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RESEARCH ARTICLE

High carbohydrate diet decreases microbial diversity and increases IL-1β levels in mice colon

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Abstract

Western diet is known to contribute to intestinal dysbiosis and the progression of inflammation. Although the Turkish diet has different macronutrient contents, the intestinal inflammatory disease incidences in Türkiye are comparable to Western countries. Thus, we hypothesized that high carbohydrate diets also contribute to inflammation of the colon. We compared diets with different macronutrient compositions and investigated their effects on colonic microbiota, cytokine, histology, and tight junction protein levels. High carbohydrate diet caused the lowest microbial diversity and is accompanied by the highest expression of interleukin-1β and claudin-1. A low carbohydrate diet with zero fiber resulted in the lowest inflammatory markers as well as the lowest occludin and claudin levels. Overall, our results indicate that carbohydrate and fiber contents of the diets are important contributors to colon health.

Keywords Inflammation · Microbiota · Tight junction protein · Carbohydrate · Dietary fiber

Introduction

Dietary composition changes with geography, culture, and time. In line with the popularity of different diets and advances in science and technology, effects of diets on health are studied at several levels. It is known that the nutrient composition of the diet is closely mirrored at the metabolic level (Liu and Sabatini, 2020). Intestinal cells have critical roles in the absorption of nutrients, and they are directly affected by the diet (Hou et al., 2021; Taylor et al., 2021). Stem cells are known to be affected by different diets: High fat (HF) diets increase stem cell clonogenicity and tumorigenicity, while caloric restriction increases stemness without such an effect (Hou et al., 2021; Wang et al., 2021). The intestinal epithelial cell turnover rate and villus structure of the intestine are also affected by the fiber content of the diet (Wu et al., 2018). Thus, diet is closely associated with intestinal function and health. Intestinal epithelial cells (IECs) are constantly exposed to diet, microbiota, and other foreign substances. IECs form a physical barrier that allows for selective permeability (Vancamelbeke and Vermeire, 2017), and their function is regulated by tight junction (TJ) proteins. TJ proteins permit small, water-soluble molecules while limiting bacteria, toxins, and antigens present in the lumen into the mucosal tissues and then to the circulatory system. TJ proteins involve transmembrane proteins like occludin and claudins, and peripheral proteins like zonula occludens (ZO). Claudins are the main determinants of barrier function and control ion passage while occludin is responsible for assembly of tight junctions. Furthermore, junctional adhesion molecules (JAM) play a role in the assembly and formation of functional TJ proteins (Salvo-Romero et al., 2015).

Intestines are closely associated with the immune system and are considered the body's largest immune organ. Thus, increased inflammation in the intestines will affect the whole body as non-gastrointestinal (GI) organs are also affected, causing diseases of the cardiovascular, nervous, and immune systems as well as diabetes. Previous studies indicated that increased levels of proinflammatory cytokines, especially IL-1β and TNFα, increase intestinal permeability and contribute to inflammation by allowing passage

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of lumen contents. TNFα plays a central role in intestinal inflammation and exerts proinflammatory effects in colitis, and anti-TNFα antibodies are used as an effective treatment for Crohn's disease and ulcerative colitis (Neurath, 2014).

Western diet (WD) is composed of highly processed ingredients and contains high amounts/ratios of protein, fat, and simple sugars. It is mostly consumed in developed countries and is assumed to be one of the major causes of the progression of inflammatory bowel diseases (IBD) (Knight-Sepulveda et al., 2015; Statovci et al., 2017). The incidence of IBD in Türkiye is also high (Ozin et al., 2009; Can et al., 2019), even though the average Turkish diet substantially deviates from WD: It is heavily based on refined grains (40% of caloric intake is from bread or other grains) but with lower amounts of fat and sugar. Based on these observations, we hypothesized that consumption of high amounts of refined grains and low-fiber foods could also be an important factor for inflammatory GI diseases. This is because the most prevalent commonality between WD and Turkish diets is the consumption of high amounts of refined grains and low amounts of fiber. To test this hypothesis, we compared the inflammatory states of the gut microenvironment of mice which were fed with various diets containing different carbohydrate, fat, and fiber amounts to each other as well as to the standard chow diet. Casein was the only protein source in all diets, but the carbohydrate, fat, and fiber sources differed. The zero-fiber diets included hydrogenated coconut oil as the fat source, while in high-fiber diets, the fat was mostly from lard, milk fat, and vegetable shortening. Carbohydrate sources of zero-fiber diets were comprised of corn starch and maltodextrin while the higher fiber containing test diets additionally contained cellulose and inulin. WD and high-protein (HP) diets contained corn starch and cellulose as the carbohydrate sources, and lard, milk fat, and shortening as the fat sources. WD was included in the study as it is known to promote inflammation. In contrast to the popular belief that high fat- and simple sugar-rich Western diets are held responsible for increased inflammation, our results suggest that carbohydrate, fiber, and protein contents of diets are additional determining factors in intestinal inflammation, and they should be considered as important parameters in nutrition studies.

Materials and methods

Animals and diets

5-week-old male Balb/c mice were kept at DEKAM (Experimental Researches Application and Research Center), Erciyes University (Kayseri, Türkiye). The animals were kept in three animals/cage and under controlled temperature, air humidity, and regulated light–dark cycles. Animal studies were performed upon permission of Erciyes University Animal Experimentation Central Ethics Committee (CECAE) (approval date: 13 April 2016, approval no: 04/16/067). Mice groups were fed ad libitum. The diets were purchased from TestDiet (Land O' Lakes, Inc, CA, USA), and included a standard chow diet (S) to be used as control and compared to diets high in carbohydrates (HC and HC-0F), low in carbohydrate (LC-0F and ketogenic), and western diets (WD and HP). The diets were specifically developed to mimic different types of human diets. Macronutrient compositions of experimental diets are listed in Table 1.

Each experimental group consisted of 6 mice. In order for mice to adapt to experimental diets, mice were fed with a mixed diet for a week, where standard mice chow was provided along with respective experimental diets, ad libitum. At the end of the first week, all mice groups were switched to and fed with 100% ad libitum experimental diets until the end of the study. At weeks 0, 1, 2, 3, 5, 9, 13, and 17, mice were weighed, fecal samples were taken and stored at – 80 °C. At the end of week 17, euthanization of mice was performed using ketamine (Alfamine, Egevet, Türkiye) xylazine (Rompun, Bayer, Türkiye) cocktail, and colon tissue samples were collected for protein analyses. Liquid nitrogen was used to freeze the samples, which were then

Table 1 Macronutrient composition of diets (% Cal)

Diet	Acronym	Protein	Fat	Carbohydrate	Fiber	Code
Standard chow	S	21	10	70	5.0	*
High carbohydrate zero-fiber	HC-0F	15	11	74	0	58R0
Low carbohydrate zero-fiber	LC-0F	15	59	26	0	58R2
High carbohydrate	HC	16	12	72	5.8	5TJS
Ketogenic	K	33	65	2	7.8	5TJR
Western	WD	16	39	45	6.7	5TJN
High protein	HP	38	40	22	6.8	5TJU

*CARFIL QUALITY rats and mice maintenance: cereal grains, oilseed products and by-products, cereal grain products and by-products, products and by-products of sugar production, oils and fats, milk products, minerals and high fiber materials

stored at − 80 °C. The experimental setup is schematically represented in Fig. 1.

Extraction of bacterial DNA

Fecal samples were isolated using AxyPrep Bacterial DNA Kit (Axygen Biosciences Inc, AZ, USA). The presence of DNA was checked on agarose gel electrophoresis. Concentrations of DNA were assessed using Nanodrop 2000 (Thermo Scientific, MA, USA).

DGGE analyses

V3 region of the 16S ribosomal DNA gene total bacterial community was used for DGGE studies. Primers GC357F (5′-CGCCCGGGGCGCGCCCCGGGCGGGGCGGGGGCACGGGGGGATTACCGCGGCTGCTG-3′) and 518R (5′-CCTACGGGAGGCAGCAG-3′) were used. PCR mixture included 300 ng template DNA, 0.3 mM dNTP, 0.4 µM of each primer, 1.5 U of *Taq* polymerase (GeneAll Biotechnology, Seoul, South Korea), and 1.5 mM $MgCl_2$. PCR conditions were; 94 °C for 5 min, 20 cycles of 30 s at 94 °C, 30 s at starting at 65 °C and then decreased by 0.5 °C until 55 °C, and 30 s at 68 °C. An additional 15 cycles were included in the protocol. DCode (BioRad, CA, USA) was used to analyze PCR products. An acrylamide-bisacrylamide (37.5:1) (Sigma-Aldrich, MA, USA) gel was used with urea and formamide, from 35 to 54% gradients. Gel runs were done for 5 h at 200 V in TAE buffer (Sigma-Aldrich, MA, USA) at 60 °C. A homemade ladder was used to normalize gels, which were chosen from the strongest DGGE bands. Gel patterns were analyzed by GelComparII (Applied Maths, Sint-Martens-Latem, Belgium). Dice similarity coefficients were 0.5 for optimization and tolerance. Dendrograms were constructed using UPGMA. Strong and frequent bands on DGGE were isolated and amplified using primers without GC-clamps. PCR products (194 bp) were sequenced by Medsantek (Istanbul, Türkiye). The sequences were used to identify bacteria at species/genus level using nBLAST (Altschul et al., 1990).

Western blot analyses

Total protein extractions were done using RIPA buffer (Thermo Fisher Scientific, CA, USA), and was performed on ice. Resulting tissue suspensions were centrifuged and supernatants were taken. Protein concentrations were measured using Pierce BCA Protein Assay (Bio-Rad, CA, USA). SDS-PAGE was performed and samples were transferred to PVDF (Bio-Rad, CA, USA). Following primary antibodies were used; polyclonal occludin (1:1000, 40–4700) (Invitrogen, CA, USA), polyclonal claudin 1 (1:1000, 717,800) (Invitrogen, CA, USA), polyclonal TNFα (A15635) (AFG Bioscience LLC, IL, USA), polyclonal IL-1β (A14428) (AFG Bioscience LLC, IL, USA), monoclonal GAPDH (1:1000, MA5-15738) (Invitrogen, CA, USA), HRP-conjugated secondary antibody (1:10,000, 31,460) (Invitrogen, CA, USA). Images were captured by ChemiDocMP (Bio-Rad, CA, USA).

Crypt depth measurement and immunohistochemistry analyses

Histological analyses included 6 mice from each experimental diet group, and 10 different pictures were taken from the colon sections of each mouse. Samples were washed and then incubated in 4% formaldehyde solution, followed by paraffinization and sectioning. They were then deparaffinized and stained in hematoxylin and eosin solutions. For every picture taken, 10 crypt depths were measured using Image J program.

Statistical analyses

Data are presented as means with standard errors. Significance is set at p 0.05. The sample size was determined by power analysis G*Power 3.1 (Franz Faul, Christian-Albrechts-Universität, Kiel, Germany). Statistical analyses were performed on GraphPad Prism. Analysis of the number of DGGE bands was performed by two-way ANOVA with repeated measures. For the remaining analyses, differences between treatment groups were assessed by one-way ANOVA with post-hoc Tukey test ($n \geq 3$ for protein expression, $n \geq 6$ for weight analysis).

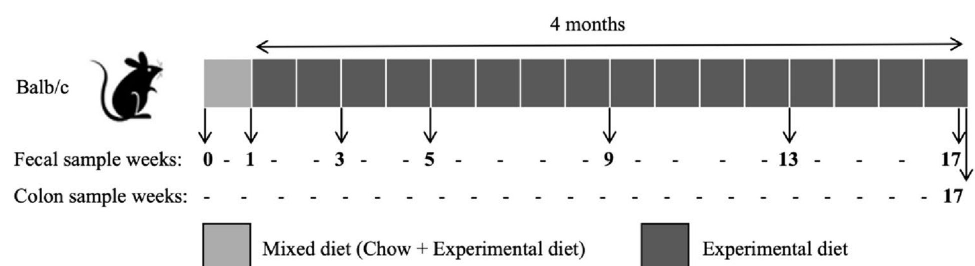

Fig. 1 Schematic representation of experimental setup of different diet types for microbiota and inflammation screening in the colon. Numbers represent the order of collected fecal samples. One box (☐) represents one week

Results and discussion

Nutritional differences are associated with disturbance of colon homeostasis, including disruption of the epithelial barrier, altered epithelial regeneration process, and mucosal immune response (Peterson and Artis, 2014). In this study, we hypothesized that not only the western diet, but also high carbohydrate and low fiber diets would result in intestinal inflammation, and the results confirmed our hypothesis. The results reveal that HC and HP diets are able to elicit significant changes in cytokine and TJP expressions, respectively, a LC-0F diet resulted in the lowest cytokine expressions, and an inflammation-associated microbiota was observed with the HC diet.

Body weight and histomorphology analyses

Adaptation period to different diets resulted in temporary changes in the body weights at the beginning of the study. The study groups started to gain weight in two weeks and there were no significant weight differences between groups at the end of the study. Hematoxylin- and eosin-stained colon sections were evaluated for morphological changes of epithelial cells and mucosal architectures and diet-associated changes between groups are presented in (Fig. 2A). The standard chow diet group showed normal crypt epithelial cells along with normal mucosal and submucosal layers. Epithelial cell structure and crypt architecture were normal in LC-0F and HC groups. In the HC-0F group, normal appearance was dominant, except for epithelial hyperplasia, which is a general feature of intestinal inflammation. The WD group showed epithelial hyperplasia similar to HC-0F group, and additionally, an irregular appearance of epithelial cells and local cell loss causing crypt damage were observed.

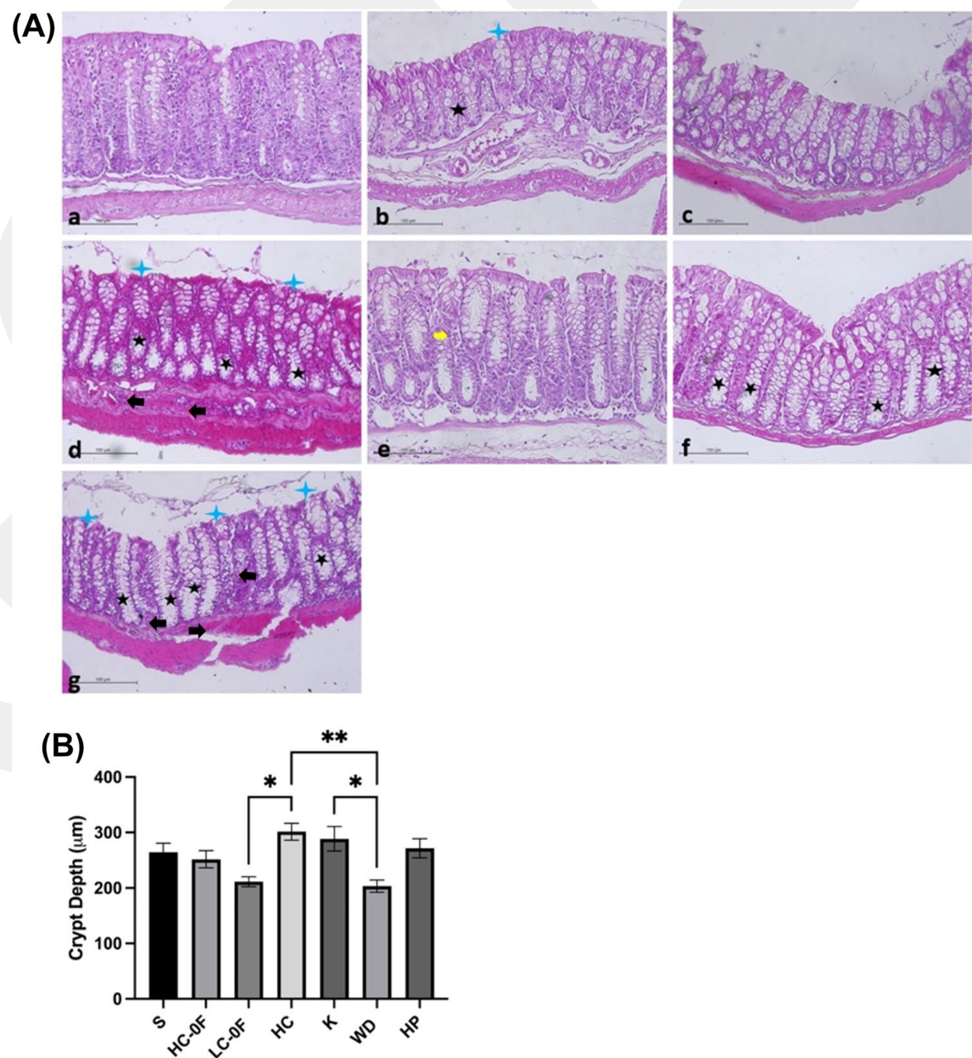

Fig. 2 Histology analysis of colon tissues show the highest epithelial damage in HP group. **A** Histomorphological analysis of colon tissues. Damaged epithelium (black star), leukocyte infiltration (yellow arrow), mucosal surface epithelium (blue star), erythrocyte infiltration (black arrow). Scale bar 100 µm. H&E-stained sections. **B** Crypt depth (µm) in diet groups. Standard chow (S), b: high carbohydrate with 0 Fiber (HC-0F), c: low carbohydrate with 0 fiber (LC-0F), d: western (WD), e: high carbohydrate (HC), f: ketogenic (K), g: high protein (HP)

The HP group displayed epithelial cell loss and disruption of the epithelial barrier, enlargement of the crypt lumen, and an irregular appearance in the mucosa. Erythrocyte infiltration in the submucosa was also evident in this group. Among all experimental groups, the HP group had the greatest damage in mucosal architecture.

There were significant differences in the crypt lengths of different groups (Fig. 2B). The crypt depths were significantly higher in HC than the LC-0F ($p < 0.05$) and WD ($p < 0.01$). The lowest crypt depths among all groups were observed for WD and LC-0F (Fig. 2B). An increased crypt depth in the gut indicates increased intestinal epithelium turnover rate and is positively correlated with gut development and health (Chwen et al., 2013; Rebollada-Merino et al., 2019). WD group mice showed significantly lower crypt depths than S, HC, K, and HP diets. In addition, the LC-0F diet caused significantly lower crypt depths than the HC and K diets, both of which had high fiber. Lower crypt depths imply lower epithelial cell turnover and may result from low butyrate concentrations, and the absence of fiber in the LC-0F diet might have caused this decrease in crypt depths (Hunt et al., 2021; Kuzmuk et al., 2005). But solely fiber content might not be responsible for the higher crypt depths, in fact, we did not observe such an effect among HC groups: HC diet resulted in significantly higher crypt depths than LC-0F and WD groups did. Thus, high carbohydrate content, independently from fiber content, might have a positive effect on crypt health. Overall, WD mice showed the lowest crypt depths among experimental groups, followed by LC-0F. Compared with those two groups, HC mice showed significantly higher crypt depths and higher epithelium turnover rates. Histomorphology and crypt depth analyses collectively indicate that HP and WD groups had the most impaired colon tissues.

Microbiota analysis with DGGE

Denaturing gradient gel electrophoresis (DGGE) was used to study microbial communities with respect to time or different environmental conditions. Interpretation of gels was done at the start and end of the study (Fig. 3). The most diverse microbial community was observed in the standard chow (S) diet group. Standard chow has a complex fiber source; thus, it is not surprising to see this group maintain a rich microbial diversity throughout the study (Fig. 3). DGGE analysis and sequencing of the most dominant bands revealed that lactic acid bacteria were present across all diet groups. The dominant phylum in the study was found to be *Firmicutes*, and *Lactobacillus murinus* was found to be the most commonly observed species between diet groups. *L. murinus* and *L. lactis* are common residents in humans and mice intestinal tracts and have been studied for their potential as therapeutics (Biddle et al., 2014; Taniguchi et al., 2021; Yuan et al., 2020). *L. murinus* was common in all experimental groups at the beginning of the study. At the end of the experiment, most of the groups still preserved this species, except for the LC-0F and WD groups, these two groups revealed a visible decrease in the *L. murinus* populations. Another dominant species across all groups was *Lactococcus lactis*. *L. lactis* was present in all diet groups except for S and this may be an indication that disruption of the microbiota might have favored enrichment of *L. lactis*. This species is frequently isolated from fermented plants and is also frequently used for fermentation in dairy industry (Song et al., 2017). *Anaerotaenia torta* was abundant in low carbohydrate groups (LC-0F and K) while *Muribaculum intestinale* and *Barnesiella intestinihominis* were abundant in HC-0F and HC diets. Similar to the literature, we noted that dysbiosis of the microbiota was associated with an increased inflammatory state of the gut. For example, *B. intestinihominis* had a high abundance in the HC diets, and we expected the HC group to have an elevated inflammatory state. Interestingly, this species is considered as an oncomicrobiotic (Daillère et al., 2016), because it stimulates the immune system, its clinical use benefited renal cell carcinoma patients (Dizman et al., 2021), and a higher progression-free survival was observed in end-stage cancer patients (Daillère et al., 2016). Similarly, *M. intestinale* abundance is increased in HC diet and this species is known to degrade complex carbohydrates. Inversely, high-fat diets (Do et al., 2018) are known to decrease numbers of *M. intestinale* (Lagkouvardos et al., 2016). The increased abundance of this species is positively associated with gut homeostasis, and it was found to protect guts against DSS-induced colitis (Chang et al., 2021). Another difference worth noting is the decrease in *Coprococcus comes* populations in LC-0F and HP diets. *C. comes* is a butyrate producer and a decrease in *Coprococcus* spp. abundance was observed with LC-0F and HP diets. Butyrate is known to affect the inflammatory state of the colon, and a lower abundance of butyrate producers would result in a higher inflammatory state. In fact, decrease in *Coprococcus* spp. is associated with symptoms of GI discomfort in both animal studies (Nagy-Szakal et al., 2017) and colorectal cancer patients (Ai et al., 2019). Additionally, *Anaerostipes caccae* population diminished in the LC-0F diet. *A. caccae* is another butyrate-producing member of the gut microbiota and is known to degrade oligosaccharides. The LC-0F diet resulted in a decreased abundance of this species in our study, in accordance with the literature, where a decreased butyrate concentration was accompanied by lower crypt depths and epithelial cell turnover rate (Kagal and Hogade, 2019).

The increase in abundance of *A. torta* in low carbohydrate diets is in line with the literature, where decreased abundance of *A. torta* was directly associated with highly inflammatory, high carbohydrate diets (Khan et al., 2020).

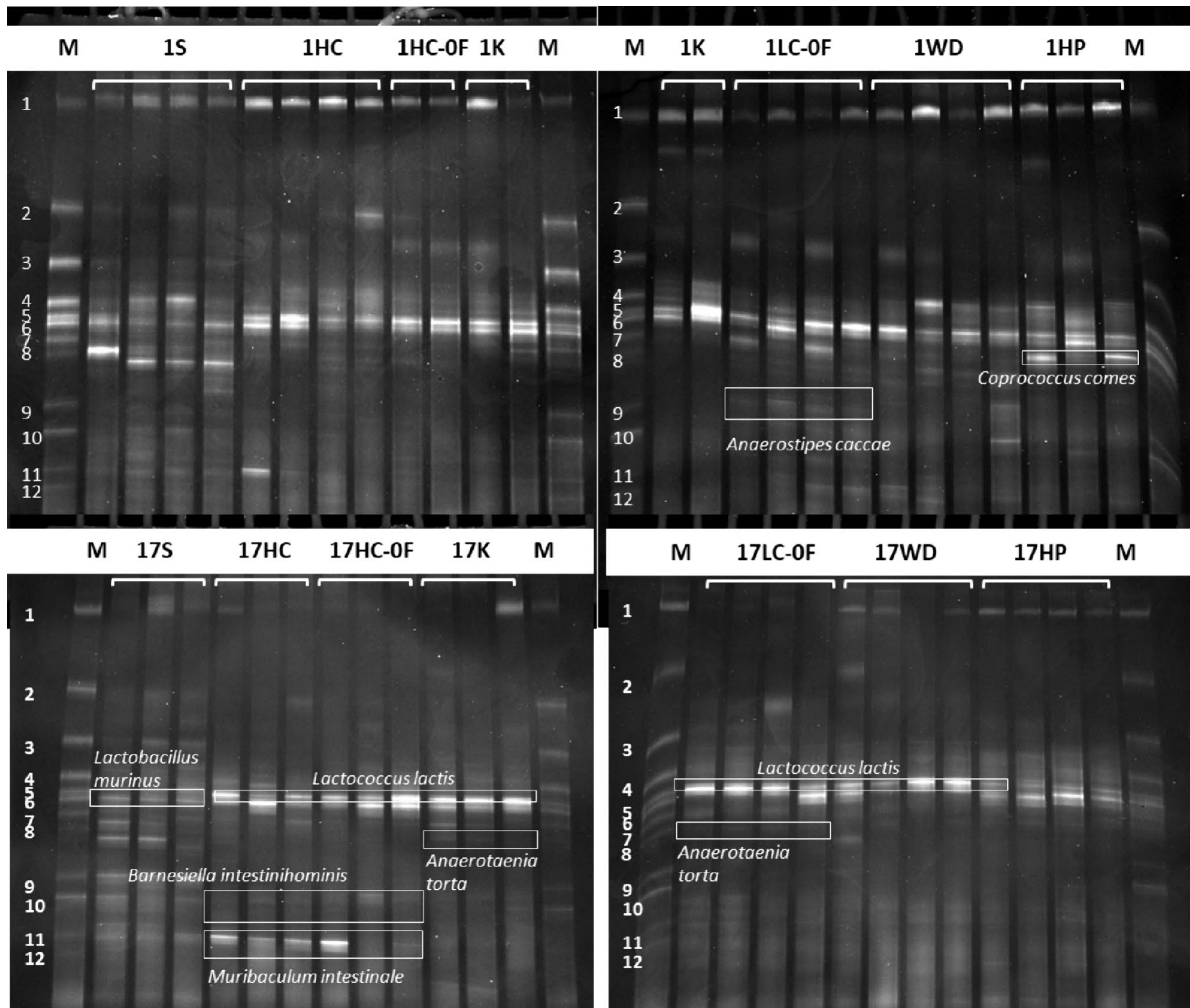

Fig. 3 DGGE fingerprinting results show *Lactobacillus murinus* as the most common and conserved species among different diet groups. DGGE gel profile of the 1st week (above) and 17th week (below) stool samples. *S* standard chow, *HC-0F* high carbohydrate with 0 fiber, *LC-0F* low carbohydrate with 0 Fiber, *HC* high carbohydrate, *K* ketogenic, *WD* western, *HP* high protein diet. The number from 1 to 12 represent marker bacterial species (1: *Lachnoclostridium pacaense*, 2: *Helicobacter japonicus*, 3: *Culturomica massiliensis*, 4: *Clostridium indolis*, 5: *Lactococcus lactis*, 6: *Lactobacillus murinus*, 7: *Coprococcus comes*, 8: *Anaerotaenia torta*, 9: *Anaerostipes caccae*, 10: *Barnesiella intestinihominis*, 11: *Muribaculum intestinale*, 12: *Paraclostridium bifermentans*). Labeled rectangles show strong bands belonging to the specific species in different diet groups (M: marker)

DGGE similarity analysis

DGGE provides a reliable analysis of the microbial community. The dendrogram presents the similarity analysis of the DGGE patterns for the first and the last week (week 17) fecal microbiota samples are shown in (Fig. 4A). The highest resemblance within the same diet groups is for the standard chow diet group, with 20% similarity between the first and the last week samples. For the remaining diet groups, the similarity is lower (< 10%), yet still evident. For example, the first week samples of different diet groups tend to cluster together, as do last week samples. HP and WD diet groups tend to cluster together and form distinct clusters from other diet groups. Another group to point out would be the zero fiber groups, namely HC-0F and LC-0F. These tend to form clusters together, independent of the carbohydrate content of the diets. Although these groups are a bit more scattered, the pattern similarity is close to 20%. The DGGE patterns showed that diversity between the groups was higher in the first week compared to that in the last week. The number of DGGE bands indicates microbial diversity for each particular diet. Figure 4B

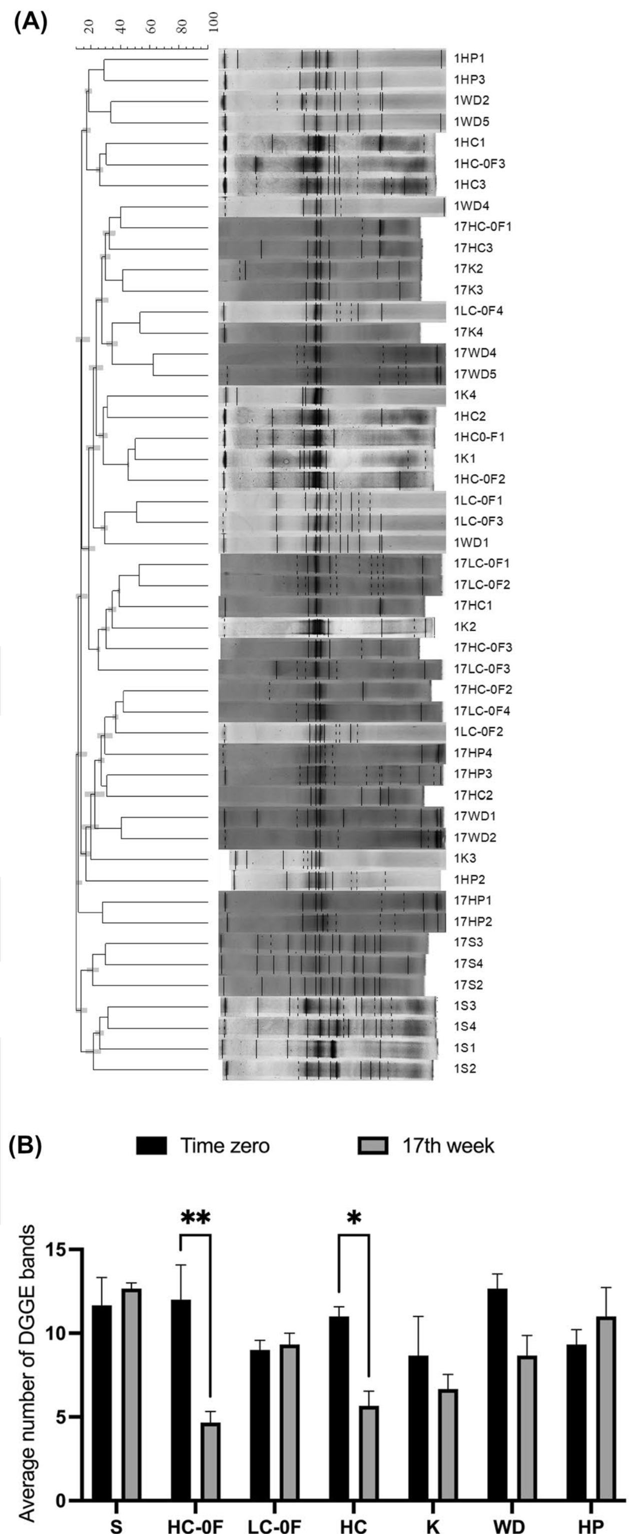

Fig. 4 Analysis of DGGE dendrogram. **A** Same diets and similar macronutrient containing diets at the beginning and the end of study tend to cluster together. Similarity (Pearson correlation coefficient-UPGMA) of the DGGE patterns between 1st and 17th week stool samples. Sample names collected at week 1 or week 17 start with these week numbers. **B** average DGGE band numbers decrease significantly in HC-0F and HC diets. Samples are from time zero and last (17th) week. *S* standard chow, *HC-0F* high carbohydrate with 0 fiber, *LC-0F* low carbohydrate with 0 fiber, *HC* high carbohydrate, *K* ketogenic, *WD* western, *HP* high protein diet. $2^{-\Delta Ct}$ values were normalized with standard chow diet. n ≥ 3 mice. *p < 0.05, **p < 0.005

provides the average number of bands for each diet for the first and last weeks of the study. The number of bands for S did not change throughout the experiment. The number of bands for LC-0F, K, WD, and HP diet remain similar as well. At the end of the study, diets that contained high amounts of carbohydrates (HC and HC-0F), resulted in significantly lower number bands when compared to the first week ($p < 0.05$ and $p < 0.01$, respectively). When the average number of bands for the latter was analyzed in more detail (data not shown), it was seen that the decrease in number of bands continued throughout the study.

DGGE patterns of experimental groups were compared to assess the state of microbial diversity caused by different diets. DGGE method is expected to reveal the most abundant species in a microbial community and although it captures a small percentage of the microbial population (Liu et al., 2010; Heiman and Greenway, 2016), it is known to as a valuable tool to observe changes in microbial community, which was also observed in our study (Fig. 4A). Fecal samples obtained from each diet group at the start and the end of the study showed that DGGE patterns in standard chow diet groups clustered together and maintained a healthy bacterial diversity throughout the study. Patterns of samples from remaining diets were also clustered together, though not as much as the standard chow diet group. The remaining diets generally grouped within the same time intervals, either at the beginning or at the end of the experiment. Similarly, we observed clustering between the samples from diets containing similar macronutrient ratios: HP clustered together with WD, K with LC-0F, and WD and zero fiber diets tended to cluster together. We also observed a decrease in microbiota diversity throughout the study for most of the diet groups. The total band number in DGGE is accepted as an indicator of microbial richness, and the reduction of bacterial diversity in the gastrointestinal tract is known to be characteristic of IBDs (Heimann and Greenway, 2016). In our study, decreased microbial diversity was evident in HC and HC-0F groups (Fig. 4B). Not surprisingly, these diets also had the highest carbohydrate content among the experimental diet groups (74%), since diets with a greater imbalance in their macronutrient content are known to result in reduced microbial diversity (Heiman and Greenway, 2016).

Cytokine and TJ protein expressions

Protein expressions of two of the pro-inflammatory cytokines, TNFα and IL-1β, were studied via western blotting (Fig. 5). No significance was observed across diet

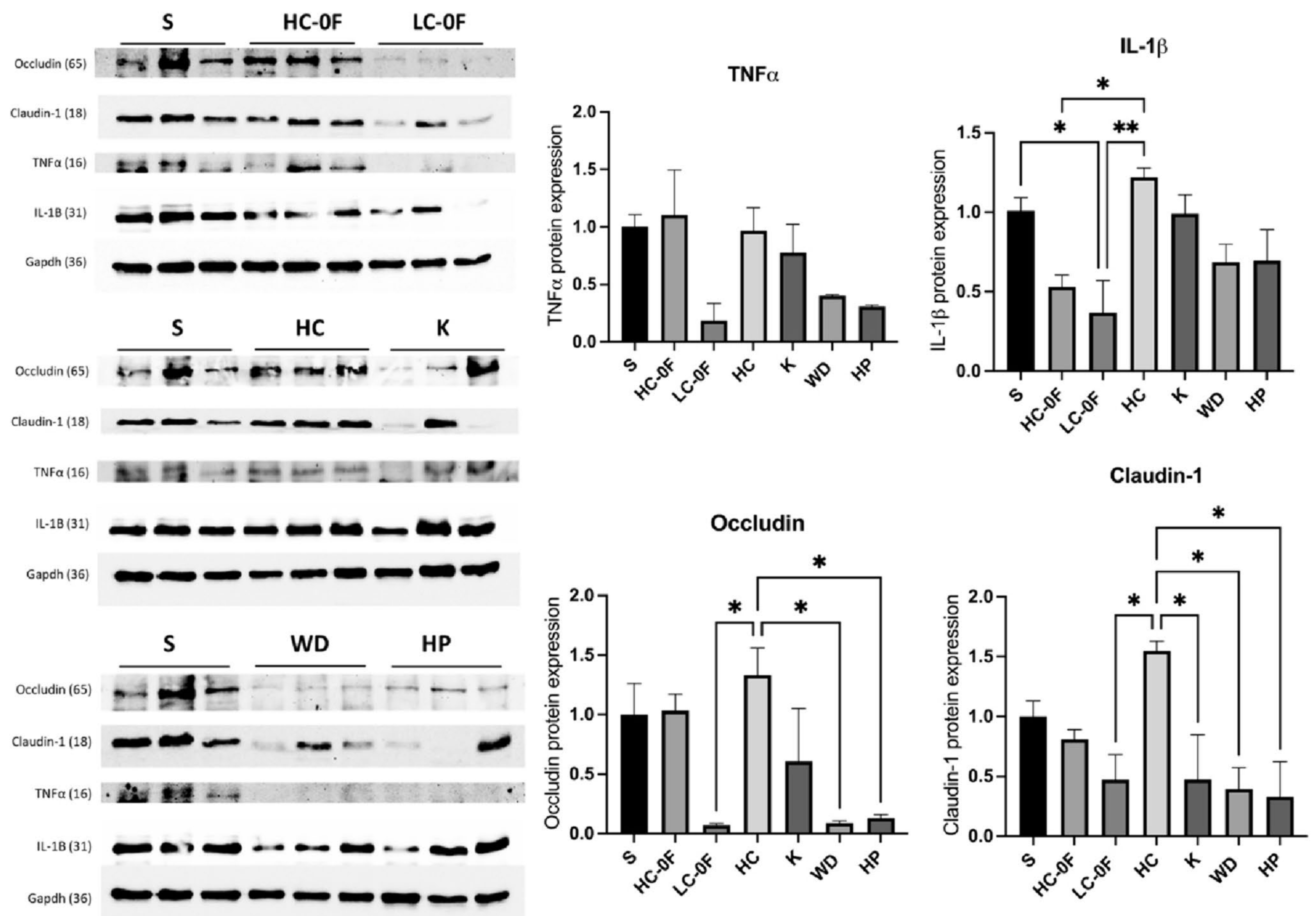

Fig. 5 Protein expressions of inflammatory cytokines TNFα and IL-1β reveal lowest inflammation in LC-0F diet. Occludin and Claudin-1 expressions are highest for HC and lowest for LC-0F, HP and WD. *S* standard chow diet, *HC-0F* high carbohydrate with 0 fiber, *LC-0F* low carbohydrate with 0 fiber, *HC* High carbohydrate, *K* ketogenic, *WD* western, *HP* high protein diet. *$p<0.05$, **$p<0.01$, ***$p<0.001$

groups for TNFα protein expression. For IL-1β expressions, the LC-0F group had significantly lower protein levels than both S ($p<0.05$) and HC ($p<0.01$) groups. In addition, the HC group had significantly higher IL-1β expressions than HC-0F ($p<0.05$) (Fig. 5). For both proinflammatory cytokines, the LC-0F group had lower protein expressions than the remaining groups (not significant). For both proinflammatory cytokines, a pronounced decrease is observed in the LC-0F diet. This and the significantly lower IL-1β levels for HC-0F than in the HC diet ($p<0.05$) may suggest that fiber content could contribute to differences in inflammatory responses. This is in contrast to the reports in the literature, where the presence of fiber is associated with a lower inflammatory state. However, it should also be noted that not all fibers produce anti-inflammatory results and that the microbiota and its by-products determine the inflammatory action. This controversial finding, however, is supported by the fact that the main treatment for IBS and IBD generally includes reduction of fiber intake (Werlang et al., 2019). In summary, it is evident that the LC-0F diet caused the lowest levels of both pro-inflammatory cytokines in the study, indicative of lower levels of inflammation.

TJ proteins maintain the integrity of the inter-epithelial barrier and are crucial in regulating the selective permeability of solutes across epithelia (Fig. 5). Impaired structure and loss of integrity of the TJ proteins cause inflammation through increased passage of foreign antigens, bacteria, and toxins into lamina propria. These foreign antigens activate inflammation, via helper T-lymphocytes and also antigen-presenting cells (Chelakkot et al., 2018). Animal model studies and human clinical studies of inflammatory diseases of the gut present conflicting results for TJ protein expression levels. It is generally reported that occludin levels decrease in an inflammatory environment, while claudin-1 levels increase (Garcia-Hernandez et al., 2017; Weber et al., 2008) not change or decrease (Li et al., 2014; Mennigen et al., 2009; Nighot et al., 2015). None of the experimental diet groups

showed significantly different TJ protein expressions than in the standard chow diet. Similar expression levels were observed for both proteins. The HC diet caused significantly higher occludin expression levels than the LC-0F, WD, and HP ($p < 0.05$) diets. Similarly, the HC group showed significantly higher claudin-1 expression levels than the LC-0F, K, WD, and HP ($p < 0.05$) diet groups did. It is especially important to indicate that while WD is held responsible for increased permeability and inflammation, we have observed even higher claudin-1 expressions for HC (Fig. 5). The low levels of claudin-1 in the LC-0F diet point to lower levels of inflammation and coincides with the fact that the LC-0F diet also had the lowest TNFα and IL-1β expressions (Fig. 5). DGGE analyses proved that the LC-0F diet resulted in a decreased abundance of butyrate producers (*C. comes* and *A. caccae*) which might play a role in regulating the TJ expression levels. In addition, *C. comes* is correlated with the production of IL-1β and IL-6 (Schirmer et al., 2016) and a decreased inflammatory response following the decreased abundance of *C. comes* correlates with the downregulation of TJ proteins. Our findings suggest that the decreased fiber intake may contribute to lowering inflammation through modulating microbiota.

Results from our study are quite important in pointing to dietary carbohydrate and fiber contents and the response of the gut microenvironment. We observed a positive correlation between bacterial diversity and levels of pro-inflammatory cytokines in our study. At the end of our study, it was observed that diets with lower bacterial diversity had higher cytokine levels, as in HC, and diets with unaffected bacterial diversity (LC-0F, WD, HP) had lower cytokine levels (Le Chatelier et al., 2013). The presence of high carbohydrate affected the expression of pro-inflammatory cytokine IL-1β and claudin-1 levels, the HC diet had increased expressions of both, in accordance with the increased inflammation seen in inflammatory diseases (Garcia-Hernandez et al., 2017; Weber et al., 2008).

Histological analysis of the colon displayed the highest epithelial damage in WD and HP diets; however, these effects were not as dramatic at the molecular level for either inflammatory or TJ levels. As for the expression of pro-inflammatory markers TNFα and IL-1β, WD did not result in an increase in these markers, meaning the inflammation is either TNFα and IL-1β independent, or that during the experiments, an adaptive response has been produced to mitigate the inflammation at the molecular level. One thing to note is that significantly low levels of occludin are observed in LC-0F, WD, and HP, and the histological analysis showed that HP and WD had the most impaired structures. However, histological analysis showed a normal crypt architecture for LC-0F, which might be associated with the low levels of both inflammatory cytokines, which in turn might help in building a normal colon architecture.

In this study, we aimed to reveal how different diets shape the colonic microbiota, important markers of inflammation, and TJ proteins. The typical Western diet was included as it was considered a primary factor in colonic inflammation. Based on the observation of similar IBD incidence in Western countries and Türkiye, despite much higher levels of refined grains in the latter, we suggested that a diet rich in refined grains may also be a significant contributor to IBDs. The effects of varying concentrations of macronutrients and fiber on the microbiota, colonic inflammatory markers, and TJ proteins resulted in varying levels of responses. In the literature, it was observed that a decrease in bacterial diversity results in inflammation of the colon, similar to what was observed in this study. Changes in microbial diversity and protein expressions of inflammatory markers coincided well with the TJ protein expressions. The HC diet resulted in significantly lower microbial diversity, higher IL-1β, and significantly higher claudin-1 levels among all diet groups. LC-0F had low crypt depth, unchanged microbial diversity, and significantly lower inflammatory markers and TJ proteins among all groups. Microbial analysis by NGS and RNAseq data would provide a more detailed analysis of the samples in future studies. This study indicates that the carbohydrate and fiber content of diets are strong determinants of the inflammatory state of the colon, and further studies are necessary to elucidate their significance for the inflammation and TJP expression in the colon.

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Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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