A Diet Low in Red and Processed Meat Does Not Reduce Rate of Crohn’s Disease Flares

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Title: A Diet Low in Red and Processed Meat Does Not Reduce Rate of Crohn’s Disease Flares

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Dr. Albenberg has received research funding from Seres Therapeutics.
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Dr. Kappelman has received research funding from and serves as a consultant for Johnson & Johnson Consumer Inc, Abbvie, Pfizer, and Eli Lilly. He is a shareholder to Johnson & Johnson Consumer Inc.

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Author Contributions:
All authors contributed to study design, analysis, drafting, and finalization of the manuscript, in particular:
Study concept and design: Lewis, Kappelman, Sandler
Acquisition of data: Lewis, Gilroy, Kappelman, Sandler
Analysis and interpretation of data: Albenberg, Brensinger, Wu, Lewis
Drafting of the manuscript: Albenberg, Brensinger, Lewis
Revision of the manuscript: Albenberg, Brensinger, Kappelman, Sandler, Lewis
Abstract:

Background & Aims: Diet may be an important factor in progression of Crohn’s disease (CD). We performed a randomized controlled trial to determine whether reduced consumption of red and processed meats decreases the risk of symptomatic relapse of CD, analyzing results from the Food and Crohn’s Disease Exacerbation Study (FACES) trial.

Methods: Adults with CD were recruited into the FACES trial from IBD Partners, an internet-based cohort of IBD patients, from November 2013 through June 2015. Individuals who were in remission (CD activity index (sCDAI) scores of 150 or less), had completed a biannual survey, and reported consumption of red meat at least once weekly were randomly assigned to groups that consumed a minimum of 2 servings/week of red or processed meat (high meat, n=118) or not more than 1 serving per month (low meat, n=96) for 49 weeks. The primary outcome was relapse of CD, defined as increase in sCDAI score by ≥70 points and to >150 or a need for CD surgery or new CD medication. A secondary outcome, moderate or severe relapse, was based on an increase in sCDAI to >219.

Results: During the trial, the high-meat groups reported consumption of 2 or more servings of red or processed meat during 98.5% of observed weeks compared 18.8% of weeks for the low-meat group. Any and moderate to severe relapse occurred in 62% of participants in the high-meat group and 42% of participants in the low-meat group. There were no significant differences in time to any (P=.61) or moderate/severe (P=.50) relapse.

Conclusions: In an analysis of data from the FACES trial, we found that among patients with CD in remission, level of red and processed meat consumption was not associated with time to symptomatic relapse. ClinicalTrials.gov Identifier: NCT0192673

KEY WORDS: Inflammatory bowel disease, IBD, prevention, quiescent disease
Background

Crohn’s disease (CD) is an inflammatory disorder of the intestines, where host genetics, environmental factors, intestinal microbiome, epithelial barrier, the gut-brain axis, and innate and adaptive immune system contribute to the pathophysiology. Current evidence suggests that environmental factors, including diet, may be important in the development and progression of CD (1). Given that diet is modifiable, it has become an attractive potential target for both prevention and treatment of CD.

The role of diet in the management of CD is one of the most common questions that patients ask their physicians, yet high quality data to answer this question are limited. Defined formula-based diets are well established to be effective for the induction of remission in CD (2, 3). Several small trials of extreme restriction diets using regular food have also demonstrated improved disease activity and prolonged time to relapse (4-6). Additionally, two small studies suggest that a semi-vegetarian diet and a diet that restricts predominantly meat and eggs may prolong CD remission (7, 8). However, these have not been adequately tested in randomized controlled trials. These data, along with epidemiologic studies linking high dietary intake of total fats, PUFAs, omega-6 fatty acids, and meat with an increased risk of CD (9), led us to hypothesize that a diet characterized by lower meat intake would be associated with a more quiescent disease course. We sought to test the hypothesis that reduced consumption of red and processed meats decreases the risk of relapse of CD in the Food and Crohn’s Exacerbation Study (FACES), a prospective randomized trial.

Methods
Study Setting and Participants

Between 11/5/2013 and 6/30/2015 participants in the Crohn’s and Colitis Foundation Partners Study (which has since been renamed IBD Partners), an internet-based cohort of more than 15,600 participants with inflammatory bowel disease (IBD), who self-identified as having CD were recruited into FACES (10). See supplemental methods for additional details. Briefly, individuals with inflammatory bowel disease (IBD) who are older than 18 years of age were recruited to join IBD Partners using foundation e-mail rosters, social media, educational and fundraising events, and the Crohn’s & Colitis Foundation website. Each participant completed a baseline survey that contained questions about demographic characteristics, treatments, disease duration, and disease activity. Follow-up surveys were completed every 6 months after baseline to capture changes in disease activity and treatment since the prior survey.

To be included in the FACES Study, subjects must have been in symptomatic remission at the time of the most recent IBD Partners survey. Remission was defined as a short Crohn’s Disease Activity Index (sCDAI) of ≤150 (11) measured based on the patient’s estimate of his/her symptoms over the prior week. The sCDAI has been previously demonstrated to closely correlate with the original CDAI (11).

Randomization, Consent, and Exclusions
Randomization took place prior to recruitment so that the consent process could be tailored to the specific treatment arm, thus avoiding contamination by allowing the subject to know what the alternative diet entailed. Anticipating a higher participation rate in the high meat arm, we utilized a 3:2 randomization schedule with a target of achieving a 1:1 participation rate. Randomization was stratified by use of anti-TNFα medications. The randomization sequence was generated by the Biostatistical Analysis Center at the University of Pennsylvania.

Once randomized, subjects received an email invitation describing the study. Those who clicked “yes” to participate were led to a screening survey that asked additional questions to assess inclusion/exclusion criteria and also baseline dietary habits. Subjects were excluded if they reported consumption of red meat less than one time per week. See supplemental methods for additional exclusion criteria. If eligibility was confirmed, subjects were directed to an online, treatment arm-specific, consent form.

**Treatments**

Participants randomized to the intervention diet (referred to hereafter as low meat group) were instructed to follow their usual diet with the additional criteria, 1) To consume not more than 1 serving per month of red meat or processed meat and 2) To consume a minimum of 16 oz. of water per day. Participants randomized to the control diet (referred to hereafter as high meat group) were instructed to follow their usual diet with the additional criteria, 1) To consume a minimum of two servings of red meat or processed meat each week and 2) To consume a minimum of 16 oz. of water per day.
Red meat was defined as all meat from livestock (12) and processed meats were any red or white meat that was prepared with smoking, salting, curing, or addition of preservatives. The definition of a serving was 3 oz., equivalent to the size of a small, lean hamburger. We included consumption of 16 oz. of water per day in both groups in order to provide a “placebo-like” intervention to the participants assigned to the control diet. Participants were instructed to follow their assigned diet for 49 weeks. Concomitant medications were continued at the discretion of the treating physician.

Assessment of Participants

We relied on participant self-report and IBD Partners records for demographic information, CD history, non-IBD related medical history, and medication history (see supplemental methods for additional details). We collected information on the participant’s usual diet, in the past month, at baseline and at week 20 using the Diet History Questionnaire II (DHQ II) from the National Cancer Institute.

Disease activity at baseline and throughout the trial was measured by the sCDAI (11). Every week, participants received one email with a link to a web-based survey that asked questions about disease status and adherence to study diet. At baseline and during weeks 9, 17, 25, 33, 41, and 49, instead of one survey, participants received a daily email for 7 days with a link to a web-based survey where they reported disease activity and current medications. At week 20, a subset of the participants was emailed a consent form to provide one stool sample that they collected and shipped from home directly to Genova Diagnostics for measurement of fecal calprotectin.
The primary outcome was symptomatic relapse of CD, defined as an increase in the sCDAI by \( \geq 70 \) points and to >150 or self-reported initiation or increase dose of an IBD medication (mesalamine, thiopurine, methotrexate, corticosteroid, anti-TNF-alpha, natalizumab) or surgery for a flare of CD. A secondary outcome of moderate to severe relapse of disease was defined the same but required an increase in the sCDAI to >219 in the absence of undergoing CD surgery or starting any new CD medication. A persistent relapse required participants to meet the definition of symptomatic relapse on two consecutive weeks.

In planning the sample size for this study, we considered a therapeutic benefit of 20% or greater with a dietary intervention to be clinically significant (13-15). We conservatively estimated the sample size requirements by using a dichotomous outcome of continued remission at all time points vs. relapse at any time point prior to the end of follow-up. Under this assumption, 97 patients per group provides a minimum of 80% power to detect a 20% absolute difference in relapse rates across the full range of possible relapse rates.

**Statistical Analysis**

Analyses were conducted using modified intention to treat, such that participants were analyzed according to the diet that they were assigned, even if they were non-adherent to the diet, except that we did not include subjects who refused participation once they learned of their assigned diet or failed to complete any follow-up surveys. Descriptive analyses utilized mean, standard deviation, median, interquartile range, counts, and percentages. Continuous and categorical variables were compared using
the Wilcoxon rank sum test and the $\chi^2$ test, respectively. Principal component analysis was utilized to define overall dietary patterns. PERMANOVA using Euclidian distances was used to compare overall dietary composition at baseline between the treatment arms. Energy-adjusted linear regression with total calorie intake as the independent variable and raw nutrient or food intake as the dependent variable was used to compare intake between treatment arms. P-values were generated by comparing the residuals using Wilcoxon rank sum.

Statistical analysis of the primary outcome, time to symptomatic relapse, utilized Kaplan-Meier survival curves to display the relapse rate among the two study groups. Stratified Cox regression was used to determine the association of the study diet with the outcome, with use of anti-TNF therapy as the stratification factor. Participants who were lost to follow-up were censored at the time of last contact. Identical methods were used for the secondary outcomes.

Subgroup analyses, using Cox regression, were conducted to further explore the potential efficacy of the dietary intervention. These included the following subgroups: treatment at enrollment with an immunomodulator (azathioprine, mercaptopurine, or methotrexate) without an anti-TNF medication, treatment at enrollment with an anti-TNF therapy, treatment at enrollment with neither an immunomodulator or anti-TNF therapy, prior CD surgery, age <18 and age 18 or older at diagnosis with CD, and baseline red meat consumption above and below the median for the study population.

Adjusted Cox regression models were used to assess for confounding by differences in baseline dietary patterns. Missing data on baseline confounders were accounted for using multiple imputation. A sensitivity analysis was conducted assuming
that all participants who agreed to participate but did not return any surveys (n=11) were considered to have relapsed at week 1. All authors had access to the study data and reviewed and approved the final manuscript.

Results

Participation rates and comparison of participants and non-participants

In total, 659 eligible subjects were randomized and invited to participate in the study, of whom 214 signed the consent form - 118 in the high meat arm and 96 in the low meat arm, one of whom was ineligible in the low meat arm due to baseline meat consumption that was too low, leaving 213 participants for analysis (Figure 1). Participants were more likely to be female, were young, had an early age at diagnosis, and were more commonly from the northeastern and less commonly from the western United States (Supplemental Table 1).

Baseline characteristics of participants

The baseline characteristics were generally well balanced (Table 1). The median sCDAI was 75.5 (IQR 44.0 – 107.0) in the high meat group and 79.0 (44.0 – 121.0) in the low meat group. The median short IBDQ quality of life score was 5.8 (5.2 – 6.3) in both groups. Similar proportions of participants reported rarely active or absence of symptoms over the prior 6 months on the Manitoba index (48.7% of the high meat group and 49.5% of the low meat group). There was also a slightly higher proportion of participants who had ever been hospitalized in the low red meat group, but the median
number of hospitalizations did not differ between the two groups. Medication use patterns were very similar between the groups.

Of the 213 participants, 190 completed a DHQ II questionnaire at week 1 to assess baseline dietary pattern. From these, 16 participants were not included in the analysis because the DHQ II questionnaire was incomplete and 9 because of implausible caloric intake estimates. Figure 2a represents a 2-dimensional principal components analysis (PCA) with each data point representing a summary of the dietary pattern of a participant using all of the raw nutrient variables within the DHQ II, showing almost complete overlap of the two treatments groups (PERMANOVA p=0.15). A similar analysis using whole foods (i.e. how often the participant consumed a particular food in the last month regardless of quantity), while generally overlapping, demonstrated somewhat more divergence between the groups (PERMANOVA p=0.006) (Figure 2b).

Further exploration of these data was conducted by comparing specific nutrients and foods of interest (Supplemental Table 2). The dietary patterns were quite similar, but differed in baseline red meat intake (mean red meat intake in ounces per day of 1.28 and 0.65 in the high meat and low meat groups, respectively, p=0.0002) (Supplemental Figure 1).

Primary and secondary outcomes

Overall, 78% of participants reached either the end of the study (week 49) or experienced an outcome. Of the 213 participants who signed consent, 11 did not complete any follow-up surveys and thus contributed no data to the analyses. The primary outcome, symptomatic relapse of CD, occurred in 62% of participants during
the course of the study, while 42% and 35% had moderate-severe or persistent recurrence, respectively. **Figure 3** shows the comparison of unadjusted time to symptomatic relapse (3a), time to moderate to severe symptomatic relapse (3b) and time to persistent relapse (3c) by arm. There were no significant differences in time to relapse for any of the outcomes (p>0.3 for all outcomes). Additionally, a sensitivity analysis, assuming that participants who did not complete any surveys relapsed at week 1, did not impact the results (data not shown).

Stratified Cox regression was used to determine the association of the baseline diet (using principle components and baseline red meat intake above and below the median) with the outcome and to assess for confounding, with use of anti-TNF-alpha therapy as the stratification factor. While this did not change the results in terms of the relationship between the study diets and any of the outcomes (**Supplemental Table 3**), baseline diet pattern in terms of nutrient intake, as measured by PC1, was strongly associated with the risk of symptomatic relapse with a hazard ratio of 13.46 (95% confidence interval 1.22 to 148.45, p=0.03). Analyses adjusted for baseline red meat consumption provided similar results to the primary analyses (data not shown). The relationship between the dietary intervention and the time to symptomatic relapse was generally similar to the primary analysis in each subgroup tested (**Supplemental Table 4**).

**Adherence to the study diets**

Adherence to the high meat diet, estimated by mean percentage of weeks consuming 2 or more servings of red or processed meat, was 98.5%; adherence to the
low meat diet defined more rigorously as consuming no red or processed meat in the prior week averaged 57.3% (Figure 4a). However, consumption of red or processed meat differed substantially between the two groups during follow-up. The median percent of weeks that participants in the low meat group reported consuming 2 or more portions of red or processed meat was far lower than that for the high meat group (2.1%, IQR 0.0 - 30.4% vs 100%, IQR 100 - 100%) (Figure 4b). We fit a logistic regression model with generalized estimating equations, clustering on patient, for predicting consuming 2 or more servings of red/processed meat in the last week. Individuals in the high meat group were much more likely to consume 2 or more servings of red/processed meat in the last week (OR=340, 95% CI 130-886, p=<0.0001). Adherence to water consumption was 91.7% and 89.0% in the high meat and low meat groups, respectively.

There were 24 participants (12 in each arm) who did not provide adequate adherence data, completing no or very few adherence surveys. 106 participants provided both baseline and week 20 DHQII questionnaires with plausible caloric intake estimates. There was no significant change in red meat consumption in the high meat group (median change -0.10 ounces per day, IQR -0.37 – 0.65, p=0.97), while in the low meat group there was a significant decrease in red meat consumption (median change 0.26 ounces per day, IQR 0.08 – 0.72, p<0.0001) (Figure 4c).

Supplemental Table 5 compares the nutrient data from the week 20 DHQII questionnaires between the two groups. The differences were generally consistent with the dietary interventions prescribed. Additionally, we examined within subject change in nutrient intake from baseline to week 20 by arm in the 106 participants (66 in the high
meat arm and 40 in the low meat arm) who completed both baseline and week 20 DHQII measures and whose caloric intake was in a plausible range at both time points. Using the Wilcoxon rank sum test, the low meat arm had statistically significantly larger decreases in intake of calories (p=0.049), carbohydrates (p=0.038), protein (p=0.049), lean meat from meat, poultry, fish (p=0.007), meat from beef, pork, veal, lamb, and game (p=0.003), and meat from franks, sausage and luncheon meats (p=0.02).

In an exploratory analysis, we fit a Cox regression model examining time-updating adherence on time to relapse in the individuals randomized to the low meat arm. The predictor was a time-updating variable with a one-week lag. For example, when looking at outcome in week 20, the predictor included percent of weeks that the individual consumed zero servings of red or processed meat during weeks 1-19. We excluded week 1 and weeks with missing adherence data were considered “non-adherent.” We found that for every 10% increase in adherence to the low meat diet, the risk of relapse increased by 8% (HR=1.075, 95% CI 0.99-1.17, p=0.0864).

**Fecal calprotectin levels**

At week 20, 18 participants in each arm submitted a stool sample for fecal calprotectin. The high meat arm had a higher median (74.5 mcg/g, IQR 37 – 133) fecal calprotectin compared to the low meat arm (36.0 mcg/g, IQR 17 – 78), but this was not statistically significant by Wilcoxon rank sum (p=0.13) (supplemental Figure 2). Additionally, there was no significant difference in the proportion of participants who had a fecal calprotectin >150 or >250 by arm (Fisher’s exact p=1.0 for both).
Discussion

In the FACES randomized controlled trial, we sought to determine whether a diet that reduces red and processed meat consumption decreases the risk of symptomatic CD relapse. In this study, participants in the low meat group reported consuming 2 or more servings of red and/or processed meat far less frequently than the high meat group. Additionally, the low meat group significantly decreased their average weekly red meat consumption during the study. Despite these clear differences in diets, there were no statistically significant differences in time to relapse for any of the outcomes suggesting that reduction of red and processed meats does not reduce the risk of symptomatic CD relapse in patients with quiescent disease.

Existing data have led to a hypothesis that diet, and particularly red meat consumption, may be associated with relapse of CD. However, nearly all of the prior data are from observational rather than interventional studies. In a study from the IBD Partners cohort, red meat was one of the foods that patients with CD reported to worsen symptoms and it was commonly avoided (16). However, dietary pattern is a complex construct since certain foods tend to be consumed together and foods also contain additives, contaminants, chemical products of preparation, etc. (17). It is possible that in the FACES study, the level of adherence to the low meat diet led to a dietary intervention which was less extreme than what is required to demonstrate a difference in time to relapse. Perhaps a diet completely devoid of red and processed meat is required to reduce the rate of CD flares and that simply reducing one’s intake is not enough. Similarly, we did not include an intervention arm without any meat as most patient directed recommendations focus on consumption of lean meats (18) and
reducing only red and processed meats is more practical for patients. Indeed, although diet is hypothesized to be an environmental risk factor for IBD pathogenesis through its effects on the gut microbiome, existing studies of dietary interventions and the gut microbiome have generally shown modest effects on gut microbiota composition, particularly in the short-term, with the exception of very extreme elimination diets (19). Alternatively, when patients reduce red and processed meat in their diet, it must be replaced with some other food. It is possible that the participants in the trial tended to replace red and processed meat with another food item that has a deleterious effect on CD.

A unique aspect of this trial was the implementation within an internet-based cohort. The growing use of the internet and social media provides investigators with an opportunity to conduct pragmatic trials in larger populations at a fraction of the cost. This study took advantage of IBD Partners, an online cohort, to identify, recruit, enroll and follow-up patients. The patients were recruited from the entire country, not from the vicinity of a major medical center and there was no direct contact with the treating physicians. Behavioral interventions, such as dietary modification, are likely the most well suited to this design, as it does not entail changing the patient’s medication regimen. Similarly, because the study design did not involve direct contact with the patient or the treating physician, we focused on prevention of relapse. Studies of interventions for active disease would require a more complex to design. This study can serve as a model for future research on diet and other environmental factors suspected of influencing IBD relapse or other chronic relapsing diseases.
Our study has several limitations. Only one third of individuals who were randomized signed the consent form. However, unlike most trials, the use of IBD Partners allowed us to demonstrate that the characteristics of the participants and non-participants were very similar. Participants were not blinded to which arm they were assigned. Another limitation was the potential for misclassification of IBD diagnosis, baseline disease activity and/or dietary pattern. We used validated measures, when possible, such as the sCDAI and FFQ. Additionally, CD diagnosis has previously been shown to be highly valid in the IBD Partners cohort (10). Another limitation is the potential for enrollment bias due to methods of recruitment, interest in participating, requirement for reading English, and the technology required to join the e-cohort (10). Finally, the outcomes were based on symptoms rather than endoscopy. Fecal calprotectin was measured in a small subset and there was no significant difference between the groups; this would be expected since calprotectin has been consistently demonstrated to predict future symptomatic relapse (20).

In conclusion, we have demonstrated the feasibility of executing a randomized controlled trial of a dietary intervention to prevent relapse of symptoms in patients with CD using IBD Partners, an internet-based cohort. This randomized controlled trial demonstrated that substantial reduction of red and processed meat consumption among patients with asymptomatic CD was not efficacious in reducing time to symptomatic relapse. Based on these results, there is insufficient evidence to recommend reduction of red and processed meat consumption solely for the purpose of improving CD outcomes, although there may be some benefit for other health conditions.
Figure Legends:

Figure 1
FACES CONSORT diagram

Figure 2
Dietary pattern before and after the intervention
a. Principle component analysis of baseline dietary patterns using nutrient variables
b. Principle component analysis of baseline dietary patterns using whole foods variables

Figure 3
Comparison of time to symptomatic relapse by arm
a. Time to any symptomatic relapse
b. Time to moderate to severe symptomatic relapse
c. Time to persistent symptomatic relapse

Figure 4
a. Comparison of adherence to the diet by treatment arm. The high meat group consumed 2+ servings of red or processed meat in 98.5% of weeks. The low meat group consumed 0 servings red or processed meat in 57.3% of weeks.
b. Percent of weeks that participants in each treatment group reported consuming two or more portions of red or processed meats
c. Change in consumption of red meat from baseline to week 20 by treatment arm
References


Table 1
Demographics and Baseline Characteristics

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<th>Low meat (n=95)</th>
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<tr>
<td>Age at baseline</td>
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<td></td>
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<tr>
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<td></td>
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<tr>
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<td>7 (7.4%)</td>
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<tr>
<td></td>
<td>Sometimes active</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25 (21.4%)</td>
<td>18 (18.9%)</td>
</tr>
<tr>
<td></td>
<td>Occasionally active</td>
<td>25 (21.4%)</td>
</tr>
<tr>
<td></td>
<td>Rarely active</td>
<td></td>
</tr>
<tr>
<td></td>
<td>26 (22.2%)</td>
<td>27 (28.4%)</td>
</tr>
<tr>
<td></td>
<td>remission/absence of symptoms</td>
<td>31 (26.5%)</td>
</tr>
<tr>
<td>Smoking history</td>
<td>never</td>
<td></td>
</tr>
<tr>
<td></td>
<td>83 (70.3%)</td>
<td>67 (70.5%)</td>
</tr>
<tr>
<td></td>
<td>former</td>
<td></td>
</tr>
<tr>
<td></td>
<td>34 (28.8%)</td>
<td>21 (22.1%)</td>
</tr>
<tr>
<td></td>
<td>current</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 (0.8%)</td>
<td>7 (7.4%)</td>
</tr>
<tr>
<td>Hx of IBD surgery</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td></td>
<td>54 (45.8%)</td>
<td>45 (47.4%)</td>
</tr>
<tr>
<td>Ever hospitalized for IBD</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td></td>
<td>74 (62.7%)</td>
<td>73 (76.8%)</td>
</tr>
<tr>
<td>Number of times hospitalized for IBD</td>
<td>Median (Q1-Q3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.0 (0.0-2.0)</td>
<td>1.0 (1.0-3.0)</td>
</tr>
<tr>
<td></td>
<td>High meat (n=118)</td>
<td>Low meat (n=95)</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>-------------------</td>
<td>-----------------</td>
</tr>
<tr>
<td><strong>Short CD Activity Index</strong></td>
<td>Median (Q1-Q3)</td>
<td>75.5 (44.0-107.0)</td>
</tr>
<tr>
<td><strong>Short IBD QOL score</strong></td>
<td>Median (Q1-Q3)</td>
<td>5.8 (5.2-6.3)</td>
</tr>
<tr>
<td><strong>Current use of aminosalicylates</strong></td>
<td>Yes</td>
<td>33 (28.0%)</td>
</tr>
<tr>
<td><strong>Current use of steroids</strong></td>
<td>Yes</td>
<td>3 (2.5%)</td>
</tr>
<tr>
<td><strong>Current use of immunosuppressants</strong></td>
<td>Yes</td>
<td>43 (36.4%)</td>
</tr>
<tr>
<td><strong>Current use of biologics</strong></td>
<td>Yes</td>
<td>59 (50.0%)</td>
</tr>
<tr>
<td><strong>Current use of antibiotics for IBD</strong></td>
<td>Yes</td>
<td>3 (2.5%)</td>
</tr>
<tr>
<td><strong>Current use of narcotics for IBD</strong></td>
<td>Yes</td>
<td>7 (5.9%)</td>
</tr>
<tr>
<td><strong>Current use of probiotics for IBD</strong></td>
<td>Yes</td>
<td>28 (23.7%)</td>
</tr>
<tr>
<td><strong>PROMIS anxiety t-score</strong></td>
<td>Median (Q1-Q3)</td>
<td>48.0 (40.3-55.8)</td>
</tr>
<tr>
<td><strong>PROMIS depressive symptoms t-score</strong></td>
<td>Median (Q1-Q3)</td>
<td>41.0 (41.0-53.9)</td>
</tr>
<tr>
<td><strong>PROMIS fatigue t-score</strong></td>
<td>Median (Q1-Q3)</td>
<td>49.8 (46.0-57.0)</td>
</tr>
<tr>
<td><strong>PROMIS pain interference t-score</strong></td>
<td>Median (Q1-Q3)</td>
<td>41.6 (41.6-52.0)</td>
</tr>
<tr>
<td><strong>PROMIS sleep disturbance t-score</strong></td>
<td>Median (Q1-Q3)</td>
<td>52.4 (50.5-54.3)</td>
</tr>
<tr>
<td><strong>PROMIS social satisfaction t-score</strong></td>
<td>Median (Q1-Q3)</td>
<td>51.8 (48.2-64.4)</td>
</tr>
</tbody>
</table>
Randomized (n = 659)

- Allocated to high red meat (n = 264)
  - Agreed to follow high meat diet (n = 118)
    - n = 101 opened email and expressed interest, but did not advance to consent forms
    - n = 45 reviewed the consent form and chose not to participate
  - No surveys completed (n = 3)
- Allocated to low red meat (n = 395)
  - Agreed to follow low meat diet (n = 95)
    - n = 179 opened email and expressed interest, but did not advance to consent forms
    - n = 120 reviewed the consent form and chose not to participate
    - n = 1 ineligible because baseline meat intake too low
  - No surveys completed (n = 8)

Analysis
- Analyzed (n = 115)
- Analyzed (n = 87)
Figure 2

a. PCA of Baseline DHQ Nutrient data

- High Meat
- Low Meat

p = 0.15

b. PCA of Baseline raw DHQ data

- High Meat
- Low Meat

p = 0.006
Figure 3

a. Time to Relapse by Arm

b. Time to Moderate/Severe Relapse by Arm

c. Time to Persistent Relapse by Arm

- Arm A – High meat
- Arm B – Low meat

p = 0.60
p = 0.52
p = 0.31
Figure 4

a. Adherence over time by Arm

b. Red or Processed meat consumption

c. Change in red meat consumption from baseline to week 20
FOOD AND CROHN’S EXACERBATION (FACE) STUDY

PROTOCOL VERSION 8.0

JAMES D. LEWIS, MD, MSCE
PRINCIPAL INVESTIGATOR

Date: V7.1
Amended:
May 22, 2013
July 23, 2013
August 22, 2013
September 3, 2013
August 14, 2014
September 29, 2014
October 30, 2014
Feb 3, 2015

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1 Specific aims

Inflammatory bowel diseases (IBD), including Crohn’s disease (CD) and ulcerative colitis (UC), are chronic and relapsing diseases. The etiology of IBD is multifactorial, including both genetic predisposition and environmental triggers. More than 100 genetic loci are linked to IBD, yet genetics explain less than 50% of the epidemiology of IBD. Environmental factors, including the composition of the gut microbiome and diet, are thought to also contribute to the etiology. Recently, we demonstrated a link between long term dietary patterns and the composition of the gut microbiome, thereby potentially identifying a connection between two commonly implicated environmental risk factors for IBD.

From a public health perspective, the most common question asked by patients about their IBD is “What should I eat?” Yet, relatively few dietary strategies have been formally tested to determine their impact on the course of IBD. As a result, dietary modification is not routinely recommended for preventing relapse.

Existing data lead to a hypothesis that diet, and particularly red meat consumption, may be associated with relapse of CD. Our research demonstrates that agrarian dietary patterns are associated with an enterotype characterized by Prevotella, a genera more commonly observed in people from rural Africa where CD is uncommon. Prevotella and related bacteria are efficient at fermenting dietary fiber, thereby leading to higher concentrations of short chain fatty acids (SCFA) which may protect against bowel inflammation. In contrast, high fat diets, through dietary induced changes in the gut microbiota, may increase bowel permeability, a hallmark of CD. High fat diets also worsen dextran sodium sulfate (DSS) colitis in rodents. A major source of hydrogen sulfide (H2S) in the bowel is bacterial fermentation of sulfur amino acids which are found in high protein foods such as meats. H2S has been proposed as contributing to bowel inflammation through a variety of mechanisms, including impaired utilization of SCFAs and direct toxic effects. However, other research suggests that H2S has anti-inflammatory properties and contributes to mucosal healing. Ingested iron, particularly iron sulfate, has also been implicated in intestinal inflammation, triggering ileitis in TNFα/WT mice, and alteration of gut microbiome.

Research in patients with IBD also points to a role for diet and particularly meat to influence the disease course. Recurrence of CD is nearly universal after ileal resection. The recurrent intestinal inflammation is dependent on exposure of the neoterminal ileum to the fecal contents and occurs within 8 days of exposure. Thus, luminal contents, including food, digestive factors, microorganisms or a combination play a key role in triggering CD inflammation. In contrast, exclusive enteral nutrition with elemental, semi-elemental, or defined formula diets has anti-inflammatory effects and is considered first line therapy for active CD in Europe and the United States. These diets are also efficacious to maintain remission. Extreme exclusion diets using regular food also have demonstrated efficacy in several small studies. Thus, diet can impact the disease course of CD, but these extreme dietary interventions are impractical for long term use. As such, there is interest in identifying less extreme but effective dietary interventions. Two recent small studies suggest that a semi-vegetarian diet and a diet that restricts predominantly meat and eggs may prolong CD remission. However, these have not been tested in appropriately designed controlled trials. Similarly, patients with UC who reported higher levels of red and processed meat consumption were more likely to relapse in a prospective cohort study, but this has not been confirmed in a separate population or tested in CD. Nonetheless, these data suggest that a diet with high levels of red and processed meat may worsen the course of IBD, particularly CD. Therefore, we will test the hypothesis that exclusion of red and processed meats reduces the risk of relapse of CD in a prospective randomized trial. The interrelated specific aims are:

**Aim 1.** To determine whether a diet with low levels of red and processed meat consumption is associated with a reduced rate of relapse of CD.

H1: Higher levels of red and processed meat consumption is associated with more frequent relapse of CD.

**Exploratory aim.** To identify other dietary patterns associated with a lower risk of relapse of CD.

2 Background

IBD affects approximately 1.5 million Americans, with peak incidence in the 2nd and 3rd decades of life. These are chronic diseases with no cure. As such, IBD is a major health problem throughout the patient’s entire life. Like many intestinal diseases, it is logical to think that dietary patterns may influence the natural history of IBD. Indeed, this is the most frequently asked question by patients. For example, the CCFA maintains an Information Resource Center that receives more than 14,000 inquiries per year, of which approximately 65% ask for dietary advice. In 2009, 6300 patients registered for a CCFA webinar on nutrition.
and IBD, the largest number for any CCFA webinar to date. (Personal communication K Arseniadis Oct 6, 2011) Furthermore, the majority of patients report intolerance to specific food items, yet less than half have seen a nutritionist and many follow nutritionally compromised diets. Thus, understanding the role of diet on the natural history of IBD is of major public health interest. Unfortunately, there have been few high quality studies that have specifically addressed this question.

### 2.1 Therapeutic strategies for IBD

Numerous medications are efficacious in the treatment of IBD (reviewed in). With the exception of mesalamine, nearly all of these medications suppress the immune system. Unfortunately, chronic immunosuppression is associated with numerous adverse effects including uncommon but potentially fatal adverse reactions. Concerns about these uncommon life threatening adverse effects strongly influence patients’ choice of medical therapies. As such, there is great interest in and need for alternative treatment strategies that are not based on immunosuppression.

Dietary interventions are an attractive alternative. Indeed, elemental, semi-elemental and defined formula diets are commonly used in the treatment of pediatric CD, particularly in Canada and Europe. Although elemental, semi-elemental and defined formula diets are effective in the treatment of Crohn’s disease, the poor palatability often requires administration via nasogastric feeding tube, often making these diets impractical for adults and for long term therapy. However, there is reason to believe that other dietary modification may also improve the course of IBD, particularly CD.

### 2.1.2 Dietary patterns are associated with incidence of IBD

Although genetics contribute to the etiology of IBD, environmental exposures are also important as IBD is believed to represent an over exuberant response to normal antigens. The bowel lumen is continually exposed to numerous antigens, including the food that we consume and the enormous population of organisms that compose the gut microbiome. There are numerous proposed mechanisms through which diet could influence the incidence of IBD, including direct dietary antigens, altering the gut microbiome, influencing gene expression, and affecting gastrointestinal permeability.

Several investigators have examined the association of dietary patterns and the risk of incident IBD (reviewed in). Most recently, the authors of a systematic review came to the conclusions that high dietary intake of total fats, PUFAs, omega-6 fatty acids, and meat were associated with an increased risk of CD and UC; high fiber and fruit intakes were associated with decreased CD risk; and high vegetable intake was associated with decreased UC risk. Taken together, these studies support a potential role of dietary patterns in the etiology of IBD. However, for most patients with IBD, the more important question is “what should I eat now that I have IBD?” There are fewer data on the role of dietary patterns to influence the natural history of IBD. Drawing from the studies of pre-illness diet, one can hypothesize that a diet characterized by greater fruit and vegetable intake and lower meat intake would be associated with a more quiescent disease course.

### 2.1.3 Hypotheses for how diet may alter the course of CD once the disease is established

Perhaps the strongest evidence for a role of intestinal contents on the course of CD comes from two studies of patients who underwent ileocolonic resection. These demonstrated that recurrence of inflammation after ileal resection is dependent on exposure of the neoterminal ileum to the fecal contents and occurs within 8 days of exposure. However, it is not known what component of the fecal stream contributes to the inflammation. It could be bacteria, other microorganisms, the digested food content, a combination of these, or something else.

Recently, we demonstrated a potential link between diet and the composition of the gut microbiome. Our research demonstrates that long term agrarian dietary patterns are associated with an enterotype characterized by Prevotella, a genera more commonly observed in people from rural Africa where IBD and particularly CD is uncommon. Prevotella and related bacteria are efficient at fermenting dietary fiber, thereby leading to higher concentrations of short chain fatty acids (SCFA) which may protect against bowel inflammation. In contrast, high fat diets, through dietary induced changes in the gut microbiota, may increase bowel permeability, a hallmark of CD. High fat diets also worsen dextran sodium sulfate (DSS) induced colitis in mice, possibly by increasing colonic epithelial non-classical NK T cells and reducing Treg cells.

A major source of hydrogen sulfide (H2S) in the bowel is bacterial fermentation of sulfur amino acids which are found in high protein foods such as meats. H2S has been proposed as contributing to bowel inflammation through a variety of mechanisms, including impaired utilization of SCFAs and direct toxic effects. However, other research suggests that H2S has anti-inflammatory properties and contributes to mucosal healing.
The role of iron in relation to the gut microbiome and the immune system is complex\textsuperscript{51, 52}. Iron catalyses the formation of oxygen radicals which can cause cellular injury and enhances NF-κB activation that perpetuate inflammation\textsuperscript{53-55}. Excess iron can increase the proportion of CD8 T cells relative to CD4 cells. Iron is also used by bacteria and has been linked to an invasive phenotype, thereby further promoting inflammation\textsuperscript{51}. Ingested iron, particularly iron sulfate, has been directly implicated in intestinal inflammation and alteration of gut microbiome in animal models\textsuperscript{13, 14}. For example, TNF\textsuperscript{ARE/WT} mice are protected from ileitis when fed an iron sulfate free diet, even with parenteral iron replacement\textsuperscript{13}. In this model, iron triggers endoplasmic reticulum stress in intestinal epithelial cells and sensitizes the epithelium toward cytotoxic T cell induced apoptosis\textsuperscript{13}. Whether heme-iron, the predominant form in meat, has the same effects is unknown.

2.1.4 Observational studies of diet and the natural history of IBD

Although there are no well-done observational studies of dietary patterns and the natural history of CD, Jowett et al. conducted a prospective study of patients with UC. Jowett observed that patients who reported higher levels of meat, eggs, protein and alcohol consumption were more likely to have a relapse of UC\textsuperscript{25}. Importantly, the association was much stronger for red and processed meats than for other meats and there was no association with fish consumption. Jowett hypothesized that these dietary patterns resulted in higher intestinal concentration of sulfate which in turn led to disease relapse. Another study found a correlation between sulfate consumption and endoscopic activity in UC\textsuperscript{56}. Jowett et al. conducted a prospective study of patients with UC. Jowett observed that patients who reported higher levels of meat, eggs, protein and alcohol consumption were more likely to have a relapse of UC\textsuperscript{25}. Importantly, the association was much stronger for red and processed meats than for other meats and there was no association with fish consumption. Jowett hypothesized that these dietary patterns resulted in higher intestinal concentration of sulfate which in turn led to disease relapse. Another study found a correlation between sulfate consumption and endoscopic activity in UC\textsuperscript{56}. In this model, iron triggers endoplasmic reticulum stress in intestinal epithelial cells and sensitizes the epithelium toward cytotoxic T cell induced apoptosis\textsuperscript{13}. Whether heme-iron, the predominant form in meat, has the same effects is unknown.

2.1.5 Dietary intervention studies to alter the course of CD

In CD, exclusive enteral nutrition with elemental, semi-elemental, and defined formula diets has been widely studied for induction of remission and is considered first line therapy in Europe\textsuperscript{17, 18}. For maintenance of remission, a diet in which half of the daily calories were from an elemental supplement resulted in a nearly 50% reduction in CD relapse rates compared to a regular diet\textsuperscript{15}. Several small trials of extreme restriction diets using regular food have also demonstrated improved disease activity and prolonged time to relapse\textsuperscript{20-22}, however such extreme restriction diets are impractical and poorly accepted. In a recent uncontrolled trial by Rajendran, food specific IgG4 levels were used to select which foods to exclude rather than excluding nearly all foods and gradually adding back selected foods\textsuperscript{23}. Eggs and beef were the most common foods with high IgG4 antibody levels and were therefore excluded by the greatest number of patients. The 29 patients on the exclusion diet experienced a significant reduction in symptoms based on a modified Crohn’s Disease Activity Index and reduction in the ESR as compared to pretreatment levels. The major limitation of this study was the absence of a control group. In another small study (n=22), Chiba et al. demonstrated superiority of the semi-vegetarian diet versus an omnivorous to maintain clinical remission (94% vs. 33%)\textsuperscript{24}. This study included patients with medically or surgically induced remission who received a lacto-ova-vegetarian diet in hospital. After discharge, the semi-vegetarian diet allowed for fish once weekly and meat once every two weeks. Eggs were allowed without limitation. It should be noted that this was not a randomized trial but rather allowed patients to choose whether or not to continue on the diet after discharge.

Taken together, studies of exclusive enteral nutrition, exclusion diets, and semi-vegetarian diets suggest that minimizing exposure of the intestinal lumen to selected food items may prolong the remission state of patients with CD. Several of the studies suggest that reducing exposure to red and processed meat may be important. However, these have been small studies in extremely select populations (e.g. those with elevated IgG4 levels or who had remission induced with elemental therapy). Furthermore, the practicality of maintaining these interventions over long periods of time is doubtful, particularly the use of elemental or semi-elemental diets for which there is the strongest evidence. As such, we will test a far more practical dietary intervention that draws on these prior experiences.

It is important to recognize that not all dietary intervention studies have suggested a benefit. Omega-3 fatty acid supplements have also been tested and were not effective in preventing CD relapse in two large placebo-controlled trials\textsuperscript{57}. One of the largest dietary trials (n=352) compared recommendations for a diet high in refined carbohydrates to one high in unrefined carbohydrates and low in sugar among patients with CD. Although there were differences in sugar and fiber intake between the study groups, rates of clinical deterioration were not statistically different\textsuperscript{58}. 2.1.6 Summary of background information

Dietary patterns are associated with subsequent incidence of IBD and preclinical research suggests that dietary patterns and specific nutrients can influence the inflammatory pathways that are activated in IBD. As such, dietary intervention studies represent an appealing approach to modify the disease course without immunosuppression. Elemental, semi-elemental and defined formula diets are first line therapy for CD in some
parts of the world and extreme exclusion diets have also been efficacious. These therapies have not had
demonstrated efficacy in UC and are generally viewed as impractical for long term management of CD. As
such, there is a need for more practical yet effective dietary interventions. We hypothesize higher levels of red
and processed meat consumption may predispose to a more severe disease course and will test this novel
dietary intervention in patients with CD.

3 Methods
3.1 Overview
The proposed study is a randomized controlled trial of a low red and processed meat diet compared to a
regular diet among patients with CD in remission. We will select from among patients enrolled in CCFA
Partners who have previously reported consuming red meat at least once weekly. At baseline, patients will
complete a semi-quantitative food frequency questionnaire assessing usual dietary patterns over the preceding
month. Disease activity will be assessed with the abbreviated Crohn’s Disease Activity Index (aCDAI)\textsuperscript{59}. Self-
reported disease status will be assessed during follow-up using an internet-based questionnaire. Repeat
assessment of adherence to the study diets will be assessed with food frequency questionnaires (FFQs)
administered after 20 weeks. One stool sample will be collected at 20 weeks for FCP testing, for those
participants that consent to do this. Follow-up duration will be for 48 weeks. Statistical analysis will compare
the time to relapse using Cox regression for patients in the two study arms. In the exploratory aim, we will
compare outcomes among patients in the highest tertile for other food items and nutrients to those in the
lowest tertile based on self-reported usual dietary patterns at baseline. Thus, the study population will be
analyzed both as a randomized controlled trial and as a prospective cohort study.

3.2 Study Organization
The study will be based out of the Center for Clinical Epidemiology and Biostatistics at the University of
Pennsylvania. The core research team will be composed of the Principal Investigator, the Project Manager,
and the Biostatistical Analysis Center.

3.2.1 Core research team responsibilities
1. Overall leadership regarding study design and conduct of the clinical trial.
3. Collaboration with CCFA Partners in the development, testing, and use of all case report forms (CRFs)
   and study procedures.
4. Collaboration with CCFA Partners in creation of the study database.
5. Development and application of quality assurance procedures including data tracking and validation,
   query processes, and maintenance of related documentation.

3.2.2 Biostatistical Analysis Center responsibilities
1. Generate the randomization sequence for each study site
2. Conduct of the statistical analyses

3.3 Study population
Patients will be recruited for this study from CCFA Partners, a cohort of more than 12,000 adult patients
with inflammatory bowel disease who have agreed to complete online surveys related to their disease\textsuperscript{60}. CCFA
Partners was initiated in 2011 through targeted recruitment of patients with inflammatory bowel disease who
had made contact with the CCFA. Subsequently, the cohort has grown through additional referrals based on
social media and other avenues. We have previously described the composition of the cohort, demonstrating
that the cohort appears generally similar to other populations with IBD, other than having a larger proportion of
women\textsuperscript{60}. Dr. Lewis also has experience using this population to gather patient preference data related to
dietary patterns\textsuperscript{61}.

3.3.1 Inclusion criteria
1. Patients must have an established diagnosis of CD. An ongoing validation study has found >95%
   accuracy for patients to correctly self-identify their underlying inflammatory bowel disease type (CD vs.
   UC) based on traditional diagnostic criteria\textsuperscript{62}.\textsuperscript{62}
Principal Investigator/Program Director (Last, first, middle): Lewis, James D

2. All patients must be in clinical remission at the time of entry into the study. Remission is defined as an aCDAI of less than or equal to 150. See §3.3.3 for further discussion of the disease activity measure. This will be measured based on the patient’s estimate of their symptoms over the prior week. In addition, we will require that the patient self-rate their disease status in the week prior to randomization as remission or minimal symptoms on a 5 point scale (remission, minimal symptoms, mildly active, moderately active, severely active).

3. Patients must have previously reported consumption of red meat at least one time per week in the CCFA Partners baseline dietary inventory.

3.3.2 Exclusion criteria

No patient will be excluded on the basis of age, sex, or race. Exclusion criteria are the following:

<table>
<thead>
<tr>
<th>Exclusion criteria</th>
<th>Rationale</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBD unspecified / Indeterminate colitis</td>
<td>This study includes only patients with established CD</td>
</tr>
<tr>
<td>Total or sub-total colectomy, ileostomy, or colostomy</td>
<td>Non-invasive disease activity measures do not accurately assess disease activity in this population</td>
</tr>
<tr>
<td>Unable to read and speak English</td>
<td>Completion of the FFQ and surveys would not be possible</td>
</tr>
<tr>
<td>No internet access</td>
<td>Completion of the follow-up surveys would not be possible</td>
</tr>
<tr>
<td>Oral or rectal steroid use within the prior two weeks</td>
<td></td>
</tr>
<tr>
<td>Perianal fistula or abscess with more than scant drainage</td>
<td>Perianal fistulas and abscesses compromise quality of life such that even if the CDAI score is low, this would not be considered a clinical remission.</td>
</tr>
<tr>
<td>Age less than 18 years</td>
<td>Improved accuracy of data collection, stability of the diet, and safety.</td>
</tr>
<tr>
<td>Pregnant or breastfeeding women</td>
<td>To avoid any risk to an unborn fetus or breastfeeding child from changing the mother’s diet</td>
</tr>
<tr>
<td>Unwilling to follow the study diet</td>
<td>Inclusion would lead to exposure misclassification and reduced statistical precision and power.</td>
</tr>
</tbody>
</table>

3.3.3 Disease activity measures

The Crohn’s Disease Activity Index is the standard disease activity index for CD clinical trials in adults. The index includes the number of liquid stools per day, abdominal pain, general well being, extraintestinal complications of CD, use of Lomotil or opiates for diarrhea, anemia, weight loss, and the presence of an abdominal mass on physical exam. The Pediatric CD Activity Index (PCDAI) is similar to the adult version, but contains information on growth, albumin, and ESR. Unfortunately, the CDAI and PCDAI are both inconvenient as both require a blood test and physical examination to calculate the score. As such, investigators recently validated the aCDAI which patients can complete without an office visit or blood draw. This disease activity measure has been validated against the full CDAI with correlation for baseline scores and score change of 0.90 and 0.96, respectively. The aCDAI uses the same scale as the full CDAI, such that scores ≤150 define remission, 150-219 mild activity, 220-450 moderate activity, >450 severe activity. **Computation is simple:** aCDAI=44+(2 x number of liquid or soft stools each day for 7 days) + [5 x sum of 7 daily abdominal pain ratings (0-3 none to severe)] + 7 x [sum of 7 daily general well being ratings (0-4 generally well to terrible)].

3.4 Intervention

Patients who meet the eligibility criteria will be randomly assigned to one of two study diets. It is important to have a control dietary intervention so that participants do not know which arm they are in. The control diet is based on the knowledge that a prior study demonstrated no therapeutic benefit or harm from altering the amount of sugar intake and that water consumption should have no effect. The intervention diet draws from the semi-vegetarian diet reported by Chiba, the IgG4 directed diets of Rajendran, and the observational data of Jowett, all of which suggest that minimizing intake of red and processed meat may be advantageous.

**Diet 1 (control diet)** – Patients will be instructed to follow their usual diet but with one special criterion:

1) To consume a minimum of two servings of red meat (e.g. beef, pork, veal, lamb, venison, or other livestock) or processed meat (e.g. bacon, sausage, hotdogs, luncheon meats) each week where a serving is defined as 3 oz, equivalent to the size of a small lean hamburger.
2) To consume a minimum of 16 oz. of water per day.
Diet 2 (intervention diet) – Patients will be instructed to follow their usual diet but with one special criterion:

1) To consume not more than 1 serving per month of red meat (e.g. beef, pork, veal, lamb, venison, or other livestock) or processed meats (e.g. bacon, sausage, hotdogs, luncheon meats) where A serving is 3 oz, equivalent to the size of a small lean hamburger.

2) To consume a minimum of 16 oz. of water per day

Our definition of red meat draws on the US Department of Agriculture definition that all meat from livestock is “red meat” as it contains more myoglobin than chicken or fish. Additionally, prior research using these definitions have shown red and processed meats to be associated with mortality, cancer and cardiovascular disease, thereby making our study consistent with other definitions. Processed meats include any red or white meat that has been prepared with smoking, salting, curing or addition of preservatives. Common examples include bacon, sausage, pepperoni, bologna, and ham. Some turkey and chicken deli meats are processed meats and others are not, depending on how they have been prepared and in particular whether preservatives have been added. We will provide participants with a list of acceptable deli meats.

We have intentionally not used total exclusion of these food items in Diet 2, recognizing that this would likely result in substantial noncompliance and that two of the prior studies from which the diet was designed focused on reduced amounts rather than complete exclusion. Furthermore, in a preliminary study, we surveyed 3,840 patient members of the CCFA with CD. Only 1% report eating red meat 1 day per month or less. The median was greater than 8 days per month. Thus, our diet represents a reduction in red meat consumption for 99% of patients with CD and an approximately 90% reduction from the median level of consumption.

We have included consumption of 16 oz. of water per day in both groups. This is anticipated to have no impact on disease course, since humans routinely consume water as a beverage and in food. However, we have added this to provide a “placebo-like” intervention to the patients who are assigned to the control diet.

### 3.4.1 Concomitant medications

All of the patient’s concomitant IBD medications will be continued at the discretion of the patient and the treating physician. Addition of new IBD medications will not be allowed, but will be part of our definition of clinical relapse. Because all patients will be in clinical remission and not receiving corticosteroids (other than low dose budesonide) at the time of enrollment, there would be little anticipation that the physician would recommend a change in medical therapy.

### 3.5 Randomization

Slightly different from most randomized clinical trials, for this study, the randomization will take place prior to recruitment. This is necessary as the consent process will be tailored to the specific treatment arm that a participant is assigned to. This is necessary to avoid contamination by allowing the participant to know what the alternative diet entails. Because it is likely that there will be a higher rate of agreement to participate in the arm where red meat is consumed twice per week than in the alternative arm, we will utilize a 3:2 randomization schedule with a target of achieving a 1:1 participation rate. We will monitor imbalance in enrollment after the first phase of recruitment and reserve the right to modify the randomization schedule if needed for the second wave of recruitment to achieve approximately 1:1 participation rate.

A stratified randomization sequence will be generated by the Biostatistical Analysis Center at the Center for Clinical Epidemiology and Biostatistics. The stratification factor will be current use of anti-TNFα therapy.

### 3.6 Blinding

Determination of the outcome is based on the patient reported symptoms used to calculate the aCDAI and is done without physician contact. Thus, this should not bias the results. Although it is conceivable that a treating physician may know that a patient is in the study and may choose to escalate therapy for disease relapse, it is unlikely that the knowledge of the diet would influence the treatment decision.

It is not possible to blind participants to which arm they are assigned, but we will not tell the participants what the alternative study arm is so as to avoid contamination of the intervention (i.e. having participants in the control arm avoiding red and processed meat).

### 3.7 Study Assessments – Plan and Methods

The sequence of study events are summarized in table 2 and §3.8 provides additional details on the data elements to be collected. Patients will be introduced to the study protocol and informed consent will be
obtained prior to proceeding with any additional study procedures. When describing the study to patients, we will state that this is a study of different dietary interventions that is designed to determine which diet is more effective at preventing relapse of CD. We will emphasize that the study diets do not require purchase of specially prepared or grown foods (such as organic foods) or use of dietary supplements, vitamins, prebiotics or probiotics. To avoid contamination of the treatment arms, patients will not be told the specifics of the two study diets prior to randomization and after randomization will only be told about their assigned diet.

### 3.8 Data to be collected and clinical measurements

**Demographics and medical history:** We will rely on patient self report and source medical records for demographic, non-IBD related medical history. Relevant non-IBD related medical history will include any medical conditions that could potentially influence both the patient’s diet and the natural history of their IBD. Although there are no conditions that are clearly known to meet this definition, we will record age, sex, race, height, weight, tobacco use, a history of diabetes, hypertension, coronary artery disease, peripheral vascular disease, chronic pancreatic or liver disease (e.g. primary sclerosing cholangitis), celiac disease, irritable bowel syndrome, rheumatoid arthritis, psoriasis, psoriatic arthritis, ankylosing spondylitis, and multiple sclerosis.

We will also obtain participants’ e-mail addresses if they consent to allow CCFA Partners to provide us with this.

**CD phenotype and prior history:** CD phenotype will be summarized using the Montreal classification\(^66\). These data have already been collected as part of the CCFA Partners intake survey. Time since last relapse will be recorded.

**Usual dietary patterns:** We will collect information on the participant’s usual diet utilizing the Diet History Questionnaire II (DHQ II), a food frequency questionnaire (FFQ) developed by the Risk Factor Monitoring and Methods Branch (RFMMB) of the National Cancer Institute (NCI). The original DHQ I was designed based on cognitive research findings, to be easy to use. It had 124 food items and included both portion size and dietary supplement questions. The DHQ II has a food list that has been updated based on more recent dietary data and consists of 134 food items and 8 dietary supplement questions. There are four different versions of DHQ II. These four versions differ by time frame (the past year versus the past month) and whether or not questions are included about portion size questions. We will utilize the questionnaire that asks about the past month and includes questions about portion sizes for this study since we are interested in most recent intake and the impact on disease course. In addition, we will be collecting these data again at the 6 month encounter.

**Stool Sample Collection:** Once a participant has reached the 20th week in the study or later, participants will be emailed a consent form to provide one stool sample that they will collect and ship from home directly to Genova Diagnostics for measurement of fecal calprotectin concentration. Participants will have to consent to provide the study team with their name and mailing address for this. Materials for collecting and shipping will be shipped to the participant’s home from Penn. The sample can be picked up by FedEx from the participant’s home. Shipping will be paid for by Genova. Participants who consent to the stool sample but do not send a stool sample to Genova will receive up to 3 reminder emails reminding them to collect and ship a stool sample.

**Current medications:** We will collect data on current medications used for IBD and other conditions.

**Primary outcome measure:** The primary outcome is relapse of CD, defined as an increase in the aCDAI by >=70 points and to >150.

Secondary outcome measure is moderate to severe relapse of disease, defined as an increase in the aCDAI to >219. Additionally, undergoing CD surgery or starting any new CD medication (steroids, mesalamine,
Azathioprine, methotrexate, anti-TNFα, anti-α4) for the treatment of CD symptoms during the interval between two visits will be considered to have identified a moderate to severe clinical relapse.

3.8.1 Adverse event reporting

Because the intervention is a dietary change that involves only the elimination of foods that patients already eat and consumption of water, we will not attribute any adverse event to the study intervention and will not collect data on adverse events.

3.9 Study Conduct

3.9.1 Obtaining informed consent

Each potential participant will be pre-screened for eligibility using the CCFA Partners database (visit 1). Those who appear to meet all eligibility criteria will then be invited by email to participate in the trial. This email will be sent directly from CCFA Partners to the patients. The invitation will introduce patients to the randomized clinical trial and will include a description of the general procedures, safety and risks. Potential participants who are interested in participating will confirm this by completing the online consent form. No steps will occur prior to informed consent; the CCFA Partners website includes consent language informing participants that by participating in CCFA Partners questionnaires, they are agreeing to be contacted about potential research studies.

We will re-send invitation emails a week later to those people who do not click on the link to the consent form, those who do click the link to the consent form but do not select “yes” or “no” to participating, and those who do consent to participate but do not select a start date to enroll. If a week later they still do not participate or enter a start date, they will be re-screened at the next 6 month CCFA module follow-up survey.

We will not re-invite participants that enroll in the study and enter a start date for enrolling, but do not complete any surveys. They will also not be re-screened for eligibility.

3.9.2 Screening

Screening will be conducted by CCFA Partners using the CCFA Partners questionnaire that members complete every 6 months. See section 3.3.1 and 3.3.2 for eligibility requirements. CCFA Partners will send an email to eligible participants informing them that they are eligible to participate in a research study conducted at the University of Pennsylvania and will provide a link to 6 additional screening questions. If they are eligible based on these questions, then they will be taken to the online consent form. Once they provide their consent to participate, a question will pop-up on the participant’s screen asking them when was the last they had active Crohn’s Disease that lasted for 7 or more days.

3.9.3 Information about study diet

If the patient is eligible based on the computed aCDAI, they will be provided with study diet information via email.

Patients will then be introduced to the web based data collection system. Follow-up data collection will be facilitated by a web based survey tool to be developed by CCFA Partners using a secure, web-based application for building and managing online surveys and databases69.

On day 1 of screening, patients will complete the NCI DHQ II (described in §3.8) and the three questions included in the aCDAI using the online system. On each of the subsequent 6 days, patients will answer only the 3 questions included in the aCDAI.

3.9.4 Follow-up encounters

Every week participants will receive one email with a link to a web-based survey to complete that asks them questions about their disease status and adherence to study diet. Every 8th week, instead of one survey, each participant will receive a daily email for 7 days with a link to a web based survey where they will report their current disease activity and any change in their current medications.) over the prior 8 weeks. With the exception of the 7th daily questionnaire, these surveys should take less than 2 minutes to complete as it includes only the 3 questions included in the aCDAI.

Additional information will be collected with the final questionnaire of each 8th week of data collection. This questionnaire will include questions about perianal fistula and medication changes to allow assessment of the outcome. To assess participant adherence to the study diet, we will ask the participant how many of their meals and snacks in the last week included red meat, pork, or processed meats?
Participants will have 72 hours to complete each survey. After the 72 hour period, the survey will no longer be accessible for the participant to complete. We will also have patients complete the NCI DHQ II again at week 20. Reminder emails will be sent if the follow-up surveys have not been completed.

3.9.5 Early withdrawal from study diet
A participant may discontinue the study diet at any time. This will not constitute a reason for early withdrawal from the study.

3.9.6 Early withdrawal from study
A participant may withdraw from the study at any time. Protocol violations will be assessed on a case-by-case basis. Any participant may be withdrawn from the study at the discretion of the Principal Investigator if it is deemed that the participant has deviated from the protocol to a sufficient extent that it might compromise the participant’s safety or the data assessment validity.

We will also withdraw women who become pregnant during the study. Participants who experience a flare of disease will be allowed to continue in the study. The limited dietary intervention presents no increased risk to participants even if they had a flare of disease.

3.9.7 Lost-to-follow-up
If a participant consents to participate but never completes any surveys, we will re-send them an invitation to participate if they still seem to meet eligibility criteria the next time they complete a CCFA Partners module.

If a participant consents to participate and participates for some time but then is lost-to-follow up, i.e., they stop completing study surveys or never completed any study surveys, we will not re-invite them to participate at the next time they complete a CCFA Partners module.

If a participant misses all of the surveys in any 8 week period, we will send an email asking if they would like to continue in the study or not. If they reply that they do not, we will withdraw them from the study. If they do not respond to this email, they will be considered lost to follow up, and no further study surveys will be sent to them.

3.9.8 Retention
In order to retain participants’ interest in study participation, a fact about Inflammatory Bowel Disease will pop-up on the participant’s screen after completing a survey. We will have 50 rotating facts that we will use.

We will email a letter to participants who miss two surveys in a row, reminding them of the importance of their participation. We will not send this email more than once in a month.

3.9.9 Protocol violations
Protocol violations include the failure to adhere to the assigned follow-up protocol.

3.10 Data Analysis
The analytic tasks required by this study take two forms: 1) data management and 2) statistical analyses.

3.10.1 Data management
Data management tasks will be performed jointly by the PI, the Project Manager, the CCFA Partners Data Coordinating Center and the Biostatistical Analysis Center (BAC). The database will be developed by the CCFA Partners Data Coordinating Center using the standard CCFA Partners web-based technology. At each study contact, data will be entered into the study database directly by the participant. The data will then be converted into a de-identified analytical data file for statistical analysis and transferred to the University of Pennsylvania.

3.10.2 Data analysis
3.10.2.1 Aim 1. To determine whether a diet with low levels of red and processed meat consumption is associated with a reduced rate of relapse of CD
All primary analyses will be conducted using the principle of intention to treat, such that participants will be analyzed according to the diet that they were assigned, even if they were non-adherent to the diet. The one exception is that we will not include patients who refuse participation once they learn of their assigned diet.
In a study of 12 UC patients who had colonoscopy once with active disease and once in remission, there was 100% correlation between FCP levels and disease activity, with all patients having FCP concentrations below 50 mg/L when in remission and above 50 mg/L when the disease was active. In addition, another 16 patients with UC who were in endoscopic remission were examined. In patients having FCP concentrations below 50 mg/L when in remission and above 50 mg/L when the disease was active, the concentration of FCP is correlated with mucosal healing as assessed by endoscopy in UC and Crohn's disease.

The concentration of calprotectin in feces can be used as an indirect measure of neutrophilic infiltrate in the bowel mucosa. This has been confirmed using radioactive labeled WBCs. In patients with Crohn's disease, there is a close correlation between four-day fecal excretion of indium labeled WBCs and four day excretion of calprotectin, one day excretion of calprotectin, and even single specimen FCP concentration. Furthermore, the concentration of FCP is correlated with mucosal healing as assessed by endoscopy in UC and Crohn's disease. This was confirmed in a second population of patients with CD.

FCP concentration is also correlated with histologic and endoscopic disease activity in patients with UC and Crohn's disease. In a study of 12 UC patients who had colonoscopy once with active disease and once with quiescent disease, there was 100% correlation between FCP levels and disease activity, with all patients having FCP concentrations below 50 mg/L when in remission and above 50 mg/L when the disease was active. In addition, another 16 patients with UC who were in endoscopic remission were examined in the same study, all of whom had FCP concentrations < 50 mg/L. Thus, it is quite evident that FCP concentration is an excellent surrogate marker of intestinal inflammation.
Perhaps most importantly, higher FCP levels are associated with earlier relapse of Crohn’s disease and ulcerative colitis. This has been reproduced in numerous studies. Based on this, we will assess the degree of mucosal inflammation after approximately 20 weeks of study diet. FCP concentrations will be compared between the groups using a t-test. If necessary due to imbalances in the baseline characteristics among patients who complete the FCP measurement, we can adjust for potential confounders using linear regression. FCP will be log transformed if necessary to meet the normality assumptions of linear regression.

3.10.2.3 Exploratory aim. To identify other dietary patterns associated with a lower risk of relapse of CD

If the dietary intervention does not influence the rate of relapse, we will pool all of the available data to explore whether other dietary patterns are associated with disease relapse. If the intervention is effective, these analyses will be performed separately by study arm and only combined if there is no effect modification.

Overall analytic strategy:

The data from the DHQ II can be summarized in terms of daily calories, food sources (e.g. meats, processed meats, eggs, etc), macronutrients (protein, fat, carbohydrates, alcohol), and micronutrients (specific amino acids, fatty acids, sugars, vitamins and elements). For these analyses, the nutrient data will be categorized in tertiles with the goal of distinguishing patients with high levels of intake from those with low levels of intake. This approach recognizes both the limitations and strengths of dietary recall data. FFQs are not generally used to make strong recommendations on specific thresholds of daily nutrient data due to substantial day to day variation in nutrient intake. However, division into tertiles or other quantiles allow for accurate separation of participants who on average have either high or low levels of intake, allows for easy visualization of the distribution of participants among levels of nutrient intake, lends itself to calculation of relative risk estimates, and does not make assumptions about linear relationships, which is ideal for a study such as the one proposed here.

Some participants may report implausible levels of caloric intake. Willett, who developed a different FFQ, recommends excluding participants with reported total energy intake outside of the range of 500 to 3,500 kcal/day for women and 800 to 4,000 kcal/day for men. We will utilize this criterion in our analysis, recognizing that these criteria were developed for a different FFQ. There are many possible ways to include diet variables in epidemiologic analyses (discussed in ). If there is an association between total energy intake and time to relapse, use of total nutrient intake or nutrient intake divided by calorie intake (assuming a null intercept) can potentially lead to bias. An alternative approach that is commonly employed is the energy-adjusted method (also known as the residual method). Energy-adjusted nutrient intake is computed as the residual from a regression model with total calorie intake as the independent variable and absolute nutrient intake as the dependent variable. The residuals provide a measure of nutrient intake independent of energy intake.

Regardless of whether we observe an association between total energy intake and relapse of disease, we will also examine nutrient intake without accounting for total energy intake as was done by Jowett. Thus there will be two sets of Cox regression models: (1) including total energy and utilizing the residual method tests whether replacing nutrient X with another nutrient in an isocaloric state is associated with a greater hazard of disease relapse; and (2) including only absolute levels of the nutrient X without accounting for total energy tests whether absolute levels of consumption of nutrient X is associated with a greater hazard of relapse.

For each of the nutrients of interest, a Cox regression model will have the nutrient or food as the independent variable and disease relapse as the dependent variable. Nutrient consumption will be categorized as low, medium and high, using cut points that divide the entire population into 3 similar sized groups. For these analyses, the nutrient data will be compared to low consumption in the primary analysis but we will also examine consumption on a continuous scale to examine for dose response (trend analysis). We will examine the variables described in §B.3.6.4 as potential confounders, retaining any variable that alters the unadjusted hazard ratio (HR) by more than 10%. Both models described above will be tested.

Nutrients to be investigated: A) food sources: fruits, vegetables, eggs, white meats, breads and cereals, and alcohol; B) macronutrients: protein, fats, carbohydrates; and C) micronutrients: sulfate containing amino acids, iron, n-3 fatty acids, n-6 fatty acids, the ratio of n-3/n-6 fatty acids, conjugated linoleic acid, linoleic acid, docosahexanoic acid, and simple sugars. Total sulfates is not currently part of the computed nutrients in the NCI DHQ II. Because each of these are prehypothesized to be associated with disease course based on prior research, some would argue that a p value of 0.05 should be considered statistically significant.
However, to account for multiple testing, we will also apply a Bonferroni correction and will report both the nominal and Bonferroni corrected p values.

Finally, we will use cluster analysis to define overall dietary patterns that may be associated with the CD disease course. The various dietary pattern clusters are then compared to each other in a similar manner to that described for the individual nutrients in the primary analysis. This approach has been used to examine dietary patterns with a variety of health states.\(^\text{88-91}\) An important distinction of the cluster analysis approach compared to the individual nutrient approach described above is that it accounts for overall dietary patterns.

### 3.11 Sample size considerations

In planning the sample size for this study we considered what level of therapeutic benefit would be considered clinically significant. Medical therapies used to prevent relapse often demonstrate therapeutic benefit of approximately 20% or greater.\(^\text{92-96}\) Therefore, we considered a therapeutic benefit of 20% or greater with a dietary intervention to be clinically significant. However, because this is an adjunct therapy with minimal if any risk, even smaller differences may be clinically important. We conservatively estimated the sample size requirements by using a dichotomous outcome of continued remission at all time points vs. relapse at any time point prior to the end of follow-up. Under this assumption, 97 patients per group (194 total patients) provides a minimum of 80% power to detect a 20% absolute difference in relapse rates across the full range of possible relapse rates. Table 3 below provides estimates of the sample size required for 80% power across a range relapse rates and effect sizes. Based on these calculations, we will target enrollment of 556 evaluable subjects to provide 80% power to detect a 7.5% reduction in the relapse rate assuming a baseline rate of 15% per year. Assuming approximately 10% loss to follow-up, we will inflate the sample size to 612. This will provide 90% power to detect a 9% to 11% decrease if the relapse rate in the control group is between 15% and 25% per year.

<table>
<thead>
<tr>
<th>Relapse rate among</th>
<th>Required sample size for 80% power</th>
<th>Absolute reduction in</th>
<th>Sample size per group</th>
</tr>
</thead>
<tbody>
<tr>
<td>control group</td>
<td>Relapse rate in intervention group</td>
<td>relapse rate</td>
<td>(total)</td>
</tr>
<tr>
<td>25%</td>
<td>10%</td>
<td>15%</td>
<td>100 (200)</td>
</tr>
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<td>25%</td>
<td>15%</td>
<td>10%</td>
<td>250 (500)</td>
</tr>
<tr>
<td>15%</td>
<td>7.5%</td>
<td>7.5%</td>
<td>278 (556)</td>
</tr>
</tbody>
</table>

### 3.12 Quality assurance measures

In addition to the data management strategies described above, the following quality assurance measures will be built into our study to assure the safety of the patients and the quality of the data.

#### 3.12.1 Manual of Standard Operating Procedures

Prior to initiation of the study, the project manager and the principal investigator will generate a manual of operations (MOP). This manual is essential to minimize variability in completion of the study protocol.

#### 3.12.2 Confidentiality and security

All data related to this trial will be recorded using the patients assigned study number. Data will be reported only in a confidential manner such that the personal identity of any subject will not be identifiable. All study data will be maintained under a double locked system, such as a locked closet within a locked office or on a password protected computer in a locked office.

### 3.13 Potential limitations

Recruitment and retention is always a concern in clinical trials. Fortunately, there are no major safety concerns with the study diet to serve as a deterrent. See section 4.2.1 for strategies to increase recruitment and retention.

Randomization minimizes the risk of selection bias and confounding. However, as with most trials there is a risk of bias from misclassification of outcomes. To minimize this, we are using a validated disease activity measure and applying standard definitions of relapse.\(^\text{59,64}\) Additionally, we will be able to validate patient reported relapse with escalation of the therapeutic regimen or development of new fistula by review of the electronic medical record. Thus misclassification of the outcome measure should be minimized.
Missing data will be minimized by using repeated emails and reminders and keeping the follow-up surveys as brief as possible. For the first 6 days of each collection periods, we will only collect the 3 questions contained in the short CDAI. Such electronic diary methods for IBD symptoms have previously been demonstrated to be feasible\cite{97}. Nonetheless, in the event of incomplete recording of information, we will take the average of all values entered and compute a weighted average for the week.

Physicians treating the patients will not be blinded, but determination of the outcome is based on the patient reported symptoms used to calculate the aCDAI and is done without physician contact. Thus, this should not bias the results. Although it is conceivable that a physician may know that a patient is in the study and may choose to escalate therapy for disease relapse, it is unlikely that the knowledge of the diet would influence the treatment decision.

It is not possible to blind participants to which arm they are assigned, but we will not tell the participants what the alternative study arm is so as to avoid contamination of the intervention (i.e., having participants in the control arm avoiding red and processed meat).

Some misclassification of baseline dietary patterns is inherent to FFQs. However, we are using a well validated instrument to measure dietary patterns\cite{98-100} and our exploratory analyses will focus on comparing widely different levels of intake (i.e., the high consumption vs. the low consumption). While the FFQ lacks precision for specific quantities, the instrument is well suited to differentiating low from high levels of intake. Adherence to the diet recommendations may not be perfect, but we will assess self-reported adherence with our surveys and plan an analysis accounting for this. To the extent that adherence is imperfect our results will more closely reflect the effectiveness in practice than efficacy. These data are equally as important.

4 Ethical Considerations

4.1 Human subjects

This study includes a randomized controlled trial of a dietary intervention among patients with Crohn’s disease that is quiescent.

4.2 Risks to subjects

4.2.1 Human Subjects Involvement, Characteristics, and Design

Involvement of human subjects: The proposed research includes a randomized controlled trial of two diets to reduce the incidence of relapse of Crohn’s disease. The control diet requires consumption of red or processed meat at least two times per week. The test diet reduces consumption of red and processed meats. Patients will be followed for 48 weeks or until they experience a relapse of their disease. Follow-up data will be obtained primarily through a web-based system that allows participants to record their disease activity.

No aspect of the study protocol alters the participants’ routine care. The study protocol does not require any change in the patients’ medications or visit schedule. There are no additional diagnostic tests such as blood tests, CT scans or colonoscopy.

Characteristics of the subject population: The study will include 612 adult patients with Crohn’s disease who are clinically in remission at the time of enrollment.

Sampling plan, recruitment, and retention: Participants will be recruited from CCFA Partners. However, participants will be provided with an incentive for participation and retention. As an incentive for participation and retention, we will provide an option for participants to obtain Target gift cards. We will provide participants a $5 Target Store gift card each time they complete the NCI DHQ II. Participants will also be entered into a daily lottery to receive a $10 Target Store gift card on each day that they complete other study questionnaires. If they do not complete a questionnaire on a certain day, they will not be entered into the lottery for that day. The winners will be predetermined by identification number and study visit. Participants who choose to participate in the lottery will be asked to provide us with their e-mail address and winners will be e-mailed a gift card.

Rationale for involvement of special vulnerable populations: There are no special exclusion criteria related to children age 18-20 as the intervention does not pose any special risks to this populations.

Procedures for assignment to study group: Allocation to study groups will be based on random assignment. The “dose” of the study diets was selected to minimize red and processed meat consumption to a level that would be less than the 5th percentile for patients with Crohn’s disease based on our preliminary study. Given that there are many vegetarians and vegans throughout the world, there is little reason to think that this level of red and processed meat consumption represents a danger to participants.
Study sites: The study will be conducted at the University of Pennsylvania and the University of North Carolina where CCFA Partners is housed.

4.2.2 Sources of Materials
The only research materials obtained from living individuals are the data to be collected. No other specimens, such as biopsies, blood, or stool samples, will be obtained. Data will be collected on Crohn’s disease history, usual dietary patterns, and current medications.

Access to the data will be limited to the research team, including the investigators, the research coordinator, and the data analysts.

Electronic data will be stored on servers within the Center for Clinical Epidemiology and Biostatistics. Access to the server is password protected. The servers are backed-up nightly to prevent loss of information.

4.2.3 Potential risks
The intervention in this trial poses little if any risk to participants. The prescribed diet is consistent with many dietary recommendations to minimize consumption of red meat. The study protocol does not change the participant’s usual healthcare in any way.

As with all research, there is the risk of loss of confidentiality of the data.

4.3 Adequacy of Protection Against Risks

4.3.1 Recruitment and Informed Consent
Recruitment will be synchronized to occur along with the biannual surveys that all CCFA Partners patients receive. When a participant in CCFA Partners appears to meet the eligibility criteria based on the responses to the biannual survey, they will receive an additional question asking if they are interested in learning more about this ancillary study, noting that that this study involves a number of dietary interventions.

Participants will not be told the details of the other intervention during the study. We will have two different versions of the online Informed Consent – one for each intervention. Participants will be provided with a link to an online videomade by the Core Research Team that explains in detail the dietary intervention to which they are randomized. Participants will be told that they can eat their usual meals and snacks but may be required to increase or limit intake of certain foods as part of the study. We will emphasize that participants will not need to purchase any special foods, take any supplements or vitamins, or cook their food in any special way.

Participants will be given an e-mail address in order to reach study staff with any questions or concerns prior to providing their consent. They will also be provided with the phone number of the Office of Research Services at the University of Pennsylvania should they have any concerns.

4.3.2 Protection against risks

Risks to privacy: Within the clinical trial, all data collected will be collected in a manner consistent with Good Clinical Practice.

Access to the data will be limited to the research team, including the investigators, the research coordinator, and the data analysts. Electronic data will be stored on servers within the CCFA Partners Data Coordinating Center and the Center for Clinical Epidemiology and Biostatistics at Penn. Access to the server is password protected. The servers are backed-up nightly to prevent loss of information.

Research involving vulnerable populations: This research is expected to not entail greater than minimal risk.

Data Safety and Monitoring Plan: As a minimal risk study, this study will not require a formal Data and Safety Monitoring Board. Monitoring of data quality will be completed by the PI or his designee (such as by a research coordinator not related to the study or by a monitor from Penn’s Office of Human Research).

4.4 Potential benefits of the proposed research to human subjects and others
Participants may benefit from participation in this trial if the dietary intervention reduces the risk of relapse of Crohn’s disease. Participants assigned to the control diet are not expected to experience any benefit or harm relative to their usual diet. However, if the study diet is demonstrated to be beneficial, it is anticipated that most patients with Crohn’s disease would elect to follow a similar diet.
4.5 Importance of the knowledge to be gained

The leading unanswered question for patients with IBD is what diet will help keep their disease in remission. Ultimately, regardless of the results, we will provide an answer to the question, “What should I eat?” If this intervention results in lower relapse rates, the answer is to reduce red and processed meat intake. If the intervention is not efficacious, healthcare providers will be able to confidently state that neither reduction of red meat or refined carbohydrates has been efficacious in controlled trials and as such, no major dietary modifications are currently recommended specifically for CD. This is a minimal risk study, so the risks to subjects are reasonable in the context of the information to be gained.

4.6 Inclusion of women

Women will be included in the study to the extent that they are representative of the population of patients with Crohn’s disease who are treated at the study sites. We will exclude pregnant or breastfeeding women. Given that there is a 1:1 or slight female predominance, we anticipate approximately 50% of participants will be females.

4.7 Inclusion of minorities

There are no exclusions based on ethnic/racial background. Minority subjects will be included in this study to the extent that they have Crohn’s disease.

4.8 Inclusion of children

This study will include children age 18 or older who have Crohn’s disease that is in clinical remission.
5 Literature cited


86. Shores DR, Binion DG, Freeman BA, et al. New insights into the role of fatty acids in the pathogenesis and resolution of inflammatory bowel disease. Inflammatory Bowel Diseases 2011;17:2192-204.


Protocol Signature Page

I will provide copies of the FACES Study protocol, any subsequent protocol amendments and access to all information furnished by the sponsor to study personnel under my supervision. I will discuss this material with them to ensure that they are fully informed about the investigational drug and the study protocol.

I agree to conduct this clinical trial according to the protocol described herein. I also agree to conduct this study in compliance with applicable federal, state and local regulations, Guidelines for Good Clinical Practice (GCP), and with the requirements of my Institutional Review Board. I understand that I may not implement this protocol without first receiving written IRB approval.

Furthermore, I understand that I can not make any changes to this protocol. (The only exception being an action needed to remove a subject from immediate harm, with subsequent notification to the study PI and IRB.)

______________________________  __________________
CLINICAL SITE INVESTIGATOR:     (Signature)           (Date)

______________________________
NAME:    (Please Print)

______________________________
INSTITUTION:

Instructions: Upon signature, please fax a copy of this form to the Project Manager and maintain the original in your regulatory file.
Supplemental methods

Participants with CD were recruited from the Crohn’s and Colitis Foundation Partners Study (has since been renamed IBD Partners), an internet-based cohort of more than 14,000 participants with inflammatory bowel disease (IBD) (1). Briefly, individuals with inflammatory bowel disease (IBD) who are older than 18 years of age are recruited to join IBD Partners using foundation e-mail rosters, social media, educational and fundraising events, and the Crohn’s & Colitis Foundation website. Each participant completes a baseline survey that contains questions about demographic characteristics, treatments, disease duration, and disease activity. Follow-up surveys are completed every 6 months to capture changes in disease activity and treatment since the prior survey.

Exclusion criteria included self-reported consumption of red meat less than one time per week, a diagnosis of IBD-unspecified or indeterminate colitis, a history of total or sub-total colectomy, ileostomy, or colostomy, a history of oral or rectal steroid use within the prior two weeks, or a history of perianal fistula or abscess with more than scant drainage. Individuals under the age of 18, pregnant or lactating females, or individuals unwilling to follow the study diet were also excluded.

CD history was summarized in terms of age at IBD diagnosis and years since diagnosis at the time of screening, history of IBD-related hospitalizations and surgeries, regularity of visits to GI physician, and Manitoba IBD index (2). The Manitoba IBD index is a patient-reported measure which captures symptom severity over a 6-month period of time (2). We also collected information on the participant’s usual diet, in the past month, at baseline and at week 20 using the Diet History Questionnaire II (DHQ II), a semi-quantitative food frequency questionnaire (FFQ) developed by the Risk Factor Monitoring and Methods Branch (RFMMB) of the National Cancer Institute (NCI). The DHQ II questionnaire was considered adequate for analysis if the questionnaire was complete and average caloric intake estimates were not implausible as defined by Willett (3) (males <800 kcal/day or >4000 kcal/day; females <500 kcal/day or >3500 kcal/day).

**Supplemental Table 1**
Demographics and baseline characteristics of nonparticipants

<table>
<thead>
<tr>
<th></th>
<th>Participants (n=213)</th>
<th>Non-Participants (n=432)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>164 (77.0%)</td>
<td>294 (68.1%)</td>
</tr>
<tr>
<td><strong>Age at baseline</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (Q1-Q3)</td>
<td>36.0 (29.0-48.0)</td>
<td>44.0 (30.5-57.0)</td>
</tr>
<tr>
<td><strong>Age at IBD diagnosis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (Q1-Q3)</td>
<td>24.0 (18.0-31.0)</td>
<td>26.0 (19.0-37.0)</td>
</tr>
<tr>
<td><strong>Race</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>191 (95.0%)</td>
<td>390 (95.1%)</td>
</tr>
<tr>
<td>Black/African American</td>
<td>4 (2.0%)</td>
<td>1 (0.2%)</td>
</tr>
<tr>
<td>Asian</td>
<td>2 (1.0%)</td>
<td>4 (1.0%)</td>
</tr>
<tr>
<td>More than one race</td>
<td>4 (2.0%)</td>
<td>13 (3.2%)</td>
</tr>
<tr>
<td>Other</td>
<td>0 (0.0%)</td>
<td>2 (0.5%)</td>
</tr>
<tr>
<td><strong>US Census Bureau region</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Northeast</td>
<td>62 (30.1%)</td>
<td>100 (24.4%)</td>
</tr>
<tr>
<td>Midwest</td>
<td>57 (27.7%)</td>
<td>112 (27.4%)</td>
</tr>
<tr>
<td>South</td>
<td>60 (29.1%)</td>
<td>107 (26.2%)</td>
</tr>
<tr>
<td>West</td>
<td>27 (13.1%)</td>
<td>90 (22.0%)</td>
</tr>
<tr>
<td><strong>Education level</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than 12th grade</td>
<td>2 (1.0%)</td>
<td>2 (0.5%)</td>
</tr>
<tr>
<td>12th grade</td>
<td>5 (2.4%)</td>
<td>27 (6.4%)</td>
</tr>
<tr>
<td>Some college</td>
<td>34 (16.3%)</td>
<td>57 (13.5%)</td>
</tr>
<tr>
<td>College</td>
<td>95 (45.7%)</td>
<td>177 (42.0%)</td>
</tr>
<tr>
<td>Graduate school</td>
<td>72 (34.6%)</td>
<td>158 (37.5%)</td>
</tr>
<tr>
<td><strong>Saw GI doctor in past year</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>28 (14.3%)</td>
<td>61 (14.9%)</td>
</tr>
<tr>
<td>1 or 2 times</td>
<td>111 (56.6%)</td>
<td>263 (64.1%)</td>
</tr>
<tr>
<td>3 or 4 times</td>
<td>37 (18.9%)</td>
<td>62 (15.1%)</td>
</tr>
<tr>
<td>5 or more times</td>
<td>20 (10.2%)</td>
<td>23 (5.6%)</td>
</tr>
<tr>
<td>Don't know</td>
<td>0 (0.0%)</td>
<td>1 (0.2%)</td>
</tr>
<tr>
<td><strong>Manitoba disease activity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constantly active</td>
<td>2 (0.9%)</td>
<td>5 (1.2%)</td>
</tr>
<tr>
<td>Often active</td>
<td>17 (8.0%)</td>
<td>13 (3.0%)</td>
</tr>
<tr>
<td>Sometimes active</td>
<td>43 (20.3%)</td>
<td>86 (19.9%)</td>
</tr>
<tr>
<td>Occasionally active</td>
<td>46 (21.7%)</td>
<td>112 (25.9%)</td>
</tr>
<tr>
<td>Rarely active</td>
<td>53 (25.0%)</td>
<td>115 (26.6%)</td>
</tr>
<tr>
<td>remission/absence of symptoms</td>
<td>51 (24.1%)</td>
<td>101 (23.4%)</td>
</tr>
<tr>
<td><strong>Smoking history</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>never</td>
<td>150 (70.4%)</td>
<td>294 (68.2%)</td>
</tr>
<tr>
<td>former</td>
<td>55 (25.8%)</td>
<td>116 (26.9%)</td>
</tr>
<tr>
<td>current</td>
<td>8 (3.8%)</td>
<td>21 (4.9%)</td>
</tr>
<tr>
<td><strong>History of IBD surgery</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>99 (46.5%)</td>
<td>190 (44.0%)</td>
</tr>
<tr>
<td></td>
<td>Participants (n=213)</td>
<td>Non-Participants (n=432)</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>----------------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td>Ever hospitalized for IBD</td>
<td>Yes</td>
<td>147 (69.0%)</td>
</tr>
<tr>
<td>Number of times hospitalized for IBD</td>
<td>Median (Q1-Q3)</td>
<td>1.0 (0.0-2.0)</td>
</tr>
<tr>
<td>Short CD Activity Index</td>
<td>Median (Q1-Q3)</td>
<td>79.0 (44.0-107.0)</td>
</tr>
<tr>
<td>Short IBD QOL score</td>
<td>Median (Q1-Q3)</td>
<td>5.8 (5.2-6.3)</td>
</tr>
<tr>
<td>Current use of aminosalicylates</td>
<td>Yes</td>
<td>59 (27.8%)</td>
</tr>
<tr>
<td>Current use of steroids</td>
<td>Yes</td>
<td>3 (1.4%)</td>
</tr>
<tr>
<td>Current use of immunosuppressants</td>
<td>Yes</td>
<td>75 (35.4%)</td>
</tr>
<tr>
<td>Current use of biologics</td>
<td>Yes</td>
<td>106 (50.0%)</td>
</tr>
<tr>
<td>Current use of antibiotics for IBD</td>
<td>Yes</td>
<td>4 (1.9%)</td>
</tr>
<tr>
<td>Current use of narcotics for IBD</td>
<td>Yes</td>
<td>14 (6.6%)</td>
</tr>
<tr>
<td>Current use of probiotics for IBD</td>
<td>Yes</td>
<td>51 (24.1%)</td>
</tr>
<tr>
<td>PROMIS anxiety t-score</td>
<td>Median (Q1-Q3)</td>
<td>48.0 (40.3-55.8)</td>
</tr>
<tr>
<td>PROMIS depressive symptoms t-score</td>
<td>Median (Q1-Q3)</td>
<td>41.0 (41.0-51.8)</td>
</tr>
<tr>
<td>PROMIS fatigue t-score</td>
<td>Median (Q1-Q3)</td>
<td>48.6 (46.0-57.0)</td>
</tr>
<tr>
<td>PROMIS pain interference t-score</td>
<td>Median (Q1-Q3)</td>
<td>41.6 (41.6-52.0)</td>
</tr>
<tr>
<td>PROMIS sleep disturbance t-score</td>
<td>Median (Q1-Q3)</td>
<td>52.4 (50.5-54.3)</td>
</tr>
<tr>
<td>PROMIS social satisfaction t-score</td>
<td>Median (Q1-Q3)</td>
<td>51.8 (48.2-64.4)</td>
</tr>
</tbody>
</table>
### Supplemental Table 2
Comparison of baseline intake of raw amounts of nutrients of interest by treatment arm

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>High meat (n=90)</th>
<th>Low meat (n=75)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>United States Department of Agriculture (USDA) database</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average daily caloric intake</td>
<td>1605.45 (1344.06, 2069.14)</td>
<td>1443.00 (1122.57, 1899.39)</td>
<td>0.02</td>
</tr>
<tr>
<td>Average daily fat intake (g)</td>
<td>71.19 (54.12, 84.75)</td>
<td>53.71 (42.23, 77.40)</td>
<td>0.06</td>
</tr>
<tr>
<td>Average daily carbohydrate intake (g)</td>
<td>193.74 (140.33, 239.25)</td>
<td>170.60 (135.05, 230.03)</td>
<td>0.02</td>
</tr>
<tr>
<td>Average daily protein intake (g)</td>
<td>65.34 (54.86, 87.02)</td>
<td>54.83 (43.97, 77.02)</td>
<td>0.93</td>
</tr>
<tr>
<td>Average daily alcohol intake (g)</td>
<td>3.17 (0.29, 9.73)</td>
<td>2.18 (0.30, 6.05)</td>
<td>0.60</td>
</tr>
<tr>
<td>Average daily saturated fatty acid intake (g)</td>
<td>20.03 (17.21, 27.75)</td>
<td>16.30 (12.05, 25.40)</td>
<td>0.12</td>
</tr>
<tr>
<td>Average daily monounsaturated fatty acid intake (g)</td>
<td>26.72 (20.27, 34.68)</td>
<td>20.78 (15.69, 29.29)</td>
<td>0.01</td>
</tr>
<tr>
<td>Average daily polyunsaturated fatty acid intake (g)</td>
<td>12.79 (10.22, 17.31)</td>
<td>12.46 (8.48, 15.13)</td>
<td>0.83</td>
</tr>
<tr>
<td>Average daily fiber intake (g)</td>
<td>14.79 (11.59, 18.35)</td>
<td>14.86 (9.66, 22.80)</td>
<td>0.23</td>
</tr>
<tr>
<td>Average daily iron intake (mg)</td>
<td>12.58 (9.75, 16.47)</td>
<td>12.25 (8.35, 16.33)</td>
<td>0.08</td>
</tr>
<tr>
<td>Average daily intake of lean meat from meat, poultry, fish (ounces)</td>
<td>3.18 (2.23, 5.22)</td>
<td>2.59 (1.64, 3.92)</td>
<td>0.18</td>
</tr>
<tr>
<td>Average daily intake of cooked lean meat from beef, pork, veal, lamb, and game (ounces)</td>
<td>1.28 (0.73, 2.04)</td>
<td>0.65 (0.34, 1.11)</td>
<td>0.0002</td>
</tr>
<tr>
<td>Average daily intake of cooked lean meat from organ meats (ounces)</td>
<td>0.00 (0.00, 0.00)</td>
<td>0.00 (0.00, 0.00)</td>
<td>0.02</td>
</tr>
<tr>
<td>Average daily intake of cooked lean meat from franks, sausages, luncheon meats (ounces)</td>
<td>0.45 (0.17, 0.83)</td>
<td>0.28 (0.07, 0.60)</td>
<td>0.17</td>
</tr>
<tr>
<td>Average daily intake of cooked lean meat from chicken, poultry, and other poultry (ounces)</td>
<td>0.87 (0.47, 1.36)</td>
<td>0.80 (0.29, 1.21)</td>
<td>0.76</td>
</tr>
<tr>
<td>Average daily intake of cooked lean meat from fish and other seafood high in omega-3 (ounces)</td>
<td>0.13 (0.04, 0.33)</td>
<td>0.11 (0.02, 0.43)</td>
<td>0.61</td>
</tr>
<tr>
<td>Average daily intake of cooked lean meat from fish and other seafood low in omega-3 (ounces)</td>
<td>0.25 (0.10, 0.47)</td>
<td>0.16 (0.06, 0.50)</td>
<td>0.57</td>
</tr>
<tr>
<td>Average daily ounce equivalents of lean meat from eggs</td>
<td>0.35 (0.18, 0.72)</td>
<td>0.31 (0.16, 0.60)</td>
<td>0.26</td>
</tr>
<tr>
<td>Average daily ounce equivalents of lean meat from soy product</td>
<td>0.01 (0.01, 0.04)</td>
<td>0.02 (0.01, 0.15)</td>
<td>0.0078</td>
</tr>
<tr>
<td>Average daily ounce equivalents of lean meat from nuts and seeds</td>
<td>0.64 (0.36, 1.45)</td>
<td>0.46 (0.11, 0.98)</td>
<td>0.99</td>
</tr>
</tbody>
</table>

University of Minnesota’s Nutrition Coordinating Center (NCC’s) Nutrition Database System for Research (NDSR)

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>High meat (n=90)</th>
<th>Low meat (n=75)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average daily total protein (g)</td>
<td>65.60 (55.19, 86.50)</td>
<td>54.24 (43.26, 75.90)</td>
<td>0.98</td>
</tr>
<tr>
<td>Average daily animal protein (g)</td>
<td>42.99 (32.78, 57.41)</td>
<td>37.66 (24.43, 45.05)</td>
<td>0.34</td>
</tr>
<tr>
<td>Nutrient</td>
<td>Group 1</td>
<td>Group 2</td>
<td>p-value</td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>---------------</td>
<td>---------------</td>
<td>---------</td>
</tr>
<tr>
<td>Average daily vegetable protein (g)</td>
<td>22.20 (17.68, 29.95)</td>
<td>20.28 (14.59, 31.14)</td>
<td>0.12</td>
</tr>
<tr>
<td>Average daily total fiber intake (g)</td>
<td>17.08 (14.21, 21.99)</td>
<td>16.88 (10.83, 23.91)</td>
<td>0.21</td>
</tr>
<tr>
<td>Average daily soluble fiber intake (g)</td>
<td>6.37 (4.37, 8.27)</td>
<td>6.03 (4.21, 8.44)</td>
<td>0.43</td>
</tr>
<tr>
<td>Average daily insoluble fiber intake (g)</td>
<td>10.93 (8.02, 13.75)</td>
<td>10.84 (6.80, 16.85)</td>
<td>0.36</td>
</tr>
<tr>
<td>Average daily Omega-3 fatty acid intake (g)</td>
<td>1.35 (1.04, 1.73)</td>
<td>1.20 (0.96, 1.73)</td>
<td>0.64</td>
</tr>
</tbody>
</table>
### Supplemental Table 3

Association between allocation to high meat versus low meat arm with outcome stratified by anti-TNF-alpha use and adjusted for baseline diet nutrient intake, as measured by PC1, and baseline red meat intake

<table>
<thead>
<tr>
<th>Treatment Arm</th>
<th>Unadjusted</th>
<th>Adjusted for PC1</th>
<th>Adjusted for baseline red meat intake above or below the median</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hazard ratio</td>
<td>95% CI</td>
<td>p-value</td>
</tr>
<tr>
<td>Symptomatic relapse</td>
<td>0.91</td>
<td>0.64-1.30</td>
<td>0.61</td>
</tr>
<tr>
<td>Moderate to severe relapse</td>
<td>0.86</td>
<td>0.56-1.33</td>
<td>0.50</td>
</tr>
<tr>
<td>Persistent relapse</td>
<td>1.30</td>
<td>0.80-2.12</td>
<td>0.29</td>
</tr>
</tbody>
</table>
### Supplemental Table 4

Association between allocation to high meat versus low meat arm with time to overall relapse by sub-group

<table>
<thead>
<tr>
<th></th>
<th>Hazard ratio</th>
<th>95% confidence interval</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants treated with an immunomodulator and without an anti-TNF-alpha at enrollment</td>
<td>0.91</td>
<td>0.41-2.04</td>
<td>0.83</td>
</tr>
<tr>
<td>Participants treated with anti-TNF-alpha at enrollment</td>
<td>0.95</td>
<td>0.54-1.66</td>
<td>0.84</td>
</tr>
<tr>
<td>Participants treated with neither an immunomodulator or an anti-TNF-alpha at enrollment</td>
<td>0.89</td>
<td>0.51-1.57</td>
<td>0.70</td>
</tr>
<tr>
<td>Participants with a history of CD surgery</td>
<td>1.14</td>
<td>0.71-1.85</td>
<td>0.58</td>
</tr>
<tr>
<td>Age less than 18 at diagnosis</td>
<td>1.31</td>
<td>0.59-2.91</td>
<td>0.50</td>
</tr>
<tr>
<td>Age greater than 18 at diagnosis</td>
<td>0.81</td>
<td>0.55-1.22</td>
<td>0.31</td>
</tr>
<tr>
<td>Baseline red meat consumption less than the median</td>
<td>0.92</td>
<td>0.53-1.62</td>
<td>0.78</td>
</tr>
<tr>
<td>Baseline red meat consumption greater than or equal to the median</td>
<td>0.74</td>
<td>0.41-1.32</td>
<td>0.31</td>
</tr>
</tbody>
</table>
Supplemental Table 5

Nutrient data from the week 20 DHQ, comparing high meat to low meat arm.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>High meat (n=70)</th>
<th>Low meat (n=42)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>United States Department of Agriculture (USDA) database</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average daily caloric intake</td>
<td>1444.22 (1191.74, 1990.79)</td>
<td>1239.48 (855.44, 1481.18)</td>
<td>0.002</td>
</tr>
<tr>
<td>Average daily fat intake (g)</td>
<td>59.27 (48.87, 75.40)</td>
<td>45.08 (32.02, 65.67)</td>
<td>0.37</td>
</tr>
<tr>
<td>Average daily carbohydrate intake (g)</td>
<td>168.35 (129.45, 223.82)</td>
<td>140.48 (106.98, 198.75)</td>
<td>0.07</td>
</tr>
<tr>
<td>Average daily protein intake (g)</td>
<td>62.37 (50.17, 80.62)</td>
<td>49.04 (33.00, 68.29)</td>
<td>0.16</td>
</tr>
<tr>
<td>Average daily alcohol intake (g)</td>
<td>2.63 (0.01, 7.58)</td>
<td>1.08 (0.00, 3.85)</td>
<td>0.42</td>
</tr>
<tr>
<td>Average daily saturated fatty acid intake (g)</td>
<td>19.42 (14.86, 23.10)</td>
<td>13.81 (9.47, 20.41)</td>
<td>0.13</td>
</tr>
<tr>
<td>Average daily monounsaturated fatty acid intake (g)</td>
<td>23.62 (19.14, 30.29)</td>
<td>15.83 (12.66, 24.33)</td>
<td>0.03</td>
</tr>
<tr>
<td>Average daily polyunsaturated fatty acid intake (g)</td>
<td>11.40 (8.86, 15.83)</td>
<td>9.82 (7.26, 14.18)</td>
<td>0.17</td>
</tr>
<tr>
<td>Average daily fiber intake (g)</td>
<td>13.40 (10.46, 17.42)</td>
<td>10.28 (7.29, 16.79)</td>
<td>0.22</td>
</tr>
<tr>
<td>Average daily iron intake (mg)</td>
<td>11.67 (8.93, 14.98)</td>
<td>9.23 (7.43, 13.22)</td>
<td>0.64</td>
</tr>
<tr>
<td>Average daily intake of lean meat from meat, poultry, fish (ounces)</td>
<td>3.45 (2.45, 4.85)</td>
<td>1.85 (1.00, 3.62)</td>
<td>0.02</td>
</tr>
<tr>
<td>Average daily intake of cooked lean meat from beef, pork, veal, lamb, and game (ounces)</td>
<td>1.32 (0.80, 2.15)</td>
<td>0.19 (0.09, 0.56)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Average daily intake of cooked lean meat from organ meats (ounces)</td>
<td>0.00 (0.00, 0.00)</td>
<td>0.00 (0.00, 0.00)</td>
<td>0.0003</td>
</tr>
<tr>
<td>Average daily intake of cooked lean meat from franks, sausages, luncheon meats (ounces)</td>
<td>0.49 (0.14, 0.77)</td>
<td>0.03 (0.00, 0.17)</td>
<td>0.0017</td>
</tr>
<tr>
<td>Average daily intake of cooked lean meat from chicken, poultry, and other poultry (ounces)</td>
<td>0.66 (0.44, 1.41)</td>
<td>0.71 (0.11, 1.59)</td>
<td>0.29</td>
</tr>
<tr>
<td>Average daily intake of cooked lean meat from fish and other seafood high in omega-3 (ounces)</td>
<td>0.10 (0.01, 0.40)</td>
<td>0.19 (0.02, 0.58)</td>
<td>0.005</td>
</tr>
<tr>
<td>Average daily intake of cooked lean meat from fish and other seafood low in omega-3 (ounces)</td>
<td>0.22 (0.07, 0.47)</td>
<td>0.19 (0.03, 0.61)</td>
<td>0.04</td>
</tr>
<tr>
<td>Average daily ounce equivalents of lean meat from eggs</td>
<td>0.33 (0.19, 0.64)</td>
<td>0.31 (0.16, 0.60)</td>
<td>0.97</td>
</tr>
<tr>
<td>Average daily ounce equivalents of lean meat from soy product</td>
<td>0.01 (0.00, 0.03)</td>
<td>0.01 (0.01, 0.08)</td>
<td>0.0009</td>
</tr>
<tr>
<td>Average daily ounce equivalents of lean meat from nuts and seeds</td>
<td>0.54 (0.19, 1.26)</td>
<td>0.51 (0.15, 1.04)</td>
<td>0.17</td>
</tr>
</tbody>
</table>

*University of Minnesota’s Nutrition Coordinating Center (NCC’s) Nutrition Database System for Research (NDSR)*

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>High meat (n=70) Median (Q1, Q3)</th>
<th>Low meat (n=42) Median (Q1, Q3)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average daily total protein (g)</td>
<td>63.83 (50.34, 82.20)</td>
<td>48.35 (33.78, 66.19)</td>
<td>0.26</td>
</tr>
<tr>
<td>Nutrient</td>
<td>Value 1</td>
<td>Value 2</td>
<td>p-value</td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>--------------------</td>
<td>--------------------</td>
<td>---------</td>
</tr>
<tr>
<td>Average daily animal protein (g)</td>
<td>43.30 (31.23, 59.82)</td>
<td>29.66 (21.31, 45.96)</td>
<td>0.03</td>
</tr>
<tr>
<td>Average daily vegetable protein (g)</td>
<td>18.89 (15.56, 24.60)</td>
<td>16.76 (12.00, 25.52)</td>
<td>0.003</td>
</tr>
<tr>
<td>Average daily total fiber intake (g)</td>
<td>15.59 (12.43, 19.98)</td>
<td>12.95 (7.61, 19.03)</td>
<td>0.17</td>
</tr>
<tr>
<td>Average daily soluble fiber intake (g)</td>
<td>5.81 (4.16, 7.45)</td>
<td>4.44 (2.62, 7.69)</td>
<td>0.54</td>
</tr>
<tr>
<td>Average daily insoluble fiber intake (g)</td>
<td>9.36 (7.34, 13.12)</td>
<td>7.70 (5.09, 12.45)</td>
<td>0.18</td>
</tr>
<tr>
<td>Average daily Omega-3 fatty acid intake (g)</td>
<td>1.21 (0.91, 1.62)</td>
<td>1.08 (0.64, 1.50)</td>
<td>0.17</td>
</tr>
</tbody>
</table>
**Supplemental Figure 1**
Distribution of baseline red meat intake, adjusted for total calorie consumption, in each treatment arm using standardized residuals

**Supplemental Figure 2**
Boxplot comparing week 20 fecal calprotectin by randomization arm
Average ounces per day cooked lean meat from beef, pork, veal, lamb, and game (standardized residuals)