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In Sprague-Dawley Rats, Consumption of Fructose or Galactose as Part of a Westernized Diet Does Not Induce Overt Inflammation in the Liver or Intestine, Whereas the Intake of Fructooligosaccharide and Fructose May Contribute to Hepatic Inflammation

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ABSTRACT

High intake of galactose and fructose induces adverse metabolic effects, but it is unknown whether simultaneous consumption of fermentable fibers attenuates them. We investigated whether dietary fructose or galactose induce and if fructooligosaccharides (FOS) mitigate local inflammation. Sprague-Dawley rats (6/group) were fed for 8 weeks control (5% fat, 35% starch) or Westernized diets: high-fat (20% fat, 35% starch), FOS (20% fat, 30% starch, 10% FOS), fructose (20% fat, 10% starch, 25% fructose), fructose+FOS (20% fat, 5% starch, 25% fructose, 10% FOS), galactose (20% fat, 10% starch, 25% galactose), galactose+FOS (20% fat, 5% starch, 25% galactose, 10% FOS). Hepatic and intestinal cytokines (IL-1β, IL-6, TNF-α, IL-10), plasma lipopolysaccharides, intestinal zonulin-1 and occludin were measured. Westernized diets high in fructose or galactose did not affect intestinal inflammation, permeability, or integrity. FOS+fructose increased hepatic IL-1β compared to high-fat, fructose, and galactose, and IL-10 relative to galactose. FOS increased liver weight compared to control and, together with fructose, further elevated it compared to control and high-fat diets. Westernized diets high in fructose or galactose did not induce hepatic or intestinal inflammation. Unexpectedly, FOS supplementation with fructose exacerbated hepatic inflammation, suggesting potential adverse interactions of fructose with fermentable fiber.

1 | Introduction

Chronic low-grade inflammation is a major contributor to noncommunicable diseases, such as obesity, diabetes mellitus, and cardiovascular diseases [1]. A high intake of sugars, particularly in the forms of sucrose and high-fructose corn syrup, has been associated with an increased risk of morbidity and mortality due to noncommunicable diseases [2, 3]. Fructose is a monosaccharide that is absorbed in the small intestine and rapidly transported to the liver, where it is phosphorylated by fructokinase and enters glycolysis as fructose-1-phosphate, bypassing the key regulatory step catalyzed by phosphofructokinase. Therefore, high fructose intake shunts hepatic metabolism toward elevated production of lipogenic precursors, thereby increasing triacylglycerol synthesis, which may progress to hepatic steatosis and inflammation [4]. High fructose intake also induces gut dysbiosis, impairs

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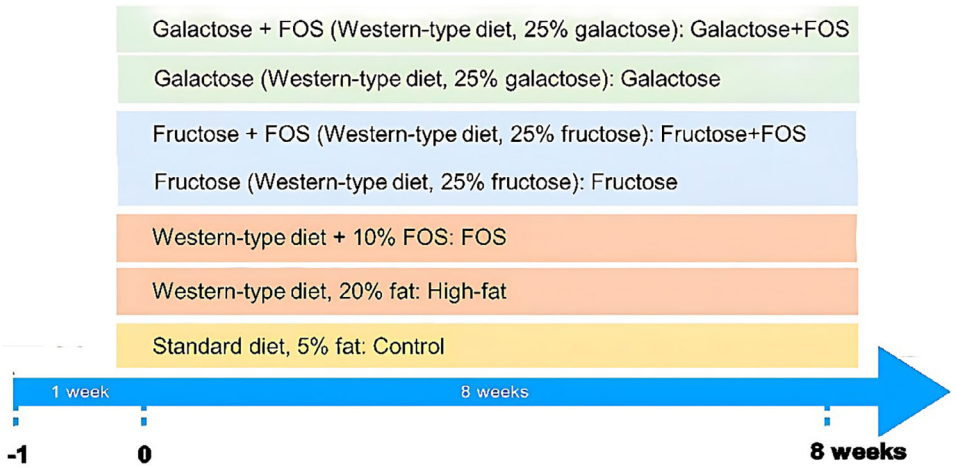


FIGURE 1 | Study design schematic for dietary interventions with sugars and added fructooligosaccharides (FOS) in rats. Sprague-Dawley rats (n=48) were acclimatized to standard chow for 12 days, then randomized to 7 groups (n = 6/group) for 8 weeks: control (5% fat, 35% starch); or isocaloric high-fat Westernized diet (20% fat, 35% starch), high-fat Westernized diet with FOS (20% fat, 30% starch, 10% FOS), fructose (20% fat, 10% starch, 25% fructose), fructose + FOS (20% fat, 5% starch, 25% fructose, 10% FOS), galactose (20% fat, 10% starch, 25% galactose), or galactose + FOS (20% fat, 5% starch, 25% galactose, 10% FOS); FOS: fructooligosaccharides.

intestinal barrier integrity, and increases intestinal permeability, allowing microbial products, such as lipopolysaccharide, to translocate into the circulation and exacerbate chronic low-grade inflammation [5]. Additionally, galactose is a ubiquitous simple sugar that is rapidly absorbed and primarily metabolized in the liver via the Leloir pathway where it is converted to glucose-1-phosphate and enters glycolysis or glycogenesis. However, at high doses, galactose metabolism results in an intermediate product, galactitol, that accumulates in the liver, resulting in hepatotoxicity and inflammation [6]. Galactose, a reducing sugar, readily forms advanced glycation end-products (AGE) that promote inflammation through receptor-mediated signaling pathways [6]. However, there is a research gap in understanding galactose metabolism at physiologically relevant high intakes, particularly with respect to its effects on chronic low-grade inflammation. Given these different metabolic pathways, fructose and galactose may have distinct metabolic and inflammatory effects.

The regular consumption of dietary fibers, on the other hand, may confer protective metabolic effects, such as lowering body weight and total cholesterol, and preserving gut function [7, 8]. However, little is known about the potential of simultaneously ingested dietary fibers to attenuate negative metabolic effects of sugar consumption. Our team previously conducted a rat trial and found that the intake of 50% galactose resulted in toxic effects, including polyuria, lens opacity, and reduction in body weight that were not attenuated by dietary fibers [9]. We have also previously shown that a Westernized diet high in fructose or galactose promotes dyslipidemia and hepatic lipid accumulation accompanied by increased serum concentrations of liver enzymes. The simultaneous consumption of FOS reduced these negative effects [10]. Systemic inflammation, however, was not observed in either trial [9, 10].

Therefore, the current work aimed to investigate whether the lipogenesis induced by a high but sub-toxic intake of fructose or galactose combined with a high-fat diet [10] progressed to local inflammation in the liver and small intestine, the main organs contributing to sugar homeostasis [11], specifically through activation of cytokine-mediated inflammatory pathways. We further explored potential protective effects of the intake of fermentable FOS.

2 | Materials and Methods

2.1 | Animals and Dietary Intervention

All animal procedures were in accordance with the Federation of European Laboratory Animal Science Association guidelines for care and use of laboratory animals, and ethical approval was received from the Regional Council (Stuttgart, Germany; RP S35-9185-99/383). This study is based on the same animal experiment previously published [10], with additional analyses focusing on cytokine-mediated chronic low-grade inflammation. Forty-two healthy, male 7-week-old Sprague-Dawley rats (250–274 g) were purchased from Janvier Labs (Le Genest Saint Isle-Mayenne; France). After 12 days of familiarization, animals were randomly divided into seven groups (n = 6 per group): The 1) control group was fed a standard rodent diet (5% fat, 35% starch) and all other experimental groups received an isocaloric Westernized diet as follows: 2) high-fat (20% fat, 35% starch), 3) FOS (20% fat, 30% starch, 10% FOS), 4) fructose (20% fat, 10% starch, 25% fructose), 5) fructose+FOS (20% fat, 5% starch, 25% fructose, 10% FOS), 6) galactose (20% fat, 10% starch, 25% galactose), 7) galactose+FOS (20% fat, 5% starch, 25% galactose, 10% FOS) (Figure 1). The 25% dose of sugars was chosen based on thorough literature research to ensure that, at the selected dose, rats do not develop opaque lenses, as observed in a previous study conducted in our lab [9, 12], and to remain comparable to the upper range of sugar consumption reported in male adults in the United States [13]. Rat feed was from Altromin Spezialfutter GmbH & Co. KG (Lage, Germany), and FOS was inulin-type from chicory root with 95% purity from Beneo GmbH (Mannheim, Germany). Further information on

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TABLE 1 | Primer sequences for quantitative real-time polymerase chain reaction experiments.

Gene	Primer sequence (5′-3′)	Annealing Temperature (°C)	PCR product size (bp)	Length of bases	Reference	Gene Code
TNF-α	F: GCCTCCTCTCTGCCATCAAG R: CTCCAAAGTAGACCTGCCCG	66	186	20	[18]	NM_012675.3
IL-1β	F: CCTTGTGCAAGTGTCTGAAG R: GGGCTTGGAAGCAATCCTTA	60	137	20	[19]	NM_031512.2
IL-6	F: CCGGAGAGGAGACTTCACAGAGGA R: AGCCTCCGACTTGTGAAGTGGTATA	66	71	24	[20]	NM_012589.2
IL-10	No information provided by the manufacturer	55	69	—	—	NM_012854
ZO-1	F: CAGGCCATTACGAGCCTCTC R: GGCTGTGGCTTGGTAGCTG	68	104	20	[21]	NM_001106266.1
OCLN	F: CATCGCTTCCTTGGTGATCT R: CAGGATTGCGCTGACTATGA	66	100	20	[22]	AB016425.1
Hprt1	F: CTCATGGACTGATTATGGACAGGAC R: GCAGGTCAGCAAAGAACTTATAGCC	64	123	25	[23]	NM_012583.2
RPLP0	F: CCCTTCTCCTTCGGGCTGAT R: TGAGGCAACAGTCGGGTAGC	66	165	20	[16]	NM_022402.2
B2M	F: CGAGACCGATGTATATGCTTGC R: GTCCAGATGATTCAGAGCTCCA	60	114	22	[24]	NM_012512.2

Abbreviations: B2M, beta-2-microglobulin; bp, number of base pairs; F, forward primer; Hprt1, hypoxanthine phosphoribosyltransferase 1; IL-10, interleukin-10; IL-1β, interleukin 1 Beta; IL-6, interleukin-6; OCLN, occludin; PCR, polymerase chain reaction; R, reverse primer; RPLP0, ribosomal protein lateral stalk subunit P0; TNF-α, tumor necrosis factor alpha; ZO-1, zonulin-1.

the diet composition can be found in our previous publication [10]. Rats were housed three per cage and kept in a controlled environment at 20±2°C, 12 h light/12h dark cycle, and 55±10% relative humidity with ad libitum access to feed and water. Feed intake and body weight were recorded weekly. After 8 weeks of intervention, rats were fasted overnight, anesthetized with carbon dioxide gas, and decapitated. Blood was collected into heparinized tubes for plasma and plain tubes for serum (Monovette, Sarstedt, Nümbrecht, Germany) and placed on ice. Tissues were excised, weighed, and snap-frozen in liquid nitrogen. All samples were immediately stored at −80°C until further analysis.

2.2 | Reverse Transcription Quantitative Real-Time Polymerase Chain Reaction (RT-qPCR) Analysis

The relative gene expression of hepatic and intestinal inflammatory markers (tumor necrosis factor alpha (TNFα), interleukin (IL) 1β (IL-1β), IL-6, IL-10), and intestinal integrity (zonulin-1 and occludin) were determined via RT-qPCR, in accordance with the Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) guidelines [14]. Primers were selected from the literature, validated with the Nucleotide BLAST database of the National Center for Biotechnology Information (https://blast.ncbi.nlm.nih.gov/Blast.cgi), and purchased from biomers.net GmbH (Ulm, Germany), except for IL-10, a ready-designed and validated QuantiTect Primer Assay purchased from Qiagen (QT00177618; Hilden, Germany) (Table 1). The reference genes hypoxanthine phosphoribosyl transferase 1 (Hprt1), ribosomal protein lateral stalk subunit P0 (RPLP0), and beta-2-microglobulin (B2M) were selected to be specific for Sprague-Dawley rats (*Rattus norvegicus*), stable in hepatic and intestinal tissues, and unaffected by the outcome of inflammation or by the experimental process of sugar feeding [15–17]. RNA was extracted from hepatic and ileum tissues according to the manufacturer's protocol (RNeasy Plus Mini Kit, Cat. No. 74134; QIAGEN, Netherlands) and diluted to a concentration of 60 ng/µL. RNA samples were stored at -80°C until further steps of analysis. According to the manufacturer's instructions, the RNA was synthesized to cDNA using the iScript cDNA Synthesis Kit (Cat. 1708891; Bio-Rad Laboratories, Germany) and incubated in a thermal cycler (MJ Research PTC-200; Bio-Rad Laboratories, Germany) for 45 min. cDNA samples were stored at -20°C. RT-qPCR was carried out with CFX 96 Real-Time PCR Detection Systems (Bio-Rad Laboratories, Germany) and set at the following respective steps: 95°C (15 s; denaturation), 55-68°C (30 s; annealing), and 72°C (30 s; elongation) for a total of 45 cycles. The total reaction volume was 10 µL (Cat. 1708882; iQ SYBR Green Supermix, Bio-Rad).

The annealing temperature of the primers (Table 1) was validated and determined according to the PCR product size and agarose gel electrophoresis. DNA from the gel was extracted according to the datasheet of the QIAquick Gel Extraction Kit (Cat. 28704; Qiagen, Netherlands) and further used to create a serial dilution for the standard curve. Samples were analyzed in triplicate, and relative mRNA expression was normalized against the geometric mean of the reference genes Hprt1, RPLP0, and B2M.

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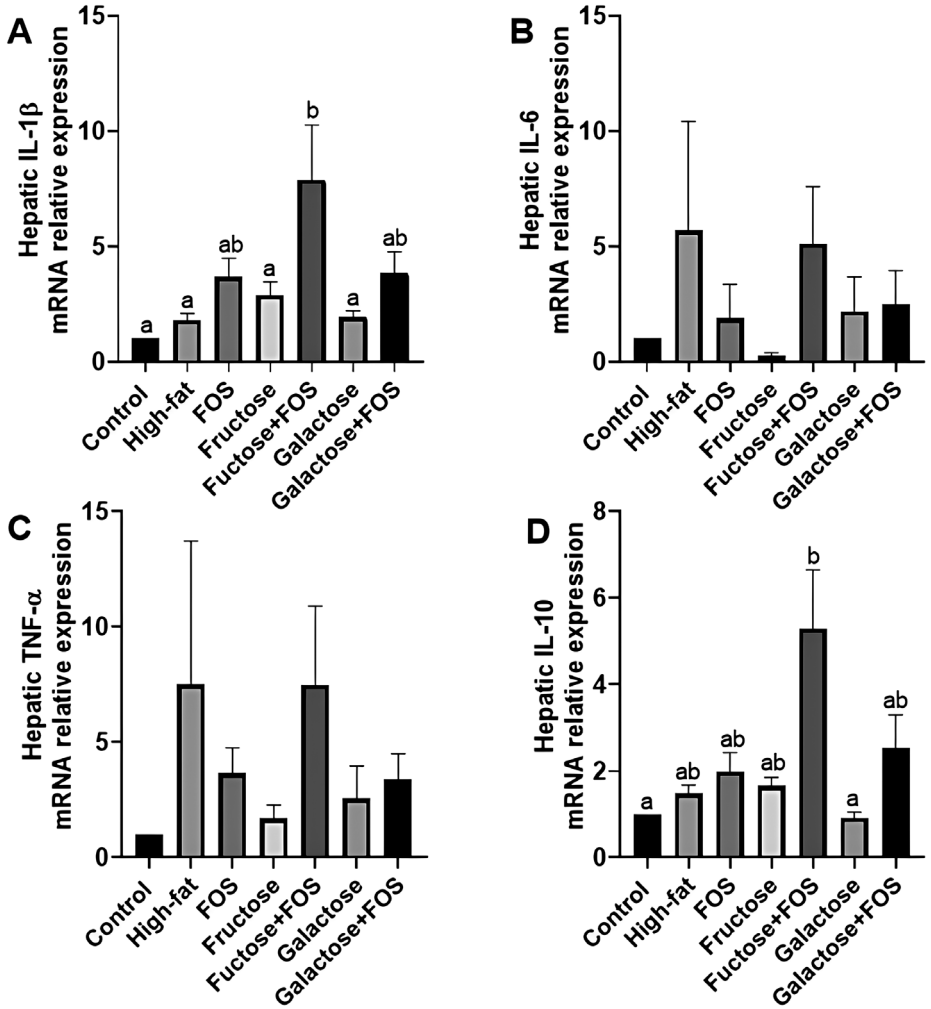


FIGURE 2 | Effects of a Westernized diet high in fructose or galactose with or without the addition of fructooligosaccharides (FOS) on mRNA expression of biomarkers of hepatic inflammation in rats after 8 weeks feeding period. (A) interleukin 1β (IL-1β); (B) interleukin-6 (IL-6); (C) tumor necrosis factor α (TNF-α); (D) interleukin-10 (IL-10). Normalization to hypoxanthine phosphoribosyltransferase 1, ribosomal protein lateral stalk subunit P0, and beta-2-microglobulin. Sprague-Dawley rats ($n = 6$/group) were acclimatized 12 days, then fed 8 weeks control diet (5% fat, 35% starch), or isocaloric Westernized diets as follows: high-fat (20% fat, 35% starch), FOS (20% fat, 30% starch, 10% FOS), fructose (20% fat, 10% starch, 25% fructose), fructose+FOS (20% fat, 5% starch, 25% fructose, 10% FOS), galactose (20% fat, 10% starch, 25% galactose), galactose + FOS (20% fat, 5% starch, 25% galactose, 10% FOS). Data are shown as mean ± standard error of the mean (SEM) from six biological replicates (individual rats) per group. Each PCR sample was measured in triplicate (technical replicates). Data of IL-1β was compared within groups by one-way ANOVA, followed by Tukey's multiple comparisons test. For the rest of the parameters, groups were compared with Kruskal–Wallis. Means with different superscripts differ significantly at $p < 0.05$.

2.3 | Enzyme-Linked Immunosorbent Assay (ELISA)

Plasma lipopolysaccharides (LPS) were quantified by ELISA following the guidelines of the manufacturer (ABIN6574100; Antibodies-online GmbH, Germany).

2.4 | Statistical Analyses

Data were analyzed using GraphPad Prism version 8.0.1 for Windows (GraphPad Software Inc., San Diego, California, USA). Normality of the data was tested with the Shapiro-Wilk test. In case the data were normally distributed, differences between the diets were computed with one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. Skewed data were analyzed using the Kruskal-Wallis non-parametric test. A p-value of < 0.05 was considered significant. All results are presented as mean ± standard error of the mean (SEM). Sample size (n = 6/group) was determined based on a power calculation for serum TNF-α, the primary outcome of the main study [10], assuming a power of 90% and a significance level of $p < 0.05$ to detect a difference of 10 pg/mL (approximately 20%) with a standard deviation of 5.2 pg/mL [25, 26]. The hepatic and intestinal inflammatory markers reported in the present analysis were secondary outcomes.

3 | Results and Discussion

This study investigated, in a Sprague-Dawley rat model, whether a Westernized diet high in fructose or galactose with or without

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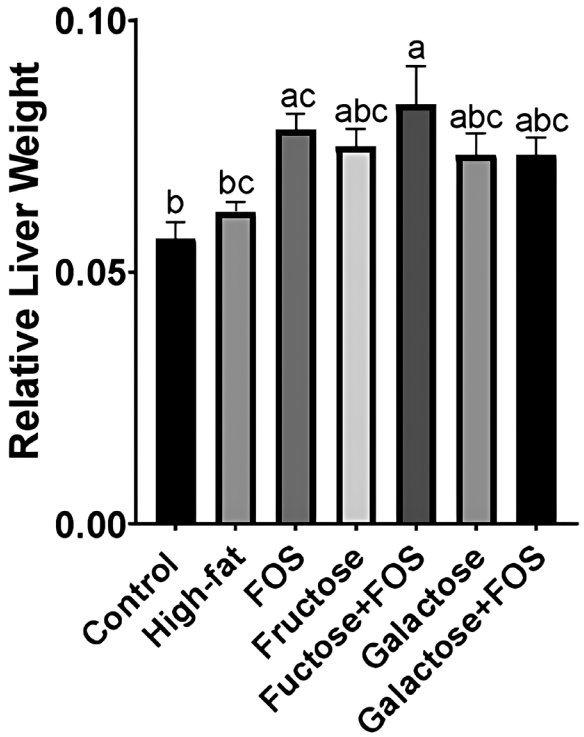


FIGURE 3 | Effects of a Westernized diet high in fructose or galactose, with or without the addition of fructooligosaccharides (FOS), on relative liver weight (ratio of liver weight divided by final body weight of the rat). Sprague-Dawley rats ($n = 6$/group) were acclimatized 12 days, then fed 8 weeks control diet (5% fat, 35% starch), or isocaloric Westernized diets as follows: high-fat (20% fat, 35% starch), FOS (20% fat, 30% starch, 10% FOS), fructose (20% fat, 10% starch, 25% fructose), fructose + FOS (20% fat, 5% starch, 25% fructose, 10% FOS), galactose (20% fat, 10% starch, 25% galactose), galactose + FOS (20% fat, 5% starch, 25% galactose, 10% FOS). Data are shown as mean ± standard error of the mean (SEM) from six biological replicates (individual rats) per group. Groups were compared by one-way ANOVA, followed by Tukey's multiple comparisons test. Means with different superscripts differ significantly at $p < 0.05$.

supplementation of fermentable FOS induces local inflammation in the liver and ileum, focusing on cytokine-mediated pathways and intestinal barrier markers. The Westernized diet model was used to predispose rats to inflammation. At the end of the study, there were no statistically significant differences in feed intake between experimental groups (Figure S1).

3.1 | Effects of a Westernized Diet High in Sugar With or Without FOS on Hepatic Inflammation

Feeding Sprague-Dawley rats a Westernized diet enriched with fructose or galactose for 8 weeks did not significantly affect the relative mRNA expression of hepatic cytokines, including the pro-inflammatory TNF-α, IL-1β, and IL-6 (which has both pro- and anti-inflammatory functions), and the anti-inflammatory IL-10, compared with rats fed a standard diet (control) or a Westernized diet without added sugars (high-fat) (Figure 2). These results indicate an absence of transcriptionally active, cytokine-driven hepatic inflammation in response to the dietary interventions.

Fructose and galactose are metabolized differently in the liver, which may result in different inflammatory and metabolic responses. Excessive fructose intake stimulates de novo lipogenesis [27], increases circulating free fatty acids, and promotes hepatic steatosis, which may precede inflammation [28]. Galactose, on the other hand, is a reducing sugar and promotes protein glycation and leads to advanced glycation end product (AGE) formation and receptor activation (RAGE) [10], processes associated with inflammation [29].

Our previous analysis showed increased liver lipids and AGE accumulation, and elevated neutrophil-derived myeloperoxidase (MPO) activity in the rats fed a Westernized diet alone and in combination with high sugar (isocaloric groups) in comparison to the control group fed standard chow [10]. However, it remained unclear whether these effects were directly attributable to the sugar intake itself or the overall hypercaloric state. The current analysis shows that these metabolic changes did not translate into significant increases in cytokine gene expression, indicating that hepatic inflammation did not progress to a chronic, cytokine-mediated state in this model.

Body weight and systemic inflammatory markers were also previously reported unchanged in the model [10], further supporting the absence of overt systemic or chronic low-grade inflammation shown in our current results. These findings align with a study on Sprague-Dawley rats showing that diets enriched in ω-6 fatty acids combined with high fructose or galactose intake increased systemic AGE and lipids but did not significantly affect inflammatory biomarkers compared with isocaloric controls [9]. Furthermore, fructose-sweetened water, either as sucrose or high-fructose corn syrup, administered for 30 days, did not significantly increase expression of hepatic IL-1β or IL-6 in adult male Sprague-Dawley rats [30]. Another study reported an increase in hepatic TNF-α and MPO activity in adult male Sprague-Dawley rats fed a diet containing 20% fructose for two weeks compared with control [31]. Therefore, the duration of dietary exposure may influence the inflammatory response, and our rats may have developed an adaptive response to the prolonged intervention. We have previously observed this adaptive capacity in Sprague-Dawley rats [32] Furthermore, the rats used in the present experiments were approximately 2 months post-weaning and undergoing sexual maturity. This developmental stage may have conferred a degree of resistance to diet-induced obesity, allowing their metabolism to adapt. An experiment with older rats could potentially have offered better translational relevance to adult human metabolic health [33]. Conversely, younger animals are often considered more prone to diet-induced obesity because they gain weight more rapidly than older animals [34]. However, the classification of "young" in animal models remains broad, encompassing 6–20 weeks of age, which creates variability and complexity in selecting an adequate age for investigating obesity and metabolic health [33[.

Meta-analyses of human trials suggest that the metabolic harm of high-fructose consumption primarily arises from sugar-sweetened beverages and overall excess caloric intake, rather than fructose itself [35, 36].

However, supplementation of FOS in the fructose+FOS group resulted in a significant increase in hepatic IL-1β expression

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compared to the Westernized diet (high-fat) and the Westernized diet plus fructose or galactose groups (Figure 2B). In addition, there was a trend toward increased hepatic TNF-α, IL-6, and IL-10 expression in the fructose+FOS group (Figure 2A,C,D), and a significant increase in IL-10 in comparison to the galactose group (Figure 2D). Hepatic TNF-α protein expression was consistent with the corresponding mRNA findings (Figure S2). Furthermore, feeding FOS alone significantly increased liver weight compared to control. Liver weight was also significantly higher in the fructose+FOS group compared with the control and high-fat groups (Figure 3). Body weight did not differ between groups, as previously reported [10]; however, this does not exclude changes in body composition, and future studies should assess visceral or epididymal fat depots to determine whether increased liver weight is associated with an increase in visceral fat. These findings are contradictory to our initial hypothesis that FOS supplementation might reduce the negative metabolic effects of dietary sugars. FOS is fermented in the colon and results in the production of short-chain fatty acids [37], which activate a molecular pathway that signals hepatic anti-inflammatory responses [38]. However, our results suggest that FOS combined with fructose may induce hepatic immune activation, as reflected by the increase in hepatic IL-1β and liver weight. A possible explanation is that there are interactions between FOS fermentation products and fructose metabolism that alter gut-liver axis signaling and immune responses. A previous study on Sprague-Dawley rats reported that a high FOS intake of 15% for 8 weeks altered the gut microbiota, specifically increasing the relative abundance of *Bacteroidetes*. This shift enhanced the microbiota's capacity to harvest energy and resulted in an increase in body weight [39]. Similarly, dysregulated microbial fermentation of the soluble fiber inulin promoted cholestasis, hepatocyte injury and liver inflammation in a microbiota-dependent manner [40]. Such dysbiotic changes may promote hepatic lipid accumulation and thus inflammation, potentially explaining our findings since the rats were on a high-fat, high-sugar diet and supplemented with FOS. Furthermore, the increased IL-10 may represent compensatory anti-inflammatory feedback. Overall, our findings support the idea that whether soluble fibers and their fermentation products are beneficial is highly dependent on internal and environmental conditions. Future work should investigate gut microbiota changes and molecular pathways to clarify these interactions. Given the pronounced sexual dimorphism of the liver, future studies should furthermore include both sexes to confirm whether the present findings, particularly the effect of FOS combined with fructose on hepatic inflammation, are also present in female rats.

3.2 | Effects of a Westernized Diet High in Sugar With or Without FOS on Intestinal Inflammation

The intestine is the largest immune organ, and dietary sugars are digested and absorbed in the upper small intestine. We hypothesized that a Westernized diet high in fructose or galactose may promote local ileal inflammation. However, in the current study, no significant effects of the diet were observed on the relative mRNA expression of cytokines TNF-α, IL-1β, IL-6, and IL-10 in the intestine (Figure 4). This is in agreement with the absence of effects of sugars on hepatic inflammation (see 3.1). A previous 12-week study feeding Sprague-Dawley rats a hypercaloric ω-6-rich diet high in fructose or galactose found no local gut effects, as evidenced by unchanged gut microbiota composition [12].

Further, we hypothesized that FOS fermentation in the gut may attenuate the adverse effects of Westernized high-sugar diets. One in vitro and in vivo Wistar rat study reported that FOS fermentation may begin in the distal small intestine [41]. This may be attributed to the small size of FOS and their degradation by the ileal microbiome [42]. Therefore, we were interested in the local effects of FOS on the ileum. However, because we did not find any adverse effects of the diets high in fat and fructose or galactose on inflammation of the ileum, we were not able to see any preventive effects of FOS.

3.3 | Effects of a Westernized Diet High in Sugar With or Without FOS on Intestinal Integrity and Permeability

The intestinal permeability and structural integrity are modulated by the diet [43]. LPS, a bacterial endotoxin, can enter the bloodstream through intestinal absorption but is normally restricted by the intestinal tight junction barrier that includes proteins such as zonulin-1 and occludin. However, a diet high in fat and sugar may facilitate LPS translocation across the intestinal membrane into the systemic circulation, resulting in low-grade inflammation [5, 44].

Feeding a Westernized diet high in fructose or galactose did not significantly affect plasma LPS concentrations compared to control or the Westernized diet alone (high-fat). However, the addition of FOS to the sugars reduced LPS numerically, but not significantly relative to feeding sugars alone. Rats in the fructose+FOS group had significantly lower plasma LPS concentrations than in the high-fat and the galactose groups (Figure 5A). Research has shown gut-liver inflammatory communication [45]. The absence of increased peripheral LPS does not exclude gut-liver inflammatory communication, as hepatic exposure is primarily determined by portal venous input, and systemic measurements may not reflect transient or compartmentalized gut-derived signals. Although there is no gold standard marker of metabolic endotoxemia, LPS remains the most widely used biomarker. For future studies focused on acute hepatic immune response, it is recommended to also measure other microbial products such as peptidoglycan fragments, microbial nucleic acids, or fungal products that may activate hepatic innate pathways without increasing LPS. Relative mRNA expression of zonulin-1 and occludin in the intestine did not differ between groups. However, adding FOS to galactose numerically increased zonulin-1 and occludin expression compared to galactose alone (Figure 5B,C). These observations align with a previous study reporting a significant increase in zonulin-1 when FOS was added to the galactose-rich diet compared to fructose or high-fat negative control, and endotoxin concentrations in the high-sugar groups were also reduced in comparison to high-fat controls [12]. In contrast, a decrease in intestinal tight junction proteins after 8 weeks of ad libitum consumption of 30% fructose-sweetened drinking water was reported in female Fischer 344 rats [46]. The discrepancy with our findings may be explained by: (i) the faster

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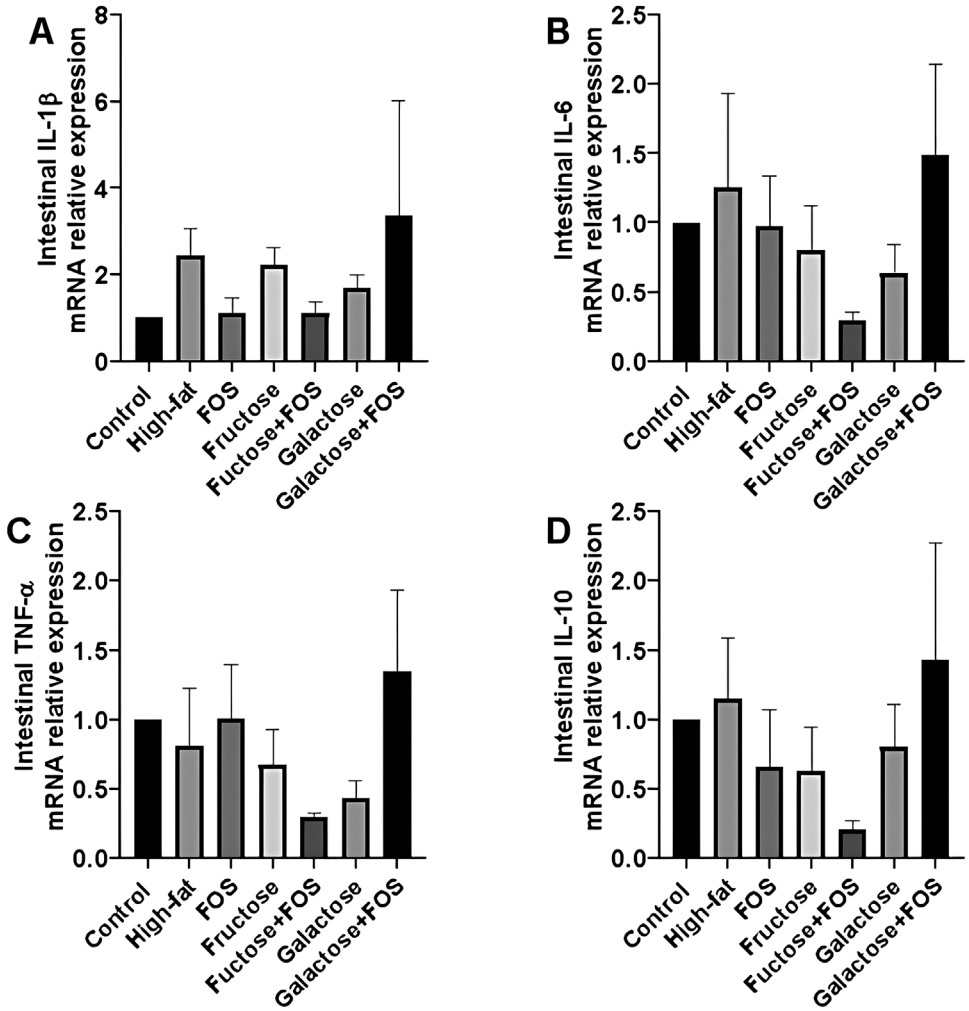


FIGURE 4 | Effects of a Westernized diet high in fructose or galactose, with or without the addition of fructooligosaccharides (FOS), on biomarkers of intestinal inflammation in rats after 8 weeks feeding period. (A) interleukin 1 beta (IL-1β); (B) interleukin-6 (IL-6); (C) tumor necrosis factor alpha (TNF-α); (D) interleukin-10 (IL-10). Normalization to hypoxanthine phosphoribosyltransferase 1(Hprt1), ribosomal protein lateral stalk subunit P0, and beta-2-microglobulin. Sprague-Dawley rats (n = 6/group) were acclimatized 12 days, then fed 8 weeks control diet (5% fat, 35% starch), or isocaloric Westernized diets as follows: high-fat (20% fat, 35% starch), FOS (20% fat, 30% starch, 10% FOS), fructose (20% fat, 10% starch, 25% fructose), fructose + FOS (20% fat, 5% starch, 25% fructose, 10% FOS), galactose (20% fat, 10% starch, 25% galactose), galactose + FOS (20% fat, 5% starch, 25% galactose, 10% FOS). Data are shown as mean ± standard error of the mean (SEM) from six biological replicates (individual rats) per group. Each PCR sample was measured in triplicate (technical replicates). Groups were compared with Kruskal–Wallis test.

absorption of sugars from drinking solutions compared to solid foods, (ii) species- or strain-specific metabolic responses, and (iii) methodological differences, as we measured tight junctions at the mRNA level whereas the comparable study reported protein expression [46]. In addition, unchanged tight-junction gene expression does not exclude functional barrier alterations, which can involve protein localization, mucus dynamics, and epithelial turnover [47].

Although sample size was determined based on a statistical power analysis, this calculation was based on serum TNF-α, the primary outcome of the study [10]; the hepatic and intestinal tissue cytokines analyzed in the present manuscript were secondary outcomes, and the study may therefore have limited sensitivity to detect smaller effect sizes for these markers.

4 | Concluding Remarks

The current study did not show a relevant impact of a Westernized diet high in fructose or galactose on hepatic or intestinal transcriptional inflammatory response or on intestinal integrity and permeability compared to both the control and the high-fat control groups. Therefore, the addition of FOS to the diet did not have protective effects. These findings should be interpreted with caution because our endpoints primarily reflect tissue-level mRNA expression and do not exclude inflammatory changes restricted to specific areas, cell populations, post-transcriptional regulation, or signaling pathways not assessed here. Interestingly, FOS exacerbated hepatic inflammation when combined with fructose, indicating that fermentable fiber-sugar interactions may, in some contexts, aggravate rather than attenuate liver inflammatory responses. Overall, our data suggest that sugar-

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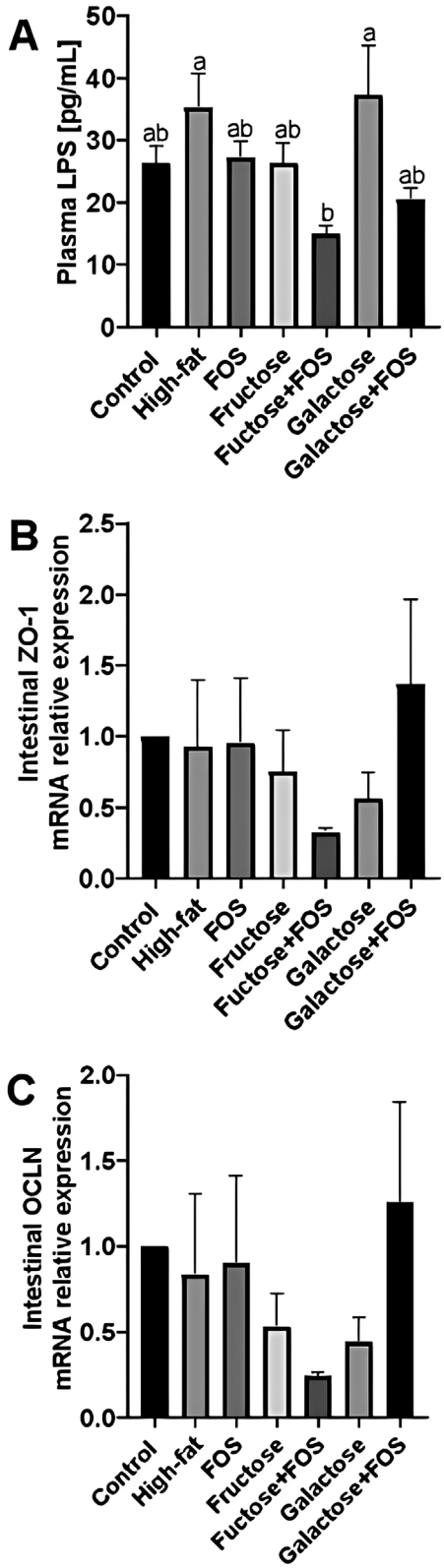


FIGURE 5 | Effects of a Westernized diet high in fructose or galactose, with or without the addition of fructooligosaccharides (FOS), on biomarkers of intestinal integrity and permeability in rats after 8 weeks feeding period. (A) lipopolysaccharides (LPS); (B) Zonulin-1 (ZO-1); (C) occludin (OCLN). Normalization to hypoxanthine phosphoribosyltransferase 1, ribosomal protein lateral stalk subunit P0, and beta-2-microglobulin. Sprague-Dawley rats (n = 6/group) were acclimatized 12 days, then fed 8 weeks control diet (5% fat, 35% starch), or isocaloric Westernized diets as follows: high-fat (20% fat, 35% starch), FOS (20% fat, 30% starch, 10% FOS), fructose (20% fat, 10% starch, 25% fructose), fructose+FOS (20% fat, 5% starch, 25% fructose, 10% FOS), galactose (20% fat, 10% starch, 25% galactose), galactose+FOS (20% fat, 5% starch, 25% galactose, 10% FOS). Data are shown as mean ± standard error of the mean (SEM) from six biological replicates (individual rats) per group. Each plasma sample was measured in duplicates, and the PCR sample was measured in triplicate (technical replicates). Groups were compared by one-way ANOVA, followed by Tukey's multiple comparisons test. Means with different superscripts differ significantly at $p < 0.05$.

driven metabolic remodeling does not uniformly translate into overt inflammatory gene induction across experimental settings and underscore the need to define the conditions under which fermentable fibers modify gut–liver signaling. These observations warrant further research into the mechanisms underlying the metabolic effects of single sugars and fermentable dietary fibers and to identify the main metabolic mediators involved in mediating the fructose+FOS interaction.

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Conflicts of Interest

The authors declare no conflicts of interest.

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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