Low dietary fiber promotes enteric expansion of a Crohn’s disease-associated pathobiont independent of obesity

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Abstract

Obesity is associated with metabolic, immunological, and infectious disease comorbidities, including an increased risk of enteric infection and inflammatory bowel disease such as Crohn’s disease (CD). Expansion of intestinal pathobionts such as adherent-invasive Escherichia coli (AIEC) is a common dysbiotic feature of CD, which is amplified by prior use of oral antibiotics. Although high-fat, high-sugar diets are associated with dysbiotic expansion of E. coli, it is unknown if the content of fat or another dietary component in obesogenic diets is sufficient to promote AIEC expansion. Here, we found that administration of an antibiotic combined with feeding mice an obesogenic low fiber, high sucrose, high fat diet (HFD) that is typically used in rodent obesity studies promoted AIEC intestinal expansion. Even a short-term (i.e., 1-day) pulse of HFD feeding before infection was sufficient to promote AIEC expansion, indicating that the magnitude of obesity was not the main driver of AIEC expansion. Controlled diet experiments demonstrated that neither dietary fat nor sugar were the key determinants of AIEC colonization, but that lowering dietary fiber from approximately 13% to 5-6% was sufficient to promote intestinal expansion of AIEC when combined with antibiotics in mice. When combined with antibiotics, lowering fiber promoted AIEC intestinal expansion to a similar extent as widely used HFDs in mice. However, lowering dietary fiber was sufficient to promote AIEC intestinal expansion without affecting body mass. Our results show that low dietary fiber combined with oral antibiotics are environmental factors that promote expansion of Crohn’s disease-associated pathobionts in the gut.

New and Noteworthy

It is commonly thought that obesity or a high fat diet alters pathogenic bacteria and promotes inflammatory gut diseases. We found that lower dietary fiber is a key factor that expands a gut pathobiont linked to Crohn’s disease, independent of obesity status in mice.
Introduction

Obesity is associated with increased risk of type 2 diabetes, cardiovascular disease, and non-alcoholic fatty liver disease. Obesity comorbidities extend beyond metabolic diseases and include increased risk and severity of infectious diseases. For example, obesity and type 2 diabetes can each increase the risk and severity of bacterial and viral infections (2, 23, 54). However, it is not clear if an obesogenic diet is sufficient to predispose to bacterial infections.

The prevalence of obesity has risen coincident with diets containing highly processed foods and foods higher in fat and refined carbohydrates. The consumption of ultra-processed foods high in refined sugars is associated with the rise in obesity and its comorbidities (12, 38). Typically, processing of foods that are high in fat and sugar results in food that is low in fiber. It is not known which dietary factor or obesity-related factor confers the risk of severity of infection from specific pathogenic bacteria.

The gastrointestinal tract is an extended metabolic site where microbes, immunity, and dietary factors directly interact. In this niche, the host must digest and absorb dietary nutrients and protect against invasive microbes providing the opportunity for dietary and microbial factors to interact within the intestine. Changes in the composition of the intestinal microbiota have been reported in obesity and linked to certain comorbidities and may be causally related (4, 22). For example, increased adiposity and higher blood glucose are transmissible via the intestinal microbiota in mouse models (4, 19, 56). Diet has a major and temporal influence on the composition of the gut microbiome; just one day of eating an obesogenic diet high in fat and low in fiber is sufficient to alter the composition of the gut microbiome in mice (19). It is unknown how ingestion of specific obesogenic dietary components influences the risk or severity of enteric infection, especially in response to resident bacteria that are associated with chronic enteric disease. Obesity is associated with chronic low-level inflammation that is often characterized by compartmentalized immunometabolism responses such as increased pro-
inflammatory cytokines, chemokines and immune cells in the adipose tissue (2). Diet-induced obesity can also promote intestinal inflammation, thereby altering the host-microbe relationship (2, 7, 19, 37). An inflammatory environment could provide a selective niche for the expansion of enteric pathogens or pathobionts that can exploit the inflammatory gut environment (10, 49). This positions obesity-related host factors or obesity-causing diets as potential modifiers of inflammatory bowel diseases (IBD), such as Crohn’s disease (CD). Associations between obesity and CD have been reported (25). Genetic risk factors involved in CD such as nucleotide-binding oligomerization domain-containing protein 2 (Nod2) also regulate obesity and type 2 diabetes risk factors in the presence of an obesogenic diet (9, 15, 41). However, it is not clear how obesity or obesogenic dietary components influence invasive, enteric pathogens that promote CD.

Adherent-invasive *Escherichia coli* (AIEC) is a pathobiont associated with intestinal lesions in CD patients. AIEC can penetrate the intestinal mucosal barrier, adhere to and invade epithelial cells and macrophages, which stimulates intestinal inflammation (5, 6, 17, 21, 57). AIEC can be a benign intestinal colonizer, but is considered a pathobiont because it can rapidly expand and promote host pathology following niche perturbation, such as antibiotic treatment or altered diet (17, 35). Antibiotic use can potentiate AIEC expansion and/or colonization through dysbiosis of commensal microbes and increased inflammation in the local gut environment (40, 52). For example, in the presence of AIEC, antibiotic treatment promotes intestinal inflammation and the oxidation of sugar substrates and nitrate species that provide a selective advantage for AIEC (40). AIEC expansion also occurs during intestinal inflammation caused by secondary infections with *Salmonella enterica* serovar Typhimurium and *Citrobacter rodentium* after a primary infection with AIEC (49).
Obesity influences many host processes that could potentiate AIEC infection, including low-grade chronic inflammation, altered mucosal immunity, and impaired intestinal barrier integrity (3, 26, 53, 61). Indeed, increased intestinal burden of AIEC has been linked with inflammation and gut dysbiosis, which are characteristics of obesity (1, 7, 46, 49). An obesity-causing diet that is high in fat and sugar leads to increased AIEC burden, in part through diet or obesity-induced changes in commensal gut bacteria (1). Recently, it was shown that combining antibiotics with a Western-style diet high in fat was associated with increased risk for markers of early IBD in humans (28). Similarly, exposing mice to these two environmental perturbations promoted inflammation and dysbiosis of commensal bacteria (28). Consumption of a Western-style diet expectedly increased obesity and impaired mitochondrial bioenergetics in the epithelium of the distal gut, which fueled expansion of Enterobacteriaceae in mice (28).

However, it is still not clear if eating more or less of specific dietary components versus pre-existing obesity promotes the characteristic Enterobacteriaceae expansion seen in CD. We aimed to determine whether obesity or dietary components in obesogenic diets alter the severity, kinetics and resolution of enteric infection with AIEC in mice.

**Methods**

**Mice**

Breeding and experimental procedures were carried out in strict accordance with the Canadian Council on Animal Care. All experiments on mice were conducted according to the protocols approved by the Animal Review Ethics Board (AREB) at McMaster University under Animal Use Protocol #16-06-02. Mice were killed by carbon dioxide asphyxiation or cervical dislocation. For all studies, mice were 8-10 weeks old before dietary intervention or the initiation of any experimental protocol. Mice were maintained on a 12-hour light/dark cycle, and experiments were performed on multiple cohorts of mice born from different parents at different times of the year. Male mice were used for experiments. Wild-type (WT) C57BL/6J mice were obtained from
The Jackson Laboratory (Bar Harbor, ME, USA) or from our in-house colony established from C57BL/6J mice received from The Jackson Laboratory (strain 000664). All mice were maintained in a specific pathogen-free facility at McMaster University.

**Diets**

Mice were fed a chow diet containing 17% kcal from fat, 29% kcal from protein, 54% kcal from carbohydrate and 13% of fiber estimated by a neutral detergent method (Teklad 22/5, Envigo, 8640) unless otherwise specified. For dietary interventions, 8-10-week-old mice were fed 60% HFD (Research Diets D12492) containing 60% Kcal from fat, 20% of Kcal from protein and 20% Kcal from carbohydrates and 6% fiber. Where indicated, mice were also fed a 45% HFD (Research Diets D12451) containing 45% Kcal from fat, 20% Kcal from protein and 35% Kcal from carbohydrate and 6% fiber. Mice were fed *sucrose and fiber matched* HSLF diet (Research Diets D12450J) containing 10% Kcal from fat, 20% of Kcal from protein and 70% Kcal from carbohydrates and 5% fiber. Mice were fed *fiber matched* NSLF diet (Research Diets D12450K) containing 10% Kcal from fat, 20% of Kcal from protein and 70% Kcal from carbohydrates and 5% fiber. For the long-term feeding models, mice were weaned onto chow diets and 8-10-week-old mice fed the specified diets for 16 weeks prior to AIEC infection. For the short-term feeding models, mice were weaned onto chow diets and fed the specified diets one day prior to infection or one day after infection, where indicated.

**Intraperitoneal glucose tolerance test**

Intraperitoneal glucose tolerance test was performed in 6-hour fasted, conscious mice. Blood glucose was measured by tail vein blood sampling using a handheld glucometer (Roche Accu-Check Performa) after injection of D-glucose (0.75 g/kg; Sigma-Aldrich). Area under the curve of blood glucose versus time was calculated using GraphPad Prism 6 software.
AIEC infection

AIEC strain NRG857c (serotype O83:H1) was grown overnight in lysogeny broth (LB) medium with shaking at 37°C. Mice were pretreated with streptomycin (2 mg/mouse) by oral gavage one day prior to infection. Mice were infected by oral gavage with 0.1 ml of phosphate buffered saline solution containing \(~2\times10^9\) colony forming units (cfu) of AIEC. The infectious dose was verified by plating of serial dilutions on LB agar supplemented with ampicillin and chloramphenicol. Body mass was measured throughout infection. Fecal pellets were weighed, homogenised (Retsch Mixer Mill) in 1 mL PBS, serially diluted, and plated onto LB agar plates supplemented with ampicillin (100 µg/ml) and chloramphenicol (34 µg/ml). Intestinal tissues were harvested into cold PBS at necropsy and were flushed with PBS to remove luminal contents, homogenized with a sterile metal bead, and plated in the same manner as feces. Plates were incubated overnight at 37°C and colonies were counted to determine cfu per gram of feces or tissue.

Histology

Ilea, ceca and colons were collected and fixed in buffered 10% formalin for 96 hours, paraffin-embedded, sectioned into 5-µm slices and then stained with haematoxylin and eosin (H&E) by Histology Services (McMaster University, Hamilton, ON). A minimum of 5 views per section were analyzed for each sample and scored according to previously defined criteria (50). Briefly, scant, moderate or dense scores were assigned for multiple variables including, but not mitted to i) lumen: necrotic epithelial cells and polynuclear leukocytes, ii) surface epithelium: regeneration, iii) mucosa: crypt abscesses and iv) submucosa: mononuclear and polynuclear leukocytes cell infiltrate. More detailed criteria are previously defined (11). Crypt length measurements and goblet cell quantification were done using ImageJ software on a Nikon microscope with at least five well-oriented crypts measured per field.
Statistical analysis

Data was assessed for normal distribution using the D'Agostino-Pearson normality test. For non-normally distributed data sets, statistical significance was determined by Mann-Whitney $U$ test for comparison of 2 data sets. A Kruskal-Wallis test with Dunn's multiple comparison was used for comparison of more than 2 non-normally distributed data sets. For normally distributed data sets, a one-way or two-way ANOVA with Tukey post-hoc multiple comparison analyses were used. All analyses were performed using Graph Prism 6.0 (GraphPad Software Inc. San Diego, CA). A P-value of 0.05 or less was considered significant.

Results

An obesity-causing diet in conjunction with antibiotic exposure promotes AIEC expansion and gut pathology in mice

To study the participation of obesity or dietary factors present in an obesogenic diet in AIEC dynamics in the host, we established a standard high-fat feeding regimen in conjunction with a previous developed AIEC infection model (8, 50). We first confirmed that diet-induced obesity worsens blood glucose control because elevated blood glucose can degrade gut barrier function and worsen outcomes from enteric infection in mice (55). We found that mice fed an obesogenic high fat diet (HFD) that contained 60% of calories from fat for 16 weeks had higher blood glucose during a glucose tolerance test, higher fasting blood glucose, higher body mass and higher adiposity compared to mice fed a standard chow diet prior to AIEC infection (Fig. 1A-D).

It is noteworthy that this 60% HFD is often used to study obesity in mice and contains higher sucrose and lower amount of fiber compared to the standard chow diet used in our experiments and many animal facilities. Mice were given a single low dose of antibiotic (streptomycin, 2 mg/mouse) one day prior to infection with AIEC. HFD-fed mice maintained a higher body mass...
compared to chow-fed mice throughout AIEC infection (Fig. 1E). HFD-fed obese mice treated with antibiotics had higher levels of AIEC in the feces and throughout the intestinal track at 17 days post-infection. Significantly elevated AIEC colony forming units (CFUs) were found in the ileum, cecum, and colon of HFD-fed mice, but there were no detectable AIEC colonies in spleen homogenates of chow-fed or HFD-fed mice (Fig. 1F). We next analyzed the time course of AIEC infection in antibiotic treated mice and found higher fecal burdens of AIEC in the HFD-fed mice every day between 1-30 days post-infection (Fig. 1G). Antibiotics are known potentiatiors of AIEC infection, but it was not clear if they were required for the exacerbated AIEC outgrowth observed in HFD-fed mice (40). We found that antibiotic pre-treatment was required to increase AIEC intestinal expansion during an obesogenic diet, since mice fed HFD for 16 weeks had similar AIEC fecal burdens to chow fed mice if mice were not given antibiotics (Fig. 1H). These data established a tractable model of HFD-induced obesity that promoted AIEC expansion in the gut in an antibiotic-dependent manner.

We next examined intestinal pathology by scoring the severity of crypt hyperplasia, immune cell infiltration, epithelial cell loss, and edema in the gut during AIEC infection. Nine days post AIEC infection, the severity of pathology was higher in the ceca of HFD-fed mice compared to chow-fed mice (Fig. 2A). In addition, HFD-fed mice had crypt elongation in the cecum compared to chow-fed mice at 9 days post AIEC infection (Fig. 2B-C). Seventeen days post AIEC infection, the severity of pathology was higher in the colon of HFD-fed mice compared to chow-fed mice (Fig. 2D) In addition, crypt elongation was observed in the ileum, cecum, and colon at 17 days post AIEC infection (Fig. 2E-F).

Diet rather than level of obesity correlates with intestinal AIEC expansion

Transgenic mice that are genetically susceptible to AIEC and fed a Western-style diet high in fat and sugar for 12 weeks have increased fecal AIEC burdens 3 days after infection (1). In order to
understand the role of obesity following long-term HFD feeding on AIEC colonization in the gut, we performed feeding studies with a second obesogenic diet in which 45% of calories were derived from fat compared to our previous results using a HFD with 60% of calories from fat. As expected, feeding mice for 16 weeks with a 45% HFD resulted in significantly higher body mass compared to the chow-fed control mice during AIEC infection (Fig. 3A). Our data also confirm the well-established result that mice fed a 60% HFD had a greater increase in body mass compared to mice fed a 45% HFD, an effect that was maintained during AIEC infection. Mice fed a 45% HFD also had elevated fecal burdens of AIEC beginning on day 1 after infection and throughout the 30-day observation period (Fig. 3B). It is well established that mice display interindividual variability in the level of obesity caused by either obesogenic diet (63). This heterogeneity in body mass in mice allowed us to compare AIEC fecal burdens of mice with different levels of obesity. Hence, we tested AIEC fecal burdens at specific time points post infection to determine if body mass correlated with fecal AIEC burden during diet-induced obesity. We found no correlation between body mass and AIEC fecal burden in HFD fed mice at days 8, 13, and 17-days post AIEC infection (Fig. 3C-E). These data suggested that an aspect of diet composition rather than the magnitude of host obesity was sufficient to promote intestinal AIEC expansion. However, it was unclear if fat content or another dietary component of obesogenic diets was contributing to the expansion of AIEC.

**Dietary components ingested during infection promote intestinal AIEC expansion independent of obesity**

To parse out the effects of overt obesity from diet, we used short-term HFD feeding in mice that were switched from a standard chow diet to a 60% HFD one day prior to AIEC infection and remained on either the HFD (or chow diet) throughout the course of infection. There were no initial differences in body weight at the onset of HFD feeding, however a slightly higher (i.e., 2-3 gram increase) body mass was observed in short-term HFD-fed mice compared to chow fed
mice during AIEC infection (Fig. 4A). Fecal burdens of AIEC were significantly higher in the HFD-fed group between days 3-9 post infection (Fig. 4B). At day 9, the differences in organ burdens were only observed in the cecum and proximal colon, where HFD mice had higher AIEC burdens (Fig. 4C). These results indicate that the constituents of the HFD may be a major factor responsible for AIEC expansion, independent of overt obesity.

If a dietary component of HFD was the factor responsible for AIEC expansion, rather than the comorbid effects of obesity, then feeding mice a short pulse of HFD would be expected to modulate AIEC loads. To test this, we compared AIEC burdens in mice fed a HFD for 16 weeks (i.e. long-term) or for one day (i.e. pulse) before AIEC infection. We also compared these HFD-fed mice to mice fed a chow diet for equal periods of time. Consistent with our previous results, mice on the long-term HFD feeding regimen had significantly higher fecal AIEC burdens compared to chow-fed controls (Fig. 5A). Mice exposed to a HFD pulse also had significantly higher AIEC fecal burdens than chow-fed control mice from day 3-9 post-infection (Fig. 5A). Importantly, the body mass of short term HFD-fed mice was significantly lower compared to mice fed a chow-diet in the long-term (Fig 5B). Thus, despite lower body mass, mice fed a HFD pulse still had significantly higher AIEC fecal burdens compared to chow-fed control mice in the long-term cohort. These data indicate that factors such as diet can regulate AIEC infectious burden independent of changes in body mass leading to obesity. Indeed, only the long-term HFD-fed mice had significantly higher adiposity compared to all other groups of mice (Fig. 5C). Overall, these data indicate that diet composition, independent of body mass or adiposity, plays a role in the regulation of AIEC infection.

Low dietary fiber promotes intestinal AIEC expansion

In addition to higher fat, obesogenic diets also contain higher sucrose and lower fiber content, each of which have been individually associated with intestinal homeostasis (27, 33). In order to
establish which component of HFD was promoting AIEC expansion, we established two defined
diet regimens that were controlled for ingredient composition but where the majority of the fat
and sucrose content was substituted with corn starch and maltodextrin. First, we compared the
60% HFD to a sucrose and fiber matched diet that has the same (high) sucrose and same (low)
fiber content, but lower fat compared to the obesogenic HFD. The goal was to test the impact
that fat content has on the AIEC infection, hence we compared the HFD to a low-fat high
sucrose, low fiber-matched diet (HSLF) using both the short-term and long-term feeding
regimens with this control diet.

From short-term feeding experiments, we first isolated dietary fat content as a composition
variable by comparing mice fed high (HFD) or low fat-containing diets (HSLF) that were
equivalent for fiber and sucrose content. Following short-term feeding and AIEC infection, both
HFD-fed and HSLF-fed mice had similar levels of AIEC in their feces for the 15-day observation
period (Fig. 6A). HFD-fed mice had higher body mass starting at day 8 post AIEC infection
compared to HSLF-fed mice (Fig 6B). The HFD mice also had significantly higher adiposity
compared to the HSLF-fed mice (16.6% versus 8.5%, respectively; Fig. 6C). The magnitude of
increase in adiposity in short-term HFD feeding compared to a HSLF diet was similar to our
results using short-term feeding of HFD compared to a chow diet (Fig. 5C). On day 17 post-
infection, there were also no differences in tissue associated AIEC burdens in any section of the
intestinal tract after short-term HFD feeding compared to a HSLF diet (Fig. 6D). We also
performed long-term feeding studies for 16 weeks using HFD or HSLF diets and found no
significant differences in AIEC fecal burden at any time during the infection (Fig. 6E), despite
increased body mass in HFD-mice throughout infection (Fig. 6F). Similarly, 16 weeks of feeding
HFD or HSLF diets did not change tissue burden or dissemination were observed at day 17 post
infection in long-term HFD- and HSLF-fed mice (Fig. 6G). These data strongly suggested that
fat content is not the key factor promoting AIEC expansion in mice fed an obesogenic diet.
We next examined the effect of fiber as a dietary variable in obesogenic diets on AIEC colonization. We used a no-sucrose, low-fat (NSLF) diet that contains the same amount of fiber as a 60% HFD but has low fat and no sucrose. Diets low in fiber have been associated with decreased levels of butyrate as well as loss of mucosal barrier integrity (16, 59). Diets higher in fiber have shown to be protective from DSS-induced colitis and mitigate aspects of metabolic disease (24, 32, 64). However, high fiber diets can potentiate enterohemorrhagic *E. coli* infections concomitant with lower commensal *E. coli* abundance (65). To assess the influence of low dietary fiber, we used the low fat, no sucrose, low fiber diet (NSLF) compared to a chow diet in our short-term feeding model. We found elevated fecal burdens of AIEC from day 1-15 post infection in the NSLF-fed mice compared to chow-fed mice (Fig. 7A) despite no differences in body mass (Fig. 7B). We also observed higher tissue burdens in the distal ileum, cecum, and proximal colon at day 17 post infection (Fig. 7C). These data indicate that ingestion of lower dietary fiber is sufficient to promote expansion of AIEC throughout the gut.

Finally, we tested if supplementation of a HFD with a specific fiber could alter AIEC colonization by comparing a 60% HFD and 60% HFD containing an additional 150 g/kg of cellulose. We found no change in fecal AIEC burden at nearly all days post AIEC infection during short-term feeding of a HFD compared to a HFD containing extra cellulose, despite mice having lower body mass between 9-24 days post-AIEC infection when fed the HFD containing extra cellulose (Fig. 8 A-B). In fact, the diet supplemented with cellulose transiently increased fecal AIEC burden 9 days post infection (Fig. 8A). Further, mice had a small increase fecal AIEC burden 3-days post infection and a small decrease in AIEC burden 7- and 10-days post infection when fed a HFD containing extra cellulose for 16 weeks despite mice being (on average) ~10 grams lighter when fed the cellulose-containing HFD (Fig. 8C-D). These data indicate that
supplementation of a HFD with ~20% cellulose is not sufficient to consistently alter AIEC fecal burden during short- or long-term dietary interventions.

Discussion

We examined how pre-existing obesity versus individual diet components altered enteric pathobiont expansion in mice. We first developed a “two-hit” model of environmental factors that altered AIEC infection. We used pre-treatment with a single antibiotic plus ingestion of an obesogenic diet and found that this combination exacerbated AIEC burdens in the intestine and feces after enteric infection. The interaction of two environmental stressors is consistent with recent work showing that antibiotic use combined with ingestion of a high fat Western-style diet worsens signs of early IBD in humans and mice (28). We found that an obesogenic HFD worsened markers of intestinal pathology during AIEC infection. Although we cannot determine directionality between increased AIEC burden and worsened intestinal pathology during HFD feeding, our results are consistent with obesogenic diets promoting both host and microbial indicators of IBD pathology and risk. We then questioned whether pre-existing obesity at the time of enteric infection or ingestion of Western-style obesogenic diet during infection was sufficient to influence AIEC expansion in the intestine. It is well established that long-term HFD feeding (for over 16 weeks) causes obesity in mice and we found that protracted HFD feeding also promoted AIEC colonization and worsened pathology in mice. We also found that short-term HFD initiated just before or just after AIEC infection was sufficient to increase AIEC intestinal expansion even in the absence of overt obesity.

HFD feeding influences many factors beyond obesity including, metabolic inflammation, poor blood glucose control, and insulin resistance (13, 29). HFD feeding also changes the composition of the commensal intestinal microbiota (4, 36). In fact, as early as one day of HFD-feeding can transiently alter the composition of gut microbial populations (19, 47, 48). Our
results showing that AIEC expansion induced by HFD-feeding only occurs when combined with the use of antibiotics implies a role for the commensal microbiota as a mediator in the interaction between these two environmental factors. Although, we did not define the precise role of the commensal microbiota, others have found that antibiotics plus a HFD combine to impair mitochondrial metabolism in the intestinal epithelium, and promote an inflammatory gut environment that allows expansion of Enterobacterales (28). It is logical for future studies to investigate if a similar mechanism opens an intestinal niche for AIEC colonization, expansion or hinders host control of this pathobiont.

The commensal microbiota reside at the crossroads of intestinal immunity and nutrient acquisition and this relationship could modify AIEC colonization. The microbiota can ferment dietary components, specifically non-digestible fibers, that escape digestion in the upper gastrointestinal tract into SCFAs such as acetate, propionate, and butyrate. Butyrate is a primary energy source for colonocytes and can mitigate weight gain and intestinal inflammation (30, 31, 58). During obesity, there is a bias towards acetate production in multiple body sites, including the intestine (43). HFD-feeding increases intestinal acetate levels (43). As AIEC is known to use acetate as alternative carbon source (18), future testing of intestinal acetate in AIEC expansion is warranted. In addition, reduced fermentation may generate a favourable environment for AIEC expansion. The concept of bacterial fermentation and production of specific SCFAs that can lower the pH of cecum and colon also warrants investigation during AIEC infection since an acidic environment can confer pathogen resistance to the host. The immunologic or microbial mechanisms linking ingestion of an obesogenic HFD to pathobiont expansion remain unknown at this time and should be the focus of future work.

Our data altering the timing and fat content of HFDs pointed to a dietary factor influencing AIEC expansion independent of obesity status. Hence, we used multiple defined control diets to
assess the impact of specific dietary components. We found that dietary fiber is one standalone factor that influences AIEC intestinal burden. It is noteworthy that a majority of studies using animal models of diet-induced obesity compare well-defined and controlled HFDs with a chow diet (60). Chow diets used by many institutions can have inter-batch variability, often lack a defined composition, and vary in their ingredient sources (42, 60, 62). One of the major differences between many chow diets and defined diets is the fiber content. Many chow diets are considered a fiber-rich diet (42). Fiber type and quantity are known to affect intestinal development and microbial populations which may confound metabolic research comparing defined diets to regular chow diets (24, 34, 45, 51). Dalby et al., found that low- and high-fat refined diets caused similar changes to the microbial populations and SCFA concentrations indicating that these features were independent of dietary fat content or obesity (14). If the low-fat refined diet were not used, these differences may have been attributed to the high fat percentage and increased weight gain. Our data add to this concept by showing that low fiber content in HFD (and as an independent factor) influences host-microbe dynamics and expansion of an intestinal pathobiont. While the diets used in the current experiments were well controlled, one confounding variable is amount of dietary maltodextrin. Maltodextrin is a resistant starch, which is defined as the portion of starch that escapes digestion in the small intestine (20). Hence, resistant starches such as maltodextrin can act similar to dietary fiber and are commonly considered as part of the dietary fiber content of foods (20, 44). A recent in vitro study showed that maltodextrin, irrespective of chain length, promoted AIEC growth and biofilm formation (39). This study also found that sucrose did not confer a growth advantage to AIEC, which supports our hypothesis that the low fiber content, not high sucrose in the diet can promote AIEC growth.

The types of fibre contributing to AIEC expansion remain unclear. We only tested a single type of insoluble fiber and found that increased dietary cellulose only caused a transient increase or
decrease in AIEC burden, but cellulose supplementation was not sufficient to alter the magnitude of AIEC burden consistently during obesogenic HFD feeding. Cellulose supplementation did lower body mass in mice, which is consistent with all the other results showing that changes in body mass do not necessarily coincide with changes in AIEC burden. Soluble fibers can be fermented by the microbiota into SCFAs, while insoluble fibers provide bulk for waste disposal (48, 62). HFD supplemented with inulin increases mucosal immunity and increases intestinal microbial diversity compared to HFD supplemented with cellulose (64). Interestingly, while HFDs supplemented with inulin or cellulose reduced body mass, only the diet supplemented with inulin showed improved glucose and insulin tolerance (64). These findings warrant careful examination of supplementation with different types and amounts of dietary fibers and AIEC intestinal expansion and relevance to CD. A recent study used over 40 custom diets consisting of different proportions of macronutrients to assess their impact on the resolution of chemically induced colitis. Of the macronutrients tested, high protein diets, particularly high in casein resulted in worsened outcomes from chemically induced colitis. A high casein diet exacerbated chemically induced colitis, including higher weight loss, fecal lipocalin-2 and colonic TNF and IL-6, and pathology. This is relevant to our results because casein comprises the largest protein component in both the HFD and defined diets used in our experiments. Intriguingly, high fiber diets containing psyllium mitigated disease activity following chemically induced colitis including weight loss, inflammatory markers, and intestinal pathology (32). These findings further highlight the need to test how different types of fibre impact AIEC intestinal expansion and modulate IBD risk.

Overall, our results are consistent with low dietary fiber as an environmental factor that participates in the multi-hit concept of IBD risk. A Western-style diet high in fat is often low in fiber. Exacerbated intestinal inflammation or worsened CD pathology and related sequelae are often attributed to higher dietary fat or to obesity, but our data show that dietary fiber is a
standalone factor that regulates expansion of an intestinal pathobiont when combined with oral
antibiotics.

Data Availability
All data are available from the corresponding author upon reasonable request.

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Figure Legends

Figure 1: Diet-induced obesity promotes AIEC expansion and colonization in the lower intestinal tract. C57BL/6J mice (8-10 weeks old) were fed a 60% HFD for 16 weeks. A) Intraperitoneal glucose (0.75 g/kg) tolerance test and area under the curve (AUC) of blood glucose and time, B) Fasting blood glucose C) Body mass and D) adiposity in chow fed and HFD fed mice prior to AIEC infection (n=18-22/group). Chow- and HFD-fed mice were pretreated with streptomycin (2 mg/mouse) one day prior to infection with AIEC. E) Body mass during AIEC infection (n=18-22/group). F) Tissue burdens at day 17 post AIEC infection (n=10-12/group). G) Fecal burdens during AIEC infection including pre-treated with the antibiotic streptomycin (n=8-10/group). H) Fecal burdens during the course of AIEC infection without antibiotic pre-treatment. Mann-Whitney test was conducted to determine significance. Values are presented as mean ± SEM. Each dot represents a mouse (except for panels A and E). *p<0.05.

Figure 2: Diet-induced obesity worsens gut pathology during AIEC infection. C57BL/6 mice (8-10 weeks old) were fed a 60% HFD for 16 weeks and pretreated with streptomycin (2 mg/mouse) one day prior to infection with AIEC. A) Overall pathology score, B) crypt length, and C) representative images of the ileum, cecum, and colon of AIEC-infected mice 9 days post AIEC infection in chow and HFD-fed mice (n=5 mice/group; 5 images/mouse). D) Overall pathology score, E) crypt length, and F) representative images of the ileum, cecum, and colon of AIEC-infected mice 17 days post AIEC infection in chow and HFD-fed mice (n=8 mice/group; 5 images/mouse). Mann-Whitney test was conducted to determine significance. Values are presented as mean ± SEM. *p<0.05.
Figure 3: Body mass does not correlate with bacterial burden during AIEC during diet-induced obesity. C57BL/6 mice (8-10 weeks old) were fed a 45% HFD for 16 weeks and pretreated with streptomycin (2 mg/mouse) one day prior to infection with AIEC (n=8-10/group). A) Body mass during AIEC infection. B) Fecal burdens AIEC infection. Mann-Whitney test was conducted to determine significance. Comparison of Body mass and AIEC fecal burden in 45% and 60% high fat diet (HFD)-fed mice assessed at C) 8 days post infection, D) 13 days post infection and E) 17 days post infection. Values are presented as mean ± SEM. Each dot represents a mouse. *p<0.05

Figure 4: Short-term feeding of an obesogenic diet promotes AIEC expansion. C57BL/6J mice (8-10 weeks old) were pretreated with streptomycin (2 mg/mouse) and fed a 60% HFD one day prior to infection with AIEC (n=8-10/group). A) Body mass during AIEC infection. B) Fecal burden of AIEC during infection. C) Tissue burdens of AIEC at day 9 post infection. Mann-Whitney test was conducted to determine significance. Values are presented as mean ± SEM. Each dot represents a mouse. *p<0.05.

Figure 5: Comparison of short-term and long-term obesogenic diet during AIEC infection. Comparison of fecal burden and body mass during AIEC infection in mice fed an obesogenic HFD for a long-term (i.e., 16 weeks) or a short-term pulse (i.e., 1 day) prior to infection compared to age-matched chow-fed mice. A) Fecal burdens during AIEC infection. B) Body mass during AIEC infection. C) Percentage of body fat (i.e., adiposity) one day prior to infection. A Kruskal-Wallis test was conducted, and Dunn’s multiple comparison test was used to determine significance for fecal burden (panel A). Two-way ANOVA was conducted and Tukey’s post-hoc test was used to determine significance for body mass during the course of AIEC infection (panel B). A One-way ANOVA was conducted and Tukey’s post-hoc test was used to determine significance for the percentage of adiposity (panel C). Values are presented as mean ± SEM.
± SEM. Significance was set at p<0.05. *denotes difference from all other groups. δ denotes difference from all chow-fed mice. Φ denotes difference from short-term HFD-fed and short-term chow-fed mice.

**Figure 6: A diet with high sucrose, low fiber, but low fat confers similar AIEC infection kinetics compared to an obesogenic high fat diet.** C57BL/6J mice (8-10 weeks old) fed a 60% HFD or low fat, high sucrose, low fiber diet for 16 weeks (long-term) or one day (short-term) prior to infection with AIEC. A) Fecal burdens and B) body mass during AIEC infection during short-term feeding (n=8-10/group). C) Percent body fat (i.e., adiposity) after short-term feeding prior to infection (a similar y-axis scale was used for comparison to Fig 5C). D) Tissue burdens 17 days post AIEC infection after short-term feeding (n=9-13/group). E) Fecal burdens and F) body mass during the AIEC infection during long-term feeding (n=6-9/group). G) Tissue burdens 17 days post AIEC infection during long-term feeding (n=8-9/group). Mann-Whitney test was conducted to determine significance. Values are presented as mean ± SEM. Each dot represents a mouse. *p<0.05.

**Figure 7: A lower fiber diet promotes AIEC expansion.** C57BL/6J mice (8-10 weeks old) were pretreated with streptomycin (2 mg/mouse) and kept on a chow diet containing ~13% fiber or fed a low fat, no sucrose diet with a lower fiber content (5-6%) one day prior to infection with AIEC. A) Fecal burdens and B) body mass during AIEC infection (n=7-13/group. C) Tissue burdens 17 days post AIEC infection (n=7-13/group). Mann-Whitney test was conducted to determine significance. Values are presented as mean ± SEM. Each dot represents a mouse. *p<0.05.

**Figure 8: Cellulose supplementation of an obesogenic diet has a minimal effect on AIEC expansion.** C57BL/6J mice (8-10 weeks old) were pretreated with streptomycin (2 mg/mouse)
and fed a HFD or HFD containing 150 g/kg of additional cellulose (HFD + cellulose) one day prior to infection with AIEC (n = 7-8/group). A) Fecal burdens and B) body mass during AIEC infection during short-term feeding. C57BL/6J mice (8-10 weeks old) were pretreated with streptomycin (2 mg/mouse) and fed a HFD or HFD containing 150 g/kg of additional cellulose (HFD + cellulose) for 16 weeks prior to infection with AIEC (n = 7-10/group). A) Fecal burdens and B) body mass during AIEC infection during long-term feeding. A Mann-Whitney test was conducted to determine significance. Values are presented as the mean ± SEM. Each dot represents a mouse. *p<0.05.
Figure 1
Figure 2

A

9 days post infection

Pathology Score

Surface Epithelium

Mucosa

Submucosa

Lumen

Chow HFD

Chow

HFD

Ileum

Cecum

Colon

B

Average Crypt Length (um)

0 100 200 300 400

Ileum

Cecum

Colon

Chow

HFD

Ileum

Cecum

Colon

C

HFD

Chow

Ileum

Cecum

Colon

D

17 days post infection

Pathology Score

Surface Epithelium

Mucosa

Submucosa

Lumen

Chow HFD

Chow

HFD

Ileum

Cecum

Colon

E

Average Crypt Length (um)

0 100 200 300 400

Ileum

Cecum

Colon

Chow

HFD

Ileum

Cecum

Colon

F

HFD

Chow

Ileum

Cecum

Colon

Figure 2
Figure 3: Graphs showing body mass and log cfu/g feces over different days post infection with 45% and 60% HFD.
Figure 4
Figure 5
**Figure 6**

A. Graph showing the log cfu/g feces over days post infection for different dietary groups.

B. Graph showing body mass (g) over days post infection for different dietary groups.

C. Graph showing adiposity (% body fat) for different dietary groups.

D. Graph showing log cfu/g tissue for different parts of the gut and different dietary groups.

E. Graph showing log cfu/g feces over days post infection for different dietary groups.

F. Graph showing body mass (g) over days post infection for different dietary groups.

G. Graph showing log cfu/g tissue for different parts of the gut and different dietary groups.
Figure 7
Figure 8
Low fiber expands gut pathobionts

**METHODS**

- Lower Fiber Diet
- Higher Fiber Diet
- Antibiotics

**OUTCOME**

- Intestinal Pathobiont Expansion

**CONCLUSION:** Low dietary fiber plus antibiotics promotes expansion of intestinal pathobionts, independent of obesity