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STRATEGIC RESEARCH AND DEVELOPMENT PROGRAM
FINAL REPORT

INSTRUCTIONS:

- This report **must be a stand-alone report**, *i.e.,* must be complete in and of itself. Scientific articles or other publications cannot be substituted for the report.
- A signed electronic copy of this report must be forwarded to Alberta Agriculture and Forestry **on or before the due date**, as per the investment agreement.
- A **detailed, signed statement of expenses incurred** during the entire funding period of the project must be submitted along with this report (refer to section D.1.a for details).
- For any questions regarding the preparation and submission of this report, please contact the AF project manager assigned to your project OR Brian Karisa at brian.karisa@gov.ab.ca

Section A: Project overview

1. Project number: 2012Q007R		
2. Project title: Personalized dietary therapies for treating Inflammatory Bowel Disease		
3. Project start date: (2012/07/01)	**4. Project completion date:** (2017/12/31)	
5. Research team information		
a) Principal investigator: (Requires personal data sheet [See Section E] only if Principal Investigator has changed since last report.)		
Name	**Institution**	**Expertise added**
Levinus A. Dieleman	University of Alberta	Gastroenterology, translational Microbiome Scientist
b) Research team members (List names of all team members. For each new team member, *i.e.*, joined since the last report, include a personal data sheet [See Section E]. Additional rows may be added if necessary.)		
Name	**Institution**	**Expertise added**
Wishart, David	University of Alberta	Metabolomics expert
Fedorak, Richard N.	University of Alberta	Gastroenterology

Madsen, Karen	University of Alberta	Gut Microbiome Scientist
Hassanzadeh Keshteli, Ammar	University of Alberta	PhD student
Valcheva, Rosica	University of Alberta	Research Associate

Section B: Non-technical summary (max 1 page)

Provide a summary of the project results which could be used by the funder(s) for communication to industry stakeholders (*e.g.*, producers, processors, retailers, extension personnel, etc.) and/or the general public. This summary should give a brief background as to why the project was carried out, what were the principal outcomes and key messages, how these outcomes and key messages will advance the livestock and meat industry, how they will impact industry stakeholders and/or consumers, and the economic benefits for the industry.

Inflammatory bowel diseases (IBD), including ulcerative colitis and Crohn's disease, are chronic intestinal conditions where patients experience recurrent symptoms of abdominal pain, weight loss and diarrhea. IBD is also associated with an increased risk of colon cancer. Many IBD patients suffer from side effects caused by medications or surgery. For reasons that are still unknown, Alberta has one of the highest rates of IBD in the world. While genes do play a role in the disease, exposure to some environmental factors appears to be very important in the disease. One of the most important environmental factors that is thought to be related to the development of IBD is diet. Although many IBD patients believe that their symptoms or even the onset of their disease, are related to certain foods, currently there is not enough scientifically proven evidence to support this claim. Certain foods are believed to have an “anti-inflammatory” effect on the body, while other foods tend to worsen inflammation. In addition, the type of food affects the composition and the function of the gut bacteria. Diet can influence the host through both direct effects on the cells of the body and also through changing the number and activity of bacteria in the gut.

In this project, we carried out a series of studies including a randomized clinical trial in patients with ulcerative colitis to determine if using a specific “anti-inflammatory diet” versus a control diet can reduce disease activity and symptoms. During the study we also measured the metabolic response of the patients in the stool, urine and blood and determined how the different diets would change gut bacteria composition. Detailed data analysis of data collected through this 6-month dietary intervention study is still ongoing, but our preliminary analysis shows that the anti-inflammatory diet can indeed prevent increases in gut inflammation in comparison to the control diet. These favorable effects are associated with changes in metabolic pathways in the patients and but also with alterations in gut bacteria.

Results from this dietary intervention study indicate the merit of an anti-inflammatory diet derived from Alberta-grown agricultural products to prevent UC flares PLUS unravel potential protective mechanisms associated with this beneficial effect.

Section C: Project details

1. Project team (max ½ page)

a) Describe the contribution of each member of the R&D team to the functioning of the project.
b) Describe any changes to the team which occurred over the course of the project.

As the PI of the project, Dr. Dieleman led the project. He has also participated actively in study design recruitment of patients, chart reviews, data analysis and interpretation and drafting manuscripts/abstracts.
Dr. Wishart was involved in performing metabolomic assays on the samples collected during the intervention.
Dr. Madsen was involved in study design, data analysis, performing microbial assays and drafting manuscripts/abstracts.
Dr. Hassanzadeh Keshteli was involved in study design, patient recruitment, laboratory assays, data analysis and manuscripts/abstracts drafting.
Dr. Valcheva had an active role in laboratory assays, microbiome analysis and drafting manuscripts/abstracts.

2. Abbreviations:

Define ALL abbreviations used.
UC: Ulcerative colitis; FCP: fecal calprotectin

3. Background (max 1 page)

Describe the project background and include the related scientific and development work that has been completed to date by your team and/or others.

Inflammatory bowel disease (IBD) is a chronic relapsing- remitting immune disorder of unknown etiology that afflicts millions of individuals throughout the world with debilitating symptoms, which impair performance and quality of life (1). IBD is precipitated by a complex interaction of environmental, genetic, and immunoregulatory factors. Despite anti-inflammatory drugs and surgery there is currently no cure. Higher rates of IBD are seen in northern, industrialized countries (2). Canada has among the highest frequencies of

ulcerative colitis (UC) and Crohn's disease (CD) in the world. The prevalence of IBD currently in Canada is nearly 0.7%, equating to more than 1 in every 150 Canadians and with Alberta as one of the highest prevalences (3). IBD is mostly prevalent in young adults and currently is not curable, with patients usually requiring lifelong medication and multiple devastating surgeries (4). The economic costs of IBD in Canada are estimated to be $2.8 billion in 2012 (almost $12,000 per IBD patient) (3).

The primary cause of IBD results from an imbalance between protective bacteria versus disease-inducing intestinal bacteria, which, in combination with genetic susceptibility, initiates a chronic intestinal inflammation (5). Several studies have been conducted on the effect of environmental factors on the onset of UC, however little is known about the effect on established disease. The effect of diet as an environmental factor on susceptibility and UC onset is more difficult to assess and study outcomes are often inconsistent or of low statistical significance. Although some foods are strongly associated with the onset of UC and only a few with the disease course, the complex role of nutrition in the etiology of UC is not well understood. It is suggested that disease risk may be enhanced or reduced due to dietary patterns rather than individual foods (6). A systematic review of retrospective cohort studies of pre-diagnosis dietary intake revealed that high intake of total fat, total polyunsaturated fatty acids, n-6 fatty acids and meat was associated with an increased risk of UC. In contrast, high vegetable intake was associated with a decrease in UC risk (7). A large prospective cohort study among middle aged women in France, found high animal protein intake increased the risk of UC (8). In addition, high meat and alcohol intake were identified as possible triggers of disease relapse in another 1-year cohort study (9). Dietary interventions with prebiotics and probiotics may have positive effects on IBD disease activity, predominantly through the “normalization” of intestinal microbial biodiversity (10, 11). In addition, the role of other dietary factors (such as n-3 fatty acids (12), polyphenols (13)) in the management of IBD is still controversial. However, it should be noted that retrospective dietary assessment is subject to recall biases and does not clarify the effect on established disease. So far, very few prospective studies have been conducted to investigate the efficacy of dietary interventions in controlling IBD-related symptoms.

Metabolomics is the systemic identification and quantitation of all metabolites in a given organism or set of biological samples (14). Similar to other “omic” approaches that are used to investigate the pathophysiology of different diseases, studying metabolomics has the potential to reveal the underlying multifactorial mechanisms of diseases, including IBD (15), especially if measured before disease relapse occurs. Other investigators have shown that urinary, serum, and fecal metabolomic profiles of IBD patients differ from healthy controls (15,16). In addition, it has been suggested that metabolomics studies have the potential to identify novel biomarkers that could be useful for surveillance and early detection of IBD relapse (15).

Considering the potential role of diet in the management of disease activity in IBD, as well as the lack of appropriate studies in this field, we decided to design an anti-inflammatory

dietary pattern and evaluate its effectiveness in maintaining disease remission in a group of adult ulcerative colitis patients. This project also enabled us to develop a number of small studies including a prospective cohort study to explore the dietary, clinical and metabolomic parameters that occur BEFORE clinical relapse in ulcerative colitis patients.

4. Objectives and deliverables (max 1 page)

a) State the original objective(s) and expected deliverable(s) of the project.
b) Indicate any modifications to the objective(s) and deliverable(s) that occurred over the course of the project.

A): In our prospective cohort pilot study, we aimed to explore dietary determinants of disease relapse in UC patients. In addition, we aimed to identify biomarkers in urine and serum that could predict future disease relapse. Studying these biomarkers (metabolites) would help us understand the pathophysiology of disease activity in UC patients in more detail.

B): The randomized placebo-controlled study evaluated if the proposed Alberta anti-inflammatory diet was effective for the prevention of relapses in ulcerative colitis as well as determine their protective mechanisms (effects on inflammatory biomarkers, metabolome, and gut microbial composition).

Deliverables: Most UC patients claim that their disease activity is highly affected by their dietary intake. Findings from this study will benefit UC patients who experience several courses of disease flare-ups and want control their disease more efficiently. These findings will have the potential to help experts in the field develop effective personalized and rational dietary recommendations for prevention of active disease and its unfavorable consequences in UC patients.

5. Research design and methodology (max 4 pages)

Describe and summarise the project design, methodology and methods of laboratory and statistical analysis that were actually used to carry out the project. Please provide sufficient detail to determine the experimental and statistical validity of the work and give reference to

relevant literature where appropriate. For ease of evaluation, please structure this section according to the objectives cited above.

A) Prospective cohort pilot study:

Patient Cohort: This prospective cohort pilot study was performed at the University of Alberta in Edmonton, Alberta, Canada. Using a convenient non-probability sampling method, adult UC patients who were able to read and write in English were recruited from the IBD clinic at the University of Alberta. The diagnosis of UC was confirmed using a combination of clinical, endoscopic and histological criteria. All patients were included if they were in clinical remission at the time of enrollment, determined by a validated partial Mayo score of less than 3 (17). Subjects were excluded if they had used oral corticosteroids in the previous four weeks, used any biological agents for UC management within 3 months before the enrollment, or had a history of colectomy. Written informed consent was obtained from all participants and the study protocol was approved by the Health Research Ethics Board-Biomedical Panel, University of Alberta, Edmonton, Canada (Pro00032213).
Demographic, clinical and dietary information: At baseline visit participants' demographic and clinical information was obtained and participants completed a food frequency questionnaire (FFQ) that assessed their food intake in the past 12 months. Anthropometric assessments (as described below), clinical information, and urine and blood samples were collected for metabolomics analyses, and stool was collected for fecal calprotectin (FCP). Twelve months after the baseline visit, patients were followed-up by a telephone interview and their clinical files were reviewed to determine if they had experienced a clinical UC relapse (defined as partial Mayo score of 3 or more) during the past 12 months. Comparisons were made between patients who a) remained in clinical remission versus b) those who experienced a clinical relapse.
In addition, long-term dietary intake was assessed using the National Cancer Institute's self-administered Diet History Questionnaire II (DHQ) (18, 19). This validated semi-quantitative FFQ included questions about 134 food items and accounts for seasonal intake of a variety of foods, portion size and frequency of intake for each food item. Body weight was measured to the nearest 0.01 kg (Health o Meter Professional 752KL medical scale) and height was measured (HM200P Portstad portable stadiometer, Charder Electronic Co, Ltd) Body mass index (BMI) was calculated as weight in kilograms divided by height in meters. squared. Waist circumference was measured at the narrowest part of abdomen over light clothing using a non-stretch measuring tape and recorded to the nearest 0.1 cm. Waist to height ratio was calculated as the ratio of waist circumference/height. Body composition (i.e. total fat mass and body fat percentage) was determined by air displacement plethysmography (BodPod) (COSMED Concord, CA, USA).
Sample Collection and Analysis: Subjects were provided with appropriate materials and instructions to collect morning urine and stool samples. In addition, fasting blood samples were collected from each participant at baseline. Urine and serum samples were assayed using a combined direct infusion (DI-)/liquid chromatography (LC-) tandem mass

spectrometry (MS/MS) (AbsolutIDQ p180 kit, Biocrates Life Sciences AG, Innsbruck, Austria) and nuclear magnetic resolution (NMR) spectroscopy, using the previously described protocol (20, 21) in order to identify and quantify metabolites. All metabolomic assays were performed at the Metabolomics Innovation Centre (Edmonton, Canada). Fecal calprotectin (FCP) was measured in stool samples using an enzyme-linked immunosorbent assay with monoclonal antibodies specific to calprotectin (Bühlmann Laboratories AG, Basel, Switzerland). FCP levels above 150 µg/g stool were used to define "high FCP" due to its association with increased risk of UC relapse, as we recently reported (22).

Metabolomic Analysis: For the metabolomic analysis, concentrations of urinary metabolites (µmol/L) were normalized by creatinine (mmol/L) and reported as a ratio (µmol/mmol). Concentrations of identified metabolites were normalized using logarithmic transformation and pareto scaling. Metabolites with a p-value less than 0.1 in the u univariate analyses) were selected for generating the logistic regression model. Multivariate statistical analysis was performed using partial least squares discriminant analysis (PLS-DA). A 10-fold cross-validation technique was used to ensure that the logistic regression models were robust. Permutation analysis using random resampling (n=2000) of the two groups of patients (i.e. clinical relapse versus remission) was conducted to determine the probability that the observed separation was a result of chance or not, and a p-value that represents the probability of a random finding was generated. To identify the major metabolites that were responsible for the discrimination between patients with clinical relapse and patients in clinical remission, variable importance in projection (VIP) values were used. The VIP value indicates the contribution of each feature to the regression model. MetaboAnalyst 3.0 (23) was used for the metabolomic statistical analysis.

Statistical analysis: Categorical and numerical variables are presented as percentage and median (interquartile range (IQR), respectively. Fisher's exact test and Mann-Whitney U test were used to compare categorical and numerical variables between two groups of UC patients (clinical relapse versus remission in the previous 12 months). To test the relationship between overweight/obesity and clinical relapse, we used binary logistic regression analysis after adjusting for age and gender. A receiver operating characteristic (ROC) curve was constructed in order to calculate the accuracy of FCP for predicting UC patients who developed a clinical relapse (partial Mayo score>2 (17)) versus those who remained in clinical remission during the 12-month follow-up. Statistical Package for the Social Sciences, version 16.0 (SPSS Inc, Chicago, IL, USA) was used for statistical analysis. A two-tailed P value of less than 0.05 was considered to be statistically significant.

B) Dietary intervention study

Patients:

This was a randomized controlled clinical trial study, started in September 2014.

Inclusion criteria: adult (18-75) ulcerative colitis patients in clinical remission who had experienced at least one flare during the previous 18 months. Patients could be on oral 5-ASA drugs and/or azathioprine or 6-mercaptopurine as long as the dose has been stable for 2 weeks (between 2-2.4 gram daily for oral 5-ASA) or 2 months (for azathioprine or 6-

mercaptopurine and anti-TNF drugs). Exclusion criteria: subjects were excluded if they had taken took steroids or antibiotics within 4 weeks of enrollment, were pregnant or lactating, had a history of colectomy, had significant systemic disorders or had a gastrointestinal infection. Written informed consent was obtained from all participants and the study protocol was approved by the Health Research Ethics Board-Biomedical Panel, University of Alberta, Edmonton, Canada (Pro00035413).
Patients that met the inclusion criteria (assessed by a gastroenterologist and study coordinator at screening visit) were asked to come to the Clinical Research Unit, University of Alberta at baseline, month 1, month 3 and month 6. At month 2, 4 and 5 there were telephone interviews. Details of clinic visits and telephone interview were as follows:

Screening Visit

- Assessment of inclusion of exclusion criteria
- Obtainment of Informed Consent
- Medical History
- Pregnancy Test
- Partial Mayo scoring

Visit at Month 0 (baseline), 1, 3, 6 (or at relapse)

- Focused Physical Exam and Medical History
- Partial Mayo scoring
- Fecal samples for Calprotectin and luminal microflora analysis and metabolomics
- Blood and urine samples for metabolomic analysis, inflammatory markers and other tests specified in the Appendix 1.
- Dietary counseling
- Questionnaires specified in the Appendix 1.
- Sigmoidoscopy (at month 6 or at relapse)

Visit at Month 2, 4, 5

- Telephone interview by the dietician
- Partial mayo scoring
- Questionnaires specified in the Appendix 1

Dietary counselling and assessment:
Patients were randomized to either Alberta Anti-inflammatory Diet (AID) or Canada's Food Guide (CFG) ("Control") groups. Patients randomized to the AID diet received menu plans and dietary recommendations in order to increase their intake of fiber, omega-3 fatty acids, antioxidants, prebiotics and probiotics all from foods. They were also recommended to decrease intake of red meat, processed meat and added sugar. Patients who were randomized to CFG group as our Control diet received dietary recommendations based on Canada's Food Guide. Each dietary counselling session took about one hour and all dietary recommendations were provided by a registered dietitian. We used 24h dietary recalls at baseline and then once a month to assess patients' adherence to the two diets. We also used

a food frequency questionnaire (18, 19) at baseline to assess participants' long-term dietary intake.

Laboratory assays:
Subjects were provided appropriate materials and instructions to collect morning urine and stool samples at baseline and month 6 or at relapse visits. In addition, fasting blood samples were collected from each participant at baseline and month 6/relapse visits. Urine samples were assayed using a combined direct infusion (DI-)/liquid chromatography (LC-) tandem mass spectrometry (MS/MS) (AbsolutIDQ p180 kit, Biocrates Life Sciences AG, Innsbruck, Austria) and gas chromatography (GC-) MS in order to identify and quantify metabolites. For metabolomic assays on serum samples we performed nuclear magnetic resolution (NMR) spectroscopy and DI- LC- MS/MS. On stool samples, we used NMR to identify and quantify metabolites. All metabolomic assays were performed at the Metabolomics Innovation Centre (Edmonton, Canada) using a previously described protocol (20, 21). Fecal calprotectin (FCP) was measured in stool samples using an enzyme-linked immunosorbent assay with monoclonal antibodies specific to calprotectin (Bühlmann Laboratories AG, Basel, Switzerland). Routine laboratory investigations on blood samples (baseline, month 3, month6/relapse) were performed at the University of Alberta Hospital (UAH) Laboratory (Edmonton, Canada) included complete blood count (CBC), iron studies (i.e., ferritin, iron, total iron-binding capacity (TIBC), and transferrin saturation), albumin, alanine aminotransferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (AP), gamma-glutamyl transpeptidase (GGT), C-reactive protein (CRP), vitamin B12 and 25-hydroxyvitamin D. On stool samples at baseline and month 6/at relapse we extracted DNA to determine bacterial composition using 16s rRNA sequencing performed at Genome Quebec facility in Montreal, Quebec, Canada.

Statistical analysis:
For normally distributed variables, one-way analysis of variance (ANOVA) or Student's t-test were used. For non-normally distributed variables Kruskal-Wallis or Mann-Whitney U tests were applied. To compare changes in FCP between the two groups, repeated measures ANOVA was used. For metabolomic analysis, a similar approach for analysing metabolomic data in the pilot study was used. To explore which microbial changes were responsible for the changes in the FCP levels, Spearman's rank correlation test was used.

6. Results, discussion and conclusions (max 8 pages)

Present the project results and discuss their implications. Discuss any variance between expected targets and those achieved. Highlight the innovative, unique nature of the new knowledge generated. Describe implications of this knowledge for the advancement of agricultural science. For ease of evaluation, please structure this section according to the objectives cited above.

Prospective cohort pilot study:

In this small pilot study, we identified potential predictors of clinical relapse in UC patients. We found that a history of higher dietary poultry and maltose intake, and high BMI, body fat mass, and waist circumference at baseline were associated with persistent UC clinical remission during a 12-month follow-up. Of significant interest, we found that the baseline serum and urinary metabolomic profile of patients who relapsed during follow-up was significantly different from those patients who did not develop a relapse. Details of findings of the pilot study are as follows:

Subject Demographics: Twenty UC patients in clinical remission were recruited with a mean age of 42.7 ± 14.8 years; 11 (55%) were females. Two (10%) patients were current smokers. Eleven patients (55%) were diagnosed to have pan-colitis and 13 (65%) subjects were on either oral or rectal 5-aminosalicylic acid (5-ASA) medications. Twenty three percent of patients were on no UC-related medication. (Table 1 of Appendix 2)

UC relapse and demographic, clinical, and anthropometric parameters: Patients were followed for 12.1 ±1.9 months and during this time 7 (35%) patients experienced a clinical relapse. The comparison between different demographic, anthropometric, and clinical characteristics of patients (at the time of recruitment) with clinical relapse versus those who were still in clinical remission at the time of follow-up is presented in Table 1 of Appendix 2. There was no significant difference between these two groups of patients in terms of age, gender, and UC-related factors (age at diagnosis, months since last relapse, UC previous extent, and UC medication) at baseline. However, UC patients who developed a clinical relapse within 12 months had significantly lower BMI, waist circumference, waist to height ratio, and fat mass compared to patients with no clinical relapse. Six out of 9(66.7%) patients with normal BMI (18.5 – 24.9 kg/m2) had a clinical relapse, whereas only 1 out of 11(9.1%) patients with overweight/obesity (BMI>25 Kg/m2) at baseline relapsed during the follow-up (relative risk (RR): 7.3, 95% confidence interval (CI): 1.1-50.3, P=0.02) , which finding was still statistically significant (P=0.03) after adjusting for age and gender.

Effect of dietary intake: There was no statistically significant difference between intake of different macro-, micronutrients as well as food groups at baseline in patients with clinical relapse versus remission within 12 months of follow-up. However, poultry and maltose intake was significantly higher in patients who remained in remission (Table 2 of Appendix 2). There was also a positive correlation between maltose intake and total grain (r=0.50, P=0.03) and whole grain intake (r=0.47, P=0.04) suggesting that the main source of maltose in our patients was grain or grain products.

Fecal calprotectin (FCP): The median (IQR) level of FCP at baseline in UC patients with clinical relapse and remission at 12 months of follow-up was 195.9 (41.2-347.9) and 23.3 (12.9-84.5) µg/g, respectively (P=0.05). Five (71.4%) patients with high FCP versus 2 (15.4%) patients with normal FCP at baseline relapsed during the follow-up period (RR: 4.6, 95% CI: 1.2-18.1, P=0.02). ROC curves for FCP as a predictor of clinical relapse in UC is presented in Figure 1 of Appendix 2. An FCP concentration of 124 µg/g resulted in a sensitivity of 71.4%, a specificity of 84.6%, a positive predictive value (PPV) of 71.4, and a negative predictive value (NPV) of 84.6% in predicting UC clinical relapse.

Metabolomic analysis: Using the described metabolomic assays, we identified and quantified 216 and 247 metabolites in serum and urine samples, respectively. After conducting univariate analysis, 16 candidate metabolites were candidate for further statistical analysis based on the P-value of < 0.1. As presented in Figure 2 of Appendix 2, UC patients who experienced clinical relapse versus who stayed in clinical remission during follow-up could be discriminated in different clusters from each other by their metabolomic profile at baseline. Using the permutation testing, we showed that this separation was statistically significant (P=0.04). The R2 and Q2 of the model was 0.84 and 0.59, respectively. VIP values of six metabolites were above 1.0, showing their important role in the discrimination between metabolomic profiles between the two UC groups. The median (IQR) levels and VIP scores of these metabolites are presented in Table 3 of Appendix 2. In comparison to UC patients who were still in remission during follow-up, those study patients with a future clinical relapse had significantly higher levels of trans-aconitate (urine), 3-hydroxybutyrate (serum), acetoacetate (serum), acetone (serum), and lower levels of acetamide (urine) and cystine (urine).

Dietary intervention study:

Fifty-three UC patients in clinical remission were recruited. Twenty six patients were randomized to the AID group and 27 patients were randomized to the CFG diet group as the control group. Their mean age was 41.4±14.7 years and 64.2% of patients were females. The UC patients had either pan-colitis (47.2%) or left-sided colitis (41.5%). There were no statistically significant differences between the two groups in terms of clinical and demographic characteristics.

During the 6-month follow up, 5 (19.2%) patients in the AID group and 8 (29.6%) patients in the CFG group relapsed during the trial (P=0.38).

Changes in quality of life score, CRP and other laboratory parameters between the two diet groups from baseline to month 6 or clinical relapse were not statistically significant (**Table 1**).

Over the 6-month intervention, the AID group showed no significant increase in FCP, whereas patients following CFG had a statistically significant increase (**Figure 1**).

Table 1. Comparison of changes in quality of life, body mass index and laboratory parameters between the two diet groups from baseline to month 6 or clinical relapse

	AID		P-value[1]	CFG		P-value[2]	P-value[3]
	Baseline	Month 6		Baseline	Month 6		
Quality of life	55.0±9.1	55.9±8.0	0.99	55.5±6.9	55.1±8.6	0.89	0.97
CRP	6.9±20.8	6.8±16.2	0.28	2.4±2.5	3.6±6.5	0.43	0.92
Vitamin D3	85.9±30.3	90.6±29.6	0.40	84.7±38.9	95.3±33.9	0.03	0.17
Vitamin B12	369.5±225.3	386.6±252.5	0.79	456.0±226.9	469.3±270.6	0.51	0.88
Serum ferritin	51.9±49.1	44.9±36.1	0.09	42.5±40.3	47.7±45.1	0.48	0.10
Body mass index	26.1±5.5	26.2±5.2	0.24	25.9±6.0	26.0±5.9	0.74	0.58

[1] for comparison of baseline vs month 6/relapse levels in the AID group
[2] for comparison of baseline vs month 6/relapse levels in the CFG group
[3] for comparison of changes from baseline to month 6/relapse between AID and CFG groups

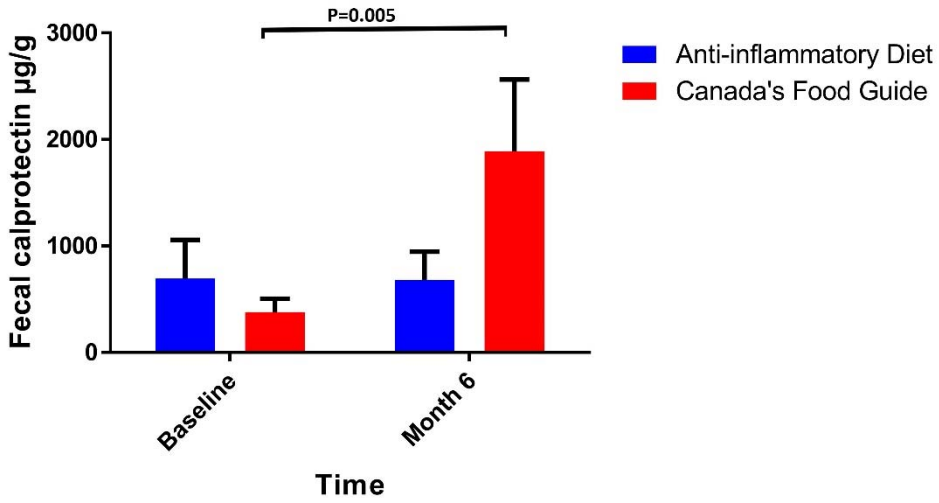

Figure 1. Comparison of changes in fecal calprotectin levels from baseline to month 6 or clinical relapse between the Anti-inflammatory Diet and Canada's Food Guide diet groups

Using the described metabolomic assays, we identified and quantified 49, 184, and 122 metabolites in stool, serum and urine samples, respectively. After conducting univariate analysis, 38 candidate metabolites were candidate for further statistical analysis based on the P-value of < 0.2. While there was no clear separation between the metabolomic profile of patients randomized to the two diet groups at baseline, we found that their metabolome was significantly different from each other at month 6 or at clinical relapse (**Figure 2**).

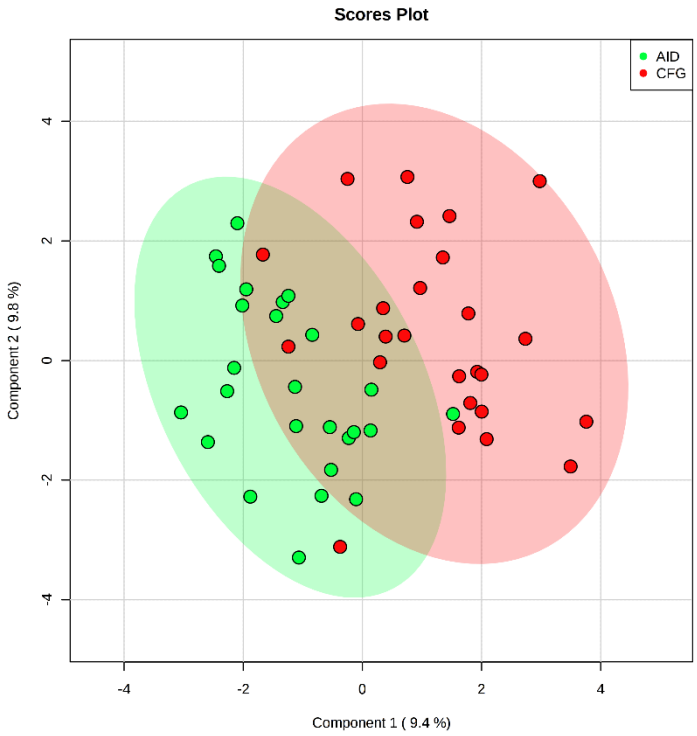

Figure 2. Partial least squares discriminant analysis plot showing a significant difference in the metabolome of patients randomized to the Anti-inflammatory Diet and Canada's Food Guide diet groups at month 6 or clinical relapse, as identified by metabolites in urine, serum and fecal samples.

To assess the most important metabolites responsible for the discrimination of the metabolomic profile at month 6 or clinical relapse between the two diet groups we used VIP scores. Metabolites with VIP scores above 1 have a major role in this discrimination. As shown in Figure 3, Patients in the AID group had higher creatinine (stool), glycerol (stool), carnosine (urine), PC ae C38:5 (urine), but lower methanol (stool), PC aa C38:1 (serum), and C16:2 (urine).

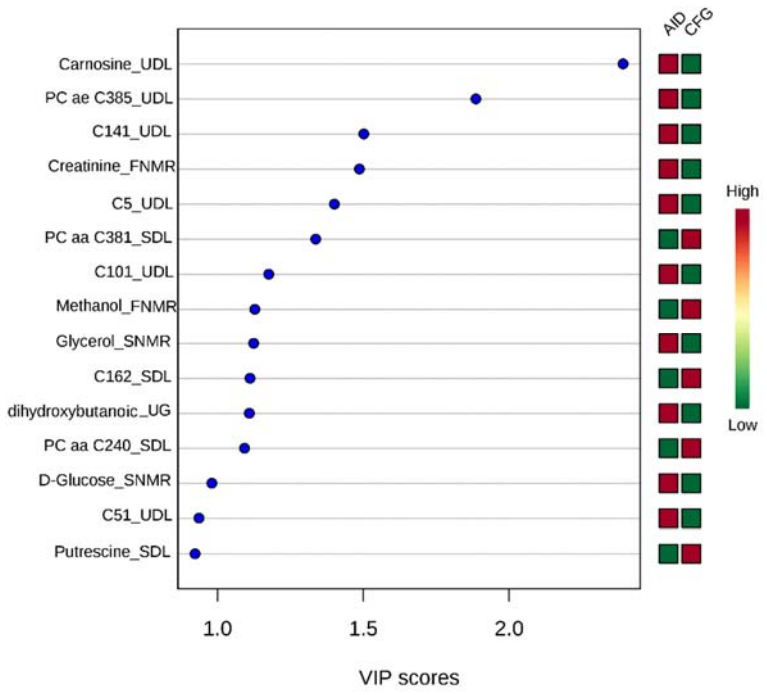

Figure 3. Variable importance in projection (VIP) plot presenting the most important metabolites in serum, urine and stool samples responsible for the separation of the metabolomic profiles from ulcerative colitis patients randomized to the two diet groups.

To explore the role of changes in gut microbial composition in correlation with increased FCP levels in patients who were randomized to the CFG group, we used Pearson correlation analysis. As shown in Figure 4, increased FCP from baseline to month 6 was significantly correlated with decreased amounts of *Coriobacteriaceae* and *Lachnospiraceae*, but increased numbers of *Veillonellaceae*, *Bacteroidaceae*, and *Enterobacteriaceae*.

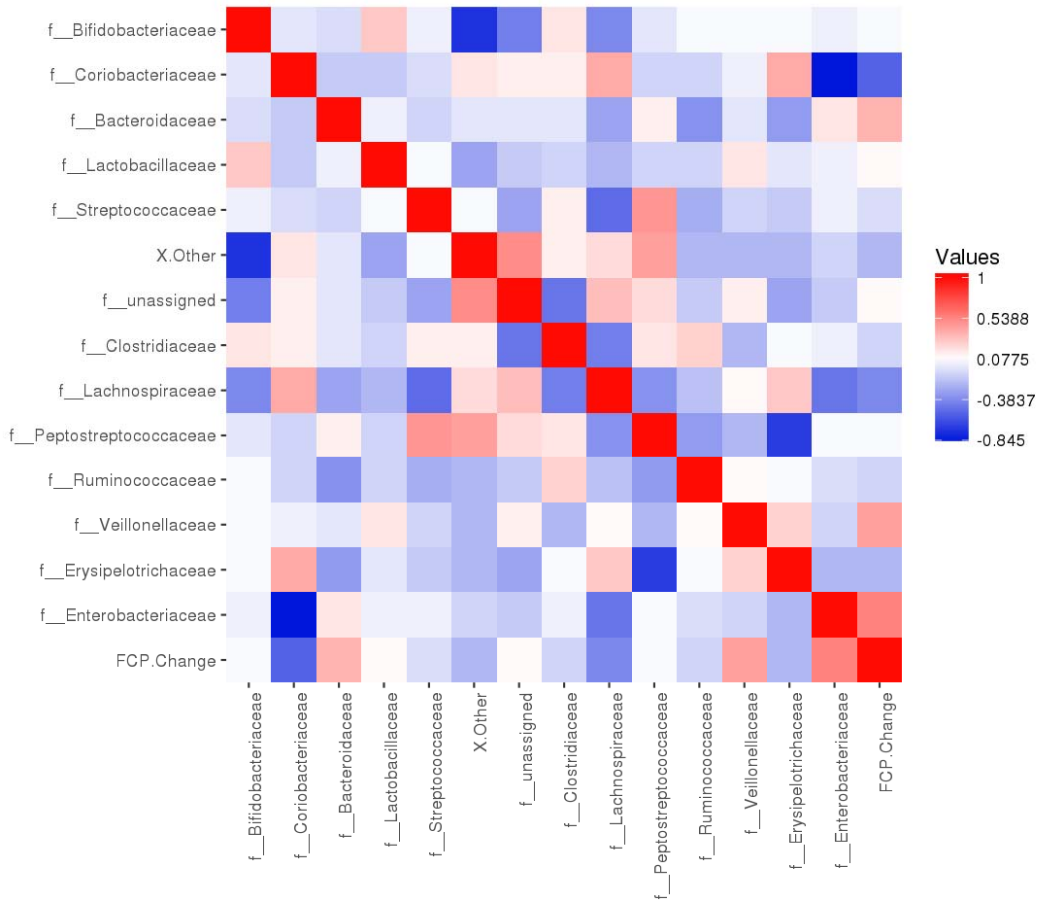

Figure 4. Heatmap plot presenting the correlation of FCP changes from baseline to month 6 with changes in gut microbial composition (family level) from baseline to month 6 in patients randomized to the Canada's Food Guide diet group.

We used 24h dietary recalls in order to assess dietary intake of patients throughout the study. As presented in Table 2, changes in dietary intake of some nutrients and food items were significantly different between the two diet groups. Patients in the AID group showed an increase in dietary intake of seafoods, yogurt, protein, fiber, zinc, selenium, phosphorus, vitamin A, niacin, and choline versus the control group.

Table 2. Comparison of changes in dietary intake of foods and nutrients from baseline to month 6 between the two diet groups.

	AID		P-value[1]	**CFG**		P-value[2]	P-value[3]
	Baseline	Month 6		Baseline	Month6		
Energy	1858.2±540.1	1973.7±557.9	0.29	1921.8±634.1	1911.5±568.5	0.45	0.26
Protein	77.7±32.2	85.2±27.9	0.12	98.1±43.7	87.7±28.0	0.11	0.03
Carbohydrate	233.3±78.9	241.9±75.8	0.29	230.6±97.6	212.4±63.4	0.12	0.08
Fat	74.3±29.2	71.3±22.1	0.93	72.6±26.9	74.2±30.6	0.83	0.58
Fiber	20.2±6.6	22.8±6.7	0.04	22.5±11.8	22.3±8.3	0.71	0.09
Vitamin A	1018.1±794.1	1134.9±731.7	0.13	1198.5±696.9	900.7±440.2	0.03	0.01
Niacin	24.8±17.5	27.2±14.7	0.23	29.2±10.0	27.8±11.1	0.27	0.06
Zinc	10.6±5.3	11.7±6.1	0.05	17.7±11.7	14.1±8.0	0.02	0.00
Phosphorus	1408.6±626.6	1561.4±548.8	0.05	1571.9±637.3	1472.5±435.0	0.36	0.02
Selenium	110.4±61.8	122.2±39.6	0.04	136.4±55.6	119.0±37.8	0.06	0.01
Choline	297.8±130.8	334.5±118.3	0.07	367.6±176.3	332.1±132.3	0.46	0.05
Seafoods	0.7±1.2	1.4±1.5	0.01	1.2±2.1	1.1±1.3	0.82	0.07
Yogurt	0.3±0.3	0.5±0.3	0.00	0.1±0.2	0.2±0.2	0.28	0.06

[1] for comparison of baseline vs month 6/relapse levels in the AID group
[2] for comparison of baseline vs month 6/relapse levels in the CFG group
[3] for comparison of changes from baseline to month 6/relapse between AID and CFG groups

Discussion and conclusions:

In our pilot prospective cohort study we identified potential predictors of clinical relapse in UC patients. We found that a history of higher dietary poultry and maltose intake, and high BMI, body fat mass, and waist circumference at baseline were associated with UC clinical remission during a 12-month follow-up. Of significant interest, we found that the baseline serum and urinary metabolomic profile of patients who relapsed during follow-up was significantly different from those patients who did not develop a relapse. The novel metabolomic findings of this study are important in several aspects. These findings can be used to help us understand the multifactorial pathophysiology of UC relapse. The identified metabolites that could predict disease relapse in this study had different microbial, host related (energy metabolism pathways) and dietary sources which highlights the role of these factors in the development of UC flare-up. In addition, these identified metabolites can be used as non-invasive biomarkers for predicting clinical relapses in UC patients after being validated in future studies with larger sample size. Furthermore, these metabolites can be applied as potential biomarkers to asses the response to different treatments (e.g. medications, dietary or lifestyle interventions) in future clinical trials.

Our prospective dietary 6 months intervention study we showed for the first time that our designed anti-inflammatory diet versus the Canada Food Guide control diet can prevent increases in fecal calprotectin levels and prevent colonic inflammation in ulcerative colitis patients who were in clinical remission at the time of enrollment. In addition, we have found that such a favorable effect was associated with specific changes in stool, urine and serum metabolites. Most of these metabolites have dietary sources (e.g. carnosine, phosphatidylcholines, glycerol, and creatinine). However, we plan to perform further analysis to study specific dietary factors that are related to the levels of these metabolites in different biological samples collected during this study. These results will also help us identify specific biomarkers that can be used to monitor dietary interventions in future studies. In addition, we aim to investigate the correlation between the relevant metabolites and gut microbial composition and function to assess if variations in the levels of these metabolites can be explained by gut microbiota. Taken together, our results from our dietary intervention study show that dietary modifications to increase intake of anti-inflammatory foods and decrease inflammatory-type foods can be effective in preventing the development of subclinical colitis, associated with specific microbial and metabolic changes in UC patients in clinical remission.

NB: Tables, graphs, manuscripts, etc., may be included as appendices to this report.

7. Literature cited

Provide complete reference information for all literature cited throughout the report.

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8. Benefits to the industry (max 1 page; <u>respond to sections *a)* and *b)* separately</u>)

a) Describe the impact of the project results on Alberta's agriculture and food industry (results achieved and potential short-term, medium-term and long-term outcomes).

The preliminary results indicate that an anti-inflammatory diet derived from Alberta grown agricultural products is effective in preventing disease relapses in patients with ulcerative colitis. This diet was composed of prebiotic fibres (flax seeds), fruit and vegetables but also omega-3 fatty acids as found in seafood. Patients were encouraged to eat these foods in that arm of the study. The details and specifics of these dietary components and especially their metabolic products found in serum, stool and urine is currently under investigation, as stated above. The results of this trial will justify a rational use of this diet to prevent disease relapses instead of the advertising of dietary therapies without any scientific evidence. The rigidity using online validated dietary questionnaires plus the analysis of protective mechanisms in our study is a unique study design that has not been done anywhere in the world. It is our expectation therefore that the results of our studies will be published in high impact journals.

b) Quantify the potential economic impact of the project results (*e.g.,* cost-benefit analysis, potential size of market, improvement in efficiency, etc.).

The economic impact of the project results will be the prevention of relapses with addition of more expensive medications, prevent colectomies, frequent invasive procedures and hospitalizations, saving millions of dollars.

9. Contribution to training of highly qualified personnel (max ½ page)

Specify the number of highly qualified personnel (*e.g.*, students, post-doctoral fellows, technicians, research associates, etc.) who were trained over the course of the project.

Ammar Hassanzadeh Keshteli is our graduate student who has been involved actively in this project. He is a PhD student under the supervision of Drs. Dieleman and Madsen at Department of Medicine, Faculty of Medicine and Dentistry, University of Alberta. In this project, we also trained several summer students including Céline Christin (Universite de Lyon, France), Floris van den Brand (Vrije Universiteit Amsterdam, the Netherlands), Tess Vader (Vrije Universiteit Amsterdam, the Netherlands) and Thomas Hoevers (Vrije Universiteit Amsterdam, the Netherlands). Rosica Valcheva is a Research Associate in Dr. Dieleman's lab and has been involved in the design and microbial analysis of the project.

10. Knowledge transfer/technology transfer/commercialisation (max 1 page)

Describe how the project results were communicated to the scientific community, to industry stakeholders, and to the general public. Please ensure that you include descriptive information, such as the date, location, etc. Organise according to the following categories as applicable:

a) Scientific publications (*e.g.*, scientific journals); attach copies of any publications as an appendix to this final report

b) Scientific presentations (*e.g.*, posters, talks, seminars, workshops, etc.)
c) Industry-oriented publications (*e.g.*, agribusiness trade press, popular press, etc.); attach copies of any publications as an appendix to this final report
d) Industry-oriented presentations (*e.g.*, posters, talks, seminars, workshops, etc.)
e) Media activities (*e.g.*, radio, television, internet, etc.)
f) Any commercialisation activities or patents

Scientific publications: Recently, we published findings from the pilot prospective cohort study in the World Journal of Gastroenterology (Appendix 2). Our abstracts in Digestive Disease Week (DDW) which is the most prestigious conference in the field of gastroenterology worldwide, have been published in the Gastroenterology journal (Appendix 3-7).

Scientific presentations:
In addition to presenting our study's results in local symposiums (Alberta Digestive Disease Summit (2013)) and university level conferences (Department of Medicine Research Day (2015-2017) (Appendix 8) and Division of Gastroenterology Research Day (2014-2017)) we have presented our findings in national (CDDW) and international (DDW) conferences as follows:

1. Keshteli AH, van den Brand F, Wishart D, Mandal R, Bjorndahl TC, Han B, Baarda J, Kroeker KI, Valcheva R, Fedorak RN, Madsen K, Dieleman LA. Dietary fat intake, fecal calprotectin and metabolomic profiles predict relapse of ulcerative colitis. Digestive Disease Week, May 3-6, 2014. Chicago, IL, USA (Appendix 9).
2. Keshteli AH, van Den Brand F, Wishart D, Mandal R, Dieleman LA, Madsen K. Metabolomic profiling can differentiate ulcerative colitis patients from control individuals. Canadian Digestive Diseases Week, February 27-March 2, 2015. Banff, AB, Canada [Oral presentation]
3. Keshteli AH, van Den Brand F, Valcheva R, Wishart D, Mandal R, Kroeker K, Fedorak RN, Madsen K, Dieleman LA. Ulcerative colitis patients with and without subclinical inflammation can be differentiated from healthy controls through metabolomic profiling. Digestive Diseases Week, May 16-19, 2015. Washington, DC, USA. (Appendix 10)
4. Keshteli AH, Valcheva R, Nickurak C, Halloran BP, van Zanten SV, Kroeker K, Fedorak RN, Madsen K, Dieleman LA. Mo1889 Adherence to an "Anti-Inflammatory Diet" for 6 Months Can Decrease Fecal Calprotectin in Ulcerative Colitis Patients: Preliminary Findings of a Randomized Controlled Trial. Canadian Digestive Disease Week, February 26 -29, 2016. Montreal, QC, Canada. [Oral presentation, Research Topics in GI Disease] (Appendix 11)
5. Keshteli AH, Mandal R, Boeckxstaens GE, Bercik P, McIntosh K, Reed DE, Wishart D, Dieleman LA, Madsen K, Vanner S. Metabolomic Profiling Differentiates Irritable

Bowel Syndrome Patients From Healthy Controls and Ulcerative Colitis Patients. Digestive Disease Week, May 21-24, 2016. San Diego, CA, USA. [Oral presentation]

6. Keshteli AH, Valcheva R, Nickurak C, Halloran BP, van Zanten SV, Kroeker K, Fedorak RN, Madsen K, Dieleman LA. Adherence to an "Anti-Inflammatory Diet" for 6 Months Can Decrease Fecal Calprotectin in Ulcerative Colitis Patients: Preliminary Findings of a Randomized Controlled Trial. Digestive Disease Week, May 21-24, 2016. San Diego, CA, USA. (Appendix 11)
7. Keshteli AH, Hoevers T, Madsen K, Hotte N, Nickurak C, Kroeker K, van den Brand F, Valcheva R, Fedorak R, Dieleman L. High fecal calprotectin levels in ulcerative colitis patients in clinical remission are associated with specific clinical and dietary intake parameters. Canadian Digestive Diseases Week, March 3-6, 2017. Banff, AB, Canada [Poster of Distinction] (Appendix 12)
8. Keshteli AH, Hoevers T, Madsen K, Hotte N, Nickurak C, Kroeker K, van den Brand F, Valcheva R, Fedorak R, Dieleman L. High fecal calprotectin levels in ulcerative colitis patients in clinical remission are associated with specific clinical and dietary intake parameters. Digestive Diseases Week, May 5-9, 2017. Chicago, IL, USA. (Appendix 12)
9. Keshteli AH, Madsen K, Nickurak C, Kroeker K, Mandal R, Wishart DS, van Zanten SV, Halloran BP, Fedorak RN, Valcheva R, Dieleman LA. Adherence to an Anti-Inflammatory Diet Prevents Increases in Colonic Inflammation in Ulcerative Colitis Patients in Remission. Digestive Diseases Week, May 5-9, 2017. Chicago, IL, USA. (Appendix 13)
10. Keshteli AH, Madsen K, Nickurak C, Kroeker K, Park H, Valcheva R, Mandal R, Wishart DS, van Zanten SV, Halloran BP, Fedorak RN, Dieleman LA. An anti-inflammatory diet prevents subclinical inflammation and associated changes in gut microbiota and metabolomic profiles in ulcerative colitis patients. Canadian Digestive Diseases Week, February 9-12, 2018. Toronto, ON, Canada [It has been accepted for an oral presentation].

Fill out the table below with the total number of each performance measure:

Number of scientific publications / presentations	26
Number of industry communications	0
Number of patents / licenses	0

N.B.: Any publications and/or presentations should acknowledge the contribution of each of the funders of the project.

Section D: Project resources

1. **Statement of revenues and expenditures:**
   a) **In a separate document certified by the organisation's accountant or other senior executive officer, provide a detailed listing of all cash revenues to the project and expenditures of project cash funds.** Revenues should be identified by funder, if applicable. Expenditures should be classified into the following categories: personnel; travel; capital assets; supplies; communication, dissemination and linkage (CDL); and overhead (if applicable).
   b) **Provide a justification of project expenditures and discuss any major variance (*i.e.*, ± 10%) from the budget approved by the funder(s).**

The Project aimed to perform a 3-year dietary study in patients with ulcerative colitis in remission in two phases: Phase 1) A pilot study to identify if patients who had experienced recent relapse (less than 18 months) differ from long-time patients in remission; and Phase 2) An interventional study to identify if the "Alberta Anti-inflammatory Diet" (study diet) will prevent disease relapse in patients who had experienced recent flare (less than 18 months) in comparison to "Canada Food Guide Diet" (control diet). The two phases (and the project respectively) were completed in a period of 5 years instead of 3 years. Given the longer project completion time, there are differences in the expenditures made per categories. The initially approved budget projected Salary expenditures of **$251,500.36** and Operational expenditures of **$98,625.00** for a period of 3 years. At the time of Project completion, $**223,584.91** were actually spent for personnel salary (including research staff and graduate students), and $**123,306.33** were spent for study supplies and materials for a period of 5 years.

The major variance in the operational budget (25% over) was produced by the following reasons:

1) The initially approved budged for the Phase 2 study was based on a proposed study population of 40 patients, however following the completion of the pilot study (phase 1) we concluded that this sample size was too small and a bigger number of patients were needed in order to reach any statistical significance. Therefore 53 patients were enrolled in Phase 2, leading to bigger operational costs for sample processing and analysis, (most importantly increased costs for metabolomics analysis)
2) Unaccounted costs associated with patient recruitment and sampling, such as Study advertisement in print media ($4,262.43), dietitian honorarium for dietary counseling ($16,741), a fee for sample deposit in CEGIIR Biobank ($1,475), and database creation and management by EPICORE (University of Alberta) ($14,885.10).

The major variance in the budget for personnel salary (10% less) was produced by the following reasons:

1) Only 1 research associate (Rosica Valcheva) was appointed to this project on 0.5 FTE rate
2) 1 graduate student (Ammar Keshteli) was initially assigned to the project. In addition, in 2015 the student was awarded with Alberta Innovates Health Solutions Graduate Studentship for PhD, therefore the Project did not further contribute to the student's stipend.
3) Other students who received training in the framework of the Project (Floris van den Brand, Tess Vader and Thomas Hoevers, Dutch medical students from the Vrije Universiteit Amsterdam, the Netherlands) were supported by other funding.

2. Resources:

Provide a list of all external cash and in-kind resources which were contributed to the project.

Total resources contributed to the project		
Source	**Amount**	**Percentage of total project cost**
Alberta Agriculture and Forestry	$352,025	29%
Other government sources: Cash	$135,000	11%
Other government sources: In-kind	$720,000	60%
Industry: Cash		%
Industry: In-kind		%
Total Project Cost		100%

External resources (additional rows may be added if necessary)		
Government sources		
Name (no abbreviations unless defined previously)	Amount cash	Amount in-kind
Genome Canada	$1,250,000	$90,000
Canadian Foundation for Innovation	$22,000,000	$720,000
AIHS (Alberta IBD Team Grant)	$5,000,000	
AIHS (Metabolomics CRIO Team Grant)	$5,000,000	
AIHS Graduate Studentship	$120,000	
Industry sources		
Name (no abbreviations unless defined previously)	Amount cash	Amount in-kind

Summary of project expenditures

Reporting period	Source	Type	Personnel	Travel	Capital Assets	Supplies	CDL*	Other	Total
Period 1 Dates: 2012/07/01 to 2013/06/30	ALMA	Budgeted	$120,500.36	$0.00	$0.00	$5,925.00			$126,425.36
		Spent	$58,845.84	$890.49		$1,410.37			$61,146.70
	Gov't	Cash							$0.00
		In-kind							$0.00
	Industry	Cash							$0.00
		In-kind							$0.00
Total Spent for Period 1			**$58,845.84**	**$890.49**	**$0.00**	**$1,410.37**	**$0.00**	**$0.00**	**$61,146.70**
Period 2 Dates:2013/07/01 to 2014/06/30	ALMA	Budgeted	$116,000.00	$0.00	$0.00	$63,495.00			$179,495.00
		Spent	$51,406.09	$0.00		$26,097.34			$77,503.43
	Gov't	Cash							$0.00
		In-kind							$0.00
	Industry	Cash							$0.00
		In-kind							$0.00
Total Spent for Period 2			**$51,406.09**	**$0.00**	**$0.00**	**$26,097.34**	**$0.00**	**$0.00**	**$77,503.43**
Period 3 Dates: 2014/07/01 to 2015/06/30	ALMA	Budgeted	$15,000.00	$0.00	$0.00	$29,205.00			$44,205.00
		Spent	$56,095.89	$2,302.90		$29,919.70			$88,318.49
	Gov't	Cash							$0.00
		In-kind							$0.00
	Industry	Cash							$0.00
		In-kind							$0.00
Total Spent for Period 3			**$56,095.89**	**$2,302.90**	**$0.00**	**$29,919.70**	**$0.00**	**$0.00**	**$88,318.49**
Period 4 Dates: 2015/07/01 to 2016/06/30	ALMA	Budgeted	$0.00	$0.00	$0.00	$0.00			$0.00
		Spent	$40,183.51	$939.73	$306.00	$17,429.10			$58,858.34
	Gov't	Cash							$0.00
		In-kind							$0.00
	Industry	Cash							$0.00
		In-kind							$0.00
Total Spent for Period 4			**$40,183.51**	**$939.73**	**$306.00**	**$17,429.10**	**$0.00**	**$0.00**	**$58,858.34**
Period 5	ALMA	Budgeted	$0.00	$0.00	$0.00	$0.00			$0.00

Dates: 2016/07/01 to 2016/12/31		Spent	$15,716.22	$0.00	$750.00	$49,758.83			$54,795.06
	Gov't	Cash							$0.00
		In-kind							$0.00
	Industry	Cash							$0.00
		In-kind							$0.00
Total Spent for Period 5			**$15,716.22**	**$0.00**	**$750.00**	**$49,758.83**	**$0.00**	**$0.00**	**$54,795.06**

CUMULATIVE ALMA CASH SPENT	$**222,247.55**	**$4,133.12**	**$1,056.00**	**$124,615.33**	**$0.00**	**$0.00**	**$352,052.00**
ORIGINAL PROPOSAL	**$251,500.36**	**$0.00**	**$0.00**	**$98,625.00**	**$0.00**	**$0.00**	**$352,052.00**

Detailed report on Salary expenditure

Employee's Position	Name			Salary (including benefits)					Encumbrance	Area
(Title)	Given	Surname		FY2013	FY2014	FY2015	FY2016	FY2017		
Research Assistant	Janis	Baarta		10,361.56	-	9,543.90	-	-	**19,905.46**	Dietary
Research Associate	Rosica	Valcheva		35,626.95	36,947.30	39,271.68	36,953.35	3,732.54	**152,531.82**	Microbiome
Term Employment	Breanne	Aylward		-	-	-	124.01	-	**124.01**	Dietary
Visiting Researcher	Celine	Christin		4,990.91	-	-	-	-	**4,990.91**	Dietary
Graduate Student	Ammar	Keshteli		7,866.42	14,458.79	7,280.31	3,106.15	1,862.70	**34,574.37**	Dietary
Graduate Student	Melissa	Silva		-	-	-	-	10,120.98	**10,120.98**	Microbiome
Sub-total				58,845.84	51,406.09	56,095.89	40,183.51	15,716.22		
Total									**222,247.55**	

Detailed report on Operational expenditures

Operational expense	Description	Reporting period					Encumbrance
		FY2013	FY2014	FY2015	FY2016	FY2017	
Patient recruitment	***Advertisement***	-	45.74	3,694.42	522.27	-	**4,262.43**
	Sample kit shipment	73.64	-	438.67	232.22	-	**744.53**
	Gift cards	-	-	450.00	800.00	905.00	**2,155.00**
	Parking reimbursement	-	-	400.00	910.00	404.00	**1,714.00**
Patients sampling	***Clinical Research Unit visits***	1,200.00	400.00	3,180.00	8,100.00	-	**12,880.00**
	Sample kits	-	15.67	2,781.68	147.85	-	**2,945.20**
	BioBank sample deposit	-	-	-	925.00	550.00	**1,475.00**
	Dietitian	-	1,596.32	2,787.16	5,185.33	7,172.19	**16,741.00**
Database Creation and Maintenance (EPICORE)		-	2,160.10	12,725.00	-	-	**14,885.10**
Software licenses		-	74.51	110.58	-	-	**185.09**
Fecal calprotectin		-	126.48	2,841.76	142.12	2,954.83	**6,065.19**
Metabolomics		-	6,000.00	-	-	35,100.00	**41,100.00**
Microbiome analysis	***DNA extraction***	-	-	-	-	1,924.74	**1,924.74**
	DNA sequencing	-	-	-	-	748.06	**748.06**
Conference registration		76.73	238.78	356.46	118.14	-	**790.11**
General lab supplies		60	15,439.74	153.97	346.17	-	**15,999.88**
Sub-total		1,410.37	26,097.34	29,919.70	17,429.10	49,758.82	
Total							**124,615.33**

Justification of Project expenditures

The Project aimed to perform a 3-year dietary study in patients with ulcerative colitis in remission in two phases: Phase 1) A pilot study to identify if patients who had experienced recent relapse (less than 18 months) differ from long-time patients in remission; and Phase 2) An interventional study to identify if the "Alberta Anti-inflammatory Diet" (study diet) will prevent disease relapse in patients who had experienced recent flare (less than 18 months) in comparison to "Canada Food Guide Diet" (control diet). The two phases (and the project respectively) were completed in a period of 5 years instead of 3. Given the longer project completion time, there are differences in the expenditures made per categories. The initially approved budget projected Salary expenditures of **$251,500.36** and Operational expenditures of **$98,625.00** for a period of 3 years. At the time of Project completion, $**222,247.55** were actually spent for personnel salary (including research staff and graduate students), and $**124,615.33** were spent for study supplies and materials for a period of 5 years.

The major variance in the operational budget (25% over) was produced by the following reasons:

1) The initially approved budged for the Phase 2 study was based on a proposed study population of 40 patients, however following the completion of the pilot study (phase 1) we concluded that this sample size is small and a bigger number of patients are needed in order to reach any statistical significance. Therefore 53 patients were enrolled in Phase 2, leading to bigger operational costs for sample processing and analysis, (most importantly increased costs for metabolomics analysis)
2) Unaccounted costs associated with patient recruitment and sampling, such as Study advertisement in print media ($4,262.43), dietitian honorarium for diet counseling ($16,741), a fee for sample deposit in CEGIIR Biobank ($1,475), and database creation and management by EPICORE (University of Alberta) ($14,885.10).

The major variance in the budget for personnel salary (10% less) was produced by the following reasons:

1) Only one research associate (Rosica Valcheva) was appointed to this project on 0.5 FTE rate
2) One graduate student (Ammar Keshteli) was initially assigned to the project. In addition in 2015 the student was awarded with Alberta XXXX? Award for PhD and the Project did not further contribute to the student's stipend.
3) Other students who received training in the framework of the Project (Floris van den Brand, Tess Vader and Thomas Hoevers, all master students at Vrije Universiteit Amsterdam, the Netherlands) were supported by other fundings.

M_Dec 7,2017_YH

UNIVERSITY OF ALBERTA

Research Services Office
222 Campus Tower
8625 - 112 Street, Edmonton, AB T6G 2E1 Canada

Statement of Award & Expenditure

For the Period Ending - June 30, 2017

Name of Grantee - Project Role	**Department**	**Reference Award Number**	
Dieleman,Levinus - Principal Investigator	270800 - MED Medicine Department	2012Q007R/QFH-11-046	
University Project Number	**Project/Grant Description**	**Start Date :**	**End Date :**
RES0011328	AgFC/MULTI 2012Q007R/QFH11046	July 1, 2012	June 30, 2017

	Reporting Period July 1, 2012 to June 30, 2017	
OPENING BALANCE	**0.00**	
AWARD		
Direct Costs	352,052.00	cr
Total Funds Available	**352,052.00**	**cr**
EXPENDITURE		
Salaries & Benefits		
Undergrad Stu Salary & Benefit		
Grad Student Salary & Benefits		
Graduate Salaries	17,116.96	dr
Graduate Student Benefits		
Postdoctoral Salary & Benefits		
Postdoctoral Fellows Salaries		
Postdoctoral Fellows Benefits		
Other Sal & Adj (all benefits)		
Other Salaries	167,271.38	dr
Other Benefits	37,859.21	dr
Professional & Technical Svcs		
Equipment	1,056.00	dr
Materials Supplies & Other Exp	124,615.33	dr
Travel	4,133.12	dr
Transfers Out		
Total Funds Expended	**352,052.00**	**dr**
Indirect Cost Expenses	0.00	
Total EXPENDITURE	**352,052.00**	**dr**
PROJECT/ GRANT BALANCE AT: December 31, 2017	**0.00**	

SIGNATURES

I hereby certify that the above statement is correct and that the expenditures conform to the general conditions imposed by the sponsoring agency, and were for the purpose for which the grant was made.

I certify that the expenditures summarized above were incurred wholly by and paid on behalf of the grantee, and that the vouchers are available for monitoring purposes.

Project Manager – Role: Dieleman,Levinus-Principal Investigator

Date

Business Officer, Research Services Office

Date

Section E: Research Team Signatures and Employers' Approval

1. Personal data sheet(s) for NEW Principal Investigator and/or team members.

Complete a personal data sheet for any NEW Principal Investigator* and/or *research team members. Any NEW Principal Investigator and/or team members MUST sign this form, as well as an authorised representative from his/her organisation of employment. (Duplicate this sheet as required)

NB: If there is a NEW Principal Investigator, please advise the funders' representative of this change in writing in addition to filling out this personal data sheet. This will allow the funder(s) to make the necessary administrative changes to the project file.

NB: Existing Principal Investigator and team members DO NOT need to complete a new form.

<table>
<tr><td colspan="4">Name:
Dr./Mr./Ms./Mrs. Last First</td></tr>
<tr><td colspan="4">Position / Organisation / Dept.:</td></tr>
<tr><td colspan="2">Address:
Street /Box #</td><td colspan="2">City Prov. Postal Code</td></tr>
<tr><td colspan="4">E-mail:</td></tr>
<tr><td colspan="2">Phone:</td><td colspan="2">Fax:</td></tr>
<tr><td colspan="2">Degrees / Certificates / Diplomas:</td><td colspan="2">Institution:</td></tr>
<tr><td colspan="4">Publications and Patents:</td></tr>
<tr><td colspan="2">Number of refereed papers:
Relevant patents obtained:</td><td colspan="2">Conference proceedings:
Other relevant publications from the past 5 yr:</td></tr>
<tr><td colspan="4">Other evidence of productivity (e.g., administrative roles, grants held, awards received, etc.):</td></tr>
<tr><td colspan="4">NEW Team Member</td></tr>
<tr><td colspan="2">Name:</td><td colspan="2">Title/Organisation:</td></tr>
<tr><td colspan="2">Signature:</td><td colspan="2">Date:</td></tr>
<tr><td colspan="4">NEW Team Member's Employer's Approval</td></tr>
<tr><td colspan="2">Name:</td><td colspan="2">Title/Organisation:</td></tr>
<tr><td colspan="2">Signature:</td><td colspan="2">Date:</td></tr>
</table>

2. **The principal investigator and an authorised representative from his/her organisation of employment MUST sign this form.**

Research team members and an authorised representative from their organisation(s) of employment MUST also sign this form.

Signatures may be scanned and submitted electronically. Original signatures should be retained by the PI and MAY be requested by the funder(s) in the future

By signing as representatives of the principal investigator's employing organisation and/or the research team member's(s') employing organisation(s), the undersigned hereby acknowledge submission of the information contained in this final report to the funder(s).

2. **The principal investigator and an authorised representative from his/her organisation of employment MUST sign this form.**

Research team members and an authorised representative from their organisation(s) of employment MUST also sign this form.

Signatures may be scanned and submitted electronically. Original signatures should be retained by the PI and MAY be requested by the funder(s) in the future

By signing as representatives of the principal investigator's employing organisation and/or the research team member's(s') employing organisation(s), the undersigned hereby acknowledge submission of the information contained in this final report to the funder(s).

Principal Investigator

Principal Investigator	
Name: Levinus A Dieleman	**Title/Organisation:** Professor, University of Alberta
Signature:	**Date:** December 15, 2017
Principal Investigator's Employer's Approval	
Name: Dr. B. Ballermann	**Title/Organisation:** Chair, DoM
Signature:	**Date:** Dec 22/2017

Research Team Members (add more lines as needed)

1. Team Member	
Name: Karen Madsen	**Title/Organisation:** Professor, University of Alberta
Signature: Karen Mad—	**Date:** December 19, 2017
Team Member's Employer's Approval	
Name: Barbara Ballermann	**Title/Organisation:** Chair, DoM
Signature:	**Date:** Dec 22/2017

2. Team Member	
Name: Ammar Hassanzadeh Keshteli	**Title/Organisation:** PhD student, University of Alberta
Signature:	**Date:** December 19, 2017
Team Member's Employer's Approval	
Name: Dr. B. Ballermann	**Title/Organisation:** Chair, Dom
Signature:	**Date:** Dec 22/2017

3. Team Member	
Name: Rossica Valcheva	**Title/Organisation:** Research Associate, University of Alberta
Signature:	**Date:** December 20, 2017
Team Member's Employer's Approval	
Name: Dr. B. Ballermann	**Title/Organisation:** Chair, Dom
Signature:	**Date:** Dec 22/2017

4. Team Member	
Name: Richard Fedorak	**Title/Organisation:** Professor, University of Alberta
Signature: Richard Fedorak	**Date:** Dec 18, 2017
Team Member's Employer's Approval	
Name: Dr. B. Ballermann	**Title/Organisation:** Chair, Dom
Signature: B. Ballermann	**Date:** Dec 22/2017

5. Team Member	
Name: David Wishart	**Title/Organisation:** Professor, University of Alberta
Signature: David Wishart	**Date:** Dec. 15/17
Team Member's Employer's Approval	
Name: Dr. Keith Tierney Assoc. Chair Research	**Title/Organisation:** Dept Biological Sciences University of Alberta
Signature:	**Date:** DEC 19th/2017

Section F: Suggested reviewers for the final report :

Provide the names and contact information of four potential reviewers for this final report. The suggested reviewers **should not be current collaborators**. The funder(s) reserves the right to choose other reviewers. Under *Section 34* of the *Freedom of Information and Protection Act FOIP)* reviewers must be aware that their information is being collected and used for the purpose of the external review.

Reviewer #1

Name:	Elena Verdu
Position:	Associate Professor
Institution:	Farncombe Family Digestive Health Research Institute, Department of Medicine, McMaster University, Hamilton, Ontario, Canada
Address:	
Phone Number:	
Fax Number:	
Email Address:	verdue@mcmaster.ca

Reviewer #2

Name:	Philip Sherman
Position:	Professor
Institution:	Department of Pediatrics, University of Toronto
Address:	
Phone Number:	
Fax Number:	
Email Address:	philip.sherman@sickkids.ca

Reviewer #3

Name:	Stephen Vanner
Position:	Professor
Institution:	Gastrointestinal Diseases Research Unit, School of Medicine, Queen's University
Address:	
Phone Number:	
Fax Number:	
Email Address:	vanners@hdh.kari.net

Reviewer #4

Name:	Jon Meddings
Position:	Professor
Institution:	University of Calgary, Dean of Medicine
Address:	3330-Hospital Drive, Calgary T2N 4N1
Phone Number:	1-403-944-6555
Fax Number:	
Email Address:	meddings@ucalgary.ca