Dietary Fiber from Oat and Rye Brans Ameliorate Western Diet–Induced Body Weight Gain and Hepatic Inflammation by the Modulation of Short-Chain Fatty Acids, Bile Acids, and Tryptophan Metabolism

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Scope: Dietary fiber (DF) induces changes in gut microbiota function and thus modulates the gut environment. How this modulation is associated with metabolic pathways related to the gut is largely unclear. This study aims to investigate differences in metabolites produced by the gut microbiota and their interactions with host metabolism in response to supplementation with two bran fibers.

Methods and Results: Male C57BL/6N mice are fed a western diet (WD) for 17 weeks. Two groups of mice received a diet enriched with 10% w/w of either oat or rye bran, with each bran containing 50% DF. Microbial metabolites are assessed by measuring cecal short-chain fatty acids (SCFAs), ileal and fecal bile acids (BAs), and the expression of genes related to tryptophan (TRP) metabolism. Both brans lowered body weight gain and ameliorated WD-induced impaired glucose responses, hepatic inflammation, liver enzymes, and gut integrity markers associated with SCFA production, altered BA metabolism, and TRP diversion from the serotonin synthesis pathway to microbial indole production.

Conclusions: Both brans develop a favorable environment in the gut by altering the composition of microbes and modulating produced metabolites. Changes induced in the gut environment by a fiber-enriched diet may explain the amelioration of metabolic disturbances related to WD.

1. Introduction

The prevalence of obesity and related comorbidities is still alarmingly high in Western countries, with little indication of an improvement in the current trend.[1] The main cause of obesity is energy imbalance developed by a "Western" type of diet and a lack of physical activity. A Western diet (WD) is characterized not only by high fat and sugar content but also by low dietary fiber (DF).[2] DF is derived from plant components that are resistant to digestion and absorption in the upper digestive tract. However, DF can undergo microbial fermentation in the colon, resulting in the production of numerous bioactive compounds and in the modification of microbial communities, which promote health. Such fibers are considered prebiotics.[3–5] Human interventions and observational studies suggest that a fiber-rich diet is linked to lower body weight, improved insulin sensitivity and glucose responses, and reduced blood cholesterol.[6–10]

DF can modulate cholesterol metabolism.[11,12] Furthermore, whole-grain fiber-rich diets induce clinical improvements related

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to bile acid (BA) metabolism and synthesis.[10] BAs are produced in the liver from cholesterol, stored in the gallbladder, and released into the small intestine to aid dietary fat digestion and absorption. They are also potent stimulators of the nuclear farnesoid X receptor (FXR), which is involved in cholesterol metabolism by the intestinal mobilization of fibroblast growth factors 15/19 (FGF15/19). This in turn activates the hepatic small heterodimer partner/FXR complex to reduce BA synthesis by inhibiting the hepatic enzyme cholesterol 7 alpha-hydroxylase (CYP7a1).[13] The presence of fiber in a meal increases the fecal excretion of BAs, stimulates their hepatic synthesis, and subsequently lowers the cholesterol pool.[12] In the intestinal lumen, BAs are subjected to microbial enzymatic biotransformation, affecting their chemical structure and potency to activate the FXR.[14,15]

Another group of microbial metabolites, known as short chain fatty acids (SCFAs), is produced by gut microbiota acting on DF by fermentation. Increased serum SCFAs were observed in individuals consuming whole-grain fiber.[16,17] SCFAs are known for their protective effects on the intestinal epithelial cells by providing energy to the colonocytes and by controlling luminal pH with the subsequent inhibition of pathogen proliferation and the modulation of microbial and host enzymatic activities.[18–20]

DF intake is associated with altered serum levels of gut-derived tryptophan (TRP) metabolites, namely, decreased serotonin (5-HT) and increased indolepropionic acid (IPA).[8,21] Indoles are produced directly by gut microbiota via TRP catabolism and have the capacity to activate intestinal nuclear aryl hydrocarbon receptors (AHRs). This stimulation initiates the interleukin-22 (IL-22)-mediated cascade of immunological responses linked to the improvement of the gut system through tight junction proteins, antimicrobial compounds, and mucin production.[22,23] 5-HT is synthesized from TRP in the epithelial enterochromaffin cells by the enzyme tryptophan hydroxylase 1 (TPH-1) and its activity potentially depends on the presence and composition of microbes.[24,25] Recent studies suggest that gut microbiota might take part in controlling glucose metabolism through gut-derived 5-HT, where circulating levels of 5-HT were inversely related to obesity outcomes and glucose tolerance.[26] These TRP metabolites are therefore emerging as the new focus of microbial activity modulation.

DF is evidently important in supporting healthy gut metabolism and thus in reducing the risk of metabolic complications of obesity. However, to date, studies investigating the differential metabolic responses to different types of fiber in the diet are lacking. For example, oat and rye brans have distinct DF compositions, where beta-glucan and arabinoxylan predominate, respectively.[27,28] In this study, we aimed to elucidate the protective mechanisms conferred by both these fibers in young mice fed a WD. We hypothesized that metabolic differences in response to oat and rye diets could be mediated through differences in pathways involving the signaling and intestinal metabolism of cholesterol, BAs, SCFAs, and TRP.

2. Results

2.1. Fiber Intake Affected Body Weight but Not Body Composition

Mice fed a WD enriched with oat or rye bran showed a significant attenuation in body weight gain compared to the mice receiving a fiber-deprived diet, with rye being more effective than oat (**Figure** 1A,B). The overall weight change differed between the groups ($p < 0.05$ for groups × time interaction), where CHOW had the lowest gain, followed by RYE, OAT, and WD. The trend in body weight increase was consistent over the period of 17 weeks, resulting in a significant gain at the end of the feeding period for each group (Figure 1A,B, $p < 0.05$). WD showed increased fat mass and decreased lean mass in the mice compared to CHOW. Interestingly, body composition analysis did not show differences in the percentage contribution from fat mass or lean mass between WD, OAT, and RYE groups (Figure 1C). It should be noted that all WDs were isocaloric (Figure 1D).

2.2. Fiber Intake Protected against WD-Induced Liver Damage by Reducing Hepatic Inflammation and Improving Gut Integrity, but Failed to Ameliorate Hepatic Steatosis

The WD increased enzymes related to liver function, namely, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) (**Figure** 2A), indicating toxicity and hepatocyte damage. Supplementation with oat and rye reduced serum concentrations of ALT and AST compared to WD, while the ALP levels remained similar to the WD group. The reductions of ALT and AST in OAT and RYE groups were found to be associated with the attenuation of hepatic inflammation in particular, tumor necrosis factor (TNF), and toll-like receptor 4 in liver mRNA expression levels (Figure 2B). Both ALT and AST correlated with liver mRNA expression for inflammatory markers, most significantly with TNF ($r = 0.98$, $p = 0.02$ and $r = 0.97$, $p = 0.03$, respectively; Table S6, Supporting Information). Moreover, colonic mucin and tight junction protein mRNA expressions, namely, Mucin 3, Occludin, and Claudin 7 (Figure 2C), were increased in both OAT and RYE compared to WD, suggesting improved gut barrier function. Furthermore, propionate and butyrate were shown to correlate with those gut barrier parameters, mainly with mRNA expression levels of Occludin (both $r = 0.95$, $p = 0.05$), while total SCFAs and acetate in particular were inversely correlated with TNF ($r = 0.99$, $p < 0.05$ and $r = -0.97$, $p < 0.05$, respectively; Table S6, Supporting Information).

Contrary to our expectations, oat and rye did not ameliorate WD-induced liver enlargement (Figure 2D). Furthermore, the concentration of liver triglycerides was elevated by WD compared to CHOW and was not modified by OAT and RYE (Figure 2E). WD developed a significant hepatic steatosis compared to CHOW (Figure 2F). However, there was a tendency for OAT and RYE groups to have smaller adipocytes than the WD group (Figure 2G). Cecal weight, an indicator of colonic microbial activity, was reduced significantly in WD compared to OAT and RYE; however, it was the highest in the CHOW group (Figure 2H).

2.3. Glucose and Cholesterol Metabolism Were Partially Improved in the OAT Group

To evaluate whether OAT and RYE groups had any effect on glucose and insulin metabolism, we first measured the fasting blood glucose in the animals. Hyperglycemia was found in
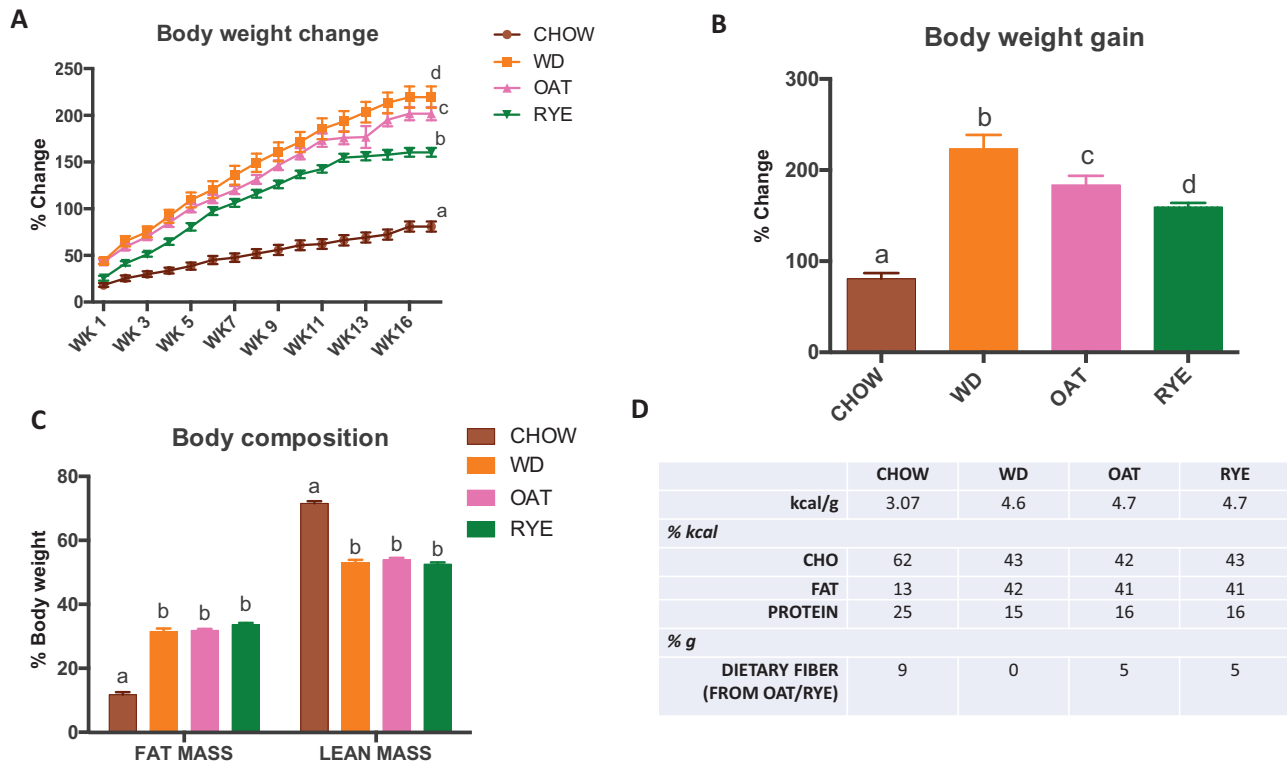
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Figure 1. Body weight and composition changes after the dietary intervention: A) changes in body weight gain over the study period (17 weeks); B) final body weight gain; C) body composition at week 15, where fat mass and lean mass are expressed as % body weight; D) energy and macronutrient contents of the diets. Data are expressed as the mean ± SEM ($n = 12$). Different letters denote statistical significance between the groups for each parameter ($p < 0.05$).

mice after WD feeding, which was alleviated significantly in the OAT group. In contrast, RYE treatment did not show any improvement in the fasting blood glucose (**Figure 3**A). The response to insulin tolerance test (ITT) varied among the groups ($p < 0.05$ for groups × time interaction) with all WD groups presenting systemic insulin resistance compared to CHOW, while supplementation with oat and rye did not improve the glucose responses in the animals at any time during the test (Figure 3B,C; $p < 0.05$). Glucose tolerance test (GTT) revealed an improved glucose clearance in OAT and RYE groups as their blood glucose levels were significantly lower than the WD at the end of GTT ($p < 0.05$; Figure 3D), although dietary regimen had no overall effect on the glucose response in the assay (Figure 3E; $p > 0.05$ for groups × time interaction).

Serum triglyceride levels were reduced in the OAT group, while neither OAT nor RYE had any effect on individual levels of low-density lipoprotein (LDL) and high-density lipoprotein (HDL). However, the HDL:LDL ratio was improved in the OAT group when compared to the WD group (Figure 3F).

2.4. Both OAT and RYE Increased Concentrations of Cecal SCFAs

To evaluate changes in microbial metabolites in response to rye and oat supplementation, SCFAs, namely, acetate, butyrate, and propionate, were measured in the cecal samples. WD significantly reduced all three SCFAs and both oat and rye restored their concentrations, especially propionate and butyrate, which were found to reach levels similar to those observed in CHOW (**Figure** 4A).

2.5. The Fate of Tryptophan Metabolism Might Depend on Microbial Activities Stimulated by OAT and RYE in a Similar Manner

Indirect measurements of TRP metabolism pathways through intestinal mRNA gene expression levels were determined in this study (Figure 4B). It was shown that WD led to a significant increase in ileal TPH-1 mRNA expression, while both oat and rye reduced it to the levels observed in CHOW. Moreover, the expression of AHR, a receptor activated by TRP-derived indoles, appeared to be inversely associated with TPH-1 expression ($r = -0.93$, $p = 0.07$; Table S6, Supporting Information). In addition, the expression of IL-22, which is released via AHR stimulation, was also reduced in WD compared to CHOW. The levels were restored by OAT and RYE, with OAT being more potent. It was noticed that the SCFA concentration was inversely associated with TPH-1, where propionate abundance in particular was highly correlated with its mRNA expression ($r = -0.99$, $p \leq 0.05$; Table S6, Supporting Information).
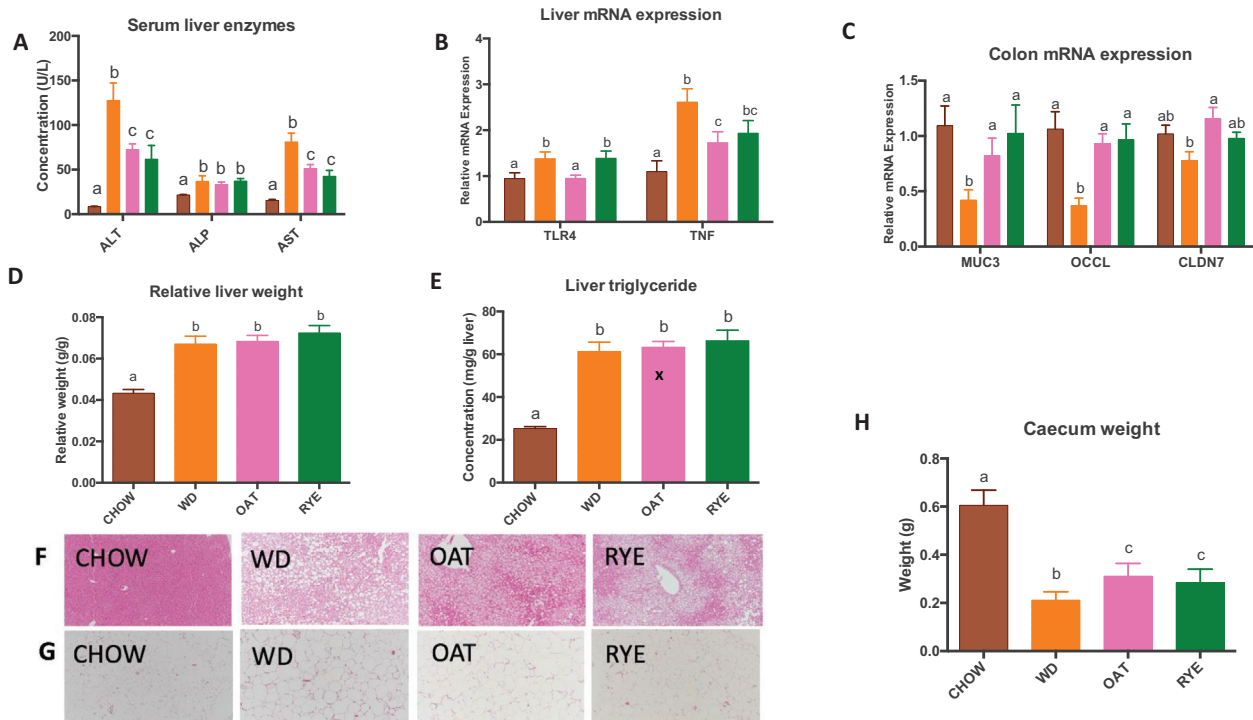
Figure 2. Liver damage and inflammation markers: A) serum liver function enzymes; B) mRNA expressions of TLR4 and TNF. Intestinal permeability markers: C) colonic mRNA expression for mucin (MUC3) and tight junction proteins (OCCL and CLDN7). Liver steatosis assessment: D) liver weight expressed relative to body weight; E) triglyceride content of the liver per milligram of tissue; F) representative images of H&E-stained sections (100×) of the liver, left lateral lobe. G) Representative images of H&E-stained sections (100×) of epididymal adipose tissue. H) Cecum weight. Data are expressed as the mean ± SEM ($n = 12$). Different letters denote statistical significance between the groups ($p < 0.05$). ALT: alanine aminotransferase; ALP: alkaline phosphatase; AST: aspartate aminotransferase; TLR4: Toll-like receptor 4; TNF: tumor necrosis factor; MUC3: mucin 3; OCCL: occludin; CLDN7: claudin 7.

2.6. The BA Profile Was Dependent on the Bran Type in the Diet

BAs were measured in the ileum and in the feces. The ileum is the last segment of the small intestine, where most of the BAs are reabsorbed and their activation of nuclear receptors is the most prominent. Ileal BA activation of the FXR leads to the recruitment of FGF15 for hepatic FXR stimulation to reduce the activity of CYP7a1, a rate limiting enzyme for BA synthesis from cholesterol.[29] In the OAT group, the proportion of a potent FXR antagonist, Tauro-β-Muricholic acid (T-βMCA), in the ileum was increased compared to all other groups and was significantly higher than that in the WD group (**Figure** 5A). Moreover, the ratio between T-βMCA and a pool of known agonistic BAs (taurocholic acid, taurochenodeoxycholic acid, taurodeoxycholic acid, taurolithocholic acid, cholic acid, chenodeoxycholic acid, deoxycholic acid, and lithocholic acid) displayed a similar pattern, being the highest in OAT (Figure 5B). This could contribute toward decreased FXR activation and increased mRNA expression of hepatic CYP7a1 (Figure 5C) compared to WD. While the OAT group showed an FXR-antagonistic profile of BAs, the RYE group had considerably higher fecal levels of conjugated BAs (**Figure** 6A,B) than the OAT group. In their conjugated forms, the BA reabsorption rate and consequently their nuclear receptor FXR activation are decreased. This is in agreement with mRNA results, which showed reduced ileal FXR mRNA expression, which

was also associated with elevated hepatic CYP7a1 mRNA expression compared to WD (Figure 5C) ($r = -0.95$, $p = 0.05$; Table S6, Supporting Information). The BA pool from all the measured compartments was the highest in WD and reduced in both fiber groups (Figure 6C).

2.7. Intestinal Microbiota Was Altered Differently by Both Bran Fibers

WD caused significant changes in gut microbiota at the phyla and genera levels compared to CHOW (Figure S1, Table S5, Supporting Information). Supplementation with either oat or rye caused a small increase in the proportion of Bacteroidetes, with RYE exerting greater effects. The OAT group, on the other hand, caused a significant decrease in the abundance of bacteria from the Firmicutes phylum. Consequently, both OAT and RYE were shown to have a higher Bacteroidetes:Firmicutes ratio than WD. In addition, the OAT group, but not the RYE group, significantly reduced the proportion of Proteobacteria and increased Saccharibacteria, to the levels observed in the CHOW group. At the genera level, OAT increased the amount of Lactobacillus, while RYE showed a significant increase in Bifidobacterium (Table S5, Supporting Information).
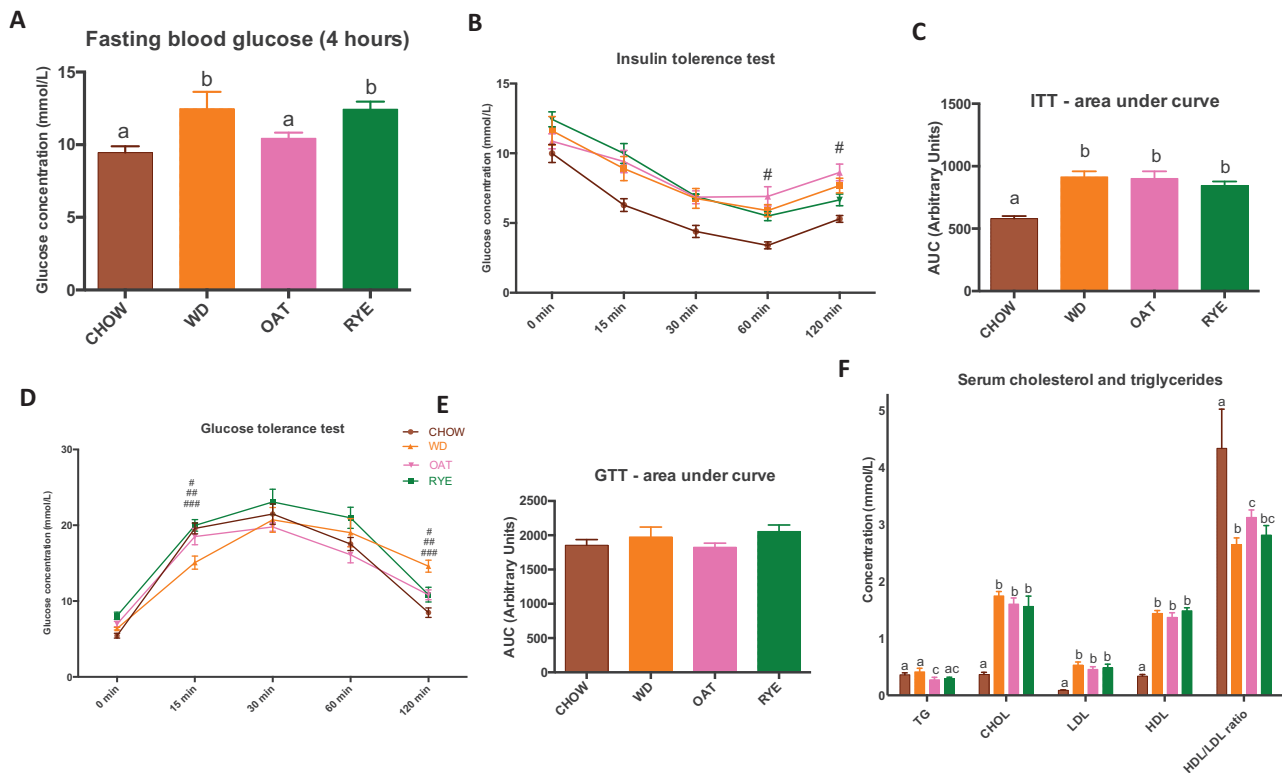
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Figure 3. Blood parameters measured after the dietary intervention: A) fasting blood glucose level (measured after 4 h of fasting); B) insulin tolerance test; C) area under curve (AUC) for ITT; D) glucose tolerance test (GTT); E) AUC for GTT; F) serum cholesterol and triglycerides. Data are expressed as the mean ± SEM ($n = 8–10$). Different letters denote statistical significance between the groups for each parameter ($p < 0.05$). One-way ANOVA with repeated measures presented as significant difference compared to WD for CHOW (#), OAT (##), and RYE (###), where $p < 0.05$. AUC: area under curve; GTT: glucose tolerance test; TG: triglycerides; CHOL: cholesterol; LDL: low-density lipoprotein; HDL: high-density lipoprotein.

3. Discussion

This study reaffirms the health benefits of oat and rye bran fibers, which were shown to successfully ameliorate a WD-induced body weight gain, reduce hepatic inflammation by the modulation of the gut microbiota, and enhance gut barrier function. In addition, we show that TPH-1 mRNA expression was downregulated in oat and rye bran–enriched diets, indicating decreased direction of the TRP to 5-HT pathway. It is conceivable that this change is also due to the fiber-related modulation of gut microbiota, which was most likely altered at the species and genera level. Our results showed increased abundance of Lactobacillus in the OAT group and of Bifidobacterium in the RYE group, which is assumed to be directly related to the beta-glucans and arabinoxylan content in each bran, respectively.[27,28,30,31]

Our main finding was reduced body weight gain by oat and rye supplementation. The positive effects of fiber on body weight gain have been reported previously in human studies and in rodents, where the improvements were associated with intestinal integrity and hepatic and systemic inflammation.[32–34] We speculated that the improvements in our study could be mediated through the restored production of SCFAs by gut microbiota acting on the fibers, as we observed significant changes in SCFA levels in response to oat and rye supplementation. Indeed, it has been suggested that SCFAs may induce or promote body weight reduction acting via specific SCFA receptors, GPR43, and

GPR41.[35–37] GPR43 and GPR41 are expressed in the intestine and the adipocytes, respectively, and promote intestinal GLP-1 secretion, thereby inducing host insulin secretion, improving leptin secretion, and stimulating the sympathetic nervous system—all activities promoting body weight reduction.[35–37] The crucial role of GPR43 was further elaborated in a study where supplementation with probiotics that stimulated SCFA production showed increased levels of plasma and cecal acetate, reduced body weight and adipose tissue mass, and improved glucose responses under HFD challenge.[38] The study showed that deletion of GPR43 in the intestine did not change HFD-induced weight gain and metabolic complications, despite similar increases in plasma and cecal SCFAs in the probiotic-supplemented group. Propionate administration to obese patients was also associated with increased GLP-1 secretion and reduced body weight.[39]

Despite the lack of improvement in hepatic fat deposition by both bran fibers in our study, mice receiving OAT and RYE were protected from the progression to NASH, most likely driven by the improved gut barrier function as observed previously.[40,41] As insulin sensitivity was not different between the WD groups despite the fiber enrichment, alterations in insulin action are unlikely to explain differences in NASH progression.

Our study also showed increased cecal concentrations of SCFAs, which, apart from their effects on body weight, might be partly responsible for the improved markers of gut barrier function observed. Increased SCFA synthesis has been reported
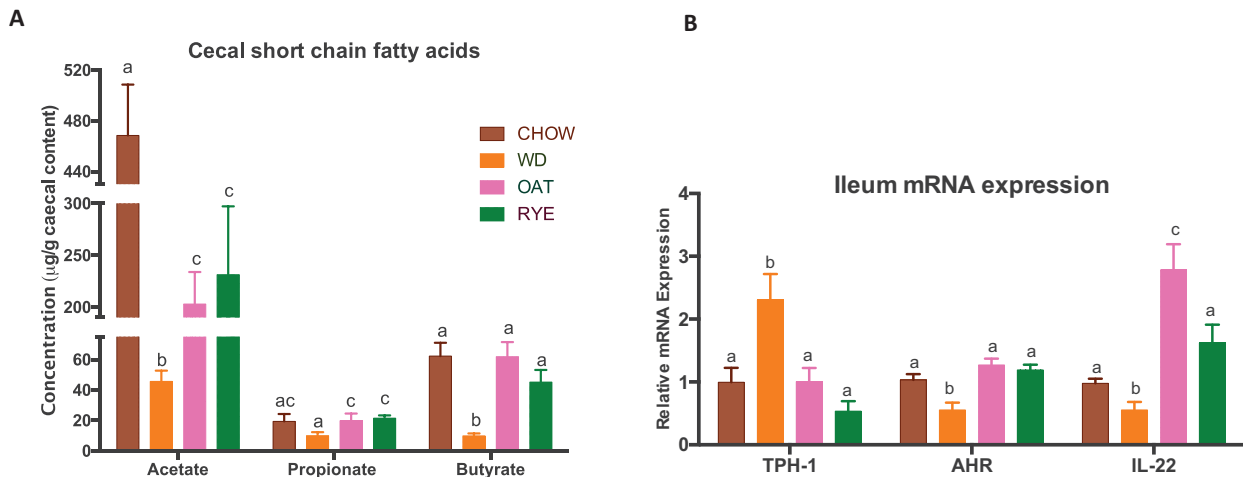
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Figure 4. Cecal short-chain fatty acid (SCFA) analysis and ileal mRNA gene expressions: A) cecal SCFAs; B) ileal mRNA gene expressions of tryptophan metabolism markers. Data are expressed as the mean ± SEM ($n = 10$). Different letters denote statistical significance between the groups ($p < 0.05$). TPH-1: tryptophan hydroxylase 1; AHR: aryl hydrocarbon receptor; IL-22: interleukin 22.
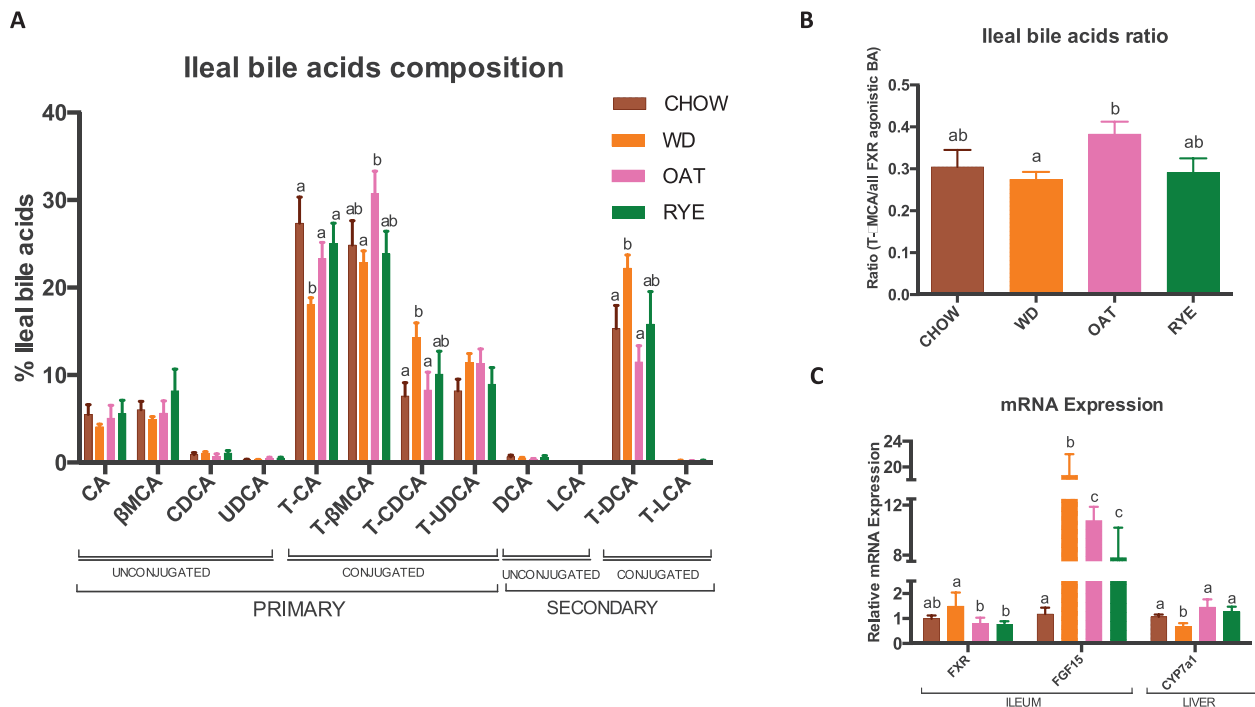

Figure 5. Ileal composition of bile acids (BAs) and mRNA expressions of genes involved in BA signaling and metabolism: A) levels of primary and secondary BAs; B) ratio between T-βMCA (FXR antagonist) and the pool of FXR agonistic BAs (TCA, TCDCA, TDCA, TLCA, CA, CDCA, DCA, LCA); C) mRNA expression level of genes involved in FXR signaling and BA metabolism. Data are expressed as the mean ± SEM ($n = 8$–10). Different letters denote statistical significance between the groups ($p < 0.05$). CA: cholic acid; β-MCA: beta-muricholic acid; CDCA: chenodeoxycholic acid; UDCA: ursodeoxycholic acid; DCA: deoxycholic acid; LCA: lithocholic acid; the letter "T" denotes a taurine-conjugated form; FXR: farnesoid X receptor; FGF15: fibroblast growth factor 15; CYP7a1: cholesterol 7 alpha-hydroxylase.

commonly in both human trials and animal studies involving oat and rye DF supplementation.[9,42] The protective effects of SCFAs on the intestinal epithelial cells are well established and their increased synthesis in the presence of DF was observed in our study and in previous reports.[18,43,44]

We postulate that a low-fiber WD potentially led to increased activity to produce and thus to increase serum 5-HT levels, and decrease alternative (protective) pathways for TRP metabolism, possibly via mechanisms involving SCFAs. We did not measure the actual serum 5-HT levels, but upregulation of the mRNA
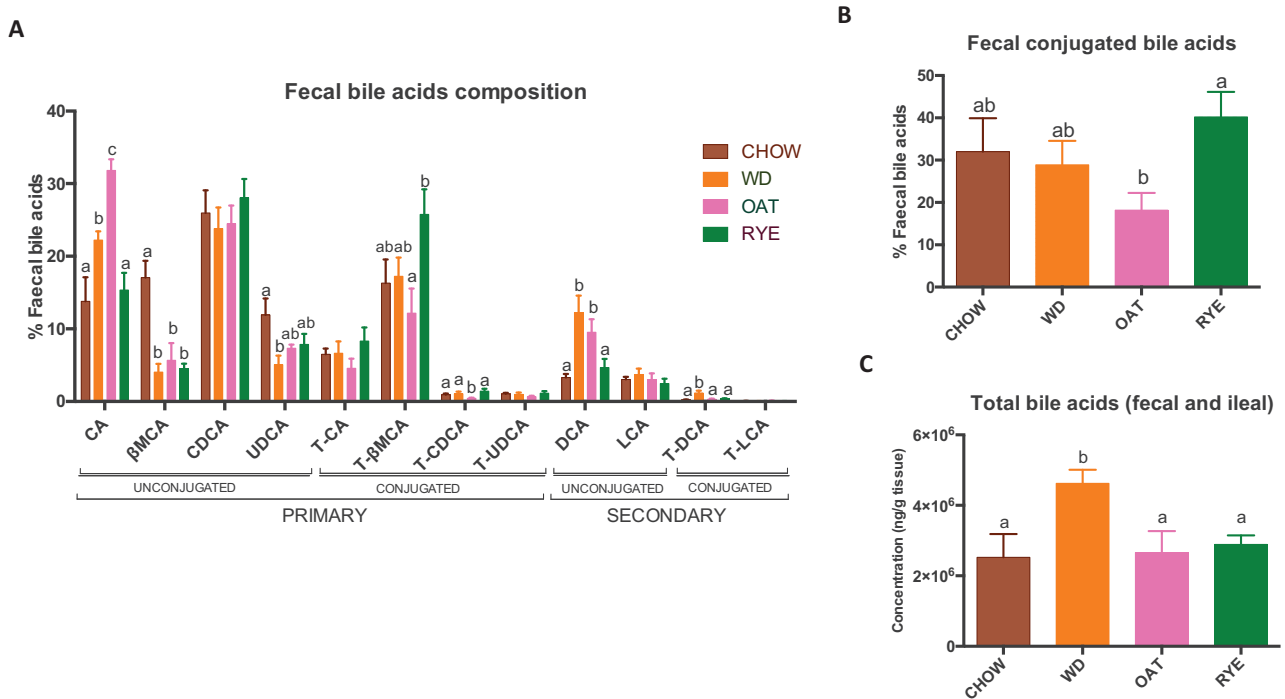
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Figure 6. Fecal composition of bile acids (BAs) and total BA pool: A) levels of primary and secondary BAs; B) proportion (%) of conjugated fecal BAs; C) BA pool measured as a cumulative of fecal and ileal BAs. Data are expressed as the mean ± SEM ($n = 10$). Different letters denote statistical significance between the groups ($p < 0.05$). CA: cholic acid; β-MCA: beta-muricholic acid; CDCA: chenodeoxycholic acid; UDCA: ursodeoxycholic acid; DCA: deoxycholic acid; LCA: lithocholic acid; the letter "T" denotes a taurine-conjugated form.

expression of TPH-1, which is responsible for 5-HT synthesis from TRP, might indicate the increased intestinal activity in fiber-deprived environments and can be corrected with the addition of either rye or oat bran, as was shown with the down-regulated mRNA expression of TPH-1 in OAT and RYE groups. Furthermore, we observed it to be inversely associated with cecal SCFA levels, in particular propionate. SCFAs might therefore take part in TRP metabolism by reducing the activity of intestinal TPH-1, and hence shunting TRP toward indole synthesis. An in vitro study suggested that SCFAs can stimulate TPH-1 production, but they were shown to inhibit its activity at high concentrations.[20] It is therefore plausible that in a healthy gut system, the adequate production of SCFAs is required to create an environment that affects not only the microbial community but also endogenous enzymatic activities. Other studies have indeed shown that TPH-1 activity is very much dependent on the presence of microbiota.[25,45] Furthermore, the genetic deletion of TPH-1 was shown to protect mice from high-fat diet–induced obesity, through reduced systemic inflammation, lowered hepatic fat deposition, and enhanced glucose tolerance and insulin sensitivity.[46] Moreover, a human observational study also strongly suggested that high fiber intake might be associated with significantly increased serum levels of the TRP metabolite IPA, which was linked with improved insulin sensitivity.[8] Endogenous TRP metabolites were elsewhere shown to activate the AHR-mediated cascade of immunoprotective responses involving IL-22.[47] Improvements against metabolic syndrome parameters by DF (inulin) in mice were associated with a re-stored activity of intestinal IL-22; despite this fact, the origin of

IL-22 activation was not extrapolated.[34] Additionally, a human intervention study showed that during the whole-grain rye bread–containing diet, plasma 5-HT levels decreased significantly when compared to the diet with white bread.[21] The authors also included an animal experiment, where rye bran supplementation in mice fed a high-fat diet resulted in significantly lower colonic 5-HT levels.[21] Collectively, these studies, as well as our own, indicate that the lack of fiber in WDs creates a luminal environment that encourages the activity of TPH-1 to synthesize 5-HT from TRP. This, in turn, results in a reduced rate of TRP conversion to indoles and consequently decreased IL-22-stimulated immunological responses. This imbalance appears to be reversed with the addition of oat and rye bran fibers. Given that both pathways of TRP metabolism, that is, 5-HT and indole production, are dependent on microbial activities, it is conceivable that these DF modulated specific changes in the gut microbiota through enhanced SCFA production, as our results also suggest.[8,21,24,25] It should be highlighted that no studies to date have investigated the link between SCFA and TRP metabolites in vivo to explain the different fates of TRP under the changing gut environment. Nonetheless, our results allow only putative conclusions in terms of fiber effects via altered TRP metabolism. It is essential that our hypothesis be further experimented to evaluate relevant metabolites, such as luminal and systemic indoles and 5-HT, as well as the effects of SCFAs on those metabolites and on the enzymatic activity of TPH-1, with subsequent impacts on the indole-mediated inflammatory responses.

The total BA pool was increased in the fiber-deprived WD group in this study, which is in agreement with observations

reported previously.[44,48] The increased excretion of BAs in OAT and RYE groups was associated with increased BA synthesis, leading to a concurrent improvement in the HDL:LDL ratio in the OAT group. It is highly possible that each of the bran fibers increased hepatic BA synthesis by diminishing intestinal FXR activation by two different routes. FXR and other nuclear receptors are activated by the binding of BAs, although their potencies vary significantly.[49] It has been shown previously that the ratio of FXR agonistic and antagonistic BAs determined the activation level of FXR and other BA-stimulated pathways.[50] Through the analysis of this ratio, our study showed that the BA composition in the OAT group favored FXR-antagonistic properties, revealing the mechanisms through which oats increased BA synthesis and improved the cholesterol and triglyceride profiles. This change in the BA profile might be linked to the unique bacterial composition that supports specific deconjugation, although deep-sequencing metagenomic analyses are needed to unravel these dependencies.

The RYE group, on the other hand, presented an elevated fecal excretion of conjugated BAs in this study. As microbial deconjugation promotes the intestinal reabsorption of BA, it can be speculated that altered microbial composition reduced BA deconjugation. This could lead to reduced BA reabsorption and FXR activation, with the downstream upregulation of BA synthesis. However, given the current understanding that BA deconjugation might convey protective mechanisms, this hypothesis requires further investigation.[51] The alternative mechanism could be through the conventional theory where BA adsorption due to the fiber present in the intestinal lumen prevents microbial activity and increases fecal excretion, resulting in the reduction of FXR activation and the downstream increase in BA synthesis.[52]

This study investigated the effects of oat and rye DF incorporated in a typical WD. In conclusion, our findings suggest that both brans have the capacity to create a favorable environment in the gut by supporting the growth of beneficial microbes, that is, Lactobacillus and Bifidobacterium genera, in a fiber-specific manner. This differentiation resulted in the generation of FXR-antagonistic BAs by oat and conjugated BAs with their increased fecal excretion by rye supplementation, which consequently improved systemic BAs and cholesterol metabolism. Furthermore, both bran fibers enhanced the production of SCFAs, leading to improved gut integrity, reduced liver inflammation, and possibly determining the pathway of TRP metabolism, in particular, TPH-1 mRNA activity and indole production.

4. Experimental Section

Animal Experiment and Diets: The handling of the mice was approved by the Committee on the Use of Live Animals in Teaching and Research (CULATR 4648-18), the University of Hong Kong. Male C57BL/6N mice aged 3–4 weeks were caged in groups of six in a controlled environment with a 12-h light/dark cycle at 22 °C ambient temperature. Food and water were provided ad libitum. After 10 days of acclimatization, mice were assigned randomly to one of the following groups ($n = 12$ per group): control standard chow (CHOW); WD; WD with 10% oat bran (OAT); and WD with 10% rye bran (RYE) for 17 weeks. Since each bran was composed of 50% DF (measured by the AOAC 2011.25 method for rye; nutritional information obtained from OatWell), oat and rye fiber concentrations in each diet amounted to 5%. Moreover, the well-characterized fermentable fractions

of each bran, beta-glucans in oat, and arabinoxylans in rye, were approximately 30% and 35%, respectively (information obtained from OatWell nutritional information and from Bender et al.), meaning that each diet delivered between 3% and 3.5% of fermentable fiber.[53] The diets (except CHOW) were purchased from Research Diets: WD (D12079B) and premix (D17041501px), which was mixed with either oat bran (OatWell) or rye bran (air-classified at VTT Technical Research Centre of Finland Ltd. to remove part of the starch and to enrich DF in the product) in-house, to achieve 5% DF concentrations (w/w). All the diets, WD, OAT, and RYE, provided the same amount of energy and macronutrients per gram of feed. Details of the ingredients and nutritional information of the brans and the diets are listed in Tables S1 and S2, Supporting Information. Food intake and body weights were recorded weekly. Body composition was analyzed using a Body Composition Analyzer (LF90 Burker, USA) at week 15. At the end of 17 weeks, mice were sacrificed and blood samples were taken from the inferior vena cava and centrifuged at $7000 \times g$ for 10 min for serum extraction. Liver, ileum, and colon tissue samples were removed and washed in saline, while the cecum was removed carefully, weighed, and its contents pushed out gently with a sterile blade. All the samples were snap frozen in liquid nitrogen and stored at −80 °C until further analyses. A portion of the liver and epididymal adipose tissues were fixed in formalin for histological analyses.

Glucose Tolerance and Insulin Tolerance Tests: Insulin tolerance tests (ITTs) and glucose tolerance tests (GTTs) were conducted in weeks 15 and 16. For GTTs, mice were fasted for 12 h. The tip of the tail was used for blood sampling at time point 0 (fasting blood glucose) and at 15, 30, 60, and 120 min after an intraperitoneal (IP) glucose injection (2 g kg⁻¹ body weight; Sigma-Aldrich, G7528, USA). For ITTs, mice were fasted for 4 h, followed by an IP insulin injection of 0.75 U kg⁻¹ body weight (Actrapid Penfil Human Insulin, Novo Nordisk) and blood glucose testing as described for GTT. Blood glucose was measured with a Roche OneTouch Ultra2 glucometer (USA) and expressed as mmol L.

Ileal and Fecal Bile Acid Measurements: BAs were extracted from the ileum and from the feces according to a method described previously, with modifications.[54] In brief, 50 mg of the ileal tissue with contents/fecal samples were homogenized in 200 μL methanol solution (50%) using a blade homogenizer (T25, ULTRA-TURRAX, IKA, Germany). Homogenized samples were mixed with 2 mL of ice-cold methanol containing the internal standard and vortexed and shaken continuously for 1 h. The mixture was then centrifuged at $12\,000 \times g$ for 10 min. The supernatant was collected into a clean vial and another extraction from the remaining residues was repeated with an additional 1 mL of ice-cold methanol. The supernatants were combined, dried completely under nitrogen gas, and reconstituted in 100 μL of methanol. The samples were then filtered through 0.45-μm polytetrafluoroethylene (PTFE) membranes to remove insoluble impurities and analyzed immediately by liquid chromatography–(quadrupole-time-of-flight)–tandem mass spectrometry (LC–(QToF)–MS/MS). Detailed conditions set for the LC–(QToF)–MS/MS analyses of the BAs are described in Section S1, Table S3, Supporting Information.

Cecal Short Chain Fatty Acid Analysis: SCFAs were extracted from the cecum as described previously, with modifications.[55] In brief, 25 mg of 1-¹³C SCFA-spiked feces was mixed with 1 mL of 0.005 м NaOH (containing 10 μg mL⁻¹ acetic acid-d₄ as an internal standard), homogenized for 45 s using a blade homogenizer (T25, ULTRA-TURRAX), and centrifuged at $13\,200 \times g$ for 20 min at 4 °C. The supernatant was collected and 0.5 mL of a 1-propanol/pyridine (3:2, v:v) mix was added, followed by 100 μL of propyl chloroformate and vortex mixing for 1 min. The samples were incubated for 1 h at 60 °C to derivatize the SCFAs. Thereafter, 0.5 mL hexane was added, mixed, and centrifuged at $2000 \times g$ for 5 min. A total volume of 400 μL of the sample extracts were taken, filtered through 0.45-μm PTFE membranes, and stored at −20 °C for gas chromatography–mass spectrometry quantitation. GC–MS (6890N GC-5973 MS, Agilent, USA) was set according to Zheng et al.'s method.[56] The SCFA concentrations were determined using the calibration curves constructed for the fatty acid determined.

Serum Biochemical Analysis and Liver Triglycerides Assay: Serum samples were analyzed for the following parameters: ALT, AST, ALP, LDL, HDL,

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total cholesterol, and triglycerides using a Cobas c111 Analyzer (Roche, USA). The liver triglyceride content was measured with a Triglyceride Colorimetric Assay Kit (Cayman Chemical, USA) following the manufacturer's instructions.

RNA Extraction and qPCR: RNA was extracted from samples of the liver, ileum, and colon using an Illustra RNAspin Mini Kit (GE Healthcare, USA) following the manufacturer's instructions. The RNA concentration was measured by a NanoDrop ND-1000 Spectrophotometer (Nano-Drop Technologies, USA) where a ratio of >1.8 (A260/A280) was accepted for the analysis. RNA integrity was checked by agarose gel electrophoresis. The cDNA was synthesized from mRNA through reverse transcription using a HiScript II Q RT SuperMix for qPCR (+DNA wiper) (Vazyme, China). qPCR was performed on cDNA (and cecal DNA) with a StepOnePlus Real-Time PCR system (Applied Biosystems, CA, USA) using an AceQ qPCR SYBR Green Master Mix (High ROX Premixed, Vazyme). The primers for the target genes were synthesized by Life Technologies (Hong Kong). The primer sequences used for the gene expression analysis are listed in Table S4, Supporting Information.

Cecal DNA and qPCR: Cecal DNA was extracted using a QIAamp PowerFecal DNA Kit (Qiagen, Germany) following the manufacturer's instructions. qPCR was performed on the DNA samples as described in Section 2.6. The abundance of microbial communities for each phylum or genus was calculated using a formula described previously and expressed as a percentage of that population within the total measured bacterial community.[57] The primer sequences are listed in Table S4, Supporting Information.[58–60]

Liver and Adipose Tissue Histology: Liver and epididymal adipose tissue samples (approximately 5 × 5 mm) were washed in phosphate-buffered saline, fixed in formalin for 24 h, and processed using a Tissue Processor (Leica ASP300S, Germany) following Feldman and Wolfe's (2014) method.[61] Paraffin-embedded blocks (Embedding Centre, Leica EG1150, Germany) were sectioned to 5-μm-thin slices (Microtome Leica RM2265, Germany) and then stained on slides (Leica ST5020 Multistainer, Germany) using the hemotoxylin and eosin (H&E) staining method.[61]

Statistical Analysis: Statistical analysis was performed using GraphPad Prism 6.0 for Mac (GraphPad Software Inc., San Diego, CA, USA). All data were expressed as the mean ± SEM. Differences among groups were determined by one-way analysis of variance (ANOVA), including Tukey's multiple comparison tests, and comparisons between two given groups were analyzed using two-tailed unpaired Student's t-tests. One-way ANOVA for repeated measures was tested for body weight, GTT, and ITT data. Pearson's correlation coefficient tests were carried out for some data. Statistical significance was considered at $p < 0.05$.

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.

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