Effects of High Intakes of Fructose and Galactose, with or without Added Fructooligosaccharides, on Metabolic Factors, Inflammation, and Gut Integrity in a Rat Model

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Scope: A high fructose and galactose intake show adverse metabolic effects in animal models and in humans, but it is yet unknown if addition of fermentable dietary fiber can mitigate such effects. This study investigate the effects of high intakes of fructose and galactose, with/without added fructooligosaccharides (FOS), on metabolic factors, inflammation, and gut integrity markers in rats.

Methods and Results: Rats (*n* = 6/group) receive different carbohydrates at isocaloric conditions for 12 weeks as follows: 1) starch (control), 2) fructose, 3) galactose, 4) starch + FOS (FOS control), 5) fructose + FOS, and 6) galactose + FOS, together with a high amount of n-6 polyunsaturated fatty acids (n-6 PUFA) in all diets except for in 7) starch + olive oil (negative control). The rats fed the galactose and galactose + FOS diets exhibit lower body weight than other groups. High-galactose diets has more pronounced effects on metabolic factors and gut permeability than high-fructose diets. High-fructose diets show less pronounced effect on these selected markers. No differences in inflammatory markers are detected for any of the diets.

Conclusions: The results suggest potential adverse effects of high galactose and fructose on metabolic factors and gut integrity markers, but not on inflammation. However, several mechanisms are at play, and general net effects are difficult to determine conclusively for the conditions tested.

1. Introduction

Global intake of sugar from foods and beverages has increased in recent decades,[1,2] accompanied by increased incidence of obesity, diabetes, and cardiovascular disease (CVD).[3] Sugars represent a direct source of energy and high intake of sugar can cause negative effects on health. Numerous studies have shown that large amounts of dietary sugars are associated with risk factors of cardiometabolic disease, including weight gain, dyslipidemia, glucose intolerance, insulin resistance, and hypertension.[4,5] The term "sugars" refers to monosaccharides and disaccharides. Among the monosaccharides, glucose and fructose are the most common, and are found in fruits and vegetables.[6] Galactose is mainly found in milk and other dairy products but can also be found in vegetables and fruits such as celery and cherries.[7] Sucrose, lactose, and maltose are the most common disaccharides found in most foods.[8] Glucose, fructose,

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and galactose are mainly consumed in the form of sucrose (table sugar), high-fructose corn syrup, and lactose.[8] Intake of sugars from fruits is an unavoidable part of a healthy diet, but added sugars can make the diet unhealthy. There is currently no clearly defined threshold at which sugar intake exerts negative health effects in humans.[9]

Both human and animal studies have shown adverse effects of high fructose intake and increased risk of development of metabolic syndrome, type 2 diabetes, and CVD.[10,11] High fructose intake leads to impairment of glucose tolerance, reduced insulin sensitivity, hypertriglyceridemia, and hypertension in animal models.[3,12] High fructose intake may also result in stimulation of hepatic de novo lipogenesis, which plays a significant role in the development of non-alcoholic fatty liver disease.[13,14] Moreover, there is evidence that high intake of fructose increases inflammation through activation of the nuclear factor-κB (NF-κB) signaling pathway and production of pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6).[15,16]

Galactose ingested in high amounts or low galactose degradation capacity is known to cause galactosemia, resulting in oxidative stress and cognitive and motor impairment.[17,18] In animal research, galactose-induced mimetic aging is an established aging model. Fructose and galactose are both reducing sugars and therefore react with amino groups, which leads to enhanced production and accumulation of advanced glycation end products (AGEs) and reactive oxygen species.[19,20] Such compounds contribute to pathological processes of age-related diseases, such as diabetes, atherosclerosis, and neurodegeneration.[21,22] Human studies have consistently demonstrated that AGEs can increase oxidative stress, impair endothelial function, and trigger inflammatory responses through activation of NF-kB, which up-regulates TNF-α, IL-6, and interleukin-1β (IL-1β) expression.[23,24]

On the other hand, intake of complex carbohydrates, such as fructooligosaccharides (FOS), a fermentable dietary fiber, has been associated with improved glycemic control and reduced risk of chronic diseases, such as obesity, diabetes mellitus, and CVD.[25,26] Gut fermentation of FOS results in production of short-chain fatty acids (SCFA), including acetate, propionate, and butyrate.[27] Beneficial effects of SCFA on health parameters include reduced inflammation and enhanced function of immune cells and the intestinal barrier.[28] A Western-type diet rich in sugars and low in dietary fiber has been shown to lead to negative metabolic effects and increased low-grade systemic inflammation as a result of impaired gut barrier integrity.[29]

In the present study, we tested the hypothesis that intake of simple sugars, such as fructose and galactose, in high amounts has pro-inflammatory and adverse metabolic effects, whereas a similar diet with added FOS, which stimulates extensive fermentation in the gut, could mitigate these adverse effects when administered as part of a diet with similar macronutrient profile. A high dose of sugars (50%), which has been shown to affect metabolic factors and inflammation in previous studies, was used [30–32] along with a high concentration of n-6 polyunsaturated fatty acids (n-6 PUFA) as part of fat in the diet, to trigger inflammation. We investigated the impact of high-sugar diets with and without additional FOS, on metabolism and low-grade systemic inflammation, through assessment of metabolic factors,

inflammatory biomarkers, and gut barrier function in a 12 week intervention study performed on rats.

2. Experimental Section

2.1. Animals and Diets

The intervention study was performed at the Institute of Nutritional Sciences, University of Hohenheim, Germany. 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 were approved by the Regional Council Stuttgart (Baden-Württemberg, Germany; trial no. V 351-18 BC).

Healthy male Sprague-Dawley rats ($n = 90$) were purchased at 7 weeks of age from Janvier Labs and had initial body weight (BW) of 250–274 g. The rats were randomly divided into seven groups of 12 animals and one baseline control group of six animals, and their metabolic status was assessed at the beginning of the experiment. Each experimental group was provided with different carbohydrates at isocaloric conditions as follows: 1) starch (control) (61.5 g/100 g), 2) fructose (50 g/100 g), 3) galactose (50 g/100 g), 4) starch + FOS (FOS control) (starch: 54 g/100 g, FOS: 15 g/100 g), 5) fructose + FOS (fructose: 50 g/100 g, FOS: 15 g/100 g) and 6) galactose + FOS (galactose: 50 g/100 g, FOS: 15 g/100 g), all together with a high amount of n-6 PUFA from safflower oil (6 g/100 g) (Sigma Aldrich), and 7) starch + olive, a negative control group that received the starch diet but with the n-6 component supplied as olive oil (**Table 1**). No information was found about the exact n-6 PUFA amounts in the olive oil provided. However, many studies have reported that olive oil contains low amounts of n-6 PUFA, typically in the range of 8–20%,[33,34] therefore olive oil was used as a control to assess the effect of high n-6 PUFA.

The starch control (group 1) was used as the basic control, since starch was added to all groups in varying amounts to obtain isocaloric conditions in all cases. The starch used was native potato starch (The Carl Roth GmbH+Co.KG, Germany), starch + FOS (group 4, FOS control) was used as a control for comparisons of groups where FOS was added to the sugars. FOS was inulin-type (DP4-5, MW: 624–679 from chicory root with 95% purity, Boneo GmbH, Germany). Starch + olive (group 7) was used as a control group to assess the effect of higher concentrations of n-6 PUFA, which were added as part of the fat in the diet of all other groups to trigger low-grade inflammation.

The rats were housed in a climate-controlled room (20–22 °C, 50 ± 10% relative humidity) with 12-h light/12-h dark cycle and fed the starch diet (control) during a 2 week adaptation period. The baseline control group was sacrificed at day 0. Six out of 12 animals per group were fed their respective diet for 6 weeks and the other six animals were fed for 12 weeks. Water was available ad libitum.

Energy intake and BW were recorded weekly. At the end of the respective feeding period, the rats were fasted for 12 h, anesthetized with carbon dioxide gas, and killed by decapitation. Blood was collected into heparinized monovettes (Monovette, Sarstedt, Germany), and feces and intestinal contents into Eppendorf tubes. Tissues (liver, kidney, brain, and small and large intestines) were excised, placed in cryotubes, and snap-frozen

Table 1. Macronutrient and micronutrient composition of the seven experimental diets.

	Starch (control)		Fructose		Galactose		Starch + FOS (FOS Control)		Fructose + FOS		Galactose + FOS		Starch + olive (negative control)	
	g	kcal	g	kcal	g	kcal	g	kcal	g	kcal	g	kcal	g	kcal
Carbohydrate														
Starch	61.5	62.0	11.5	11.6	11.5	11.6	54.0	56.5	4.0	4.2	4.0	4.2	61.5	62.0
Fructose	-	-	50.0	50.4	-	-	-	-	50.0	52.4	-	-	-	-
Galactose	-	-	-	-	50.0	50.4	-	-	-	-	50.0	52.4	-	-
FOS	-	-	-	-	-	-	15.0	7.9	15.0	7.9	15.0	7.9	-	-
Cellulose	12.5	6.3	12.5	6.3	12.5	6.3	5.0	2.6	5.0	2.6	5.0	2.6	12.5	6.3
Protein	18.0	18.1	18.0	18.1	18.0	18.1	18.0	18.9	18.0	18.9	18.0	18.9	18.0	18.1
Fat														
Safflower oil	6.0	13.6	6.0	13.6	6.0	13.6	6.0	14.1	6.0	14.1	6.0	14.1	-	-
Olive oil	-	-	-	-	-	-	-	-	-	-	-	-	6.0	13.6
Minerals and vitamins	2.0	-	2.0	-	2.0	-	2.0	-	2.0	-	2.0	-	2.0	-
Total	100 g	100 kcal	100 g	100 kcal	100 g	100 kcal	100 g	100 kcal	100 g	100 kcal	100 g	100 kcal	100 g	100 kcal

FOS, fructooligosaccharides; kcal, kilocalorie.

in liquid nitrogen. Plasma and serum were separated from the blood cells as soon as possible after collection. All samples were immediately stored at −80 °C until further analysis.

After 2 weeks of the 12 week intervention, the dietary regime for the galactose and galactose + FOS groups was modified in response to adverse effects (polyuria and lens opacity) observed in the rats in these two groups. The rats in these groups were provided with the intervention diet for 4 days, followed by 3 days on starch (control) diet, from week 3 through 12 of the study. During the study, only one rat (from starch + FOS group) died, due to volvulus.

2.2. Biochemical Analyses

Plasma AGEs, uric acid, creatinine, urea, alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), triglycerides, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein/very low-density lipoprotein (LDL/VLDL), C-reactive protein (CRP), zonulin, endotoxin, and serum insulin were measured.

Insulin (E-EL-R2466), CRP (E-EL-R0022), and creatinine (E-EL-0058) were analyzed using commercial sandwich enzyme-linked immunosorbent assays (ELISA) and urea (E-BC-K183). ALT (E-BC-K235), ALP (E-BC-K092), AST (E-BC-K236), uric acid (E-BC-K016), and triglycerides (E-BC-K238) were analyzed using a colorimetric method purchased from Elabscience Biotechnology Inc., UK. Cholesterol assay kits for assessment of HDL and LDL/VLDL colorimetric assay kits (ab65390) were purchased from Abcam plc., UK. Zonulin (MBS747447) and endotoxin (MBS2606662) were analyzed using ELISA (MyBiosource, Inc., USA). All measurements were conducted according to the manufacturer's instructions. Homeostasis model assessment-estimated insulin resistance (HOMA-IR) was calculated according to the formula: fasting insulin (uIU dL^{-1}) × fasting glucose (mg dL^{-1})/405.[35]

AGEs in plasma were measured by ultra high-performance liquid chromatography (UHPLC)-tandem mass spectrometry,[22]

and Nε-(carboxymethyl)lysine (CML), pentosidine, and lysine were quantified. In brief, 50 µL of sample were hydrolyzed with 500 µL of 0.6 M trichloroacetic acid and 50 mL 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; pentosidine (m/z) 379@316, DP 120 V, EP 11 V, CE 33 V; and lysine (m/z) 147@84, DP 80 V, EP 8 V, CE 21 V. Quantification of analytes was performed using CML, pentosidine, and lysine analytical standard calibration curves 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%.

2.3. Statistical Analysis

Statistical analysis was carried out using SAS statistical analysis software (release 9.4; SAS Institute, Cary, NC, USA). Changes in BW over the 12 week study period were analyzed by repeated-measures analysis of variance (ANOVA). Differences between diets were evaluated by one-way ANOVA, followed by Tukey's multiple comparison test. Effect of diet after 12 weeks was the primary outcome. If effects of specific diets were observed
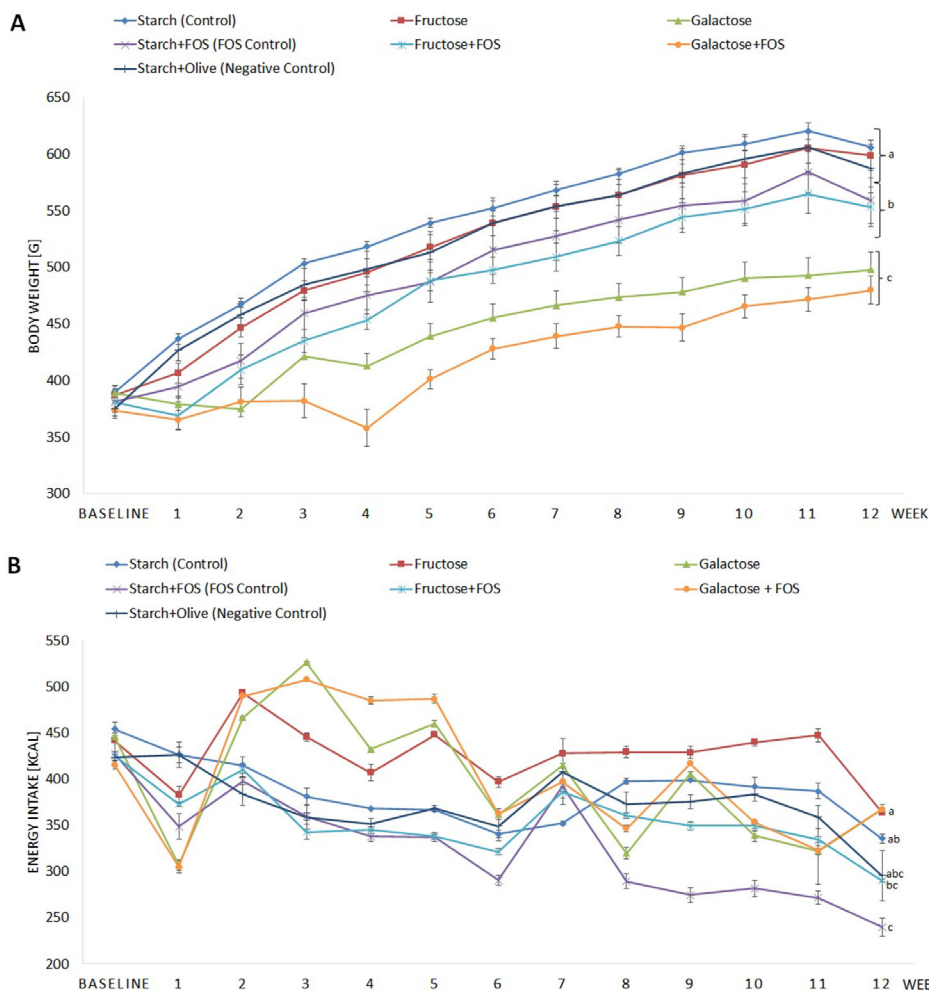
Figure 1. Effects of high-carbohydrate diets (fructose and galactose), with and without fructooligosaccharides (FOS), on A) body weight (g) and B) energy intake (kcal) in rats during the 12 week study. Body weight and energy intake analyzed by repeated-measures. ANOVA with Tukey's test was used to compare differences between groups. Means with different superscripts (lowercase letters) are significantly different ($p < 0.05$).

after 12 weeks, a secondary analysis was conducted to evaluate whether effects were evident already after 6 weeks. Assumption of normality and homogeneity of variance were tested using the Shapiro–Wilks test. Data not normally distributed were log-transformed prior to analysis. Values of $p < 0.05$ were considered significant. All results are presented as least square (LS) means ± standard error of the mean (SEM), adjusted for baseline values obtained from the baseline group to nullify pre-existing differences.

3. Results

3.1. Effects of Diet on Energy Intake and BW

After 12 weeks of ad libitum isocaloric food intake, rats on the galactose and galactose + FOS diets (groups 3 and 6) had significantly lower BW than the other groups (**Figure 1**A). These differences became evident after 3 weeks of intervention. The galactose and galactose + FOS groups had lower energy intake at the beginning of the experiment, but higher energy intake than the other

groups between 3 and 6 weeks (Figure 1B). Despite the higher energy intake after 12 week intervention, the rats in the galactose and galactose + FOS groups gained less weight than the other groups. Moreover, higher energy intake was observed in the fructose than starch + FOS (FOS control) and fructose + FOS groups, but no significant difference was observed in BW changes.

### 3.2. Effects on Blood Glucose and Insulin

No significant difference was found in blood glucose concentration between the groups after 12 weeks. Significantly higher insulin concentration was observed in the galactose + FOS group compared with the starch + FOS (FOS control) group after 12 weeks. Insulin concentration was also higher in the galactose + FOS group compared with the starch (control), fructose + FOS, and starch + FOS (FOS control) groups after 6 weeks (data not shown). HOMA-IR concentration was significantly higher in the fructose group than in all other groups except fructose + FOS and galactose + FOS (**Table 2**).

Table 2. Effects of high-carbohydrate diets (fructose and galactose), with and without additional fructooligosaccharides (FOS), on metabolic factors in rat plasma after 12 weeks.

	Diets						
	Starch (control)	Starch + olive (negative control)	Starch + FOS (FOS control)	Fructose	Fructose + FOS	Galactose	Galactose + FOS
Blood glucose [mg dL^{-1}]	61.67 ± 5.70	54.17 ± 2.10	64.00 ± 7.13	73.50 ± 7.59	70.00 ± 7.39	55.00 ± 4.86	54.00 ± 3.52
Insulin [ng mL^{-1}][a])	6.33 ± 0.82[ab]	5.42 ± 0.57[ab]	4.50 ± 0.57[b]	6.62 ± 0.70[ab]	6.74 ± 0.79[ab]	6.14 ± 0.41[ab]	7.89 ± 0.35[a]
HOMA-IR [mg dL^{-1}]	20.27 ± 0.98[b]	18.18 ± 2.23[b]	17.30 ± 2.33[b]	29.78 ± 4.02[a]	28.46 ± 3.86[ab]	20.8 ± 2.55[b]	25.87 ± 1.49[ab]

HOMA-IR, homeostasis model assessment-estimated insulin resistance. [a]) Measured in serum. Values shown are LS mean ± SEM of six rats. Groups were compared by one-way ANOVA followed by Tukey's test. LS mean with different superscripts (lowercase letters) differ significantly ($p < 0.05$).
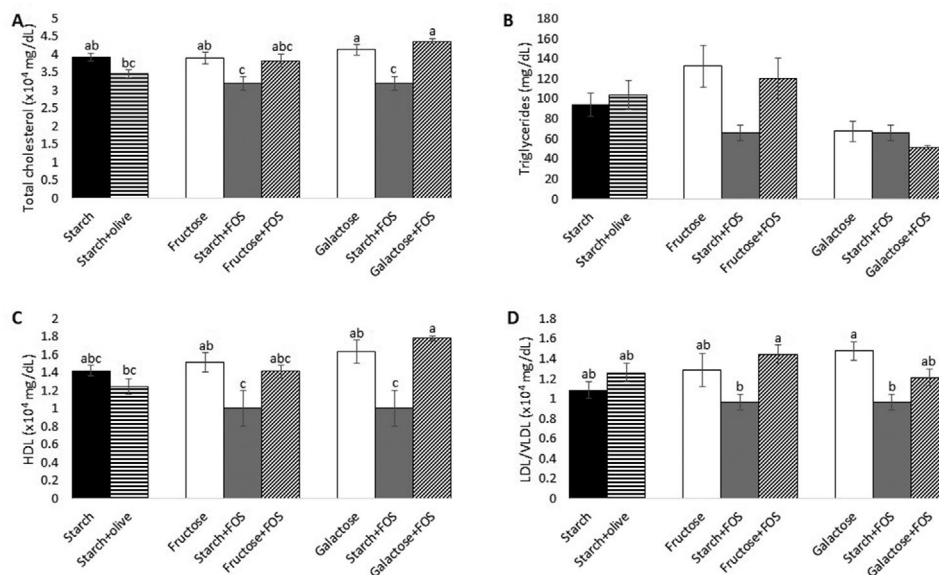
Figure 2. Effects of high-carbohydrate diets (fructose and galactose), with and without fructooligosaccharides (FOS), on lipid profiles in rat plasma after 12 weeks. A) Total cholesterol, B) triglycerides, C) high-density lipoprotein (HDL), and D) low-density lipoprotein/very low-density lipoprotein (LDL/VLDL). Values shown are LS mean ± SEM of six rats. Groups were compared by one-way ANOVA followed by Tukey's test. LS means with different superscripts (lowercase letters) differ significantly ($p < 0.05$).

3.3. Effects on Lipid Profile

Plasma cholesterol concentrations were significantly higher in the galactose and galactose + FOS groups than in the starch + FOS group after 12 weeks (**Figure 2**A). However, lower total cholesterol concentration was observed in the galactose + FOS and starch + olive (negative control) groups than in other groups at 6 week (data not shown).

Plasma HDL concentration was significantly higher in the galactose + FOS group compared with the starch + FOS (FOS control) after 12 weeks (Figure 2C). Significantly higher HDL concentration was also observed in the galactose and galactose + FOS groups than in all other groups except fructose group at 6 week (data not shown). Moreover, LDL/VLDL concentration was significantly higher in the fructose + FOS and galactose groups than in the starch + FOS (FOS control) group (Figure 2D). At 6 week, LDL/VLDL concentration was significantly higher in the galactose group than in the other groups (data not shown). In general, no clear pattern was observed for total cholesterol and
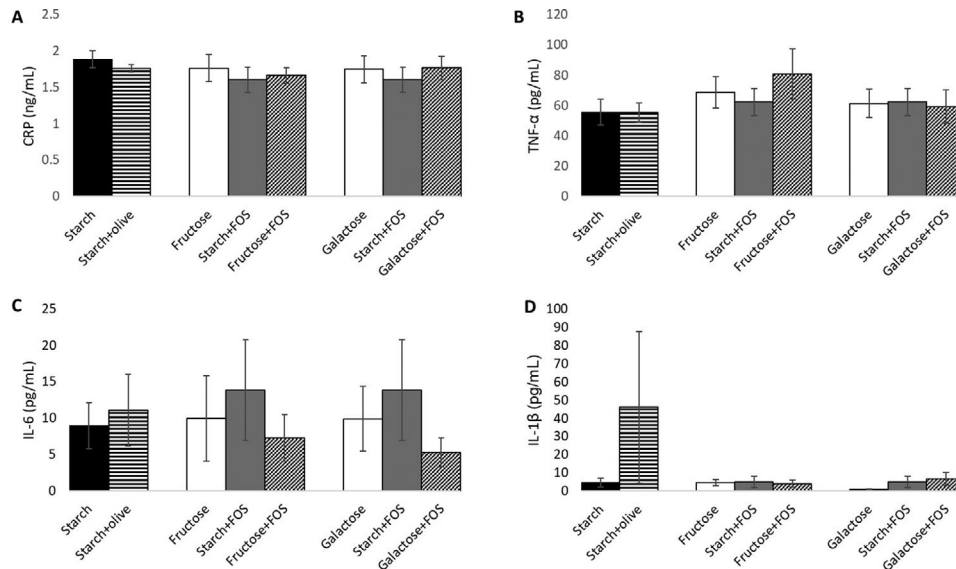
HDL concentration with addition of FOS to the diet. However, a decreasing trend in LDL/VLDL concentration was seen in the galactose + FOS group. No difference between diets was observed for triglyceride concentration after 12 weeks (Figure 2B).

3.4. Effects on Inflammatory Biomarkers

No significant differences between the diets were found for any of the inflammation biomarkers analyzed, i.e., CRP, TNF-α, IL-6, and IL-1β, after 12 weeks (**Figure 3**).

3.5. Effects of Diet on AGEs—Inflammation-Related Markers

Plasma concentrations of CML were significantly higher in the galactose and starch + olive (negative control) groups than in the starch (control), starch + FOS (FOS control), and fructose + FOS groups after 12 weeks. Significantly higher pentosidine

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Figure 3. Effects of high-carbohydrate diets (fructose and galactose), with and without fructooligosaccharides (FOS), on pro-inflammatory biomarkers in rat plasma after 12 weeks. A) C-reactive protein (CRP), B) tumor nuclear factor-alpha (TNF-α), C) interleukin-6 (IL-6), and D) interleukin-1β (IL-1β). Values shown are LS mean $\pm$ SEM of six rats. Groups were compared by one-way ANOVA followed by Tukey's test. LS means with different superscripts (lowercase letters) differ significantly ($p < 0.05$).

concentration was found in the galactose group compared with the starch (control), starch + FOS (FOS control), and fructose + FOS groups. Moreover, both CML and pentosidine concentrations were significantly higher in the starch + FOS (FOS control) group than in other groups at 6 week (data not shown). In general, lower CML and pentosidine concentration were observed in both the fructose and galactose groups after treatment with FOS (**Figure 4**A,B).

Lysine concentration was significantly higher in the galactose + FOS group compared with all other groups except starch + olive (negative control) and starch + FOS (FOS control) (Figure 4C). At 6 week, lysine concentration was significantly higher in the starch + olive (negative control) group than in all other groups except starch + FOS (data not shown). Overall, lysine concentration increased for the fructose and galactose diets with added FOS.
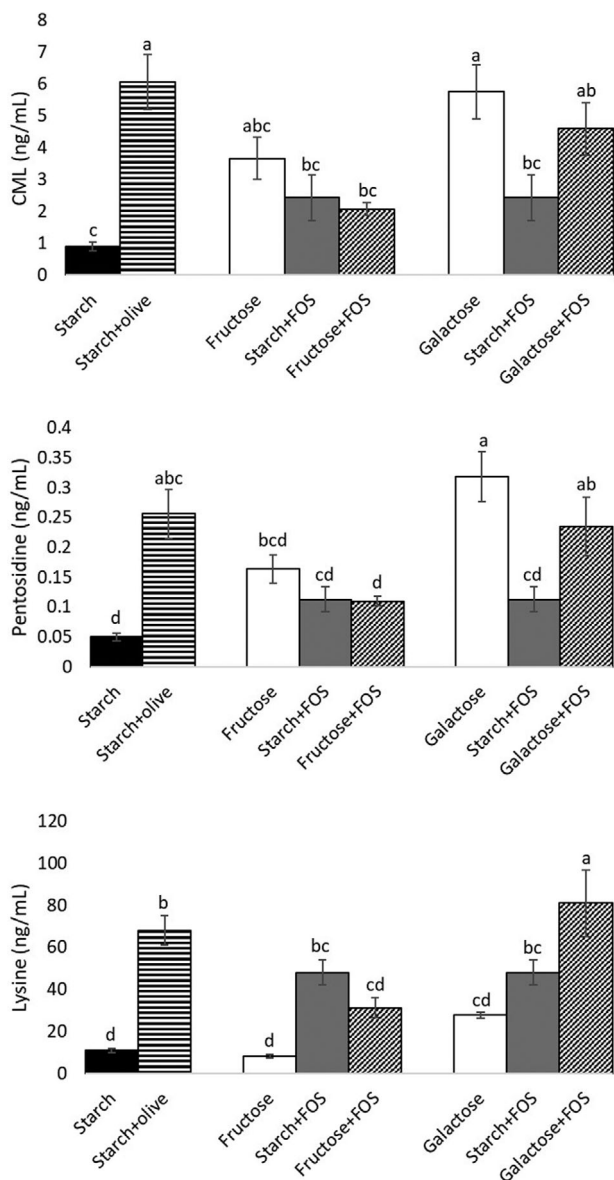
3.6. Effects on Intestinal Permeability Parameters

Significantly higher zonulin concentration was observed in the galactose + FOS group compared with the fructose and starch + olive (negative control) groups after 12 weeks. Endotoxin concentration was significantly lower in the galactose group than in the starch (control) and starch + olive (control) groups (**Figure 5**). At 6 week, zonulin concentration was significantly higher in the galactose and galactose + FOS groups than in the starch (control), starch + FOS (FOS control), and fructose groups, whereas endotoxin concentration was significantly lower in the galactose + FOS than fructose + FOS group (data not shown).

4. Discussion

This study investigated the effects of high intake of fructose and galactose, with and without additional FOS, on metabolic factors and inflammatory and gut integrity markers in rats after a

12 week intervention. A high amount of the omega-6 linoleic acid was added in all diets except starch + olive (negative control), to elicit low-grade inflammation.[36] Numerous studies have investigated the effects of diets rich in simple carbohydrates on metabolic factors and inflammation.[37] To our knowledge, this is the first animal study to evaluate the impact of high-fructose and high-galactose diets to which FOS had been added to mitigate the adverse effects of these simple sugars. In general, the intervention diets affected several metabolic factors and gut integrity markers, but no significant changes in inflammatory markers were found.

The results showed that a high-galactose diet (with or without FOS) resulted in a smaller increase in BW between weeks 3 and 12 of intervention than the other diets tested. In addition, clinical symptoms were manifested in rats in the galactose and galactose + FOS groups, with most rats in these two groups displaying polyuria and lens opacity after 2 weeks of intervention. Similarly, previous studies have shown that rats fed galactose doses corresponding to 50% of total energy in the diet did not gain weight normally.[38,39] The dose fed in those studies and in the present study (50%E) might have been high enough to induce a toxic effect of galactose,[40,41] which could have affected measurement of metabolic markers in our intervention. Another study found that intake of galactose at 12% of total energy for 3 weeks caused lower BW in rats, which was alleviated when galactose was reduced to 10% of total energy in the diet.[39] High intake of galactose is reported to result in a significant proportion of the galactose being excreted with the urine,[38,42] causing the rats fed the galactose and galactose + FOS diets in the present study to drink large amounts of water and to develop polyuria, due to the osmotic effect of galactose.[38,39] It has been shown that a high dose of galactose increases hepatic uridylation of galactose, leading to hypergalactosemia and loss of galactose into the urine.[43] The unmetabolized galactose is then converted into

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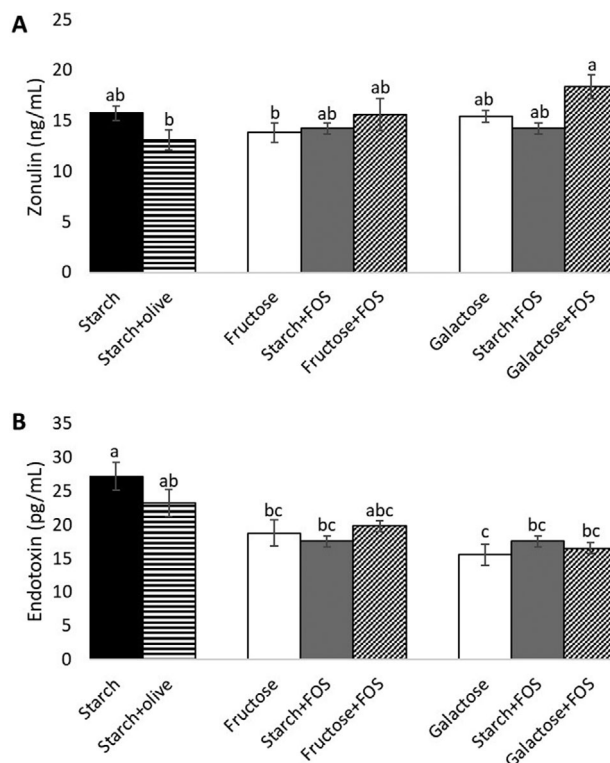**Molecular Nutrition
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Figure 4. Effects of high-carbohydrate diets (fructose and galactose), with and without fructooligosaccharides (FOS), on advanced glycation end products (AGEs) in rats. A) Nε-(carboxymethyl)lysine (CML), B) pentosidine, and C) lysine after 12 weeks. Values shown are LS mean ± SEM of six rats. Groups were compared by one-way ANOVA followed by Tukey's test. LS means with different superscripts (lowercase letters) differ significantly ($p < 0.05$).

Figure 5. Effects of high-carbohydrate diets (fructose and galactose), with and without fructooligosaccharides (FOS), on A) zonulin and B) endotoxin in rat plasma after 12 weeks. Values shown are LS mean ± SEM of six rats. Groups were compared by one-way ANOVA followed by Tukey's test. LS means with different superscripts (lowercase letters) differ significantly differ ($p < 0.05$).

galactitol by aldose reductase, which causes cataracts in animal models.[17,39,42]

Energy intake in the galactose and galactose + FOS groups was lower than in the other groups at the beginning of the intervention. However, after modification of the feeding plan for these two groups, the energy intake increased to reach a level similar to that of the other groups after 5 weeks. This may reflect the adaptation period of the galactose-fed rats to a new dietary regime.[38,44]

Adverse metabolic effects of high-sugar diets, particularly fructose and galactose, have been reported previously.[45,46] Both ani-

mal and human studies have shown that intake of fructose, compared with glucose, sucrose, and lactose, has only minor effects on blood glucose and insulin.[47] Similarly, little effect on fasting blood glucose was observed in rats fed the fructose diet that may be due to slow absorption rate and because fructose is first converted to glucose in the liver.[47] Galactose also has little impact on blood glucose due to hepatic metabolism and interference with glucose release from the liver, inducing hypoglycemia.[47,48] However, high insulin concentration was observed in rats fed the galactose + FOS diet and it could be explained by the incretin effect, i.e., insulin secretion induced by glucagon-like peptide-1 (GLP-1) and/or glucose-dependent insulinotrophic polypeptide (GIP).[49] It has been shown that galactose stimulates GIP secretion, and subsequently insulin secretion, in humans.[49,50] A diet with additional FOS could also contribute to increased incretin secretion, through gut fermentation.[51] Gut fermentation of FOS leads to increased SCFA production, which has been shown to enhance GLP-1 and GIP secretion by intestinal L-cells.[51] We measured the HOMA-IR index to assess insulin resistance.

We expected the diets high in sugar to alter concentrations of blood lipids, including total cholesterol, HDL, LDL/VLDL, and triglycerides, as reported in previous studies.[52,53] For example, it has been shown that a high-fructose diet may result in acute and chronic alteration of blood lipids,[54] and that a diet containing higher fructose than glucose can cause

lipid abnormalities, including increased total cholesterol, LDL, and triglyceride concentrations.[52] D-galactose treated mice have also been reported to demonstrate an increase in cholesterol concentration.[55] In line with previous findings, we observed higher total cholesterol concentration in rats fed the galactose and galactose + FOS diets than the starch + FOS (FOS control) diet. In other studies on both humans and animals, FOS has been reported to reduce cholesterol concentration,[56] so the increase we observed in total cholesterol concentration in the galactose + FOS group after the 12 week intervention remains unexplained. Many studies have reported decreases in HDL concentration after high fructose or high galactose intake.[52,55] In contrast, we observed a similar response in HDL as in total cholesterol concentration after high intake of fructose and galactose, for underlying reasons that are still unclear. Our results are in agreement with previous studies reporting unfavorable effects of high sugar intake in increasing LDL cholesterol [57] after a few weeks of high fructose and galactose intake.[57,58] Higher LDL/VLDL concentrations were observed in rats fed the fructose + FOS and galactose diets than the starch + FOS (FOS control) diet. Overall, a decreasing trend in LDL/VLDL concentration was observed in rats fed the galactose + FOS diet. Although numerous studies on rodents report higher plasma triglycerides due to lipogenesis induced by fructose intake,[54,59] our study did not support these findings. Reduced triglyceride concentration after ingestion of FOS and oligofructose due to inhibition of hepatic de novo fatty acid synthesis [56] could explain the trend for a lowering in triglyceride concentration observed after intake of FOS in this study.

The major AGEs from exogenous sources are methylglyoxal-hydroimidazole (MG-H1), CML, carboxyethyl-lysine, pentosidine, and pyrraline.[60] CML and pentosidine are commonly used as biomarkers of glycation processes.[61] Numerous studies have suggested that diets high in fructose and galactose increase AGE accumulation in different tissues.[19,62] Direct comparison of different carbohydrate dietary regimens on systemic AGE concentrations was performed for the first time in the present study. The results showed a tendency for higher concentrations of AGEs in rats exposed to the galactose diet compared with the fructose diet, despite lower weekly exposure to simple sugar in the galactose groups compared with the fructose groups. These findings may suggest higher reactivity of galactose in terms of evoking protein glycation compared with fructose in our experimental conditions. However, we cannot exclude the possibility that the high AGE concentrations detected in the galactose groups were a consequence of the toxic impact of galactose, which would confirm the role of AGEs as early biomarkers of sugar-induced changes in health status. We observed an increase in CML concentrations in the galactose and starch + olive (negative control) groups compared with the starch (control), starch + FOS (FOS control), fructose, and fructose + FOS groups, where systemic CML concentrations reached their highest level after 12 weeks. Pentosidine, a fluorescent lysine-arginine cross-linked AGE compound derived from pentose, was present at higher concentrations in plasma of the galactose group than in other groups in this study. Accumulation of both CML and pentosidine has been linked with pathological development such as chronic kidney disease and Alzheimer's disease.[63,64] Although pentosidine is typically formed from a pentose, treatment with D-galactose in mice for 8 weeks has demonstrated an accumulation of pentosidine

and CML in the brain.[65] Previous studies have suggested that high fructose and glucose concentrations also play an important role in facilitating the accumulation of pentosidine in the skin, aortic tracheal, and tendon tail collagen in rodents.[66,67] Lysine and arginine are major sites of glycation in many proteins, including hemoglobin and albumin. Antiglycation activity is usually assessed through estimation of carbonyl content and free lysine.[68] Significantly higher lysine concentration was found in the galactose + FOS group in this study. Overall, the analysis of AGE concentrations in groups of rats receiving different types of carbohydrates demonstrated a lowering of CML and pentosidine concentrations with added FOS.

The intestinal barrier plays a vital role in regulation of host immune function and health.[69] Zonulin, a protein involved in regulation of epithelial tight junctions in the small and large intestine, has been proposed as a biomarker of intestinal permeability.[70] Higher expression of zonulin has been found in the early stage of disease.[71] Higher expression of endotoxin in the blood indicates epithelial barrier dysfunction and promotes inflammatory processes.[72] Endotoxin, also known as lipopolysaccharides (LPS), is a major component of the outer membrane of Gram-negative bacteria. Fructose malabsorption promotes gut permeability, and higher fructose intake promotes gut inflammation and an accompanying rise in blood endotoxin concentrations due to endothelial dysfunction and decline of tight-junction proteins.[29,73] In this study, inconsistent changes in zonulin concentrations were observed in response to endotoxin expression in the plasma.

One potential reason for lower expression of zonulin and endotoxin after the 12 week intervention may be a protective effect of intestinal alkaline phosphatase (IAP). Emerging studies on the favorable effects of IAP in different organs in humans and animals have found that animal ALP consists of two classes: 1) tissue non-specific ALP, expressed in liver, kidney, bone, and plasma and 2) tissue-specific isozymes, including IAP, which play an important role in intestinal homeostasis and health through interaction with gut microbiota, diet, and gut.[74] In the intestine, IAP detoxifies endotoxin and protects the host from bacterial invasion by dephosphorylation of bacterial-derived LPS and is suggested to be a potential intestinal microbiota regulator.[75] Moreover, IAP is expressed and secreted by intestinal epithelium and remains biologically active in the intestinal lumen and mucosal membrane.[74] We analyzed ALP concentrations in plasma, and not in the intestine, and were therefore unable to confirm previous findings (Table S1, Supporting Information). However, it has been reported that around 1–2% of IAP is secreted into the blood circulation, which may widen its activity towards systemic infections.[74] Dietary fermentable fiber, including FOS, galactooligosaccharides, and raffinose, also increases colonic and intestinal activity.[76]

Our study has several limitations. First, the high dose of fructose and galactose used may have caused a mild toxic effect on the rats. Second, slight changes in the study design due to clinical symptoms as demonstrated in the rats fed the galactose and galactose + FOS diets made the interpretation of the results more challenging. Third, the results from the present rat study cannot be directly translated to humans. Nevertheless, this study has also several strengths. First, the study was large and compared the effects of different sugars with and without added fiber

under isocaloric conditions. Second, three control groups were included that allowed us to evaluate the effects of the sugars, sugar with added FOS, and the impact of added n-6 to evoke inflammation. Third, by using a rat model, we could control the conditions to minimize the environmental conditions that may otherwise have affected the results.

In summary, type of carbohydrate in the diet affects metabolic effects in a complex way and the net effects are difficult to predict. The results in the present study indicated that high-galactose and galactose + FOS diets have more severe adverse effects on metabolic factors, lipid profiles, AGEs, and intestinal permeability than high-fructose and fructose + FOS diets. However, the effects obtained for the galactose and galactose + FOS groups may have been confounded by the clinical symptoms manifested in these groups at the beginning of the intervention. We found no clear benefits of added FOS in the diet as a strategy to mitigate the adverse effects of high fructose and high galactose intake. Further studies should seek to determine the threshold dietary dose of galactose to avoid manifestation of clinical symptoms and the optimal dose for FOS to obtain significant beneficial effects. Measurement of IAP concentration could be included in future studies, as IAP plays an essential role in gut integrity, inflammation, and microbiota composition.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

N.A.M.O. designed and conducted the experiments, performed sampling and statistical analysis, interpreted the results, and wrote the manuscript. J.F. contributed to designing the study, conducting the experiments, and interpretation of results, and participated in manuscript writing. J.K. contributed to conducting the experiments. F.D.B. and C.M. contributed to sample analysis and interpretation of results. M.C. contributed to sample analysis, interpretation of results, and manuscript writing. G.Z., K.M., and A.W. contributed to designing the experiments and critically reviewed the manuscript. R.L. conceived the present study, contributed to interpretation of results and manuscript writing, and had overall responsibility for the study.

Data Availability Statement

Data available on request from the authors.

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