (400-fold magnification) from rats fed the different diets for 8 weeks. showing lipids deposition.

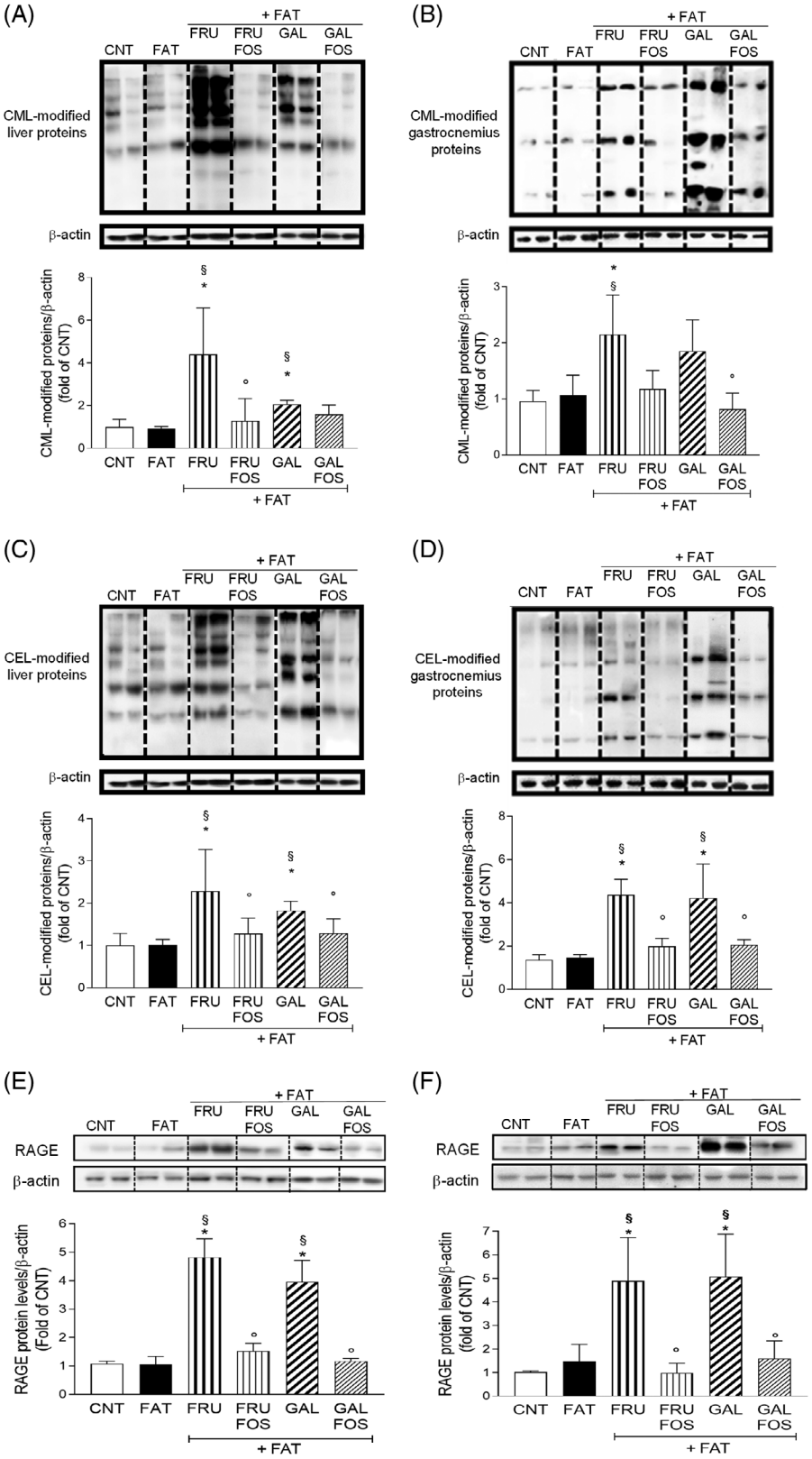

Figure 3. Representative Western blot analyses of AGE accumulation: carboxymethyl lysine (CML)-modified proteins (A, B), carboxyethyl lysine (CEL)-modified proteins (C, D), and RAGE expression (E, F) in liver (A–C–E) and gastrocnemius (B–D–F) of rats fed the different experimental diets for 8 weeks. Data are expressed as mean ± SD for $n = 6$ rats per group. * versus CNT. § versus FAT. ° versus FAT or FAT+ GAL or FAT+FRU, respectively.

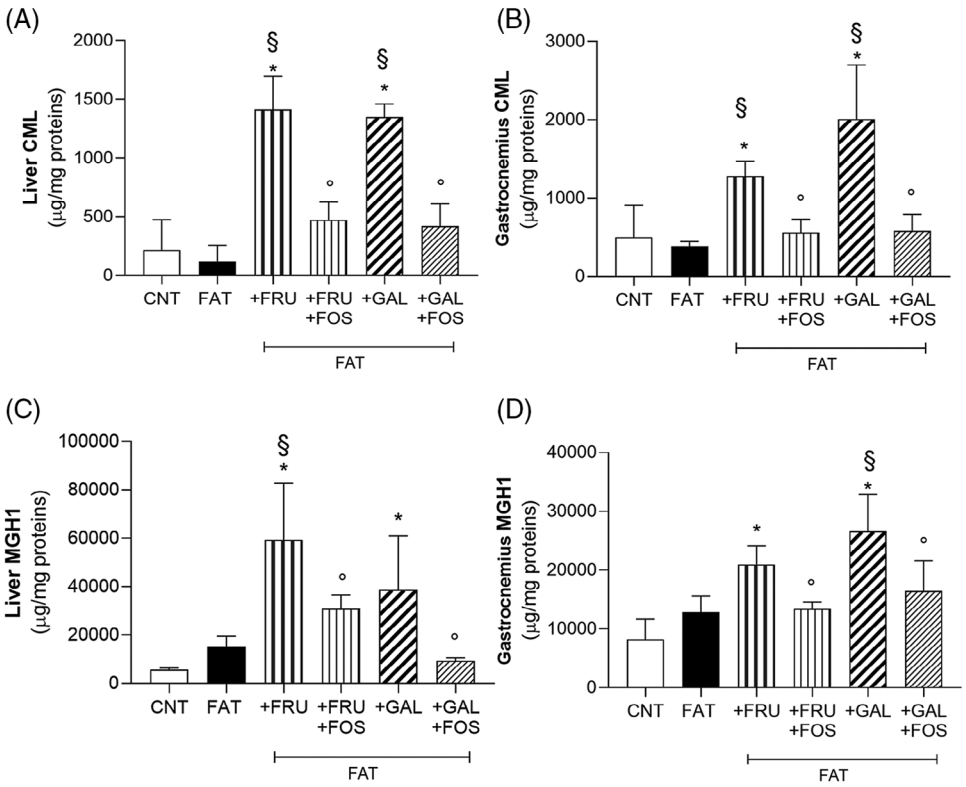

Figure 4. Quantitative analysis of carboxy methyllysine levels and MGH-1 evaluated by UHPLC-MS in liver (A,C) and gastrocnemius muscle (B,D). Data are expressed as mean ± SD for $n = 6$ rats per group. * versus CNT. ° versus FAT or FAT+GAL or FAT+FRU.

liver and gastrocnemius of rats fed fructose or galactose as part of a Westernized diet and a significant reduction of RAGE upregulation upon simultaneous FOS intake.

Quantitative analysis of AGEs, specifically of the arginine-derived MGH-1 and the lysine-derived CML, confirmed that the sunflower oil used as fat source did not affect local AGEs production, whereas the highest AGEs concentrations were observed in the liver and skeletal muscle of mice exposed to the sugar-enriched diets, with no significant differences between fructose and galactose. Besides, the anti-glycating properties of FOS were here confirmed (**Figure 4**).

3.4. Effects of Fructose, Galactose, and FOS on Markers of Systemic and Local Inflammation

The sugar- and FOS-induced changes on the AGE-RAGE axis were closely paralleled by changes in the MPO activity in both the liver and the skeletal muscle (**Figure 5A,B**). Specifically, the fat intake numerically and the addition of fructose or galactose to the Westernized diet significantly increased MPO activity, whereas the simultaneous intake of FOS with fructose or galactose significantly reduced tissue MPO activity, thus suggesting diet-related changes in local neutrophil infiltration. To extend our investigation to other types of infiltrating immune cells, which contribute to the development of metabolic inflammation, we measured the relative mRNA expression of CCL2 and CXCL10, two of the most important chemoattractant chemokines regulating the migration and infiltration of a wide range of immune cells, including T lymphocytes.[21] CCL2 expression increased significantly in liver and skeletal muscle of mice fed fructose or galactose, but not of mice fed with the fat diet only (Figure 5B). A similar pattern was observed when CXCL10 was measured, thus suggesting that the diet enrichment in either fructose or galactose may contribute to an increased local recruitment of T-lymphocytes during an early phase tissue inflammation. Most notably, FOS supplementation resulted in a reduction of local expression of both CCL2 and CXCL10, thus indicating potential beneficial effects of this nutritional approach in shaping local innate immune response as well as the adaptive immunity. The systemic markers of inflammation CRP and TNF-α were quantified in plasma, but did not differ among groups (**Figure 6**).

4. Discussion

Here we provide evidence that the addition of fructose or galactose to a Westernized diet with 20% fat from sunflower oil has adverse effects on markers of metabolic derangements at the systemic and tissue levels. Specifically, we found that the 20% fat-enriched diet exerted mild effects on blood triglycerides, not further exacerbated by adding fructose or galactose, whereas both sugars increased lipid and AGEs accumulation in both the liver and skeletal muscle.

MPO is the most abundant proinflammatory enzyme stored in the azurophilic granules of neutrophil granulocytes and it is considered a good biomarker of neutrophil infiltration.[22] MPO

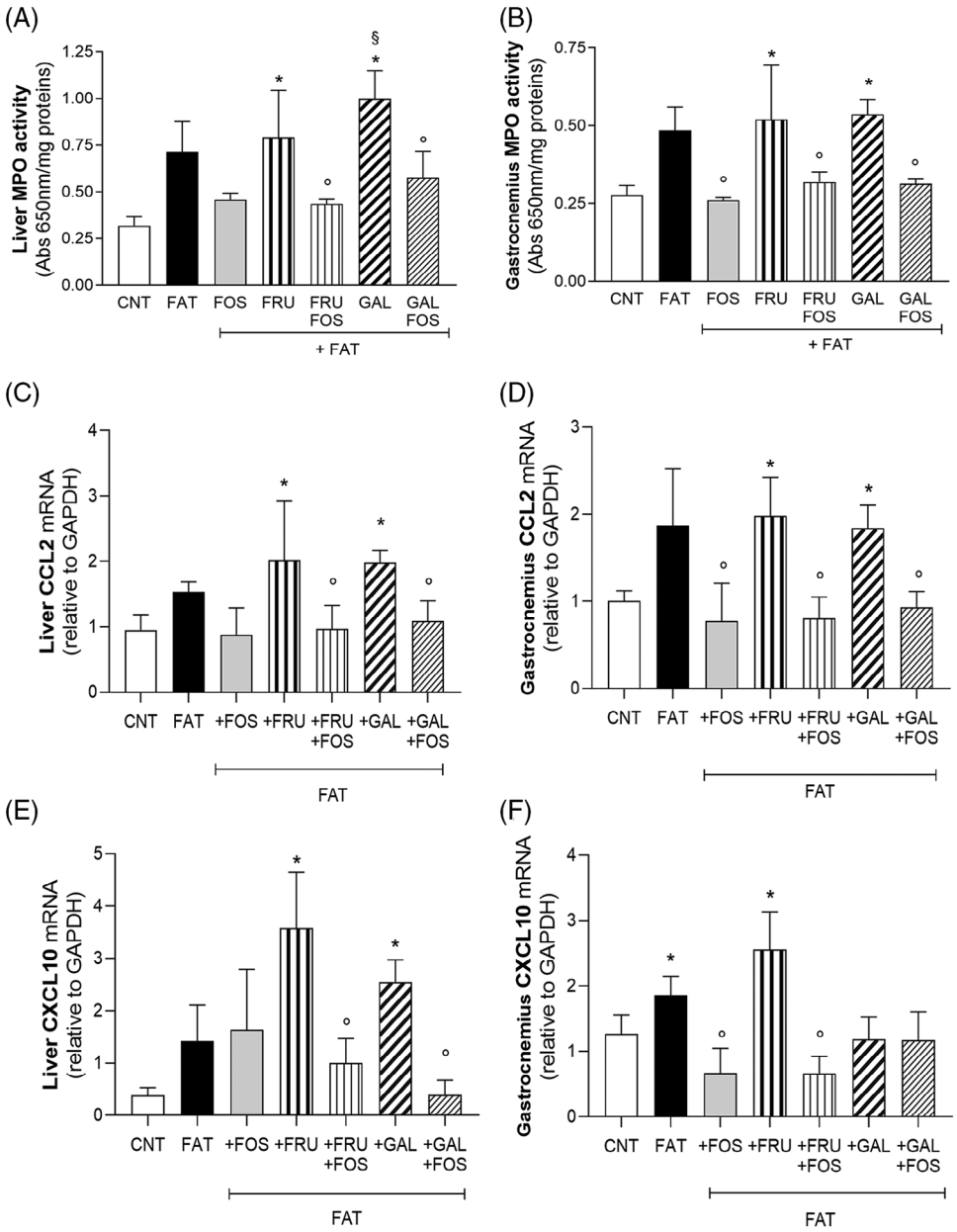

Figure 5. Myeloperoxidase (MPO) activity (A,B) and mRNA levels of CCL2 and CXCL10 in liver (C,E) and gastrocnemius muscle (D,F). Data are expressed as mean ± SD for $n = 6$ rats per group. * versus CNT. ° versus FAT, FAT+GAL or FAT+FRU.

activity significantly differed from that in the control groups when diets were enriched in either fructose or galactose; FOS supplementation counteracted the MPO activation induced by the sugars. We also documented similar changes in the local accumulation of the chemoattractant chemokines CCL2 and CXCL10, which are known to contribute to the T-cell recruitment to sites of inflammation. Although virtually all cell types may release chemokines, innate and adaptive immunity cells, including polymorphonuclear neutrophils, represent the major source of them and chemokines derived by neutrophils, including CCL2 and CXCL10, may exert key role in shaping the innate and adaptive immune response.[23] Our data confirm previous findings supporting the hypothesis that although both hepatocytes and myocytes may contribute to the local inflammatory state, the majority of pro-inflammatory mediators are secreted by the immune cells infiltrating the tissue, which are, in turn, further attracted by the overaccumulation of chemokines.[24] The recently documented role of simple sugars in suppressing immuno-protective gut microbiota may also contribute to the development of local inflammation here documented. In fact, dietary sugars can increase the inflammatory tone of the intestine indirectly by depleting intestinal microbes that regulate the protective functions of intestinal immune cells, mainly CD4 T cells subtypes, such as T helper 17, which are known to contribute to the protection from obesity and metabolic syndrome.[17] These changes in the gut microbiota composition, due to exposure to different

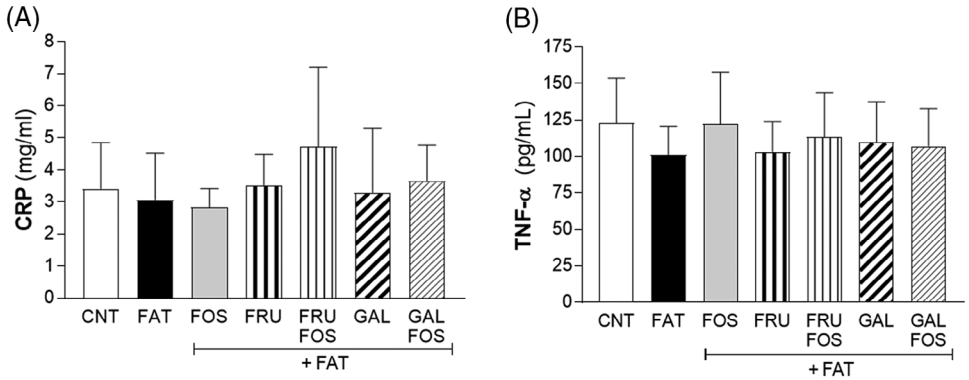

Figure 6. Effects of the diets with and without fructooligosaccharides (FOS) on CRP (A) and TNF-α (B) plasma levels in rats at the end of the 8 weeks' study. Data are expressed as mean ± SD for $n = 6$ rats per group. * versus CNT. °versus FAT. FAT+GAL or FAT+FRU. § versus FAT.

macronutrients, may correlate with markers of metabolic inflammation in specific organs. For instance, a recent in vivo study[25] demonstrated that CCL2 was significantly upregulated in the skeletal muscle of mice fed a high-fat high-sugar diet when compared with mice fed with a diet enriched in fat only and the same authors showed specific correlations between dietary sugar-driven changes in specific bacterial genera abundance and several markers associated with glucose metabolism and inflammation. As a matter of fact, in our study we did not find significant changes in circulating inflammatory markers, namely CRP and TNF-α, among the different groups, suggesting more of an organ-specific inflammatory tone rather than a systemic inflammation. The lack of parallelism between the systemic and tissue inflammatory profile could be due to the mild impact of the dietary insult we used, which may only evoke early markers of local metabolic inflammation. Our findings are in agreement with previous studies showing that the local recruitment and degranulation of neutrophils is a primary event preceding the release of systemic mediators and acute-phase proteins.[26]

Interestingly, we found that the intake of either fructose or galactose was enough to evoke accumulation of AGEs, which may be due not only to the well-known non-enzymatic Maillard reaction, but also to an accelerated glycolysis. In fact, entering the glycolytic process, both galactose and fructose lead to the production of the glycolysis reactive intermediates dihydroxyacetone phosphate and glyceraldehyde-3-phopshate.[27] These intermediates non-enzymatically generate increased concentrations of glyoxal (GO) and methylglyoxal (MGO), which are the specific precursors of the AGEs detected in our experimental conditions.[28] The sugars-induced AGEs overaccumulation was associated with an upregulation of the AGE receptor RAGE. RAGE is a member of the immunoglobulin superfamily and the binding of AGEs to RAGE mediates cellular responses, including innate immune response, promoting chemotaxis, and the secretion of inflammatory mediators.[29] We and others have previously described the crucial role of the AGE-RAGE pathway as a key cascade at the interface between inflammation and the development of metabolic derangements[30,31] For instance, AGEs induce a deregulated activation of triacylglycerol and cholesterol synthesis interfering through multiple mechanisms on the transcriptional activity of the SREBP lipogenic transcription factors, resulting in intracellular lipid accumulation with reduced muscle efficiency and hepatic fibrosis.[32,33] Thus, the sugar-induced exacerbation of lipid derangements, that we observed mainly in the liver, might be explained at least in part by the related accumulation of AGEs with relevant consequences at systemic levels, as documented by significant increase in plasma cholesterol as well as in AST and ALT concentrations when either fructose or galactose were added to the diet. Interestingly, the administration of FOS resulted in significant improvement of the diet-induced metabolic derangements at both the systemic and tissue levels. FOS supplementation has already been demonstrated to lower serum and liver lipids, as well as markers of metabolic inflammation, thus resulting in improvement of diet-induced liver steatosis and inflammation.[34] Here, for the first time, we also demonstrated that the supplementation with the prebiotic FOS effectively prevented AGEs accumulation and RAGE overexpression, thus highlighting a new perspective in the molecular mechanisms underlying the local protective effects of FOS. We previously reported alterations in microbiota composition caused by the diet, which may lead to the selection of specific microbial populations that use AGEs as energy fuel and affect gut epithelial barrier integrity, thus facilitating the local accumulation and systemic spread of either diet-derived or dietary AGEs.[35] Accordingly, clinical studies have shown that prebiotics may restore microbial balance within the gastrointestinal tract by interfering with the absorption and/or production of AGEs by the human host.[36] Hence, the restoration of microbial population diversity by prebiotics, including FOS, and the preservation of gut barrier function may prevent the formation or accumulation of AGEs in the intestinal lumen and most of all their distribution to distal organs to participate in inflammation and dysmetabolism.

We thus propose here that the positive outcomes of FOS supplementation to the sugar-enriched diets may at least partly be due to its ability to interfere with the process of protein glycation and, thus, AGEs accumulation. This is one of the first studies specifically aimed to compare the metabolic effects evoked by two of the most widely consumed simple sugars, fructose and galactose, and the impact of dietary fibers supplementation in mitigating these effects. The experimental protocol used allowed us, for the first time, to demonstrate that the supplementation with the prebiotic FOS effectively prevented sugars-induced AGEs accumulation and RAGE overexpression, thus highlighting a new perspective in the molecular mechanisms underlying

the local protective effects of FOS. Nevertheless, we acknowledge that other mechanisms might also be involved, since FOS administration protected against lipid accumulation and inflammatory responses also when rats were fed a Westernized diet enriched in fat only and in the absence of AGEs accumulation and RAGE upregulation. Furthermore, the intake of fructose and galactose in our rats was approximately 23% of the energy intake and thus exceeds that reported for a US population, which was around 10% of the daily energy (ca. 49 g d^{-1}) for fructose[4] and much lower for galactose (up to only 3.6 g d^{-1}).[7] However, the observed protective effects of FOS consumption, even in the presence of high intakes of simple sugars, strengthens the important role of fermentable dietary fibers for human health and further corroborates evidence from clinical studies[37] and support dietary recommendations for a high intake of fiber.

In conclusion, our results confirm the role of fructose and galactose as important triggers of diet-induced AGEs production and metabolic derangements that may act in synergy when added in high, yet nutritionally relevant amounts, to a Westernized diet high in fat. Furthermore, our findings show that the concurrent administration of the prebiotic FOS to a high-fat high-sugar diet exhibits protective effects against AGEs accumulation, thus, preventing hepatic steatosis and inflammation, which supports the importance of dietary fibers in the prevention of cardiometabolic diseases and their risk factors.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

F.A., D.C., J.F., and M.C. contributed equally to this work. J.F., M.C., R.L.: Conceptualization; J.F. and M.C.: supervision, writing, reviewing and editing. D.C. and F.A.: Investigation, data curation, writing and original draft preparation. R.M., E.A., N.S., F.D.B.: Investigation, data collection, and analysis. M.A., C.E., R.L.: Supervision. Validation, critical review and revision. All of the authors revised the manuscript and approved the final version.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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