

ORIGINAL ARTICLE

Dietary interventions to prevent high-fructose diet-associated worsening of colitis and colitis-associated tumorigenesis in mice

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Abstract

Diet is believed to be an important factor in the pathogenesis of inflammatory bowel disease. High consumption of dietary fructose has been shown to exacerbate experimental colitis, an effect mediated through the gut microbiota. This study evaluated whether dietary alterations could attenuate the detrimental effects of a high-fructose diet (HFrD) in experimental colitis. First, we determined whether the procolitic effects of a HFrD could be reversed by switching mice from a HFrD to a control diet. This diet change completely prevented HFrD-induced worsening of acute colitis, in association with a rapid normalization of the microbiota. Second, we tested the effects of dietary fiber, which demonstrated that psyllium was the most effective type of fiber for protecting against HFrD-induced worsening of acute colitis, compared with pectin, inulin, or cellulose. In fact, supplemental psyllium nearly completely prevented the detrimental effects of the HFrD, an effect associated with a shift in the gut microbiota. We next determined whether the protective effects of these interventions could be extended to chronic colitis and colitis-associated tumorigenesis. Using the azoxymethane/dextran sodium sulfate model, we first demonstrated that HFrD feeding exacerbated chronic colitis and increased colitis-associated tumorigenesis. Using the same dietary changes tested in the acute colitis setting, we also showed that mice were protected from HFrD-mediated enhanced chronic colitis and tumorigenesis, upon either diet switching or psyllium supplementation. Taken together, these findings suggest that high consumption of fructose may enhance colon tumorigenesis associated with long-standing colitis, an effect that could be reduced by dietary alterations.

Introduction

The incidence of inflammatory bowel diseases (IBD) has increased worldwide (1–3). Increased consumption of a western diet has been suggested to contribute to the rising incidence of IBD (4–6). In addition to being high in saturated fat and low in fiber, a western diet is enriched in fructose (7–9). This has led

to the hypothesis that diets high in fructose could contribute to the rising incidence of IBD (10–12). In support of this possibility, we and others found that a high-fructose diet (HFrD) exacerbated experimental colitis in several mouse models (13,14). Dietary fructose-induced changes in the fecal microbiota, which

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Abbreviations

AOM	azoxymethane
CRC	colorectal cancer
DSS	dextran sodium sulfate
HFrD	high-fructose diet
H&E	hematoxylin and eosin
IBD	inflammatory bowel disease

included a loss of probiotic bacteria, were causally linked to the worsening of colitis (13). Among its effects, dietary fructose has been found to alter the integrity of the gut barrier (13–16).

A well-recognized complication of long-standing colitis is an increased risk of colorectal cancer (CRC) (17–21). The risk for IBD patients to develop CRC increases with the extent of colitis, severity of inflammation, and duration of disease (22–24). Repetitive tissue destruction and renewal along with oxidative damage can induce mutations and tumor initiation, predisposing to colitis-associated CRC (19,25–27). Because a HFrD causes worse acute experimental colitis, it is reasonable to posit that excess dietary fructose would also exacerbate chronic colitis leading to an increase in colitis-associated colorectal tumors. Developing strategies to attenuate colitis and thereby reduce the risk of colitis-associated CRC represents a significant unmet need. Chemoprevention strategies utilizing anti-inflammatory agents, e.g., 5-aminosalicylates have been tested but the benefits remain uncertain (28–30). In a recent epidemiological study, a diet imbalance with high consumption of soft drinks and sugar and low consumption of vegetables was associated with increased risk of ulcerative colitis (31). The possibility that this reflected the ability of vegetable-derived fiber to protect against the harmful effects of fructose was considered. Consistent with this possibility, psyllium fiber was recently reported to suppress acute experimental colitis (32).

The current study tested the ability of two potentially practical dietary alterations to attenuate HFrD-mediated exacerbation of experimental colitis and colitis-associated tumorigenesis. We found that either switching from a HFrD to a fructose-free diet or providing supplemental psyllium fiber protected against the worsening of acute and chronic colitis caused by a HFrD. Additionally, these two approaches suppressed the enhanced inflammation-associated colorectal tumor development resulting from high-fructose feeding. These findings suggest that altering dietary patterns in IBD patients could reduce disease severity and the risk of developing colorectal cancer.

Materials and methods

Diet composition and induction of acute experimental colitis

Mice were fed AIN-93G purified diet (control) containing a mixture of carbohydrates (64 kcal%) or a HFrD (based on AIN-93G) in which all carbohydrate content comes from fructose. These two diets contain cellulose (5% w/w) as a source of fiber. Additional HFrD diets that were tested contained different fibers including psyllium, pectin and inulin (5% w/w). The details of the composition of the different diets are shown in [Supplementary Table 1](#), is available at [Carcinogenesis Online](#).

To induce colitis, mice were administered 1% dextran sodium sulfate (DSS; MP Biochemical) in drinking water for 7 days. For comparisons of colitis severity during DSS administration, measurements including body weight, rectal bleeding and diarrhea were carried out as described previously (33). Briefly, diarrhea was assessed by measuring the softness or appearance of the stool on a scale from 0 to 3. Bleeding was determined by detection of heme in stool using the Hemocult Sensa test (Beckman Coulter, Brea, CA) or evidence of gross bleeding on a scale from 0 to 3. At the end of the experimental period, mice were euthanized and colons

were excised, length measured then they were flushed with ice-cold phosphate-buffered saline. Colons were then fixed in 10% formalin or 4% paraformaldehyde for 4–6 h, Swiss-rolled, paraffin-embedded then sectioned. Sections were stained with hematoxylin and eosin (H&E) to determine the severity of histological injury, using a scoring system that was adapted from (34). All animal studies were approved by the Institutional Animal Care and Use Committee of Weill Cornell Medicine.

Chronic colitis and colitis-associated colorectal tumor model

C57BL/6J mice were purchased from the Jackson Laboratory and administered a single intraperitoneal injection of azoxymethane (AOM) (10–12.5 mg/kg; Sigma-Aldrich) at 9 weeks of age. At 12 weeks of age, 1% DSS was administered in drinking water for 5 days followed by regular drinking water for 14 days. This cycle was repeated twice using 0.5% and 0.25% DSS for the second and third rounds, respectively. Measurements including body weight, rectal bleeding and diarrhea were carried out after the initiation of DSS as described above.

At the end of the experimental period, colons were excised and flushed with phosphate-buffered saline and fixed in 4% paraformaldehyde. Whole mounts were stained with methylene blue for gross enumeration of tumors then Swiss-rolled, paraffin embedded, and subjected to histologic analysis by a gastrointestinal pathologist, following H&E staining. Colon sections were evaluated using pathology scores for chronic DSS-induced colitis models, as described previously (35).

16S rRNA analysis

Feces were collected from individual mice by placing them into empty cages. Feces were snap-frozen in liquid nitrogen and stored at -80°C until shipped. One fecal pellet per mouse was shipped to Molecular Research LP (Shallowater, TX) for 16S rRNA profiling. DNA extraction from feces was carried out using the Powersoil DNA Kit (Qiagen) per manufacturer's instructions. Following DNA extraction, the 16S rRNA gene V4 variable region PCR primers 515/806 with barcode on the forward primer were used in a 30 cycle PCR using the HotStarTaq Plus Master Mix Kit (Qiagen) under the following conditions: 94°C for 3 min, followed by 30–35 cycles of 94°C for 30 s, 53°C for 40 s and 72°C for 1 min, after which a final elongation step at 72°C for 5 min was performed. Pooled samples were purified using calibrated Ampure XP beads, and the pooled and purified PCR product was used to prepare illumina DNA library. Sequencing was performed using an Illumina HiSeq. Sequence data were processed using the MR DNA analysis pipeline (Shallowater, TX). In summary, sequences were joined, depleted of barcodes then sequences < 150 bp or with ambiguous base calls were removed. Sequences were denoised, operational taxonomic unit generated, and chimeras removed. Operational taxonomic units were defined by clustering at 3% divergence (97% similarity). Final operational taxonomic units were taxonomically classified using BLASTn against a curated database derived from RDP11 and NCBI (www.ncbi.nlm.nih.gov, <http://rdp.cme.msu.edu>).

Statistical analysis

To evaluate differences in study end points including colon length, histologic score, tumor number and tumor size between two experimental groups, the non-parametric Wilcoxon rank-sum test was used. The non-parametric Kruskal-Wallis test was used to evaluate differences in the above end points across multiple groups. Differences between groups of interest were further assessed using Wilcoxon rank-sum test. *P*-values were adjusted for multiple comparisons by using the Bonferroni-Holm method. These end points were summarized graphically using box-plots with default summary statistics in R (e.g. center line, median; box limits, upper and lower quartiles; whiskers, the most extreme data points within $1.5\times$ interquartile range from the box; points, data points lying beyond the extremes of the whiskers). Differences in tumor incidence in mice between or across experimental groups were examined using the Fisher's exact test. For relative mouse body weight and the occurrence of bleeding (score ≥ 1), severe bleeding (score ≥ 2), diarrhea (score ≥ 1) or severe diarrhea (score ≥ 2) measured over time, linear and generalized linear mixed effects model were used where appropriate. Contrasts for parameters of interest including the rate of change in weight and average proportion

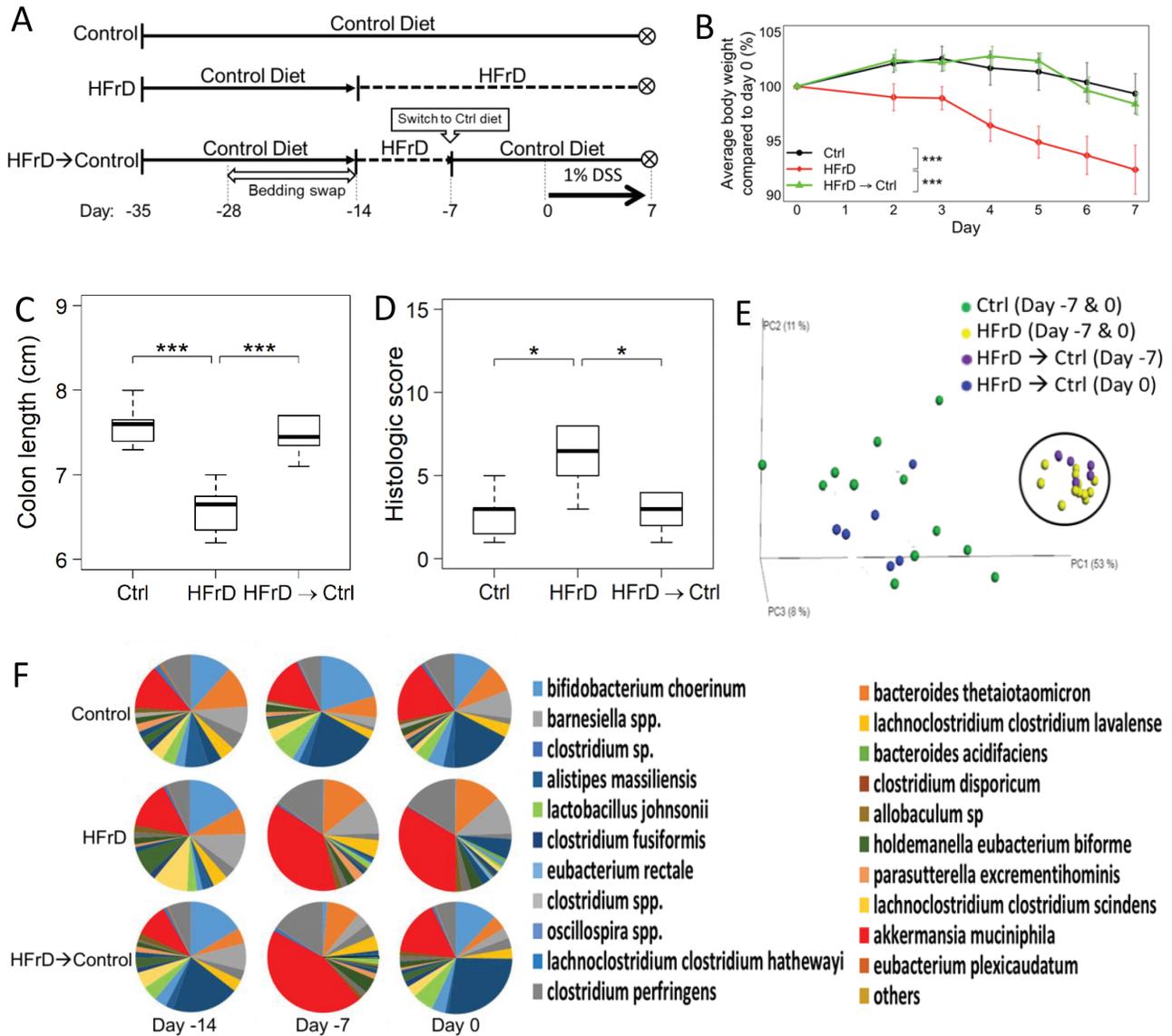


Figure 1. Switching from a HFrD to a control fructose-free diet protects against HFrD-induced worsening of colitis in association with normalizing the gut microbiota. (A) Experimental design showing groups and treatments of mice given control, HFrD or switched from HFrD to control diet. (B–D) Measurements of colitis severity were carried out in mice described in (A) during and following DSS exposure including body weight determined by calculating percent change on a given day compared with the weight on day 0 (according to (A)) during DSS exposure (B), colon length measured following harvesting of colon at necropsy (C) and histologic score evaluated on H&E-stained sections of the colon (D) ($n = 7$ – 8 /group for B–D). * $P < 0.05$, *** $P < 0.001$. For (C) and (D), components of box and whisker plots include center line, median; box limits, upper and lower quartiles; whiskers, the most extreme data points within $1.5 \times$ interquartile range from the box. (E) and (F) Fecal samples were collected from individual mice in each group on days -14 , -7 , and 0 (according to (A)) and one pellet was used for 16S rRNA profiling. Data are shown by principal coordinate analysis (E) and the percent abundance of different bacteria in each group (F) ($n = 6$ /group for E and F). Black circle in (E) indicates clustering of samples from the HFrD group on days -7 and 0 and the HFrD → Ctrl group on day -7 .

of mice with bleeding, severe diarrhea or severe diarrhea were examined using simultaneous tests of general linear hypotheses. P -values were adjusted for multiple comparisons by controlling the experiment-wise error rate using the ‘single-step’ implementation in the R *multcomp* package. All the above described analyses were carried out in R.

Results

Switching from a HFrD to a fructose-free control diet protects against the procolitic effects of dietary fructose

Given previous work demonstrating that feeding a HFrD for 1 week to mice worsens acute colitis (13), we first tested whether removing the fructose-containing diet after 1 week of

consumption could reverse these effects. This approach was employed to mimic a potential clinical scenario in which IBD patients remove excess fructose from their diet. To test this, mice were fed a control diet for 21 days (days -35 to -14 in Figure 1A), 14 days of which bedding was swapped between cages in order to homogenize the microbiota (days -28 to -14 in Figure 1A). Mice were then either continued on control diet or given HFrD for 7 days (days -14 to -7 in Figure 1A), after which, half of the mice given HFrD were switched back to control diet for an additional week, whereas the remaining mice were continued on their original diets (days -7 to 0 in Figure 1A). All mice were then challenged with 1% DSS in drinking water for 7 days (days 0 to 7 in Figure 1A) to induce colitis while being continued on their respective diets, and colitis severity was assessed. As

shown in [Figure 1B–D](#), mice fed the HFrD manifested markedly worse colitis compared with mice fed the control diet. However, mice switched from HFrD to control diet were protected from the procolitic effects of the HFrD, as demonstrated by attenuated weight loss, lack of colonic shortening and reduced histological injury ([Figure 1B–D](#)). Given our recent report showing the importance of the microbiota in HFrD-induced worsening of colitis, we next tested whether microbial changes paralleled the diet-mediated changes in colitis severity (13). Hence, we carried out 16S rRNA analysis of feces from mice fed the control diet for 28 or 35 days (Control group on days –7 and 0 in [Figure 1A](#)), HFrD for 7 or 14 days (HFrD group on days –7 and 0 in [Figure 1A](#)) or HFrD for 7 days then switched to control diet for 7 days (HFrD → Control group on days –7 and 0 in [Figure 1A](#)). As expected, principal coordinate analysis showed a marked shift in microbial populations following 7 or 14 days of HFrD feeding ([Figure 1E](#)). Consistent with the improvement in colitis ([Figure 1B–D](#)), the changes in the microbial population induced by HFrD were rapidly reversed by switching mice back to control diet for 7 days ([Figure 1E](#)). When examined at the species level, the HFrD led to profound changes in the microbiota including increased abundance of *Akkermansia muciniphila* and decreased abundance of *Lactobacillus johnsonii*, species both suggested to be important in HFrD-induced worsening of colitis (13) ([Figure 1F](#); [Supplementary Table 2](#), is available at *Carcinogenesis* Online). Remarkably, when the mice were switched from a HFrD to a control diet, the changes in these populations and others were rapidly reversed, and mimicked what was found in mice fed control diet during the entire experiment ([Figure 1F](#); [Supplementary Table 2](#), is available at *Carcinogenesis* Online). Taken together, these results demonstrate that removing fructose from the diet led to a rapid reversal of the procolitic effects of the HFrD, which were paralleled by normalization of the microbiota.

Psyllium supplementation protects mice from HFrD-induced exacerbation of acute colitis

The above results indicate that removing fructose from the diet can rapidly prevent the procolitic effects of a HFrD. However, given how ubiquitous fructose is in both processed foods and sugar sweetened beverages, eliminating fructose from the diet may not be practical for many IBD patients. With this in mind, we next tested whether using a dietary supplement, a potentially more practical approach, would have a similar protective effect. To this end, we tested the efficacy of three different types of fiber including inulin, pectin and psyllium when compared with standard diet containing cellulose as its fiber source, on acute colitis severity during HFrD feeding ([Supplementary Table 1](#), is available at *Carcinogenesis* Online). Mice were fed these four isocaloric diets for 1 week and then challenged with 1% DSS in drinking water to induce colitis while being continued on their respective diets, and colitis severity was assessed. As shown in [Supplementary Figure 1](#), is available at *Carcinogenesis* Online, diet containing psyllium as its fiber source was most effective at protecting against the procolitic effects of a HFrD. We next determined to what extent psyllium protects against the detrimental effects of a HFrD. To test this, all mice were fed a control diet for 21 days (days –28 to –7 in [Figure 2A](#)), 14 days of which bedding was swapped between cages in order to homogenize the gut microbiota. Mice were then either continued on control diet containing cellulose, given HFrD-containing cellulose or HFrD-containing psyllium for 7 days (days –7 to 0 in [Figure 2A](#)). All mice were then challenged with 1% DSS in drinking water for 7 days to induce colitis while being continued on their

respective diets, and colitis severity was assessed (days 0 to 7 in [Figure 2A](#)). As shown in [Figure 2B–F](#), psyllium supplementation strongly protected against DSS-induced colitis during HFrD feeding. To determine whether the protective effect of psyllium on colitis was associated with microbial changes, we carried out 16S rRNA analysis of the feces from mice prior to DSS administration (day 0 in [Figure 2A](#)). These data showed that although feeding a HFrD induced a shift in microbial species, supplementation with psyllium caused a distinct shift altogether ([Figure 2G](#); [Supplementary Table 3](#), is available at *Carcinogenesis* Online).

A HFrD exacerbates chronic colitis and increases colitis-associated colorectal tumor burden

The above findings provide two potential methods to attenuate acute colitis enhanced by high-fructose feeding. Therefore, we next sought to test whether these interventions protect against chronic colitis and the tumorigenesis associated with this condition. This concept is especially relevant given the increased risk of developing colon cancer in patients with long-standing IBD (17–21). Before evaluating these approaches in this setting, we first determined whether long-term administration of a HFrD would exacerbate chronic colitis and result in more colorectal tumors. To test this, the two isocaloric diets that were used in the acute studies described above were utilized: control diet (AIN-93G purified diet) containing a mixture of carbohydrates and a HFrD in which all carbohydrate content comes from fructose ([Supplementary Table 1](#), is available at *Carcinogenesis* Online). As shown in [Figure 3A](#), mice were fed the control diet for 21 days. Mice were then given one intraperitoneal injection of the colon carcinogen AOM (day 0 in [Figure 3A](#)). Fourteen days after receiving AOM, the mice were either continued on control diet or given HFrD for 7 days (days 14 to 21 in [Figure 3A](#)). The mice then received three rounds of decreasing concentrations of DSS (one round = 5 days of DSS followed by 14 days of regular drinking water to induce colitis) ([Figure 3A](#)). Mice were given regular drinking water after the last round until sacrifice ([Figure 3A](#)). Colitis severity (body weight, diarrhea) was assessed both during the experimental period and at the end of the study (colon length, histologic score). As shown in [Figure 3B–E](#), each of these endpoints revealed that mice fed a HFrD manifested worse chronic colitis compared with mice fed the control diet.

To evaluate whether the exacerbation of chronic colitis mediated by a HFrD was associated with a greater colorectal tumor burden, colons were harvested at the end of the experimental period (day 92 in [Figure 3A](#)). Methylene blue staining of harvested colons and subsequent H&E staining of colonic sections suggested more tumors in the mice fed the HFrD ([Figure 4Aa](#) and [Ab](#)). Direct counting of tumors on methylene blue-stained whole mounts revealed a significant increase in tumor incidence, number per mouse and size ([Figure 4B–D](#)). The differences in tumor number between groups were confirmed by counting tumors using H&E-stained sections of colons (0.17 ± 0.58 versus 1.0 ± 1.21 ; $P = 0.02$). Taken together, these findings demonstrate that feeding a HFrD to mice enhances chronic colitis and the development of colitis-associated tumors.

Switching back to a control diet after HFrD feeding attenuates chronic colitis and tumor development

Having established that feeding a HFrD enhances chronic colitis and colitis-associated tumorigenesis ([Figures 3](#) and [4](#)) and switching from a HFrD to a control diet abrogates the acute procolitic effects of a HFrD ([Figure 1](#)), we next investigated whether dietary fructose removal attenuates chronic colitis and

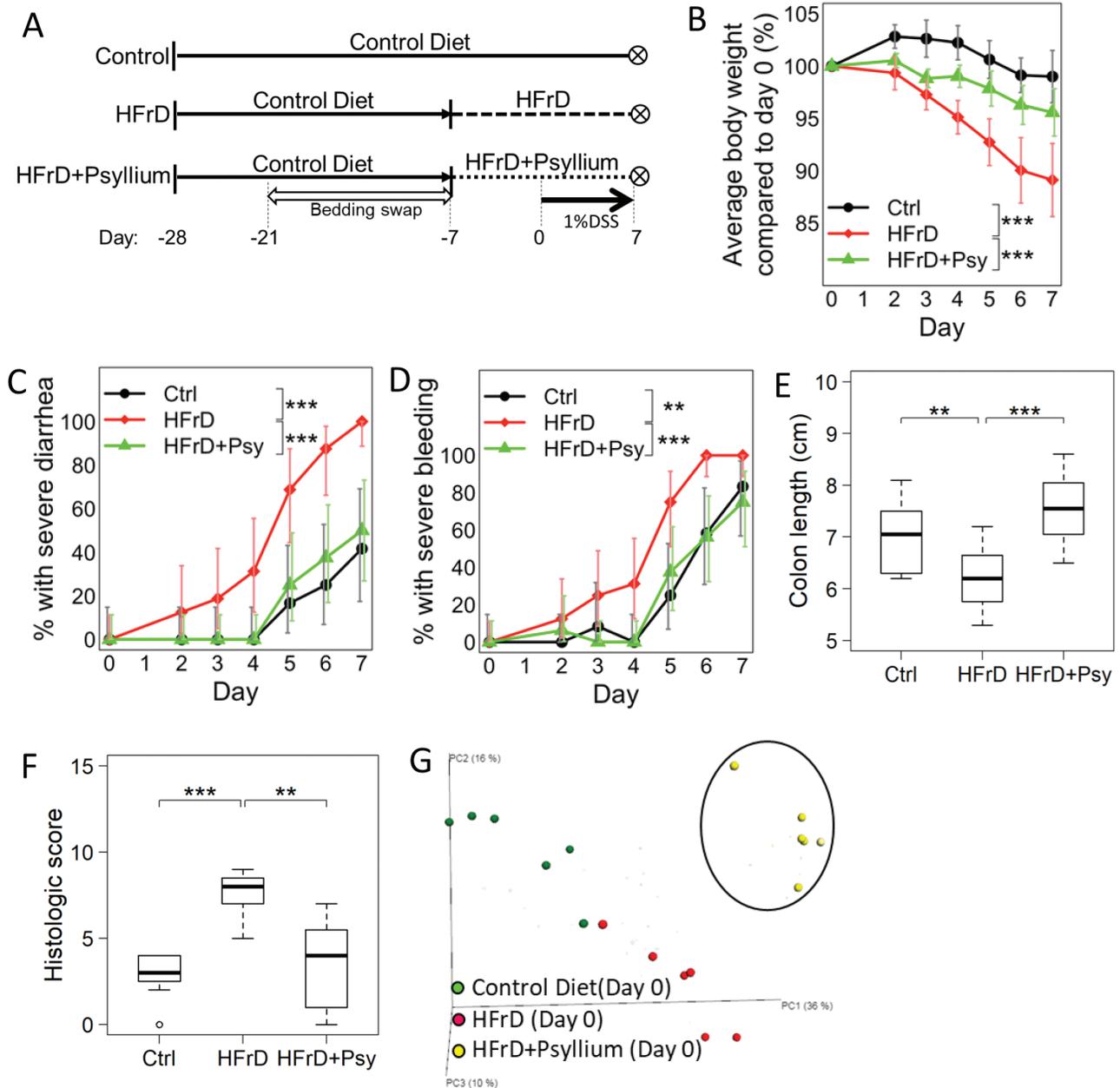


Figure 2. Psyllium protects against HFrD-induced worsening of experimental colitis. (A) Experimental design showing groups and treatments of mice given control, HFrD or HFrD supplemented with psyllium. (B–F) Measurements of colitis severity were carried out in mice described in (A) during and following DSS exposure including body weight determined by calculating percent change on a given day compared with the weight on day 0 (according to A) during DSS exposure (B), percent of mice with severe diarrhea as defined by having a score of ≥ 2 determined by assessing softness of stool (C), percent of mice with severe bleeding as defined by having a score of ≥ 2 determined by presence of blood in stool (D), colon length measured following harvesting of colon at necropsy (E) and histologic score evaluated on H&E-stained sections of the colon (F) ($n = 12\text{--}16/\text{group}$ for B–F). ** $P < 0.01$, *** $P < 0.001$. For (E) and (F), components of box and whisker plots include center line, median; box limits, upper and lower quartiles; whiskers, the most extreme data points within $1.5 \times$ interquartile range from the box; points, data points lying beyond the extremes of the whiskers. (G) Microbial abundance in feces collected on day 0 (according to A) is shown by principal coordinate analysis ($n = 6/\text{group}$ for G). Black circle indicates clustering of samples from mice fed HFrD containing psyllium.

associated tumorigenesis. To test this, mice were first fed control diet for 21 days then administered AOM (Figure 5A). Fourteen days after receiving AOM (day 0 in Figure 5A), the mice were either continued on control diet or given HFrD for 7 days (days 14 to 21 in Figure 5A). One percent DSS was then given for 5 days to induce colitis followed by 7 days of regular drinking water (days 21 to 33 in Figure 5A). Half of the mice given HFrD diet were then switched back to a control diet (day 33 in Figure 5A), whereas the other half continued to receive the HFrD for the remainder

of the study. Mice were continued on regular drinking water for an additional 7 days (days 33 to 40 in Figure 5A) then given two more rounds of DSS while being kept on their respective diets and colitis severity was evaluated. This design was intended to mimic the clinical scenario in which a patient with colitis might discontinue fructose consumption after having active disease for a period of time. As shown in Figure 5B–F, although mice given HFrD for the entire period manifested worse chronic colitis, those mice switched from a HFrD to a control diet after

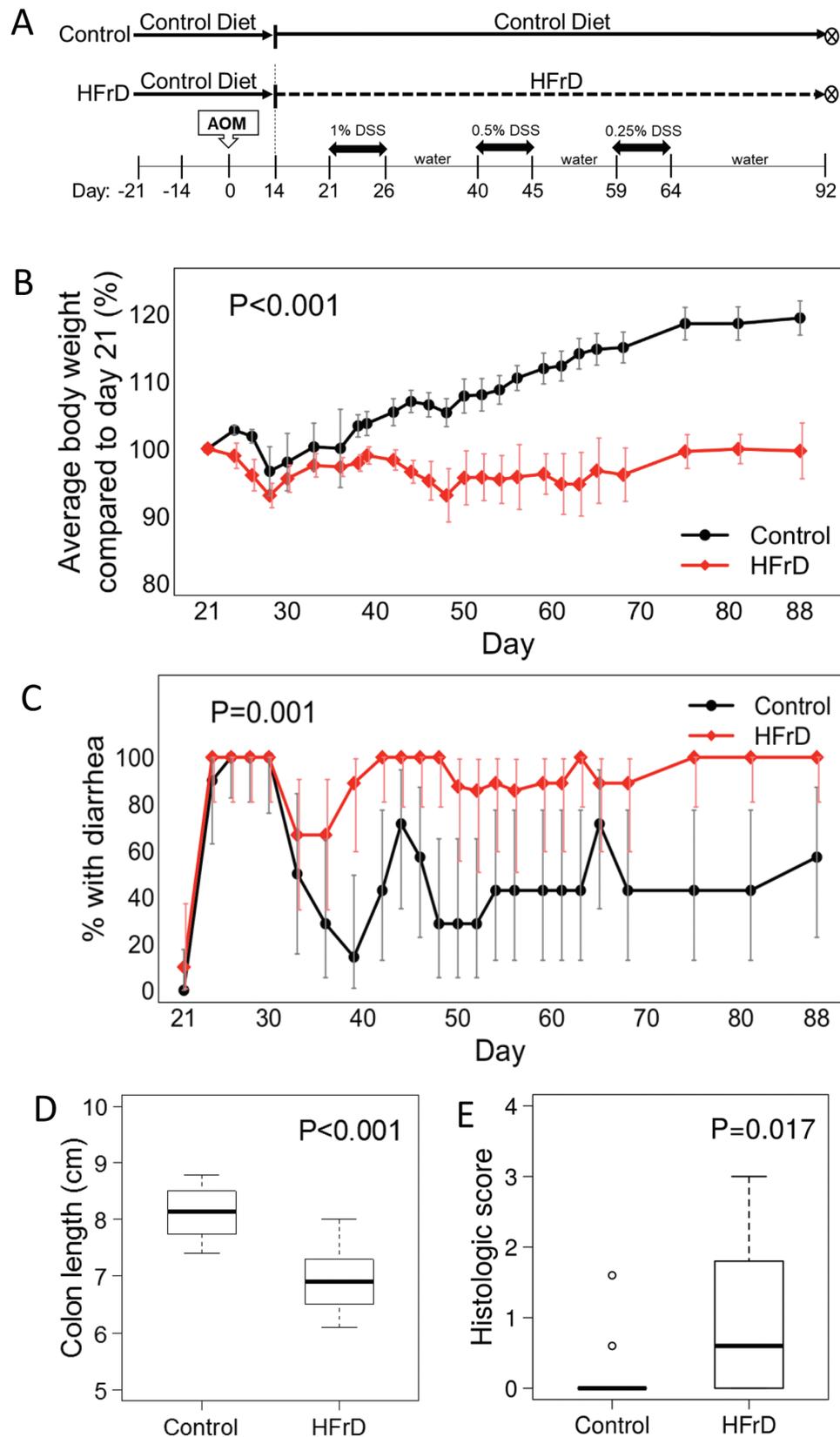


Figure 3. HFrD feeding increases the severity of chronic colitis. (A) Experimental design showing groups and treatments of mice given control or HFrD. (B–E) Measurements of colitis severity were carried out in mice described in (A) during and following DSS exposure including body weight determined by calculating percent change on a given day compared with the weight on day 21 (according to A) (B), percent of mice with diarrhea as defined by having a score > 0 determined by assessing softness of stool (C), colon length measured following harvesting of colon at necropsy (D) and histologic score evaluated on H&E-stained sections of the colon (E) ($n = 12\text{--}16/\text{group}$ for B–E). For (D) and (E), components of box and whisker plots include center line, median; box limits, upper and lower quartiles; whiskers, the most extreme data points within $1.5\times$ interquartile range from the box; points, data points lying beyond the extremes of the whiskers.

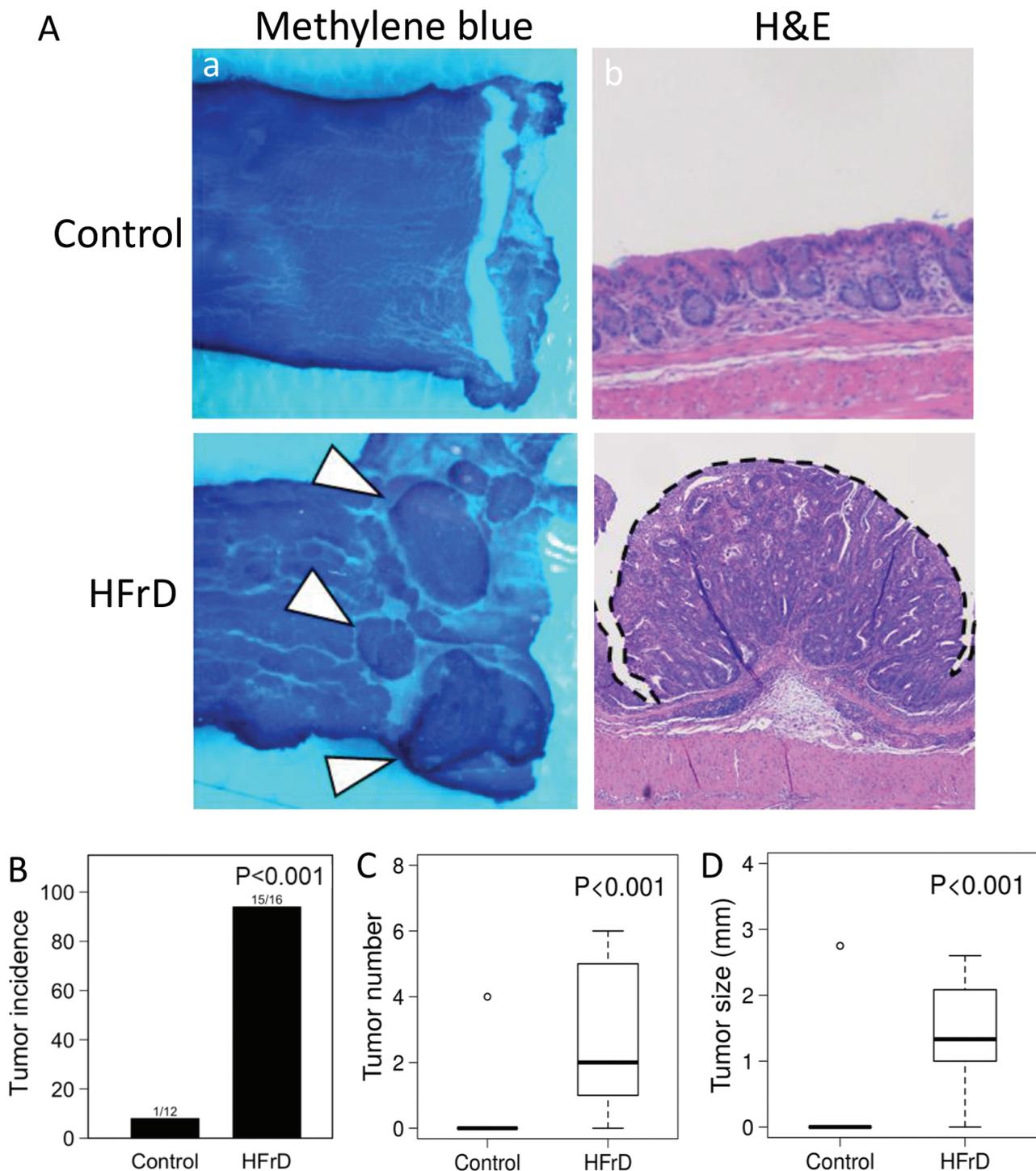


Figure 4. Feeding a HFrD increases colitis-associated tumorigenesis. (A) At the end of the study described in [Figure 3A](#), tumor burden was assessed in mice using methylene blue staining on whole mount colons (a) and H&E staining on colonic sections (b) (20 \times). Arrowheads indicate tumors in a. Dashed line indicates tumor in (b). (B–D) Tumor incidence (B), number per mouse (C) and size (D) were quantified in mice given control or HFrD at the end of the study described in [Figure 3](#) ($n = 12$ – 16 /group for B–D). For (C) and (D), components of box and whisker plots include: center line, median; box limits, upper and lower quartiles; whiskers, the most extreme data points within $1.5\times$ interquartile range from the box; points, data points lying beyond the extremes of the whiskers.

one round of DSS exposure developed less severe chronic colitis than mice that were maintained on the HFrD during the entire experimental period. In line with this amelioration of chronic colitis, switching from the HFrD to the control diet also attenuated the inflammation-associated pro-tumorigenic effect of high-fructose feeding, including reductions in tumor incidence, number and size ([Figure 5G–I](#)).

Supplementation with psyllium attenuates chronic colitis and colitis-associated tumor burden in mice fed a HFrD

Given the marked ability of psyllium to attenuate HFrD-induced worsening of acute colitis ([Figure 2](#)), we next tested whether this agent can reduce chronic colitis and protect against

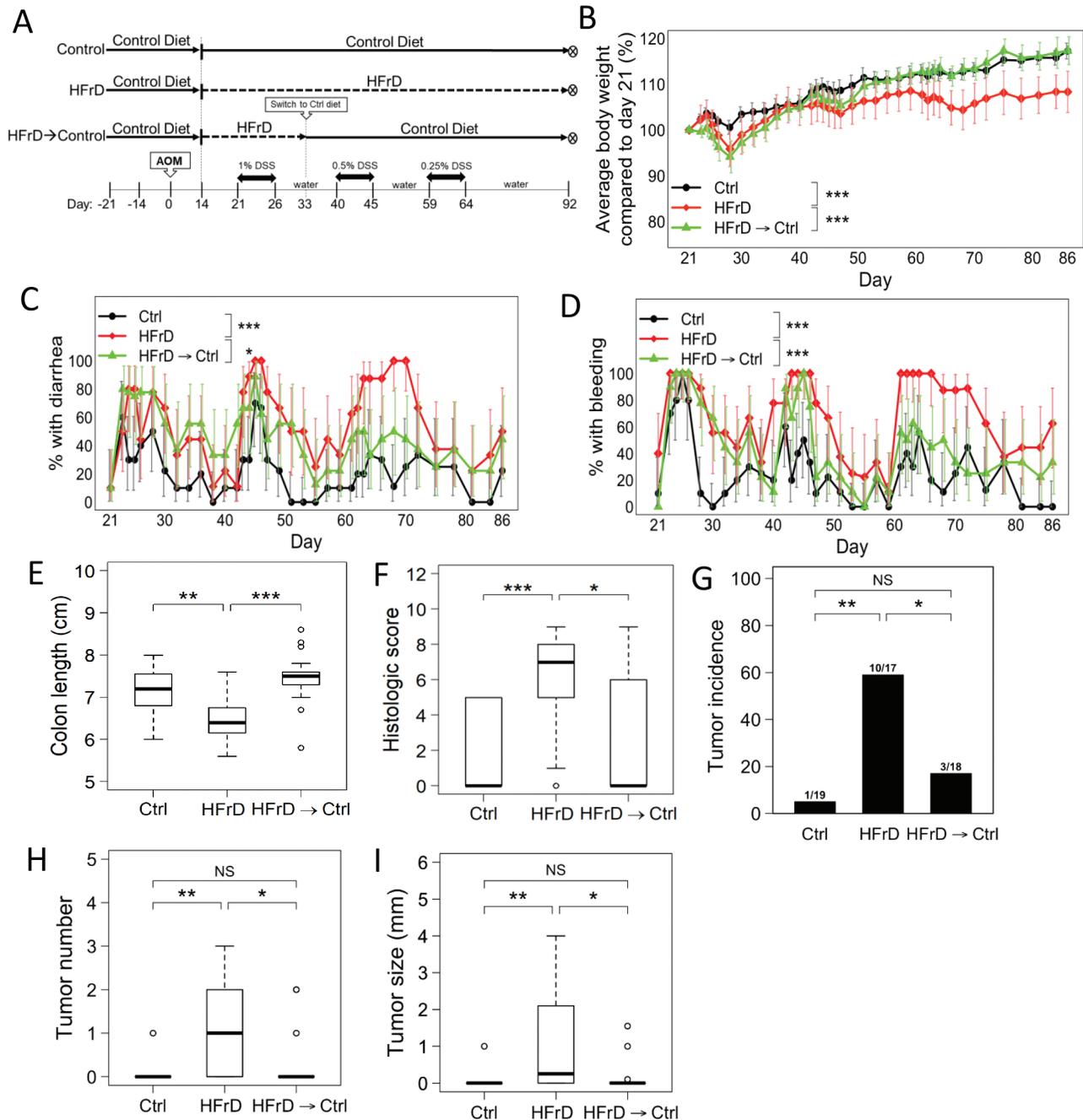


Figure 5. Switching to a control diet attenuates HFrD-mediated enhancement of chronic colitis and colitis-associated tumorigenesis. (A) Experimental design showing groups and treatments of mice given control, HFrD or switched from HFrD to control diet. (B–F) Measurements of colitis severity were carried out in mice described in (A) during and following DSS exposure including body weight determined by calculating percent change on a given day compared with the weight on day 21 (according to A) (B), percent of mice with diarrhea as defined by having a score > 0 determined by assessing softness of stool (C), percent of mice with bleeding as defined by having a score > 0 determined by presence of blood in stool (D), colon length measured following harvesting of colon at necropsy (E) and histologic score evaluated on H&E-stained sections of the colon (F) ($n = 10/\text{group}$ for B–F). (G–I) Tumor incidence (G), number per mouse (H) and size (I) were quantified at the end of the experimental period shown in (A) ($n = 17\text{--}19/\text{group}$ for G–I). (G–I) Tumor incidence (G), number per mouse (H) and size (I) were quantified at the end of the experimental period shown in (A) ($n = 17\text{--}19/\text{group}$ for G–I). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. NS = not significant. For (E), (F), (H) and (I), components of box and whisker plots include: center line, median; box limits, upper and lower quartiles; whiskers, the most extreme data points within $1.5\times$ interquartile range from the box; points, data points lying beyond the extremes of the whiskers.

colitis-associated tumorigenesis. Mice were fed control diet for 21 days and then administered AOM (days -21 to 0 in Figure 6A). Fourteen days after receiving AOM (day 0 in Figure 6A), the mice were either continued on control diet or given HFrD for 7 days (days 14 to 21 in Figure 6A). One percent DSS was then given for 5 days to induce colitis followed by 7 days of regular drinking

water (days 21 to 33 in Figure 6A). Half of the mice given HFrD diet were then given HFrD-containing psyllium instead of cellulose (day 33 in Figure 6A), whereas the other half continued to receive the HFrD for the remainder of the study. Mice were continued on regular drinking water for an additional 7 days (days 33 to 40 in Figure 6A) and then given two more rounds of

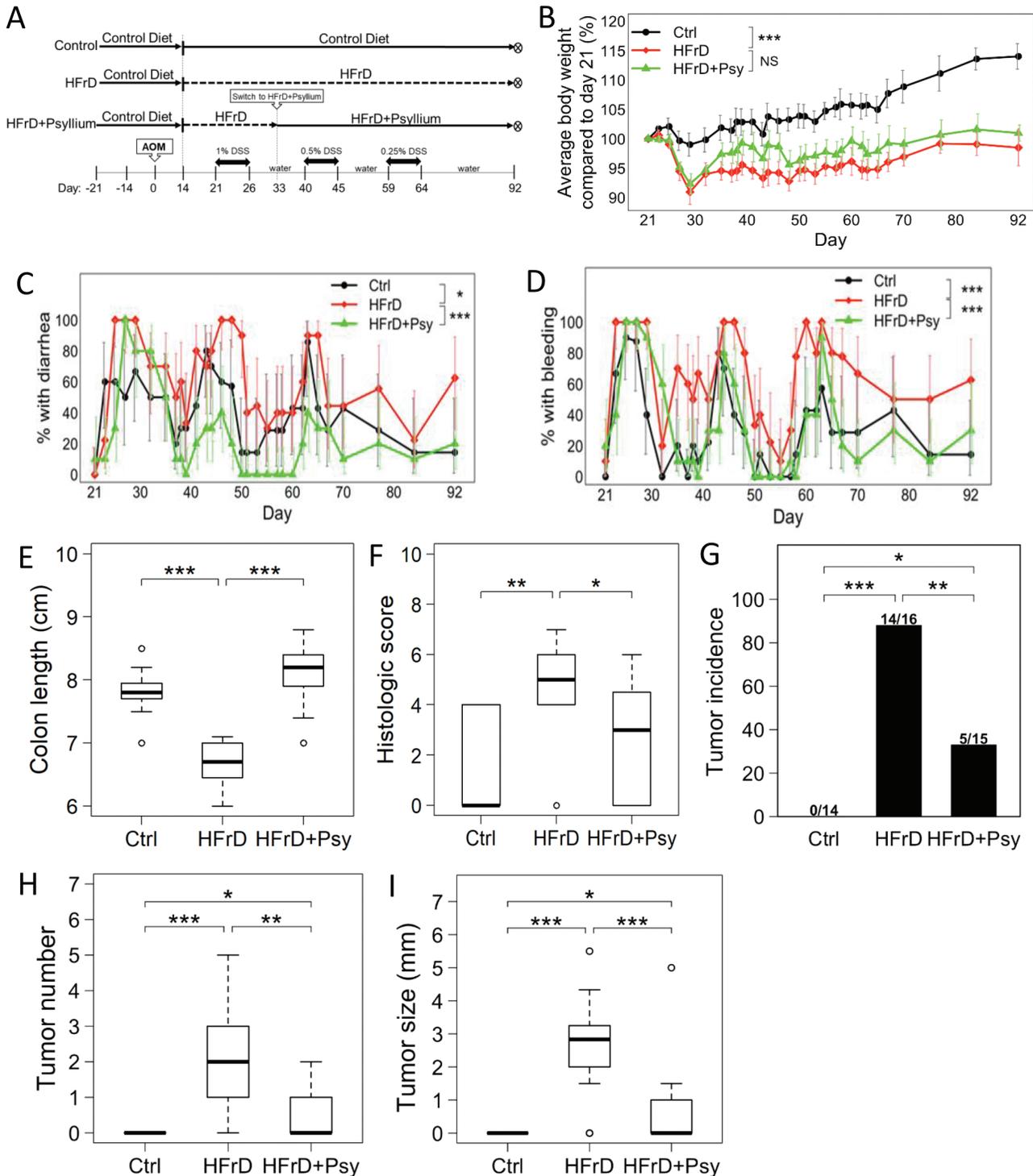


Figure 6. Psyllium supplementation protects against HFrD-induced worsening of chronic colitis and enhanced tumor development. (A) Experimental design showing groups and treatments of mice given control, HFrD or HFrD supplemented with psyllium. (B–F) Measurements of colitis severity were carried out in mice described in (A) during and following DSS exposure including body weight determined by calculating percent change on a given day compared with the weight on day 21 (according to A) (B), percent of mice with diarrhea as defined by having a score > 0 determined by assessing softness of stool (C), percent of mice with bleeding as defined by having a score > 0 determined by presence of blood in stool (D), colon length measured following harvesting of colon at necropsy (E) ($n = 12\text{--}16/\text{group}$ for B–E) and histologic score evaluated on H&E-stained sections of the colon (F) ($n = 8/\text{group}$ for F). (G–I) Tumor incidence (G), number per mouse (H) and size (I) were quantified at the end of the experimental period ($n = 14\text{--}16/\text{group}$ for G–I). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. For (E), (F), (H) and (I), components of box and whisker plots include: center line, median; box limits, upper and lower quartiles; whiskers, the most extreme data points within 1.5 \times interquartile range from the box; points, data points lying beyond the extremes of the whiskers.

DSS while being kept on their respective diets. Measurements of colitis severity were carried out which showed that psyllium supplementation of the HFrD attenuated colitis severity (Figure 6B–F). Consistent with its ability to suppress chronic colitis in HFrD-fed mice, supplementary psyllium also reduced colon tumor incidence, number and size (Figure 6G–I).

Discussion

Fructose consumption has increased in association with rising rates of IBD (1,2,36,37). Murine studies have shown a direct connection between high dietary fructose intake and worsening of colitis, including a role for gut microbiota in mediating this effect (13,14). The current study demonstrates that dietary alterations including removal of fructose from diet following HFrD feeding as well as feeding supplemental psyllium, attenuate acute colitis. Moreover, high-fructose feeding enhanced chronic colitis and increased colitis-associated tumor development, effects that were attenuated by either switching back to a control diet or feeding psyllium. Taken together, our findings suggest that high-dietary-fructose consumption may be a risk factor for tumor development in IBD patients, a potential risk factor that could be abrogated by dietary alterations.

The current study demonstrates that the detrimental effects of high-fructose feeding on colitis severity are completely reversed by switching mice back to a control diet (Figure 1B–D). Interestingly, this protection occurred in parallel with a normalization of HFrD-induced shifts in gut microbial populations (Figure 1E and F). These findings suggest that reversal of fructose-induced microbial changes is key to the protection afforded by dietary fructose removal. In an initial study demonstrating that feeding a diet high in fructose exacerbated experimental colitis, alterations in microbial populations were also observed (14). A recent report by our group showed that high-fructose feeding induced bacterial alterations but further found through bacterial ablation experiments that these microbial changes were responsible for the worsening of colitis (13). Moreover, we showed abundance, localization and metabolic changes in specific bacterial species that were likely contributors to the observed phenotype (13). Many of the species that changed in abundance as a result of HFrD feeding in this previous study, also changed in the same direction in the current work, including reduced *L.johnsonii* and increased *A.muciniphila* (Figure 1F and Supplementary Table 2, is available at *Carcinogenesis* Online). Impressively, when mice were switched back to a control diet, both of these microbes reverted back to near their levels while on control diet (Figure 1F and Supplementary Table 2, is available at *Carcinogenesis* Online). The observed normalization in the abundance of these species might be especially important for the protection afforded by dietary fructose removal given their roles in deconjugation of bile acids (*L.johnsonii*) and degradation of colonic mucus (*A.muciniphila*) (38–40). Importantly, the overall findings of these experiments suggest that if dietary fructose worsens colitis in humans, altering the diet patterns of patients could be an effective preventive approach.

Given that changing patient behavior, including diet, can be challenging and the fact that fructose is abundant in processed foods and sugar sweetened beverages (36,37,41), intervention with a supplement might have greater utility to attenuate the detrimental effects of dietary fructose. To this end, we found that supplementation of the HFrD with psyllium markedly attenuated fructose-induced worsening of colitis (Figure 2A–F). Psyllium has previously been reported to be a key dietary factor

that attenuates experimental colitis (32). This protective effect was observed in both DSS-induced and T cell transfer models of colitis (32). The protection afforded by psyllium in this previous study appeared to be at least in part, microbially mediated (32). In line with these findings, our work suggests a role for the microbiota in psyllium-mediated protection against HFrD-induced exacerbation of colitis, given that psyllium feeding resulted in a unique shift in fecal bacterial populations (Figure 2G and Supplementary Table 3, is available at *Carcinogenesis* Online). In addition to this distinct pattern overall, examination at the species level revealed particular changes that might be related to the protection afforded by supplemental psyllium. For instance, *A.muciniphila* increased in abundance in HFrD-fed mice, an effect that was attenuated when diet was supplemented with psyllium. This species is known to degrade colonic mucus and impair gut barrier function (13,39,42). A similar normalization in abundance was observed for *Bacteroides acidifaciens*, a microbe previously associated with experimental colitis (43,44). Whether microbial products derived from utilization of fiber could be playing a role in the protective effect of psyllium warrants further investigation.

It is well appreciated that chronic inflammation is a risk factor for developing neoplasia, especially in the gastrointestinal tract (17–21). In fact, individuals with long-standing IBD are at increased risk for developing CRC (17–21). In light of the recent work reported by our group and others showing that feeding a HFrD to mice exacerbates acute experimental colitis, it stands to reason that this diet could also enhance chronic colitis and consequences thereof (13,14,17,18). In fact, the current work demonstrates that HFrD feeding worsens experimental chronic colitis which predisposes mice to colorectal tumor development. If elevated fructose consumption in humans worsens IBD severity, it is possible that such a diet could increase the risk of developing IBD-related cancer. Although it is likely that fructose-related worsening of inflammation is the mechanism by which greater tumor development occurs in mice, it is possible that direct utilization of fructose by colon tumors could enhance their development and growth (45). In order to test whether increased colon tumor burden resulting from HFrD feeding could be abrogated through intervention, the effects of dietary fructose removal and psyllium supplementation were tested. Impressively, both of these approaches markedly reduced tumor development under these conditions. Of note, each of these interventions was initiated after an initial DSS exposure while on HFrD diet. This scenario could be envisioned for IBD patients who have experienced flares while consuming a potentially harmful diet and elect to alter their dietary patterns to reduce their risk of developing colon cancer.

Supplementary material

Supplementary data are available at *Carcinogenesis* online.

Supplementary Figure 1. Psyllium is an optimal fiber source to protect against HFrD-induced exacerbation of colitis. Measurements of colitis severity, including body weight (A), bleeding (B) and colon length (C) in mice given a HFrD supplemented with cellulose, inulin, pectin or psyllium for 1 week, then administered 1% DSS for 1 week, while being continued on their respective diets ($n = 8/\text{group}$ for A–C). *** $P < 0.001$. For (C), components of box and whisker plots include: center line, median; box limits, upper and lower quartiles; whiskers, the most extreme data points within 1.5x interquartile range from the box.

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