THE UNIVERSITY OF SYDNEY

Study Protocol

FULL STUDY TITLE

Influence of Extra Virgin Olive Oil intake on Disease Activity and Gut Microbiota Profile of Community Dwelling Adults with Ulcerative Colitis in Comparison to Healthy Subjects

SHORT STUDY TITLE

***COLONiC**: Consequences of OLive Oil replacemeNt on ulcerative Colitis*

CONFIDENTIAL

STATEMENT OF COMPLIANCE FOR NON DRUG OR DEVICE CLINICAL TRIALS

This document is a protocol for a clinical research study. The study will be conducted in compliance with all stipulations of this protocol, the conditions of ethics committee approval, the NHMRC National Statement on Ethical Conduct in Human Research (2007) and the Handbook for Good Clinical Research Practice (GCP)

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1. GENERAL INFORMATION

Study Protocol: COLONiC: Consequences of OLive Oil replacemeNt on ulcerative Colitis

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2. Resources

Resources necessary for the project to be conducted

The University of Sydney, Faculty of Health Sciences Cumberland Campus has the following resources which will be used during the study:

- Keiser pneumatic resistance training machines
- Treadmill
- Dual energy x-ray absorptiometry (DXA) scanner (Lunar Prodigy, GE Medical Systems, Madison, WI)
- Consumables for blood collection and storage
- Consumables for stool collection and storage
- -80 degree freezer
- Respiratory gas analysis system (Medgraphics PFX Ultima, USA),
- Axivity AX3 accelerometer
- 12-lead electrocardiogram

With considerations to the high risk of osteoporosis in individuals living with IBD including those with UC, the use of the DXA to assess bone mineral density as part of this project is of great importance and is usually included in standard of care. Identifying clinical features of participants with UC would further inform us of any potential risk associated with strength assessment and physical testing procedures. With consideration to apparently healthy participants without UC, assessment of bone mineral density would also allow us to identify any underlying issues and potential risks associated with strength assessment and physical testing procedures. It should be noted that the use of the DXA for this study has received Radiation Safety Approval as of 15/01/2019; approval number RSC1901.

2. SYNOPSIS

Ulcerative Colitis (UC) is a progressive, chronic condition defined by acute bouts of inflammation accompanied by severe gastrointestinal symptoms and periods of remission. Prevalence is increasing globally while mechanisms of the disease are poorly understood. Environmental factors including diet have been proposed as a determinant for UC aetiology; however evidence beyond epidemiological studies and animal studies has been limited. Particularly, the role of specific dietary elements and lifestyle interventions with the potential of facilitating clinical remission and improve symptoms has not been well characterised. Despite this gap in research, diet has been consistently identified to be a significant factor influencing disease progression and symptoms in those with UC. This highlights the need of robustly designed trials investigating the impact of dietary components and disease expression of UC in the active phase of the disease.

Separate lines of research have identified the Mediterranean Diet (Med Diet) as a dietary pattern of interest with consideration to inflammatory processes. The protective effects of the diet have been partially attributed to the use of olive oil as a primary fat source, and preliminary evidence has demonstrated its protective effects in experimental UC models. Dietary intake of olive oil appears to attenuate disease aetiology in animal models of colitis and modulate the gut microbiome, which has been hypothesised to influence mucosal healing and symptomatic improvements. Translation of these studies into human trials

however have been limited, with the majority of trials identified being uncontrolled clinical trials and retrospective studies. As such we will conduct a novel study examining the effects of chronic consumption of extra virgin olive oil on the gut biota and symptoms of community dwelling UC cohorts in comparisons to healthy controls. Secondary outcomes include markers of inflammation, macro and micronutrient intake, nutrition status and quality of life.

We hypothesise that replacement of usual dietary fats with extra virgin olive oil will improve disease activity scores in those with Ulcerative Colitis relative to the usual care control group. Additionally, the impact of extra virgin olive oil consumption will be distinct between those with active UC and healthy controls. Using results from past diet studies in UC, the effect size of diet was estimated to be 0.917. Sample size was calculated at an alpha of 0.05 and beta 0.8, resulting in an estimated sample size of 60 participants.

The COLONIC will be a novel study identifying the impact of dietary fat types on the disease expression of UC and changes to the gut microbiota in comparisons to healthy subjects without IBD.

3. RATIONALE / BACKGROUND

Ulcerative Colitis (UC) is an inflammatory bowel disease (IBD) mainly affecting the superficial mucosa of the large intestine (colon) and rectum. It is a chronic, progressive condition characterised by periods of acute inflammation and remission (1, 2). Typically associated with developed countries such as North America and Northern Europe, UC is emerging as a global disease. Prevalence has increased in recent years and developing nations, where the condition is uncommon, are reporting higher incidences in line with industrialization (3, 4). Interestingly several studies on UC prevalence in second generation migrants found comparable rates with the native population of the country they migrated to (5-7). This suggests that environmental factors significantly contribute to UC aetiology, and may hold clues for future adjunctive non-pharmacological approaches (8).

Diet as a modifiable risk factor in the development (2, 9) and progression (10, 11) of UC has been documented however poorly understood. Particularly, elements of the diet which promotes mucosal healing and facilitates remission in active disease remains uncertain, with a high degree of variability across studies (12). Consequently, evidence based dietary guidelines for the management of UC is severely lacking (13).

Separate lines of research have identified the protective role of a Mediterranean Diet (Med Diet) in inflammation (14) including in IBD (15). Part of this protective property may be attributed to the consumption of olive oil, contributing to a higher proportion of Oleic Acid (OA) in the diet (16, 17). Olive oil has demonstrable effects in animal models of colitis (18, 19) and effectively modulates the gut microbiota (20, 21), however well-designed human studies are limited.

As such, we will examine the effects of olive oil on the disease activity of UC and identify potential changes to the gut microbiome in human cohorts. We hypothesise that chronic olive oil consumption has marked effects on UC symptoms, gut microbiome profile, and inflammatory

markers. Additionally, we also hypothesise that the changes in the gut microbiome in those with UC will be distinct from the changes observed in healthy subjects without IBD.

4. AIMS

1. To conduct the first RCT of the effects of chronic extra virgin olive oil consumption on the human gut microbiota in both healthy and diseased states
2. To conduct the first RCT of the impact of extra virgin olive oil on the expression of UC in those with mild-moderate disease states
3. To assess the relationship between inflammation, gut microbiota changes and UC symptoms in response to a short term dietary prescription
4. To investigate the course of the alteration and persistence of the responses to dietary change by characterizing the gut microbiota and biological markers of the disease during the intervention period and any sustained or residual effects 4-weeks post-intervention.

Primary Hypotheses

1. Replacement of dietary fats with extra virgin olive oil will positively influence disease activity scores and disease symptoms
2. Dietary fat replacement will modulate the gut microbiota profile of those with UC and healthy controls.
3. Distinct changes in the gut biota will occur between participants with UC and healthy controls once exposed to the diet intervention, indicating the importance of gut microbiota in modulating disease outcomes.
4. The impact of short term dietary changes has longer term effects post cessation of the diet.

Secondary aims of the study will be to determine the effect of extra virgin olive oil on inflammatory markers of UC and quality of life measures, and characterization of dietary strategies in individuals living with UC. Adherence to the intervention protocol, weekly health status and adverse events also constitute secondary study outcomes.

5. PARTICIPATING SITES

Clinical Laboratories, and institutions involved in the research

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6. RESEARCH PLAN / STUDY DESIGN

6.1 TYPE OF STUDY

A randomized, controlled, parallel clinical trial.

6.2 STUDY DESIGN

Participants will be divided into two groups; individuals with Ulcerative Colitis (n: 30) and healthy controls (n: 30). Participants within these two groups will be randomised after screening in a 1:1 ratio to nutrition intervention or usual care. We will use a concealed, computer-generated sequence of randomly permuted variable blocks of four or 6, stratified for presence/absence of disease and sex, generated by an online program created by a statistician not otherwise involved in the study, available at www.randomization.com. Randomisation will occur at the completion of the baseline assessment and will be concealed by a statistician not otherwise involved in the study. The baseline assessor will confirm the participant's assignment at the end of the study with this staff member and communicate the study allocation to the participant to discuss next steps.

Intervention arms

Treatment Group: Replacing free fats with extra virgin olive oil

The treatment groups for both participants with UC and healthy controls will be instructed to modify their current dietary intake by replacing a portion of their fat intake with extra virgin olive oil (EVOO) over a 4-weeks. We will aim to replace all free fats or fats added into meal preparation which includes but is not limited to cooking oil, fat spreads, butter, and oil based dips and sauces. For the purposes of this study, we will aim to ensure that energy intake between participant's usual diet and the intervention remains the same. As such, average energy contributions of free fats in the diet will be calculated, which will then be converted to the quantity of EVOO required to be consumed daily. Kitchen weight scales and marked bottles will be used to assist estimating the participant's daily requirement. The study dietitian will provide information on strategies to incorporate the oil into the participant's daily meals and a checklist for daily intake to assist with the behaviour change. If the participant is fasting for any reason during the study, the intervention will resume once the participant is able to consume meals again. No top ups from previous days will be required. For participants with shared meal preparation responsibilities in the household, additional bottles of EVOO will be provided. The EVOO used for this study will be Australian produced EVOO currently available for sale as a food item in accordance with Australian food regulations and will not be a special product or formulation. Upon completion of the 4 weeks of the intervention, all remaining EVOO bottles will be collected and participants will be requested to return to consuming their regular diet. During this 4-week washout period, participants will resume their usual intake of free fats to identify any sustained effects of the intervention.

Both during active intervention and the washout period, participants will be requested to maintain all their daily activities including physical activity, sleep and exercise. All of the documentation of changes in health care, habitual exercise or diet noted for the control group below will also be recorded in the intervention group.

Control Group: Usual Care

Participants will continue to receive usual care as documented at baseline. Any new medical therapy for UC commenced during the study period will be recorded, as well any change in chronic treatments for UC, surgeries, other new medical conditions or treatments, or other interventions employed for their known or purported health benefits (e.g., pre- and pro-biotic supplementation, acupuncture, hypnotherapy, and counseling). Participants will be asked to maintain their habitual lifestyle regarding diet and exercise throughout the study, but any changes they do make will be recorded.

6.3 PARTICIPANTS & DATA COLLECTION

We will recruit men and women aged ≥ 18 years with Ulcerative Colitis who are able to provide written informed consent will be recruited from gastroenterologists as well as via community/social media advertising in the greater Sydney area.

Participants with UC Inclusion Criteria:

1. Aged ≥ 18 in 2018
2. Ulcerative colitis (UC) >3 months' duration of any extent.
3. Stable medication. No changes to medication or therapies in the preceding 4 weeks.

Participants with UC Exclusion Criteria:

1. Prior or concomitant fecal microbiota transplantation or any rectal preparations currently.
2. Antibiotic/anti-tuberculosis treatment for any reason during the study period or in the preceding 4 weeks.
3. Probiotic supplements or biological/monoclonal antibody agents during the study period or in the preceding 12 weeks. Food based probiotics such as yoghurt and kefir will be allowed.
4. Significant prior gastrointestinal surgery (e.g., colon resection, colectomy, appendectomy) or clinical evidence of any comorbid chronic disease that may interfere with the patient's ability to enter the trial and undertake the intervention planned.
5. Coexistent serious autoimmune disease such as rheumatoid arthritis or systemic sclerosis.
6. Planned major surgery within the first 3 months after randomization.
7. Pregnancy or female planning pregnancy within the first 3 months after randomization.
8. Participation in another clinical trial for with concurrent participation is deemed inappropriate.
9. Daily consumption of olive based product(s) in any form (including oil, pickles, tapenade, spreads, and supplements) as determined by the diet history and medication list

10. Currently on a special diet in which participation is deemed inappropriate (e.g. low residue diet, liquid diet, low fat diet)

We will also recruit men and women aged ≥ 18 years with no significant chronic or acute disease who are able to provide written informed consent to participate in the study as the healthy control group. Recruitment will be achieved through community/social media advertising in the greater Sydney area alongside the recruitment strategy for participants with UC.

Healthy Control Inclusion Criteria:

1. Aged ≥ 18 years in 2018
2. Willing to be randomized into one of the two study arms and participate in the intervention prescribed
3. Stable medication. No plans to modify current medication or supplement regiment during the study period and no changes to medication in the preceding 4 weeks.

Healthy Control Exclusion Criteria:

1. Those in remission from UC as defined by an assessment by a gastroenterologist.
2. History of fecal microbiota transplantation or any rectal preparations including enemas.
3. Antibiotic/anti-tuberculosis treatment for any reason during the study period or in the preceding 4 weeks.
4. Probiotic supplements or biological/monoclonal antibody agents during the study period or in the preceding 12 weeks. Food based probiotics such as yoghurt and kefir will be allowed.
5. Chronic or acute disease requiring medical or therapeutic intervention including but not limited to diabetes, IBD, chronic kidney disease, cancer, IBS.
6. Coexistent serious autoimmune disease such as rheumatoid arthritis or systemic sclerosis.
7. Significant prior gastrointestinal surgery (e.g., colon resection, colectomy, appendectomy) or clinical evidence of any comorbid chronic disease that may interfere with the patient's ability to enter the trial and undertake the intervention planned.
8. Planned major surgery within the first 3 months after randomization.
9. Pregnancy or female planning pregnancy within the first 3 months after randomization.
10. Participation in another clinical trial for with concurrent participation is deemed inappropriate.
11. Daily consumption of olive-based product(s) in any form (including oil, pickles, tapenade, spreads, and supplements) as determined by the diet history and medication list
12. Currently on a special diet in which participation is deemed inappropriate (e.g. low residue diet, liquid diet, low fat diet) or under the supervision of an Accredited Practising Dietitian or other relevant healthcare professional

6.4 SAMPLE SIZE

Power Calculations:

At the time of writing, there were limited published papers identifying the effects of diet on active Ulcerative Colitis. We identified three studies on dietary intervention; 1 randomized controlled trial of a single food component (22) and two uncontrolled trials of a comprehensive diet intervention (23, 24). Only the two uncontrolled studies had enough information to allow for the calculation of an effect size. Hedges' bias corrected effect sizes were calculated from published means and standard deviations of a single group, pre-post study involving a dietary intervention over 8 weeks (23) and 6 weeks (24) (Table 1).

The effect size of a dietary intervention in active Ulcerative Colitis calculated from these studies were 0.917 (23) and 2.638 (24) respectively. With consideration to the current study design which includes adult populations and the use of the same scoring tool, we have selected the paper by Konijeti, et. al (24) to estimate sample size.

Table 1

Citation	Outcome	Baseline	Post dietary intervention	Mean difference	Pooled SD	Effect size	Sample size (n total)
Suskind et al., (23)	Pediatric Ulcerative Colitis Activity Index	28.3 (8.8)	6.7 (2.9)	- 21.6	6.6	0.917634	48
Konijeti et al., (24)	Partial Mayo Score	5.8 (1.2)	1.2 (2.0)	- 4.6	1.6	2.638281	16

The Partial Mayo Score is a validated 9-point, non-invasive method of assessing disease severity in Ulcerative Colitis comprising of stool frequency, bleeding, and subjective assessments of disease activity. Remission is set at ≤ 1, Mild Disease between 2-4, Moderate Disease between 5-6, and Severe Disease ≥ 7 (25).

Based on the available evidence, we hypothesize that EVOO consumption would elicit a similar reduction to average Mayo Score in the intervention group compared to those randomized into the usual care group. Due to the single dietary intervention used in this study and accounting for the uncontrolled study cited, a conservative approach to estimate effect size was implemented.

We calculated effect size based on a 2 point (22%) reduction in the Partial Mayo Score (less than half of the mean difference cited in literature), with alpha 0.05 and beta 0.8. Effect size was calculated to be 1.25, with a sample size of 12 participants per group (24 individuals with Ulcerative Colitis randomized to either the intervention or usual care) required to determine changes in disease activity post intervention. Accounting for a 16% dropout rate which occurred in both studies selected, we will aim to recruit 28 participants with Ulcerative Colitis.

With consideration to those without a history of Inflammatory Bowel Disease, we will also aim to recruit an equal number of participants. Due to the limited evidence available on the effects of EVOO consumption on the gut microbiome and inflammatory markers in apparently healthy individuals, the recruitment number of apparently healthy controls are not based on any hypotheses on the differences between those living with UC and non-IBD cohorts subjected to EVOO consumption or dietary intervention. In total, we will aim to recruit 56 participants for this study; 28 individuals with UC and 28 individuals without a history of IBD.

Expected duration of the study and start times

It is expected that the study will take four years to complete. This includes 6 months to set up the study, including recruitment and training of the study personnel, procuring study resources, and preparation of the recruitment and study materials.

It is expected that recruitment will begin no later than April 2019. We are aiming for a conservative estimate of 1 participant per week, and means all participants will be recruited by June 2020, however additional time has been allocated to account for delays related to other investigations, work and travel arrangements, and recent changes to therapy.

The baseline assessments will start in April 2019 and end latest by August 2020.
The post-intervention assessments will start in May 2019 and end by November 2020, with additional time allocated to account for unexpected delays in recruitment and assessment.
The main outcome publication will be prepared by early to mid-2021. Table 2 illustrates the overall project schedule.

Table 2. Colonic Project Schedule

	2018	2019				2020				2021
	Oct-Dec	Jan-Mar	Apr-Jun	Jul-Sep	Oct-Dec	Jan-Mar	Apr-Jun	Jul-Sep	Oct-Dec	Jan-Mar
Study protocol and ethics approval	X	X								
Set-up of the study	X	X								
Recruitment of participants			X	X	X	X	X			
Baseline assessments			X	X	X	X	X	X		
Post-intervention assessments				X	X	X	X	X	X	
Preparation of main outcome publication								X	X	X

The research team will assess all outcomes at baseline, mid and post-intervention. Data collection and entry will be performed using both paper based forms and online via RedCap data managing system.

6.5 STATISTICAL ANALYSIS

Intention-to-treat analysis regardless of dropout or study adherence is the primary analytic strategy. Primary and secondary continuous outcomes will be analyzed via linear repeated measures mixed models for testing of Group X Time interaction, with covariate adjustment over baseline and 4 weeks to characterize the intervention effects. In addition, outcomes at 5-8 weeks will identify and characterize any residual effects of the intervention during the washout phase and will be analyzed with respect to the intervention changes to determine the time course, if any, of residual effects. Covariates selected *a priori* based on the literature include age, sex, macronutrient intake, energy intake, and habitual physical activity level; other potential confounders identified at baseline may be added as required. A mediation analysis will be conducted via the PROCESS macro for SPSS version 2.13.2 to determine the indirect effect of dietary change on objective and subjective outcomes mediated by hypothesized beneficial alterations in the gut microbiota, while accounting for any potentially direct effects of EVOO.

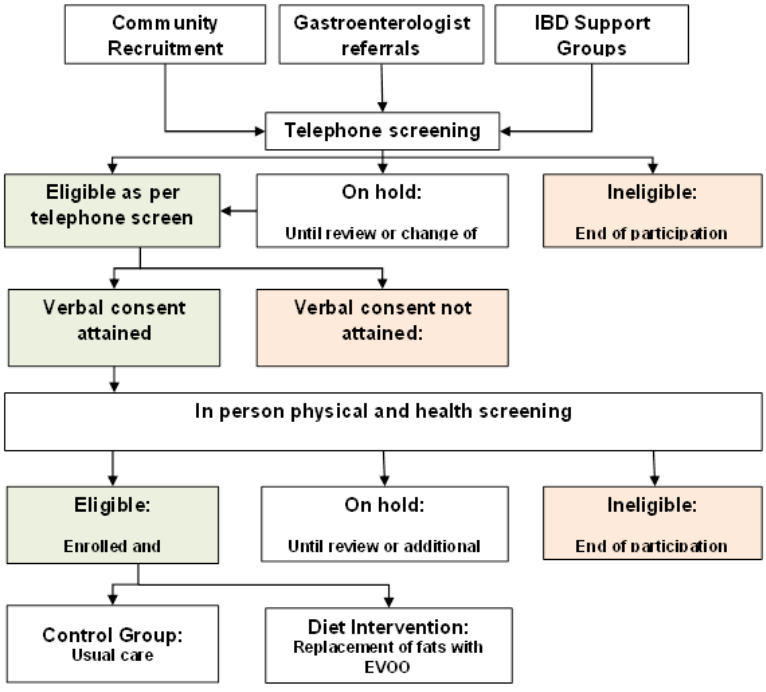
7. ETHICAL CONSIDERATIONS

7.1 RECRUITMENT AND SELECTION OF PARTICIPANTS

Potentially eligible participants will be recruited from the greater Sydney area. The study will be advertised broadly via flyers distributed through doctor's surgeries, gastroenterologist clinics, and offices of health care facilities. The study will also be advertised via social media (e.g., Facebook and Twitter), university intranet and websites, and local radio. Permission will be obtained from each of these sites prior to advertising the study. All advertising material will have the contact number and email address of our research team on it, and interested participants will contact us directly if they wish to hear more about the study and wish to schedule a telephone screen for eligibility. We will avoid real or perceived coercion by indicating in the recruitment letters of invitation and telephone screen that this is completely voluntary and will in no way influence their health care and treatment. The screening and initial contact will be done by the student overseeing the study. We will not be contacting people by email addresses without their permission after initial telephone screening and will not be sending letters or calling people directly who have not previously given us permission to do so or have called us in response to one of our recruitment materials.

Potentially eligible participants will undergo a two-stage screening process which includes a. telephone screen and in-person physical and health screen. Both participants with UC and healthy controls recruited will undergo the same screening process as illustrated in Figure 1.

Figure 1. COLONIC Screening Procedure



Telephone screen

Initial screening	
Presence of Inflammatory Bowel Disease *(only for participants with UC)*	Participant confirms that have been diagnosed with Ulcerative Colitis (UC) at least 3 months or greater in duration. Confirmation of disease activity via the Partial Mayo Scoring Index for UC
Confirmation of eligibility	Participants confirm that they do not meet any of the exclusionary criteria.
Medical history and medication review	Review of medical history and current medications and/or therapies.
Medical contact details	Contact details of treating general practitioner (GP) and gastroenterologist are collected.
Confirmation of inclusion criteria with GP and relevant specialists	A letter is sent to the participant's GP and relevant specialists for confirmation of inclusion criteria and to rule out any exclusionary study criteria, unstable diseases, or contraindications to participation in the study.

Participants who meet the study criteria as assessed during telephone screening. Those deemed to be eligible and are still interested in participating will be requested to attend an in person physical and health assessment at the University of Sydney Cumberland Campus C Block Clinic at a nominated date. Prior to any assessments at the clinic, participants will be briefed on the Participant Information Statement (PIS) with a copy provided to the participant. This will be followed by a written, witnessed, and informed consent on participating in the COLONIC Study.

Assessments will be completed over 2 days, spread at least between 7 days, with the first assessment to include a physician health screen, a variety of questionnaires and physical performance measures. Upon completion of this first assessment, participants will be fitted with an Axivity AX3 accelerometer to wear over a 7-day period. Participants will also be provided with instructions on completing a 3-day food diary and stool collection kit for home collection. All provided samples and diaries will be collected during the second assessment.

In-person Physical and Health screen

This screening takes place over two days (at least 7 days apart). Findings obtained from the assessments over the 2-day period may make the person ineligible for participation or placed on-hold for further investigation. Note that day 2 of the assessment will be done while the participant is in a fasted state.

DAY 1 - Physical and Health Screen	
Physician screening and exercise stress test	Physician health screen, resting electrocardiogram (ECG), exercise stress test with ECG.
Inflammatory bowel disease activity *(only for participants with UC)*	UC disease activity including subjective physician assessment, via the Partial Mayo Scoring Index for UC.
Muscle strength	1 repetition maximum tests for the chest press, seated row, triceps push down, lateral pull-down, leg press, leg extension, leg flexion, hip abduction and hip extension.
Muscle power	Chest press, seated row, triceps push down, lat pull-down, leg press, leg extension, leg flexion, hip abduction and hip extension.
Questionnaires	Baseline measures: Demographic, physical activity and sedentary behaviour, nutritional status, food related questionnaires, food choice questionnaires, and functional status questionnaires.
Physical activity and sleep assessment	Participants will be fitted with an Axivity AX3 accelerometer to wear for 7 days and provided with a sleep diary. Paffenbarger Physical Activity Questionnaire will be completed in person.

DAY 2 – Physical and Health Screen (Participants arrive in a fasting condition)	
Stool sample collection	First bowel motion of the day to be collected. Can be completed either at home using the supplied collection kit or in clinic prior to assessment 2.
Anthropometric measures and body composition	Weight, height, waist circumference, DXA scan. DXA assessments to measure bone mineral density will be completed at baseline only for all participant groups.
Blood test	Lipopolysaccharide (LPS), C-reactive protein (CRP), tumor necrosis factor and interleukins 1, 6, 10 and 12, full blood count. Save serum and plasma for other markers.
Collection of forms provided at assessment 1	Review of electronic and/or paper based 3-day food diary and sleep diary. Axivity
Questionnaires	Inflammatory Bowel Disease (IBD) Questionnaire, Depressive Symptoms will be assessed via the Hospital Anxiety and Depression Scale (HADS) and Patient Health Questionnaire (PHQ-9), fatigue will be assessed via the IBD Fatigue Scale.

Individuals deemed eligible as assessed during in-person physical and health screen, and who are still interested to participate, are enrolled and randomised into the study.

Figure 2 illustrates an overview of the study flow from screening of participants until completion of the study.

Figure 2. COLONIC Study Scheduling

2 – 3 Weeks	**Stage 1 Screen**	**Telephone Screen (Duration ~60 minutes)**
	Participant Information Sheet & Consent Form	
	Baseline Clinic Visit 1	**In person physical and health screen (duration ~4-5 hours)** - Participant Information Sheet (PIS) & informed consent - Questionnaires (demographic, nutrition status) - Physician Screen - Functional Assessment - 3-day Weighted Food Diary Instruction - Stool Collection Instructions - 7-day Axivity Monitor Attachment *Location: The University of Sydney, Faculty of Health Sciences, Lidcombe*
	Baseline Clinic Visit 2 (must be at least 8 days after Clinic 1)	**In person physical and health screen (duration ~5-6 hours)** - Fasted assessments - Anthropometry - Collection and review of assessment 1 forms *Location: The University of Sydney, Faculty of Health Sciences, Lidcombe*
	Randomisation into 1 of 4 Groups (UC Usual Care, UC Intervention, Control Usual Care, Control Intervention)	
4 Weeks	**Intervention (Week 1-4)**	**Intervention**: Replacement of usual free fats with the study fats **Usual Care**: Maintain all usual daily activities **Weekly teleconference for all study groups** (duration 15-20 minutes) Review new symptoms, changes to current therapy, lifestyle, visits to a healthcare professional
2 Weeks	**Mid-Point Assessment (Week 5, over 2 days)**	**Repeat a portion of assessments in Clinic Visit 1 and Clinic Visit 2** - Fasted assessments - Nutrition assessment & food diaries - Stool collection - Questionnaires *Location: The University of Sydney, Faculty of Health Sciences, Lidcombe*
4 Weeks	**Washout Period (Week 6-9)**	**Intervention 1 and 2**: Collection of all unused study fats, resume usual daily behaviour prior to study **Usual Care**: Maintain all usual daily activities **Weekly teleconference for all study groups** (duration 15-20 minutes) Review new symptoms, changes to current therapy, lifestyle, visits to a healthcare professional
2 Weeks	**Final Assessment Clinic Visit 1**	**In person physical and health screen (duration ~3-4 hours)** - Physician review (as required) - Questionnaires (food intake, IBD symptoms, affect) - 3-day Weighted Food Diary Instruction - Stool Collection Instructions - 7-day Axivity Monitor Attachment *Location: The University of Sydney, Faculty of Health Sciences, Lidcombe*
	Final Assessment Clinic Visit 2 (must be at least 8 days after Clinic 1)	**In person physical and health screen (duration ~2-3 hours)** - Fasted assessments - Anthropometry - Collection and review of assessment 1 forms *Location: The University of Sydney, Faculty of Health Sciences, Lidcombe*
End of Study Participation		

During each week of the study the participants in both the intervention and control groups will be provided with a questionnaire to complete which will be used to assess their health status and reporting of events throughout the study period. The Partial Mayo Scoring Index for Ulcerative Colitis will be incorporated in these weekly health checks to subjectively assess disease activity and allow participants to report any changes to their symptoms.

7.1.1 Eligibility Criteria

We will recruit men and women aged ≥ 18 years with Ulcerative Colitis who are able to provide written informed consent will be recruited from gastroenterologists as well as via community/social media advertising in the greater Sydney area.

Participants with UC Inclusion Criteria:

1. Aged ≥ 18 in 2018
2. Ulcerative colitis (UC) >3 months duration of any extent.
3. Stable medication. No planned changes to current medication and therapies during the study period, and no changes to medication in the preceding 4 weeks.

Participants with UC Exclusion Criteria:

1. Prior or concomitant fecal microbiota transplantation or any rectal preparations currently.
2. Antibiotic/anti-tuberculosis treatment for any reason during the study period or in the preceding 4 weeks.
3. Probiotic supplements or biological/monoclonal antibody agents during the study period or in the preceding 12 weeks. Food based probiotics such as yoghurt and kefir will be allowed.
4. Significant prior gastrointestinal surgery (e.g., colon resection, colectomy, appendectomy) or clinical evidence of any comorbid chronic disease that may interfere with the patient's ability to enter the trial and undertake the intervention planned.
5. Coexistent serious autoimmune disease such as rheumatoid arthritis or systemic sclerosis.
6. Planned major surgery within the first 3 months after randomization.
7. Pregnancy or female planning pregnancy within the first 3 months after randomization.
8. Participation in another clinical trial for with concurrent participation is deemed inappropriate.
9. Daily consumption of olive based product(s) in any form (including oil, pickles, tapenade, spreads, and supplements) as determined by the diet history and medication list
10. Currently on a special diet in which participation is deemed inappropriate (e.g. low residue diet, liquid diet, low fat diet)

We will also recruit men and women aged ≥ 18 years with no significant chronic or acute disease who are able to provide written informed consent to participate in the study as the healthy control group. Recruitment will be achieved through community/social media

advertising in the greater Sydney area alongside the recruitment strategy for participants with UC.

Healthy Control Inclusion Criteria:

4. Aged ≥ 18 years in 2018
5. Willing to be randomized into one of the two study arms and participate in the intervention prescribed
6. Stable medication. All drugs to be held at constant dose during the study and no changes to medication in the preceding 4 weeks.

Healthy Control Exclusion Criteria:

1. Those in remission from UC as defined by an assessment by a gastroenterologist.
2. History of fecal microbiota transplantation or any rectal preparations including enemas.
3. Antibiotic/anti-tuberculosis treatment for any reason during the study period or in the preceding 4 weeks.
4. Probiotic supplements or biological/monoclonal antibody agents during the study period or in the preceding 12 weeks. Food based probiotics such as yoghurt and kefir will be allowed.
5. Chronic or acute disease requiring medical or therapeutic intervention including but not limited to diabetes, IBD, chronic kidney disease, cancer, IBS.
6. Coexistent serious autoimmune disease such as rheumatoid arthritis or systemic sclerosis.
7. Significant prior gastrointestinal surgery (e.g., colon resection, colectomy, appendectomy) or clinical evidence of any comorbid chronic disease that may interfere with the patient's ability to enter the trial and undertake the intervention planned.
8. Planned major surgery within the first 3 months after randomization.
9. Pregnancy or female planning pregnancy within the first 3 months after randomization.
10. Participation in another clinical trial for with concurrent participation is deemed inappropriate.
11. Daily consumption of olive based product(s) in any form (including oil, pickles, tapenade, spreads, and supplements) as determined by the diet history and medication list
12. Currently on a special diet in which participation is deemed inappropriate (e.g. low residue diet, liquid diet, low fat diet) or under the supervision of an Accredited Practising Dietitian or other relevant healthcare professional

7.2 INFORMED CONSENT

During the telephone and in-person screening process, participants will be informed of the purpose of the screening, and what will happen to their data. Oral consent will be sought only to the answering of questions on our telephone screen. If participants respond that they are willing to answer the questions, this will constitute their verbal consent for those specific

questions only. If they refuse to answer the questions in its entirety, they are thus considered to have refused to give consent for the study, and the telephone screen will end. The telephone screen answers will be recorded directly in REDCAP by the research staff conducting the screen. These data will be exported to EXCEL/SPSS for tracking of study recruitment procedures and reporting of CONSORT flow of participants.

Post telephone screen, participants will be provided with a form outlining their intent on participating in the COLONIC Study which will need to be sent to their nominated healthcare provider(s). This step is critical to ensure that their participation in this study is not contra-indicated to the current care or therapy received, in addition to clarifying any ongoing therapies or treatment. If a participant is on hold prior to the in-person assessment, additional information from the participant's nominated healthcare provider(s) may be requested. Participants will be consulted and briefed prior to any communication being established, and all relevant health information will be kept confidential. Those deemed to be eligible and are still interested in participating will then be requested to attend an in person physical and health assessment at the University of Sydney Cumberland Campus C Block Clinic at a nominated date.

Prior to any assessments at the clinic, participants will be provided a copy of the Participant Information Statement (PIS) and an overview of the study schedule, assessments, and commitments will be provided including information on data confidentiality and withdrawals. This will be followed by a written, witnessed, and informed consent on participating in the COLONIC study by signing the Participant Consent Form (PCF).

7.3 CONFIDENTIALITY AND PRIVACY

Data will be collected over the phone (telephone screening) and in person (assessments), using researcher-administered questionnaires, and physical testing. Data gathered from the screening process, assessment, and training sessions will be entered by the research staff into a database created in REDCap. Data will be entered in a re-identifiable form using unique identity numbers. This code will be used on all electronic data forms, electronic databases and hard copy data forms. No forms will contain the names of participants, or other identifying information.

All participant information will remain re-identifiable, so that information that is relevant to their medical care can be communicated to appropriate medical professionals. Participants will only remain re-identifiable to persons directly involved with the study, namely the Primary and Associate Investigators or HDR students who are conducting the intervention/ assessments. All data will be de-identified prior to depositing in a repository at the end of the study, as required by the National Health and Medical Research Council.

During screening, participants will be assigned a Screening ID. Participants who are found eligible for the study and consent to participate will be randomised by an investigator who is otherwise not involved in the study. Participants will then be issued with a Study ID Code. The Screening ID Code and Study ID code will be recorded in the randomisation database.

The investigator performing the randomisation will only be provided with Screening ID, age, and gender of the participant to perform the randomisation procedure. Once randomised, participant ID codes will not be removed, even if they choose to withdraw from the study.

7.4 DATA STORAGE AND RETENTION

Electronic copies of information will be stored in two places:

1. CIA Prof. Maria Fiatarone Singh's Research server maintained by the University of Sydney. This carrier will be used to store contact information, progress databases, digital copies of consent forms, medical letters, audio and video recordings, and correspondence and manuals of procedures. This is a password protected server only accessible to researchers involved in the project. Server back-up is performed by the university central server. All documentation will be password protected. REDCAP Digital is a secure web application hosted by The University of Sydney for building and managing databases. All data entered into the database will be stored on a secure server of the University of Sydney. As such, no project data is ever transmitted at any time by REDCap to another institution or third party.

Access to the database will be provided only to research staff working on the study. User privileges will be used to limit the viewing and/or editing access that research members have within the database. All access rights will be managed by the study coordinator at the University of Sydney. Furthermore, all data entry forms will use participant codes rather than names or other potentially identifying information.

Hard copies of the following information will be stored in the locations below:

1. Original copies of participant consent forms will be stored in a locked filing cabinet in secure lockable K block office K221 at the Cumberland Campus of the University of Sydney.

2. A back up hardcopy of assessment data (analogous to REDCAP digital data) will be stored in a locked filing cabinet in secure, lockable K block office K221 at the Cumberland Campus of the University of Sydney.

A complete dataset of REDCAP data will be downloaded, copied and stored securely on Prof. Maria Fiatarone Singh's server at the University of Sydney. Hardcopy data will continue to be stored in a secure, lockable office in K block, K221.

Data collected in this study will be retained indefinitely in de-identified form with consideration of the novel nature of the study and potentially significant clinical importance of our findings. Additional projects involving long-term follow up study participants to investigate factors related to progression of inflammatory bowel disease, which may take an unknown number of years, may be considered. In the event that such a study is considered, the appropriate ethics amendment and consent forms will be required in consultation with HREC as has been done with previous longitudinal studies. There is also the potential for outcomes from this study to be pooled with other concurrent studies or future studies on other chronic gastrointestinal conditions which will also require the relevant ethics application or amendment.

8. SAFETY CONSIDERATIONS

8.1 ADVERSE EVENTS

Adverse events will be monitored through the administration of a weekly questionnaire and reporting of events throughout the study period. If participants report any adverse events, these must be reported to the University of Sydney HREC. These must be reported even if they may be unrelated to the intervention.

Serious adverse events resulting in hospitalisation or death must be reported to the University of Sydney HREC within 24 hours, even if they may be unrelated to the intervention. In both cases, participants will be followed-up by phone or in-person until resolution of the adverse event.

8.2 Risks of the study

Gastrointestinal Discomfort and Adverse Reactions to Foods:

This research study involves a slight variation of the participant's dietary intake. In the unlikely event that a severe adverse reaction to the intervention food item occurs, participants will be requested to cease the intervention for follow up with the study Dietitian and PhD candidate (Mr Kenneth Daniel) in consultation with the study Physician. Participants with a history of food allergy or sensitivity to the intervention food item (olive oil) and any food items or supplement derived from olives will be identified during screening and will not be eligible for the study. Participants with a special dietary requirement resulting in their inability to adhere to the intervention will also be excluded.

The use of a fat based food item for isocaloric replacement to their normal diet in this study was determined in part due to the lower likelihood of resulting in gastrointestinal discomfort to the participant compared to other food items e.g., dairy, red meat, insoluble fibre. The study model proposed was chosen as it had the potential to cause the least amount of burden to participants as opposed to a comprehensive dietary change, in addition to being a novel model of identifying the impact of specific dietary constituents. Participants will receive one on one counselling with the study Dietitian throughout their enrolment in the study, and individualized support will be provided. Dietary assessment and intervention is a vital part of this study as it is the topic investigated, and as such will need to be included.

Muscle Soreness and Injury:

As with any exercise testing there are potential risk of injury. Prior to any physical testing participants will undergo a physician screen. Additional medical information will also be sought from the participants nominated GP or specialist overseeing their health to ensure that there are no contra-indications to exercise testing. A physician supervised stress test will also be performed as part of the screening procedure.

The exercise testing and stress test may result in some muscle soreness and fatigue. There is also a small risk of musculoskeletal soreness or injury during the physical function test,

however this is rare during the type of testing proposed for this project. To minimize risk of injury, participants will be supervised by at trained and experienced health professional during all testing procedures. Any activities which are contra indicated to the participant will also not be performed, with a note justifying the decision recorded in the assessment sheet. Any events of episodes during testing will be followed up by the study physician. Exercise testing is required to identify any potential changes to functional capacity and strength during the study, including any changes occurring as a result of improved gastrointestinal symptoms and inflammation.

Activity monitoring:

There is a small chance that wearing the activity monitor on the lower back under the bandage might cause some slight skin irritation from the tape residue and location. We will ensure that the tape is applied neatly and without tensing the skin to minimise any discomfort due to the tape application.

Blood sampling:

An experienced professional will collect the blood samples from the participant's vein. Rarely will blood leak from a vein, but this is easily rectified by applying pressure over the vein. All procedures will be performed under sterile conditions with highly experienced and qualified professionals. Blood sampling will be performed while the participant is lying down to avoid light-headedness or fainting during the procedure, and participants will be requested to move their legs after periods of lying down. Sharps and biological waste will be disposed of in their corresponding bins which are located throughout the testing site in C block.

Blood collection is necessary to this study to determine physiological response to the intervention and provide further insights to any associated biochemistry in relation to the gut microbiota which would not be achieved just by tracking the dietary changes alone. In the unlikely event that participant distress occurs from this process, information to counselling can be provided. Blood test results from the study may be forwarded to the participants nominated healthcare professional for further investigation if deemed appropriate upon receiving informed consent from the participant (e.g., assessments for vitamin D, in which deficiency often occurs in this cohort).

Radiation:

This research study involves exposure to very low amounts of radiation from the DXA x-rays. The effective dose of radiation from this study is very low at about 0.02 milisieverts (mSv) done once at baseline. For comparisons, about 2 mSv (100x higher) is experienced each year by the public from natural sources as part of everyday living. No harmful consequences have been demonstrated at this level and the risk is minimal. Participants will be requested to inform the researchers if they have participated in any other research study in the past 5 years where they have been exposed to radiation. Participants will also be advised to show the Participant Information Statement from this study to research staff of any other study they may wish to volunteer for in the next 5 years. DXA is included as part of this research due to the insights it provides on a participant's bone density and risk of fracture, in addition to allowing the team to assess body composition in response to the intervention. Osteoporosis is a known extra-

intestinal manifestation of IBD, and as such accurate assessments of bone mineral density is valuable as standard of care with considerations to fracture risks and quality of life. Due to complications in food absorption in relation to the chronic condition we are investigating, individuals with IBD are at a higher risk of developing osteopenia and osteoporosis. Identifying this risk benefits both the research team and the participant's current care, and results will be communicated with the participants nominated healthcare professional for follow up if consent has been provided by the participant. Radiation safety approval received on 15/01/2019, RSC number RSC1901.

Adverse Effects:

During each test procedure, and at regular intervals throughout the program, we will ask participants to inform us of any side effects that they may experience. Participants will be advised that they must contact the study staff immediately if there are any unusual health experiences, injury or bad effects. This notification should take place whether or not they believe that the problem is related to the program or from some other cause. Prior to any testing, the study physician will review participant's medical history to make sure they are medically ready for the study procedures.

In the event of an injury or other misadventure, we will contact the participant's GP and /or gastroenterologist, and may recommend an appropriate course of action in consultation with the GP as necessary. In the event of any adverse event participants will be able to contact the study physician Professor Maria Fiatarone Singh on 02 9351 9755.

9. OUTCOMES AND SIGNIFICANCE

9.1 OUTOMES

Table 2 illustrates the COLONIC study primary and secondary outcomes. Table 2 illustrates the outcomes that will be assessed and the time points at which each outcome will be assessed (Baseline, 4 weeks, 8 weeks).

Primary Outcomes	**Time Point**
Disease Activity for Ulcerative Colitis Partial Mayo Score for Ulcerative Colitis	Baseline, 4 weeks, 8 weeks
Microbiome Dysbiosis Evaluation of gut bacterial profiles. Participants will be required to produce a stool sample into a plastic bag, ensuring no fluid or urine comes in contact with the sample. Participants will be provided with a collection kit with instructions. The fecal sample will be stored at the University of Sydney freezers at -80ºC if produced onsite, or stored in participant's freezers then transported to the University of Sydney during the clinic date. It will then be transported to Medlab Clinical (66 McCauley St., Alexandria, NSW 2015) in batches where they will be stored at -80ºC and then analyzed	Baseline, 4 weeks, 8 weeks

once there are enough samples to make the run financially viable (approximately every 10 samples).	
Secondary Outcomes	
Nutrition 3-day weighted food records, 1 Month Retrospective Food Frequency Questionnaire (FFQ), Probiotic foods and nutraceutical inventory, Risk of Malnutrition via the Mini Nutritional Assessment (MNA) Diet quality as determined by the CSIRO Diet Index Polyphenol content of the diet as determined by FoodWorks Oleic acid intake as determined by FoodWorks Diet Inflammatory Index (DII) Plasma Oleic Acid levels Faecal SCFA content Plasma Hydroxityrosol sulfate and Tyrosol as determined by ultra-HPLC-ESI-MS/MS Self-reported symptoms diary for food intolerances and adverse reactions Food Choice Questionnaire Food Related Quality of Life Questionnaire for IBD (FR-QoL-29)	Baseline, 4 weeks, 8 weeks

Anthropometry Waist, upper-arm and calf circumferences, height, weight, Resting Heart Rate	Baseline
Body composition Whole body and regional lean and adipose tissue, Bone density in the lumbar spine and hip. Dual-energy X-ray absorptiometry (DXA)	Baseline
Inflammatory profile Cytokines/adipokines including tumour necrosis factor and interleukins 1, 6, 10 and 12. C-Reactive Protein Erythrocyte Sedimentation Rate Faecal calprotectin TNF α Faecal IgA and IgA coating bacteria analysis Faecal Cytokines LPS in blood Serum levels of Folate Serum levels of Calcium Serum levels of vitamin D Blood cell count	Baseline, 4 weeks, 8 weeks
Neuropsychological outcomes Inflammatory Bowel Disease (IBD) Questionnaire, Depressive Symptoms via will be assessed via patient health questionnaire (PHQ-9) and Hospital and Anxiety Depression Index (HADS), Quality of life via the SF-36® Health Survey, Fatigue via the IBD Fatigue Scale. Sleeping patterns via the PSQI	Baseline, 4 weeks, 8 weeks
Physical activity participation Objective physical activity 7-day physical activity monitoring using Axivity MEMS 3-axis accelerometer and physical activity logs	Baseline, 4 weeks, 8 weeks
Maximal aerobic capacity (VO_2peak) Graded treadmill test to voluntary fatigue with indirect calorimetry	Baseline
Muscle strength 1-repetition maximum tests for the chest press, seated row, shoulder press, lat pull-down, leg press, leg extension, and leg flexion.	Baseline
Adverse events All adverse events related and not related to the intervention. Gathered using weekly health status check and reporting of events throughout the study period.	Weekly throughout the study

9.2 OUTCOMES AND SIGNIFICANCE

Current evidence on the human gut microbiota in UC typically focuses on the risk of developing the condition, while studies identifying what occurs during active disease is lacking, especially when exposed to a dietary stimulus. Similarly, robust intervention and dietary support for community dwelling UC cohorts appear to be lacking despite the high level of need demonstrated in literature.

EVOO as a component of the Mediterranean diet has been suggested to have anti-inflammatory properties, which may have implications for conditions involving chronic inflammation such as UC. Animal models have demonstrated the robust effects of EVOO intervention and its effects on the gut biota, while the benefits of EVOO consumption in human cohort has been suggested however clinical trials are lacking. As such, the COLONIC Study will be the first randomized controlled trial identifying the effects of EVOO on the human gut microbiota in an apparently healthy population in comparison to those with chronic gastrointestinal conditions. Furthermore, we will also be the first trial identifying the effects of these changes in response to diet and its contribution to UC disease etiology in community dwelling cohorts. This will allow for future investigations on the role of nutrition and food components in modulating the gut microbiota with potential consequences in modifying disease outcomes.

10. TIMELINES / MILESTONES

Table 4 illustrates the expected project timeline and milestones.

Table 4. COLONIC Study Timeline

	2018	2019				2020				2021
	Oct-Dec	Jan-Mar	Apr-Jun	Jul-Sep	Oct-Dec	Jan-Mar	Apr-Jun	Jul-Sep	Oct-Dec	Jan-Mar
Study protocol and ethics approval	X	X								
Set-up of the study	X	X								
Recruitment of participants			X	X	X	X	X			
Baseline assessments			X	X	X	X	X	X		
Post-intervention assessments				X	X	X	X	X	X	
Preparation of main outcome publication								X	X	X

Study duration: 4 years (2018-2021)	It is expected that the study will take four years to complete. This includes 6 months to set up the study, including recruitment and training of the study personnel, and preparation of the recruitment and study materials.
October 2018 – January 2019	Study protocol will be drafted and ethics approval obtained.
April 2019 – June 2020	It is expected that recruitment will begin in early-mid April 2019. We are aiming for a conservative estimate of 1 participant per week, and means all participants will be recruited by June 2020. Additional time from the initial forecast has been allocated for delays related to health changes and scheduling conflicts.
April 2019 – September 2020	It is expected that the baseline assessments will start early-mid June 2019 and end by September 2020
June 2019 – October 2020	The final assessment will start in mid-June 2019 and end by October 2020.
July 2020 to March 2021	The main outcome publication will be prepared early to mid 2021.

11. FUNDING/SUPPORT BEING SOUGHT OR SECURED

Funding will be sought for analysis of blood and stool (gut microbiome). Until funding is secured to perform the analyses, these samples will be stored in -80 degree freezers.

Grants that will be targeted include:

- Kenneth Rainin Foundation
  This encourages collaboration among health researchers from across disciplines to advance the study of inflammatory bowel disease. Awards are worth up to USD 300,000 each.
  http://krfoundation.org/health/grants/synergy-award/

- Crohn's and Colitis Foundation of America
  These provide established researchers with funds to generate sufficient preliminary data to become competitive for funds from external sources. Awards are worth up to USD 115,830 per year for one to three years.
  http://www.ccfa.org/science-and-professionals/research/grants-fellowships/senior-research-awards.html

- Innovations in inflammatory bowel disease research
  Crohn's and Colitis Foundation of Canada | Foundation Canadienne des Maladies Inflammatoires de l'Intestin
  These support innovative projects that have the potential to improve the diagnosis, therapy and prevention of inflammatory bowel disease. Grants are worth up to CAD 50,000 for a maximum period of one year.
  http://www.ccfc.ca/site/c.ajIRK4NLLhJ0E/b.6326399/k.4BD2/Opportunities_For_Researchers.htm

- International Organisation for the Study of Inflammatory Bowel Disease
  These support members of the organisation in conducting research relevant to inflammatory bowel disease. Grants are worth up to USD 200,000 for a maximum period of one year. Preference will be given to small grants of up to USD 50,000. Salaries to cover principle or co-investigator salaries will not be covered.
  http://www.ioibd.org/grants/

- Crohn's and Colitis Foundation of America
  These support innovative proposals that lead to improvements in the prevention, diagnosis or therapy of Crohn's Disease or Ulcerative Colitis.
  http://www.ccfa.org/science-and-professionals/broad/funding/

12. PUBLICATION POLICY / DISSEMINATION OF RESULTS

Primary and secondary outcomes from the study will be published in appropriate scientific journals. All scientific papers, including authorship will be agreed upon by consensus by the PI and AI's.

Dissemination of results will also occur by presenting results at scientific conferences, and in presentations to the public. In addition, participants may elect to receive a one-page summary of the study findings.

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