Clinical Trial Protocol

Therapeutic Intervention and Clinical Trial Research – Project Description

This report has been complied with reference to the NHMRC Project Description guidelines and prepared in accordance with the Good Clinical Practice Guidelines (GCPs) as per the publication titled "Australian Clinical Trial Handbook: A simple, practical guide to the conduct of clinical trials to International standards of Good Clinical Practice (GCP) in the Australian context".

1.0 Title	
1.1 Project title	The influence of gene variants on physiological responses to a Mediterranean diet - a nutritional genomics focus
1.2 RMIT HRETH #	*Not yet allocated*
1.3 Version	Version 1, prepared January 11th, 2019.
1.4 Public Registry	Trial registration has been undertaken via the Australia and New Zealand Clinical Trials Registry (ANZCTR) on Thursday 24th January 2019, and is currently awaiting registration number to be assigned.

2.0 Project Team Roles and Responsibilities	
2.1 Chief investigator	Dr Jessica Danaher **Affiliation and position** Level B Lecturer / Early Career Development Fellow in Nutrition, School of Sciences, College of Science, Engineering and Health, RMIT University. **Research activities Dr Jessica Danaher will be responsible for** Throughout this project, Dr Danaher will oversee supervision of a PhD student, clinical intervention, the collection and processing of data, and some biochemical analysis including genetic variant, hormone, and gene expression testing, to ensure successful completion of all phases of the project. She will manage relationships with additional Chief Investigators of the project, who will be responsible for supervising the PhD student when undertaking metabolomics and microbiome analyses. Dr Danaher will also oversee the development of research publications (including a doctoral thesis) and conference presentations arising from this research.
2.2 Co-investigator(s)	Dr Daniel A. Dias **Affiliation and position** Level B Lecturer, School of Health and Biomedical Sciences, College of Science, Engineering and Health, RMIT University. **Research activities Dr Daniel Dias will be responsible for** Throughout this project, Dr Dias will oversee supervision of a PhD student, the clinical intervention, and the collection and processing of data. He will be responsible for supervising the PhD student when undertaking

	metabolomics-based analyes. Dr Dias will also oversee the development of research publications (including a doctoral thesis) and conference presentations arising from this research.
	Professor Rob Moore **Affiliation and position** Professor of Biotechnology, School of Health and Biomedical Sciences, College of Science, Engineering and Health, RMIT University. **Research activities Professor Rob Moore will be responsible for** Throughout this project, Professor Moore will oversee the PhD student when undertaking microbiome analysis. Professor Moore will also oversee the development of research publications and conference presentations arising from this research.
	Ms Suzi Ristevski **Affiliation and position** PhD student, School of Sciences, College of Science, Engineering and Health, RMIT University. **Research activities Ms Suzi Ristevski will be responsible for** Ms Ristevski will be responsible for the project design and execution. She will be undertaking participant recruitment, obtaining consent, data collection, sample collection and data analysis. She will also develop research publications (including a doctoral thesis) and present her work and reputable national and international conferences.
2.3 Peer review process	Peer review of the research proposal has been undertaken by Dr Lisa Newman, an academic who has expertise to ensure that the investigators comply with the criteria of the project, confirm that investigators have required expertise and that any conflicts of interest are declared; gives consideration to ideas and concepts that may sound implausible, and challenge "popular thinking." (Appendix E).

3.0 Resources	
3.1 Trial site	RMIT University Bundoora West Campus Plenty Road Bundoora, VIC 3083 Ph: 9925 6117
3.2 Resources necessary for the project to be conducted	Clinical testing and data collection will be performed at the RMIT Bundoora Campus Clinical Suites (Building 203). This facility includes dual-energy x-ray absorptiometry (DEXA) equipment for body composition measurements, and will be made available to the researchers by RMIT.

	Storage and experimental analysis of collected specimens will occur at the RMIT Bundoora Campus Biosciences Laboratories (Building 223). This will include the facilitation of measurement of metabolites (metabolomics) associated with glucose, fat and protein metabolism using gas chromatography-mass spectrophotometry. The laboratory/premises and equipment to be used in this research have been subjected to OHS assessments and are deemed safe for use. Relevant risk assessments will be undertaken, and standard operating procedures will be generated, prior to experimental procedures in accordance with RMIT OHS requirements. Additional resources/equipment that will be used for the project include: - Blood sampling items (including syringes, needles, adhesives, cleaning swabs and blood collection tubes) - Oral glucose tolerance testing drinks - Hormone (ghrelin and insulin) kits - Gene expression analysis (including extraction chemicals, microarray kits, real-time-PCR consumables) - Gut bacteria analysis (including faecal sample collection kits, extraction chemicals, 16S rRNA profiling and metagenomics consumables) - Genetic variant testing (including buccal swabs, extraction chemical, 10 x gene variant kits and associated consumables - Food scales - Food frequency questionnaire access - Food hampers of example food products recommended throughout the dietary intervention.
3.3 Funding support	Funding to the value of **$129,287.13** (AUD) has been obtained from the Allen Foundation Inc for this research project (May 31st 2018 – May 31st 2021) This philanthropic grant is subject to reporting of annual progress reports (due on May 31st over the three-year funding period).
3.4 Is the research of intervention sponsored? NO *If YES, provide details of the sponsorship and any contractual or financial agreements entered into by the researchers:* Not applicable	
3.5 Are there any clinical laboratory(ies) and/or technical department(s) and/or institutions involved in the trial? NO	

4.0 Background
4.1 Does this research involve the investigation of a product (therapeutic device, drug, supplement or other type of substance)?

NO

4.2 Does this research involve the use of a physical intervention (non-device)?

NO

4.3 Does this research involve the use of a psychological, social or behavioural intervention?

YES

If YES, provide details about the intervention(s):

This project utilises two dietary interventions (behavioural), consisting of a Mediterranean diet and a general healthy eating diet (control diet). Each participant will be randomly assigned to either a Mediterranean diet (intervention) or a general healthy eating diet (control diet), for the duration of 8-weeks, followed by a cross-over (total duration 16-weeks).

Participants will receive sample meal plans, recipes and dietary advice for each dietary intervention. Nutrition advice will be provided by an Accredited Practising Dietitian (Ms Suzi Ristevski and Dr Jessica Danaher). This task will be facilitated by providing nutrition education material (including sample menu plans and recipes) to each participant.

For the Mediterranean diet component, a food hamper will also be provided with relevant food items to be used during the 8-week period. Each Mediterranean meal plan will adhere to the Mediterranean diet developed in the Mediterranean Diet Model in Australia: Strategies for Translating the Traditional Mediterranean Diet into a Multicultural Setting study (George et al. 2018) and the validated protocols used in the "PREvencion con DIEta MEDiterrannea" (PREDIMED) trials (Martinez-Gonzalez et al, 2012). Key features of this diet include a high plant-based (fruit, vegetables, legumes and wholegrains) and high dietary fat intake (predominantly monounsaturated fatty acids derived from extra virgin olive oil). Moderate amounts of fish, nuts and dairy are also recommended, along with red wine as being the alcoholic beverage of choice.

The general healthy eating diet will be in accordance with The Australian Guide to Healthy Eating. Food groups and portion/serve sizes will be described, with a focus on high fibre, low fat eating and reduced intake of discretionary foods.

An ad libitum approach for each diet will be advised, based on previous literature (Papamiltiadous et al. 2016; Jacka et al. 2017**;** Itsiopoulos et al. 2018).

Reference details listed in section 4.10

4.4 Does this research involve this use of a therapeutic device?

NO

4.5 Provide a summary of research relevant to the trial

Genetic predisposition can influence different responses to the same diet type between individuals. Therefore, personalised nutrition recommendations by health professionals, which take these differences into account, is a more suitable tool to prevent and treat nutrition-related chronic diseases than traditional "one-size-fits all" recommendations.

Nutritional genomics is a relatively new field of research assessing the mechanisms by which nutrients and dietary patterns interact with our genome at different stages. Metabolism of each nutrient involves the activity of several enzymes, each one encoded by a gene with allelic variants; each potentially contributing to the absorption and utilisation of nutrients, and ultimately influencing an individual's nutritional requirements (Murgia & Adamski 2017). More than one allelic

variant is also generally responsible for affecting a person's predisposition to a nutrition-related disease.

Allelic variant information is not the only factor complicating the translation of nutritional genomics into nutritional practice; the discovery of other levels of control to dietary phenotyping, including the environment-modulated epigenome (e.g. microRNA activity) and the intestinal microbiome, are other complicating factors (Murgia & Adamski 2017). Although complex, all of these factors must be taken into consideration when identifying sub-groups that would benefit from particular dietary recommendations.

The Mediterranean diet is considered a dietary pattern with beneficial effects on human health, including protective effects against nutrition-related diseases (i.e. cardiovascular disease and type 2 diabetes) (Corella et al. 2013; Ros et al. 2014; Salas-Salvadó et al. 2011). The Mediterranean diet comprises an abundant consumption of olive oil, a high intake of vegetables/fruit/legumes/whole grains/nuts/seeds, and a moderate intake of red wine with meals (Fitó & Konstantinidou 2016).

High adherence to the Mediterranean diet can attenuate the incidence of stroke in carriers of the risk-T allelic variant of the Transcription Factor 7-Like 2 (TCF7L2) genes rs7903146 polymorphism (Corella et al. 2013) and negate the association between risk variants in the Fat Mass and Obesity-Associated (FTO), Melanocortin-4 Receptor (MC4R) genes and type 2 diabetes (Ortega-Azorín et al. 2012). Additionally, following a Mediterranean diet over a long-term (3 years) period resulted in the greatest body weight reduction in the obesity risk variant (AA genotype) of FTO's rs9939609 polymorphism, despite carriers of the risk variant having higher body weight than their non-risk counterparts at baseline (Razquin et al. 2010). Whilst these findings suggest a protective role of the Mediterranean diet in the link between nutrition-related diseases and their predisposing genes, further studies are necessary to determine the mechanisms involved.

Some health benefits of the Mediterranean diet have been attributed to its richness in antioxidants, which have the capacity to modulate gene and protein expression, and influence inflammation. An 8-week Mediterranean-based dietary intervention induced changes in the expression of inflammation-related microRNAs in the white blood cells of overweight subjects with metabolic syndrome (Marques-Rocha et al. 2016). Additionally, high adherence to a Mediterranean diet over 8-weeks improved metabolic syndrome features induced by weight loss, suggesting a good combined strategy to treat obesity (Hermsdorff et al. 2009). Metabolomic techniques, which allow the characterisation of a large number of metabolites present in a biological sample at one time, have also shown that an 8-week Mediterranean diet can produce significant changes to the plasma metabolic profile of individuals with metabolic syndrome (Bondia-Pons et al. 2015). Furthermore, high-level adherence of foodstuffs consistent with the Mediterranean diet has been associated with beneficial microbiome-related metabolomic profiles (De Filippis et al. 2015), with associations between gut microbiota and nutrition-related diseases, such as obesity, an emerging topic of research interest. There is a lack of evidence on how allelic variants of genes associated with nutrition-related diseases influence these outcomes.

Reference details listed in section 4.10

4.6 Provide a rationale for the trial (i.e. how the research will fill any gaps, contribute to the field of research or contribute to existing or improved practice)

The Mediterranean diet is a well-researched dietary pattern which has shown beneficial influences on health, including protective effects against metabolic diseases. However, there is limited evidence on how specific genetic variations may influence an individual's response to the Mediterranean diet on an epigenetic, gut microbiome and metabolic level. It is important to understand these factors to assist with developing dietary interventions and guidelines which are tailored to responsive individuals.

This project will provide data for several publications, each focused-on genes associated with metabolic diseases. This project can provide important information on genetic sub-groups that would benefit from particular dietary recommendations, explore the possible underlying mechanisms and interactions between genes and diet, and contribute to scientific knowledge that can facilitate the translation of nutritional genomics into nutrition practice.

The findings will form the foundation for evaluating if nutritional genomic screening and personalised nutrition recommendations in healthcare settings, may be an effective strategy to treat and prevent metabolic diseases, thereby reducing the social, health and economic burden of these diseases on society.

4.7 Please describe the research question / aims / objectives /hypothesis of this project

This project is designed to determine if specific genetic sub-groups associated with metabolic disease risk benefit from a Mediterranean diet. This research may lead to personalised nutrition recommendations using genomic information to promote health.

The aims of the proposed project are to:

(i) Determine the influence of an 8-week Mediterranean dietary intervention on gut bacteria, levels of metabolites (involved in glucose, fat and protein metabolism) present in blood, and gene modifiers (whether genes are turned on or off) in blood. This will be compared to a person's habitual diet (baseline results) and a general healthy eating diet following The Australian Guide to Healthy Eating (control diet).

(ii) Determine the potential of key genetic variants related to metabolic disease (including the Fat Mass and Obesity-Associated (FTO) gene, Transcription Factor 7-Like 2 (TCF7L2) gene, and Apolipoprotein E (APOE) gene) to influence gut bacteria, blood metabolite and gene modifying outcomes in response to the dietary interventions.

(iii) Determine whether differences between genetic variants exist for body composition, key hormones (ghrelin and insulin), glucose tolerance, and dietary intake (food frequency and nutrient composition) in response to the dietary interventions.

It is hypothesized that variations in key genes associated with metabolic disease impacts on gut bacteria, blood metabolites and gene modifiers following an 8-week Mediterranean dietary intervention.

4.8 Please describe the expected outcomes of this project

Primary Outcomes:

This study will reveal if the genetic background of individuals affects their response to a Mediterranean diet by measurement of key physiological factors, including:

- Plasma metabolites associated with glucose, fat and protein metabolism
- Gene expression
- Gut bacteria diversity and composition
- Circulating hormones (ghrelin and insulin)

Secondary Outcomes:

- Body composition
- Glucose tolerance
- Food frequency and nutrient intake
- Mood state

4.9 Statement of compliance	This trial will be conducted in compliance with the Clinical Trial Protocol, the National Statement on Ethical Conduct in Human Research (2007), the Good

	Clinical Practice Guidelines, and any conditions set by RMIT University's Human Research Ethics Committee.

4.10 References to literature that are relevant to the trial

1. Bondia-Pons, I., Martinez, JA., de la Iglesia, R., et al. (2015). "Effects of short- and long-term Mediterranean-based dietary treatment on plasma LC-QTOF/MS metabolic profiling of subjects with metabolic syndrome features: The Metabolic Syndrome Reduction in Navarra (RESMENA) randomized controlled trial." Mol Nutr Food Res, 59(4):711-728.

2. Corella, D., Carrasco, P., Sorlí, JV., et al. (2013). "Mediterranean diet reduces the adverse effect of the TCF7L2-rs7903146 polymorphism on cardiovascular risk factors and stroke incidence: a randomized controlled trial in a high-cardiovascular-risk population." Diabetes Care, 36(11):3803-3811.

3. De Filippis, F., Pellegrini, N, Vannini, L., et al. (2015). "High-level adherence to a Mediterranean diet beneficially impacts the gut microbiota and associated metabolome." Gut, 65(11):1812-1821

4. Fitó, M. & Konstantinidou, V (2016). "Nutritional genomics and the Mediterranean diet's effects on human cardiovascular health." Nutrients, 8(4):218.

5. Hermsdorff, HH., Zulet, MÁ., Abete, I., & Martínez, JA. (2009). "Discriminated benefits of a Mediterranean dietary pattern within a hypocaloric diet program on plasma RBP4 concentrations and other inflammatory markers in obese subjects." Endocrine, 36(3):445-451.

6. Marques-Rocha, J. L., Milagro, F.I., Mansego, M.L., et al. (2016). "Expression of inflammation-related miRNAs in white blood cells from subjects with metabolic syndrome after 8 wk of following a Mediterranean diet-based weight loss program." Nutrition, 32(1): 48-55.

7. Murgia, C. & Adamski, MM (2017). "Translation of nutritional genomics into nutrition practice: The next step." Nutrients, 9(4): 366.

8. Ortega-Azorín, C., Sorlí, JV., Asensio, EM., et al. (2012). "Associations of the FTO rs9939609 and the MC4R rs17782313 polymorphisms with type 2 diabetes are modulated by diet, being higher when adherence to the Mediterranean diet pattern is low." Cardiovasc Diabetol,11:137.

9. Razquin, C., Martinez, JA., Martinez-Gonzalez, MA., et al. (2010). "A 3-year intervention with a Mediterranean diet modified the association between the rs9939609 gene variant in FTO and body weight changes." Int J Obes (Lond), 34(2):266-272

10. Ros, E., Martínez-González, MA., Estruch, R., et al. (2014). "Mediterranean diet and cardiovascular health: Teachings of the PREDIMED study." Adv Nutr, 5(3):330S-336S.

11. Salas-Salvadó, J., Bulló, M., Babio, N., et al. (2011). "Reduction in the incidence of type 2 diabetes with the Mediterranean diet: results of the PREDIMED-Reus nutrition intervention randomized trial." Diabetes Care, 34(1):14-19.

12. George, S., Kucianski, T., Mayr, H., et al. (2018). "A Mediterranean Diet Model in Australia: Strategies for Translating the Traditional Mediterranean Diet into a Multicultural Setting." Nutrients, 10: 465-482.

13. Papamiltiadous, E., Roberts, S., Nicoll, A., et al. (2016). "A randomised controlled trial of a Mediterranean Dietary Intervention for Adults with Non-Alcoholic Fatty Liver Disease (MEDINA): study protocol." BMC Gastroenterology, 16: 14-20.

14. Itsiopoulos, C., Kucianski, T., Mayr, H., et al. (2018). "The AUSralian MEDiterranean Diet Heart Trial (AUSMED Heart Trial): A randomized clinical trial in secondary prevention of coronary heart disease in a multiethnic Australian population: Study protocol." American Heart Journal, 203: 4-11.

15. Jacka, F., O'Neil, A., Opie, R., et al. (2017). "A randomised control trial of dietary improvement for adults with major depression (the 'SMILES' trial)." BMC Medicine, 15: 23-36.

5.0 Project Design	
5.1 Research Project Setting	The clinical sessions will be undertaken at the RMIT Bundoora Campus Clinical Suites (Building 203).
5.2 Trial design	The study will use a randomised, controlled, cross-over design.

5.3 Overview of the research design and theoretical approach

Please note: Specific details of the methodology (how data will be collected and analysed) is provide in sections 5.8 and 5.9.

Inclusion and exclusion criterion, and methods for participant recruitment are detailed in Section 5.4. All eligible participants will be asked to provide written consent based on RMIT approved Human Research Ethics documents. All eligible participants will be asked to read the information to participant's form and provide written consent based on RMIT approved Human Research Ethics documents (Appendix B). Participants will be provided the opportunity to attend a familiarisation session (up to 1 hour) to discuss the study protocols and consent with the researcher.

Clinical Sessions (approximately 3 hours each):

Eligible participants will be asked to attend the Clinical Trial Suites at RMIT University Bundoora (Building 203) and asked to bring the signed consent form. Participants will be asked to complete a personal information and medical questionnaire (Appendix C).

During the initial (baseline) clinical session participants will perform a standard buccal swab (this will not be repeated in subsequent clinical sessions). Each buccal swab will be used for genetic profiling using Single Nucleotide Polymorphism arrays.

During the clinical session participants will be weighed, measured for height and have their hip to weight ratio recorded. Blood pressure will be measured using a sphygmomanometer. Participants will have their body composition measured using Dual-Energy X-ray Absorptiometry (DEXA).

Participants will then be asked to wear a respiratory mouth piece and nose clip (for approximately 10 mins) to have their respiratory gases measured every 30 seconds via an open-circuit sampling system. Respiratory data will be used to determine resting metabolic rate and each participant's daily energy requirements. Following this, participants will perform a standard oral glucose tolerance test (OGTT) to determine glucose handling. Blood samples will be taken prior to, at 30-mins, 60-mins and 120-mins following glucose ingestion for subsequent measure.

Habitual dietary intake will be measured using a 3-day food record (2 weekdays and 1 weekend day). Participants will be asked to complete a food frequency questionnaire. Dietary quality will be assessed using the Healthy Eating Index (Basiotis et al. 2002). Mood State will be analysed using the Profile of Mood State questionnaire and the Hospital Anxiety and Depression Scale questionnaire (Appendix H (i) and H (ii)). These questionnaires will be administered whilst the participants are undertaking the OGTT. Stool samples will be collected by participants using a stool collection kit.

Following the initial (baseline) clinical session participants will be eligible to commence the dietary intervention and will be randomly assigned to either the 8-week Mediterranean dietary intervention or the 8-week General Healthy Eating intervention (Control). This dietary intervention

timeframe has demonstrated physiological changes on a molecular and metabolic level (Bondia-Pons et al. 2015; Marques-Rocha 2016).

A post intervention clinical session (as described above) will be repeated at the end point of the first 8-week dietary intervention period. The participant will then cross-over to the 8-week dietary intervention they have not yet completed. Total duration of the combined dietary intervention period will be 16 weeks. A final (third) clinical session will be repeated at week 16 (see Figure 1.0).

Dietary Intervention Mediterranean (8-weeks):

Each participant will receive a detailed sample Mediterranean meal plan, recipes, and education material to assist with adherence to a Mediterranean diet for the 8-week period. This will be explained by an Accredited Practising Dietitian. This task will be facilitated by providing a calibrated scale, a menu plan to each participant, along with a food hamper with food items that will be used as part of a Mediterranean diet.

Each Mediterranean meal plan will adhere to the Mediterranean diet developed in the Mediterranean Diet Model in Australia: Strategies for Translating the Traditional Mediterranean Diet into a Multicultural Setting study (George et al. 2018) and the validated protocols used in the PREDIMED trials (Martinez-Gonzalez et al. 2012). This includes using extra virgin olive oil (minimum 3-4 tablespoons per day), eating vegetables with every meal (100g leafy greens, 100g other vegetables, 100g tomatoes per day), at least two serves of fruit per day, legumes at least twice per week, at least three serves of fish per week, red meat less often, one serve of nuts daily, two serves of dairy daily (milk and yoghurt preferable), cheese in moderation (three days per week preferably feta, 1 serve equalling 30 grams), choice of wholegrain breads and cereals, consuming 3 eggs per week, sweets to be consumed in moderation (preferably homemade), red wine is optional (0-2 standard glasses per day, with meals, instructions to not get intoxicated). Advice on alcohol will be provided to those who already consume alcohol in their habitual diet.

Dietary Intervention General Healthy Eating (8-weeks):

Each participant will be provided with general healthy eating advice in accordance to the Australian Guide to Healthy Eating (AGTHE). This task will be facilitated by providing the participant with relevant education material, a guide to food groups and portion/serve sizes, with a focus on high fibre, low fat eating and reduced intake of discretionary foods. This diet has been chosen as a control diet based on previous literature and to also reflect current dietetic advice provided in practice to the Australian population (Papamiltiadous et al. 2016; Itsiopoulos et al. 2018).

Dietary intake will be measured using a 3-day weighed food record during the intervention mid-point (week 4) and the week before the end of each dietary intervention (week 7/prior to the post intervention clinical session). Adherence to the Mediterranean diet will be assessed using the Mediterranean Diet Adherence Screener Score (MEDAs) tool (Appendix H (iii)).

An ad libitum approach for each diet will be advised, based on previous literature (Papamiltiadous et al. 2016; Jacka et al. 2017**;** Itsiopoulos et al. 2011; Itsiopoulos et al. 2018; Ryan et al. 2013).

Participants will be instructed to maintain their habitual physical activity patterns during the intervention, with physical activity patterns monitored and evaluated weekly by informal individual support-orientated phone calls throughout the 8-week intervention. Food recall strategies will be adopted by researchers throughout these phone calls to monitor and evaluate dietary compliance.

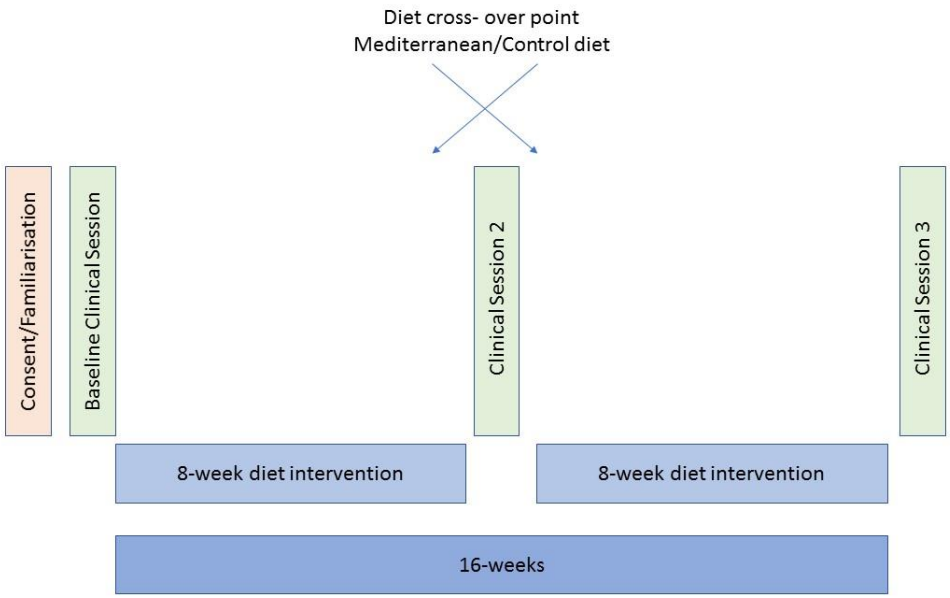

Figure 1.0: A schematic overview of the 16-week research project. The consent/familiarisation session will take approximately one hour, and the clinical sessions will take approximately three hours each. All clinical sessions will involve questionnaires, body composition testing, blood pressure, and blood and stool sample collection. Buccal Swab/DNA testing will only occur at the baseline clinical session.

References:

1. Basiotis PP, Gerrior SA, Juan WY, Lino M. The Healthy Eating Index: 1999– 2000. Washington DC: US Department of Agriculture, Center for Nutrition Policy and Promotion; 2002.

2. Bondia-Pons, I., Martinez, JA., de la Iglesia, R., et al. (2015). "Effects of short- and long-term Mediterranean-based dietary treatment on plasma LC-QTOF/MS metabolic profiling of subjects with metabolic syndrome features: The Metabolic Syndrome Reduction in Navarra (RESMENA) randomized controlled trial." Mol Nutr Food Res, 59(4):711-728.

3. Jacka F.N., O'Neil A., Opie R., et al (2017). "A randomised controlled trial of dietary improvement for adults with major depression (the "SMILES" trial)." BMC Medicine, 15:23.

4. Marques-Rocha, J. L., Milagro, F.I., Mansego, M.L., et al. (2016). "Expression of inflammation-related miRNAs in white blood cells from subjects with metabolic syndrome after 8 wk of following a Mediterranean diet-based weight loss program." Nutrition, 32(1): 48-55.

5. Trichopoulou, A., Costacou, T., Bamia, C., et al. (2003). "Adherence to a Mediterranean diet and survival in a Greek population". N Engl J Med, 348(26):2599-608.

6. Zulet M.A., Bondia-Pons, I., Abete, I., et al. (2011). "The reduction of the metabolic syndrome in Navarra-Spain study: a multidisciplinary strategy based on chrononutrition and nutritional education, together with dietetic and psychological control". Nutr Hosp, 26(1):16-26.

7. Ros, E., Martínez-González, MA., Estruch, R., et al. (2014). "Mediterranean diet and cardiovascular health: Teachings of the PREDIMED study." Adv Nutr, 5(3):330S-336S.

8. Salas-Salvadó, J., Bulló, M., Babio, N., et al. (2011). "Reduction in the incidence of type 2 diabetes with the Mediterranean diet: results of the PREDIMED-Reus nutrition intervention randomized trial." Diabetes Care, 34(1):14-19.

9. George, S., Kucianski, T., Mayr, H., et al. (2018). "A Mediterranean Diet Model in Australia: Strategies for Translating the Traditional Mediterranean Diet into a Multicultural Setting." Nutrients, 10: 465-482.

10. Papamiltiadous, E., Roberts, S., Nicoll, A., et al. (2016). "A randomised controlled trial of a Mediterranean Dietary Intervention for Adults with Non Alcoholic Fatty Liver Disease (MEDINA): study protocol." BMC Gastroenterology, 16: 14-20.

11. Itsiopoulos, C., Kucianski, T., Mayr, H., et al. (2018). "The AUSralian MEDiterranean Diet Heart Trial (AUSMED Heart Trial): A randomized clinical trial in secondary prevention of coronary heart disease in a multiethnic Australian population: Study protocol." American Heart Journal, 203: 4-11.

5.4 Population to be studied	*Description and number:* This trial will recruit n = 120 healthy males and females aged between 18 - 55 years who are deemed eligible to participate as indicated by not meeting one or more of the exclusion criterion (see section below). For withdrawal of subjects see section 5.9.2. *Inclusion criteria:* Individuals with a body mass index greater than 18.5 kg/m^2, males and females aged between 18 - 55 years who are deemed eligible to participate as indicated by not meeting one or more of the exclusion criterion. *Exclusion criteria:* Participants will be excluded from participating within the project for the following reasons: Individuals with presence of psychiatric disorders, pathologic eating disorders, chronic diseases related to the metabolism of energy and nutrients (i.e. hyperthyroidism), difficulty in changing food habits, participants with special dietary needs (e.g. exclusion of whole food groups), and/or unable or unwilling to give informed consent. These exclusion criterion are set as they may influence an individual's ability to comply with the recommendations of the nutritional intervention. Additional exclusion criterion includes: weight instable in the previous 3 months (evident by a loss or gain of more than 10% of total body weight), pregnant or potentially pregnant, post-menopausal, taking contraindicated mediation (e.g. antibiotics, thyroid, hyperlipidemic, hypoglycemic, hypertensives, psychotropic drugs or appetite suppressants) or the use of any dietary supplement that might interfere with the results of the study. These additional exclusion criteria are set as the may exert an independent influence on microbiome and metabolic outcomes, anthropometric, biochemical, clinical, body composition (DEXA) analysis and/or dietary outcomes monitored throughout the intervention. *Sample size and statistical or power issues* Statistical power for a sample size of at least 120 participants to detect a medium effect size with 80% power was calculated upon previously published data on cross-over (Itsiopoulous et al. 2011 (n=27); Ryan et al. 2013 (n=12)) and single arm studies (Di Renzo et al. 2018 (n=188, split on FTO genotype); Jacka et al. 2017 (n=67); Papamiltiadous et al.

	2016 (n=94); Parletta et al. 2007 (n=152)). In these studies, a 6 week to 3-month Mediterranean diet - control intervention was able to have a significant influence on a variety of physiological and psychological outcomes. Based on allowances for attrition, including predicted dropout rates, sample size was inflated by 20% for the current project (to n=120 overall), which takes a randomised cross over approach and accounts for genotypic influences on data outcomes.
5.5 Participant recruitment strategies and timeframes	Volunteers will be recruited from the RMIT University staff and student population, and also the local community (surrounding Melbourne Suburbs) by word of mouth and electronic (i.e. RMIT Facebook and Twitter) (Appendix F iii) and print advertisement (i.e. flyers) (Appendix F ii). Researcher contact details (phone and email) will be provided on electronic and print advertisment material. Volunteers willing to participate in the study will be provided with a NHMRC 'Information to participants' form approved by RMIT HREC (Appendix B). This will be provided through email or mail based on volunteer preference. Volunteers will have the opportunity to recieve further information over the telephone, email and/or face-to-face. The principle research student Suzi Ristevski, and Chief Investigator (Dr Jessica Danaher) will be the primary contacts for potential participants. Individuals expressing interest in participating in this study will be initially interviewed/screened over the phone or in person to determine suitability to participate in this study (please refer to section 5.4 for the inclusion and exclusion criteria). Particpants believed to meet eligibility criteria will be invited to attend a familiarization session (section 5.6). (See Appendix F (iv) for an example of email correspondence). Volunteers will be given time to think about participation (i.e. talk to family or friends) and they can consider participation during the time the study runs (i.e. there is no set timeframe). Volunteers willing to participate in the study will be asked to sign an Informed Consent form and reminded that participation is completely voluntary, and that they can withdraw from the study at any time.
5.6 Approach/es to provision of information to participants and/or consent	Participants will have the opportunity to be familiarised with the study purpose, design and the risks and benefits associated with the study. This is also an opportunity for participants to ask questions and to discuss the information. Participants will be asked to read and sign the informed consent document (if agreeing to particpate). It will be explained that participation is purely voluntary and that they can withdraw from the study at any time. How their privacy will be protected will also be discussed, along with the dissemination and publication of the project outcomes. Participants will be forwarned about potential genetic results (as per the National Statement guidelines on genetic data dissemination) (see Appendix D for an Ethical Defensible Plan).

	Competence to give consent will be determined by an individuals ability to show that they understand the information. This will be determined by using a 'teach-back' method, where the researcher will prompt the individual to recall information provided. The determination of competence to give consent will be made by the Chief Investigator (Dr. Jessica Danaher) and PhD student Ms Suzi Ristevski. Participants will be informed of risks associated with the study via the Information to Participants Sheet (Appendix B). No specific cultural group is either included or excluded in this project. Participant Information Sheets and Consent Forms will be provided in English as a default language. In circumstances where a participant does not speak English as their first language, assistance will be sought by RMIT University's "Languages, Translating and Interpreting" services to provide that participant with translated versions of these documents, specific to their first language. There is no specific dependent or unequal relationship, however as investigators are staff of RMIT, and this study will be advertised and run at RMIT, it is possible that students of the investigators may volunteer to participate. To ensure that participants' voluntary consent is not compromised by the relationship the following steps will be taken: 1) Student volunteers willing to participate in this study will be provided with a RMIT HREC "Information to Participants" approved document and consent forms, which all other participants for this study will also receive. This will be provided through email or mail based on volunteer preference. 2) The participant will be reminded prior to commencement of the intervention that participation is completely voluntary and that they can withdraw from the study at any time. In the case of a potentially unequal or dependent relationship existing (i.e.: teacher/student), an investigator who does not have a relationship with the volunteering participant will be responsible for the procedures undertaken (i.e.: scheduling dates to attend sessions, taking biological samples etc).
5.7 Reimbursing of participants	Participants will be offered compensation in the form of a giftcard (to assist with grocery shopping and/or travel expenses) for the time commited to the project. A maximum of $75 per person will be provided in installments. At the end of each of the three testing sessions, particpants will receive a $25 gift card. Particpants will also be provided with a food hamper incorporating main items from the Mediterranean diet at the start of the Mediterraenean intervention. This hamper will be valued at approximately $50 per person.
5.8 Methodological Approach / Research Techniques	

Buccal Swab/DNA Collection: Cells from inside of a person's cheek will be collected using a standard buccal swab. This is a relatively non-invasive way to collect DNA samples. Each swab will be used for genetic profiling using Single Nucleotide Polymorphism arrays as per manufactures instructions. Genetic data will be coded and re-identifiable. Participants will be advised that their material and data will not be released for use without their consent, unless required by law (as per the National Statement section 3.5).

Oral Glucose Tolerance Test (OGTT): Participant will ingest 1.75g of glucose per kilogram body weight (maximum 75g glucose) at each clinical session. Blood samples will be taken prior to, and at 30-mins, 60-mins and 120-mins following glucose ingestion. Insulin sensitivity will be calculated using the composite index (Matsuda & DeFronzo 1999) and homeostasis model assessment of insulin resistance calculated as per Matthews et al. (1985).

Body Composition Assessment: Body composition will be determined using a calibrated Lunar Prodigy Pro Dual-Energy X-ray Absorptiometry (DEXA) at each clinical session. The test involves having the subject lie down on their back in a standardised position. A low dose of radiation will then scan their entire body for approximately six minutes. The DEXA segments regions of the body (arms, trunk, and legs) into three compartments for determination of fat, muscle and bone composition.

Blood Samples: Details on how blood will be sampled, and the frequency, volume and treatment of samples are detailed in section 5.9. Serum will be assayed in the RMIT Biosciences Laboratory (Building 223) for a standard clinical chemistry profile (including glucose, triglycerides, cholesterol). Serum concentrations of inflammatory cytokines/chemokines and hormones (including ghrelin, insulin) will be determined using commercially available enzyme-linked immunoabsorbent assays in accordance with manufacturer's instructions.

Plasma metabolic profiling of metabolites associated with glucose, fat and protein metabolism will be performed using Gas Chromatography-Mass Spectrophotometry (GC-MS) as per methods described by Danaher et al. (2016). Plasma microRNA expression will be performed using microarray analysis and real-time-PCR.

Stool Samples: Stool samples will be collected by participants using a collection kit that includes a toilet hat (a specimen collector which hangs on the toilet seat), instructions for collecting/mailing the specimen, a collection tube, examination gloves, alcohol wipes, a postage-paid return mailing envelope, and biohazard mailing bag. Participants will be recommended to mail their samples to researchers immediately post collection. Samples can be stored at -80C prior to microbiome analysis.

Indirect Calorimetry: Expired air will be directed via a ventilometer into a mixing chamber and analysed for O2 and CO2 content. This involves participants wearing a respiratory mouthpiece and nose clip to have their respiratory gases measured in 30 sec intervals (for approximately 10 mins in total). Prior to each trial the gas analyser will be calibrated using commercially prepared gas mixtures. Respiratory data will be collected prior to the OGTT to determine resting metabolic rate. This data will be compared to calculations using Harris–Benedict equations, with adjustments for weight and physical activity levels applied, and used to inform nutritional (energy) requirements.

Dietary Intake and Quality Testing: Three-day weighed food records (2 weekdays and 1 weekend day) (Appendix I (i)) will be maintained in the week prior to the intervention, at the midpoint of the intervention, and during the week before the end of the intervention. Food records will provide information about baseline intake and adherence to the prescribed diet, and will be evaluated using a scientifically validated program (FoodWorks, Xyris Software) to determine macronutrient and micronutrient intake.

A validated online 120-item semi-quantitative food frequency questionnaire (Australian Eating Survey) (O'Brien et al. 2014) will be administered. Participants will self-report frequency of food

consumption for the previous 6 months at baseline and for the previous 8 weeks the post intervention clinical session. This questionnaire is not publicly available.

Adherence to the Mediterranean diet will be assessed using the Mediterranean Diet Adherence Screener Score (MEDAs) tool (Appendix H (iii)). method assigns a score of 0 or 1 according to the daily intake of nine components: high ratio of MUFA/SFA, moderate intake of alcohol, high intake of grains, vegetables, fruit and nuts, legumes and fish, low intake of meat and meat products, and moderate intake of milk and dairy products. The highest score of nine points reflects maximum adherence.

Diet quality will be assessed using the Healthy Eating Index via DIAL Software (Basiotis et al. 2002). The program gives different values between 0 and 100 considering the servings per day of food groups, macronutrients and selected micronutrients. The final value is classified into five categories (>80 points, "excellent diet"; ranging to 0 to 50 points, an "inadequate diet").

Mood State will be assessed using two validated questionnaires, the Profile of Mood State (POMS) questionnaire and the Hospital Anxiety and Depression (HADS) questionnaire. The POMS questionnaire uses 65 words or statements that describe feelings people have, whereas the HADS questionnaire consists of 14 questions (7 items belonging to depression and 7 items belonging to anxiety categories) which are scaled (Appendix H (i) and H (ii)).

References:

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7. Martı´nez-Gonzalez, M., Garcı´a-Arellano, A., Toledo, E., (2012). "A 14-Item Mediterranean Diet Assessment Tool and Obesity Indexes among High-Risk Subjects: The PREDIMED Trial." Plos One, 7 (8), e43134.

5.8.1 Participant Commitment	Approximately 16 weeks. This includes the two 8-week nutritional intervention periods (Mediterranean Diet and control diet).
5.8.2 Project Duration	The project will commence as soon as ethics has been approved, and the proposed project conclusion date is December 31st, 2020.
5.8.3 Participant Follow up	Not applicable.
5.8.4 Discontinuation criteria	Participants may be discontinued from participating in the case that they enrol meeting inclusion criteria; however, their circumstances change to meet exclusion criterion during participation. For example, a participant requiring antibiotic mediation during their participation will be discontinued as this medication can exert an independent influence on the gut microbiota.

5.9 Biological Sample Collection

5.9.1 What will be sampled and how?

Blood will be collected via IV catheterisation. Participants will rest in a supine position while a flexible 20-gauge Teflon catheter is inserted into an antecubital vein and attached to a three-way stopcock. This will allow for repetitive blood sampling during the oral glucose tolerance test (OGTT). The catheter will be kept patent by flushing it with isotonic saline after collection of each blood sample. Venous blood will be collected in EDTA, lithium heparin and serum-separating vacutainers. Blood samples will be collected on 3 clinical sessions. In the initial (baseline) clinical session and at a clinical session which immediately follows each of the 8-week dietary intervention periods (Mediterranean and control diet).

Oral Glucose Tolerance Test (OGTT): During each of the three clinical sessions, blood will be collected 4 times. A 20ml sample will be taken prior to glucose ingestion, and 20ml samples will be taken at 3 time points post ingestion (at 30 minutes, 60 minutes and 120 minutes). Thus, the maximum amount of blood taken per OGTT will be 80ml, with a total of 240ml taken throughout the research project. This compares with 500ml that is withdrawn during a standard donation at the blood bank.

Stool samples: Stool samples will be collected by participants. Participants will be provided with a stool collection kit (DNA Genotek OMNIgene-GUT Stool kit: https://www.dnagenotek.com/us/products/collection-microbiome/omnigene-gut/OMR-200.html) and related material. This includes a toilet collection hat, disposable gloves, collections spoons, a leak-proof primary receptacle with screw cap, leak-proof secondary receptacle with absorbent material and screw cap, padded envelope marked for mailing biological material and instructions for collecting/mailing the specimen. An infographic of how

to collect the sample (Appendix I (ii)) and a short questionnaire about the collection time and date will be included in the collection kit.

Stool samples will be collected at three time points: within a week of the initial (baseline) clinical session, and at the end of each 8-week nutrition intervention (Mediterranean and control).

Buccal Swab/DNA Collection: Cells from inside of a person's cheek will be collected using a standard buccal swab at the baseline clinical session. Two swabs will be taken. This is a relatively non-invasive way to collect DNA samples and is not a certifiable activity. Each swab will be used for genetic profiling using Single Nucleotide Polymorphism arrays as per manufactures instructions. Genetic data collected will be coded. Participants will be advised that their material and data will not be released for such uses without their consent, unless required by law (as per the National Statement section 3.5).

5.9.2 Impact of and response to particpant withdrawal

Participants have the right to withdraw at any time during the project, and the researchers will respect their decision. Participants will be informed of their right to withdraw their consent for their data to be used in the study, including time limitations to this (i.e. no data can be withdrawn following publication). Participants may withdraw at any time by informing a member of the researcher team by phone or email.

5.10 Data Management – Sample storage and disposal

Bio-specimens:

Plasma and serum samples will be decanted and aliquoted into de-identifiable labelled tubes, snap frozen in liquid nitrogen, and stored in a -80°C freezer for subsequent analysis.

Stool samples can be stored by participants in a provided biohazard mailing bag at room temperature until being mailed (or alternatively brought with them to their clinical session). It is recommended samples are sent to researchers immediately where possible. On arrival to the laboratory samples will be stored in a −80 °C freezer for subsequent analysis.

DNA samples will be stored in the freezer at (-20°C). Genetic data collected will be coded in a re-identifiable manner. Participants will be advised that their material and data will not be released without their consent, unless required by law (as per the National Statement 3.3). Additional information on genetic testing, to be supplied to participants as per Section 3.5.12 of the National Statement, will be provided in the Information to Participants form (Appendix B).

Blood and DNA samples will be taken by Dr Jessica Danaher and PhD student Suzi Ristevski. Stool samples will be collected by participants. Dr Jessica Danaher and Ms Suzi Ristevski are qualified in IV Cannulation and Phlebotomy (qualification attached in Appendix G). DNA sample collection using buccal swabs is not a certifiable procedure. Participants do not require qualifications for stool collection. Participants will receive instructions for collecting/mailing the stool specimen, and additional verbal guidance of the process will be provided by researchers prior to collection.

Human bio-specimens will be coded and stored in RMIT Laboratories (in which security assess is required). Once the analysis has been conducted on the samples they will be destroyed. The analytical data attained from these samples will be retained for at least seven years.

Paper and electronic data:

A paper-based personal information and medical questionnaire (Appendix B) will be completed by participants at the baseline clinical session. Paper-based food records will be kept by participants prior to, and twice throughout the dietary intervention. All hardcopy records will be kept in a locked filing cabinet at RMIT.

Direct electronic data entry will be used when collecting biochemical, DEXA and respiratory gas measurements at clinical sessions. Direct electronic data entries, in addition to electronic data generated via experimental techniques (e.g. GC-MS chromatogram outputs for metabolomics and dietary analysis outputs via Foodworks Software), will be kept in password protect files on the RMIT desktops of the project investigators.

The scientifically validated Australian Eating Survey is a 120-item questionnaire which will be completed by participants online and password protected. The data export is available upon request by investigators at conclusion of the study by emailing eatingsurvey@newcastle.edu.au. Data will be provided as a SPSS file for Windows Version 24 software. This data is compared to the Nutrient Reference Values and endorsed by the National Health and Medical Research Council. These outputs will be kept in password protect files on the RMIT desktops of the project investigators.

Dr Jessica Danaher will be responsible for the security of confidential data. All hardcopy data will be kept in a locked cabinet file at RMIT. All electronic data will be kept in password locked protected files on RMIT host servers. This data will be held for 15 years following publication (clinical trials data).

5.11 Data Analysis (including accounting for confounding factors and statistical power calculations)

Multivariate data attained through metabolomic techniques will be analysed as per statistical methods described by Danaher et al. (2016). Univariate data will be analysed using SPSS for Windows Version 24 software. Differences between allelic variant / genotype groups and individual metabolites, microRNA and gut microbiota will be examined using unpaired repeated-measures two-way ANOVA's. Where univariate analysis reveals any significant main effects for time, subsequent pairwise comparisons will be performed to detect differences over time. Where a genotype by time interaction is detected, multiple comparisons with Tukey's post hoc tests will be completed to identify differences. One-way ANOVA's will be performed for participant characteristic and indirect calorimetry data, with unpaired t-tests completed if interactions between factors are found. Linear regression and covariant analysis (ANCOVA) will be used to determine the effect of potential covariants (confounding factors) on allelic representation of dependent variables. Data will be expressed as mean ± SEM unless otherwise stated. The level of probability will be set at $p < 0.05$. Overall power of the data set will also be assessed via partial eta-square.

1. Danaher, J., Gerber, T., Wellard, R.M., et al. (2016). "The use of metabolomics to monitor simultaneous changes in metabolic variables following supramaximal low volume high intensity exercise" Metabolomics, 12(1):1-7.

5.12 Data Linkage	Participants will be de-identified and assigned a code by the researcher. They will only be re-identifiable by the researcher to minimise privacy breaches. The re-identification is also important in the context of returning genomic research findings as per 3.3.58 of the National Statement. Data will not be linked to any public medical records.
5.13 Outcome measures	The main outcomes to be measured involve revealing if the genetic background of individuals affects their response to a Mediterranean diet by measurement of key physiological factors, including: - Blood metabolites associated with glucose, fat and protein metabolism

	- Gene expression - Gut bacteria diversity and composition - Circulating hormones (ghrelin and insulin) Secondary outcomes that will also be measured include: - Body composition - Glucose tolerance - Food frequency and nutrient intake - Mood State
5.14 Will participants in the study be requested to modify any other treatments or interventions they may be receiving? NO	
5.15 Will participants in the study be requested to modify their regular daily activities (e.g. dietary intake, sleep, exercise)? YES *If YES, provide details:* The participants will be modifying their regular dietary intake as described in sections 4.3 and 5.3.	
5.16 Are any medication(s)/treatment(s) not permitted before and/or during the trial? YES *If YES, provide details:* Volunteers taking contraindicated prescription medication (e.g. antibiotics, thyroid, hyperlipidemic, hypoglycemic, hypertensives, psychotropic drugs or appetite suppressants) will be excluded from participating (or have their participation discontinued) as these medications may exert an independent influence on microbiome, metabolic, anthropometric, biochemical, clinical and/or dietary outcomes monitored throughout the intervention.	
5.17 Procedures for monitoring compliance	Participants will receive weekly support-orientated phone calls to ensure that procedures are being followed and how they are tolerating the intervention. To monitor compliance to the dietary protocol, validated questionnaires will be used as described in section 5.8.
5.18 Are there any likely or expected negative side-effects of the intervention? NO	

6.0 Assessment of Safety and Participant Monitoring
Are there likely or expected adverse events associated with the intervention? YES *If YES, provide details of these and the safety parameters in situ:* **<u>6.1 Low probability PHYSICAL risk:</u>**

1) Small risk of minor bruising or infection associated with blood sampling.

2) Small risk of a vaso-vagal attack associated with blood sampling.

3) Participants will be exposed to low dose radiation

6.1.2 How will these PHYSICAL risks be minimised:

Blood Sampling: To minimise risk of bruising and infection, blood samples will be performed by a qualified phlebotomist. The area will be swabbed with disinfectant prior to the insertion of the cannula to reduce the risk of infection. Compression will be applied to the area following the withdrawal of the cannula to reduce risk of bleeding and haematoma. After use, the cannula and blood drawing syringes will be disposed of properly and safely into biohazard bins to eliminate further risk of infection.

Radiation (DEXA Scans): The DEXA body composition test will involve participants being exposed to a low dose of radiation for approximately six (6) minutes per scan (thus 18 minutes in total throughout the entire study). Radiation exposure for the whole body scan is approximately 0.01mSv per scan. The radiation from a total body composition scan is equivalent to less than half a day of natural background radiation. The maximal permissible x-ray dose for non-occupational exposure is 500 mSv per year. Total radiation dose will be approximately 0.03mSv for the entire study. At this dose level, no harmful effects of radiation have been demonstrated as any effect is too small to measure. The Chief Investigator (Dr Jessica Danaher) has a current radiation license and the appropriate qualifications to run DEXA scans (see Appendix G (iv)). The PhD student Ms Suzi Ristevski is also enrolled to complete this qualification in March 2019, and an amendment to this application will be submitted following certification.

6.1.2 How will these PHYSICAL risks be managed if an adverse event were to happen?

Blood Sampling: In the case of an adverse reaction, infection or bruising associated with blood sampling, the subject would be asked to contact one of the listed investigators and advised to see their own medical professional for assessment at the investigators expense.

In the event of a vaso-vagal attack, the participant will be placed in a supine position with their feet elevated. A staff member qualified in First Aid will monitor the participant and their heart rate. If the participant is not recovering within 3 minutes, an ambulance will be called. In the event of a cardiac arrest, CPR will be performed, and urgent medical assistance sought.

Dr Jessica Danaher will be the first aid person on call, as she will be responsible for undertaking and overseeing blood sampling and thus will be with participants at the time in which they are exposed to physical risks. Dr Jessica Danaher is certified in First Aid (Level 2) and CPR via St John Ambulance Victoria (see Appendix G (iii)).

6.2 Low probability PSYCHOLOGICAL risks:

1. There is a small risk that blood and faecal sampling may induce procedure related stress and anxiety.

2. There is a small risk that body composition (DEXA) analysis may induce procedure related stress and anxiety.

3. There is a small risk that there may be embarrassment related to revealing dietary intake and providing stool samples.

4. There is a small risk of elevated stress and anxiety regarding learning about a potential to reveal information about genetics, health and disease for the individual, and also to relatives.

6.2.1 How will these PSYCHOLOGICAL risks be minimised:

Psychological Risks from Experimental Techniques: To minimise the risk of stress, anxiety and/or embarrassment caused by blood and stool sampling procedures, body composition impedance analysis, and dietary analysis, the participant will be guided through each procedural step (in a verbal and/or written form) as they are being performed. If participants become uncomfortable, the test and procedure will be immediately stopped. The participant will also be reminded prior to commencement of the project, and at data collection points, that participation is completely voluntary, that they are under no obligation to continue, and that they can withdraw from the study at any time.

To minimise stress and anxiety regarding potential genetic information, an Ethically Defensible Plan has been developed as per the National Statement 3.2 and 3.3 in the event that significant health results arise from the research (see Appendix X).

6.2.2 How will these PSYCHOLOGICAL risks be managed if an adverse event where to happen?

Psychological Risks from Experimental Techniques: In the case of psychological stress, the supervising staff, will demonstrate empathy and respect, problem solving and create options to likely empower the participant and minimise distress, anxiety and/or embarrassment.

An independent counselling service will be offered to participants if they wish to seek counselling as a result of their involvement in the study through RMIT University's counselling services (Ph: 9925 5000).

6.3 How will the investigators monitor the health and well-being of participants throughout the duration of the project?

During the project, participants will be monitored by phone and email. Support and guidance will be offered throughout the project. Researchers will be contactable within office hours (9am-5pm) via phone and email throughout the duration of the project.

6.4 Will the investigators monitor the health and wellbeing of participants for any period after completion of the study?

NO

6.5 Identifying potential benefits

6.5.1 Outline potential benefits to the participant(s)

The researchers cannot promise or guarantee that volunteering participants will gain any benefit from participating in this research, however, they may gain a better understanding about their health. All participants will receive three oral glucose tolerance tests (test for diabetes and insulin resistance) and body composition assessments (including bone density) during the course of the study and may receive information regarding results of these tests if desired. Participants will also receive sample dietary meal plans designed by a dietitian and weekly lifestyle change support (via phone calls, and access to recipes/blogs curated by academics via email).

Participants may also gain personal satisfaction for their contribution to scientific knowledge and evidence based nutritional practice.

6.5.2 Outline the potential benefits to the wider community

Whilst the science of nutritional genomics continues to demonstrate potential individual responses to nutrition, the complex nature of gene, nutrition and health interactions provide a challenge for healthcare professionals to analyse, interpret and apply this information to patient recommendations. This project can provide important information on sub-groups that would benefit from particular dietary recommendations, explore the possible underlying mechanisms and interactions between genes and diet, and contribute to scientific knowledge that can facilitate the

translation of nutritional genomics into nutrition practice. Overall, the findings may offer additional support for nutritional genomic screening in health care settings, as an effective strategy to treat and prevent nutrition-related chronic diseases, thereby reducing the social, health and economic burden of these diseases on society.

The greatest risk associated with participating in this study will be donating blood samples and body composition analysis (DEXA). However, the investigator obtaining blood samples is a trained and highly experienced phlebotomist, and the radiation exposure is low (equivalent to less than half a day of natural background radiation) with a certified investigator operating DEXA equipment. Consequently, it is the researcher's view that the potential benefits of subjects participating in this study, for the wider community, outweigh the potential risks.

7.0 Results, Outcomes and Future Plans	
7.1 Plans for return of results or findings of research to participants	The decision tree in the National Statement 3.3.30 was used to determine whether to return the results/findings of the research to the participant. Individual genetic results will be disclosed to the individual, however with caution and emphasis that more research and knowledge is required to translate findings into practice. An Ethical Defensible Plan has been devised in accordance with the National Statement 3.2 and 3.3 (see Appendix D).
7.1 Plans for dissemination and publication of project outcomes	The results and outcomes of the study will be presented in research publications (including a doctoral thesis), along with presentations related to the project. Participants will also be offered a summary of the project outcomes.
7.2 Other potential uses of the data at the end of the project	There are no other plans for potential uses of the data at the end of the project.
7.3 Project closure processes	The RMIT University HREC will receive progress reports throughout the duration of the project, and will receive a final report at the conclusion of the project.
7.4 Plans for sharing and/or future use of data and/or follow-up research	Researchers intend to disseminate the project outcomes to contribute to scientific knowledge, academic, professional and to nutrition and dietetic practice. The outcomes may be of public interest and may assist with future development of precision nutrition.
7.4.1 Anticipated secondary use of data	There is no anticipated secondary use of data.

8.0 Direct Access to Source Data/Documents

8.1 Statement of compliance	The investigators will permit trial-related monitoring, audits, IRB/IEC review, and regulatory inspections(s) by providing direct access to source data/documents.

9.0 Ethics	
9.1 Ethical considerations	All ethical considerations related to the trial are documented in detail in the NHMRC Human Ethics Application Form.
9.2 Data Handling and Record Keeping	Dr Jessica Danaher will be responsible for the security of confidential data. All hardcopy data will be kept in a locked cabinet file at RMIT. All electronic data will be kept in password locked protected files on RMIT host servers.
Will the intervention be administered or undertaken in a way that is different to standard practice or to that for which it has been approved? NO	

10.0 Use of placebo(s)
10.1 Does the research involve the use of a PLACEBO/CONTROL ONLY group (i.e. group that does not undertake the intervention/treatment at any stage of the research)? NO *If Yes, will participants in the placebo/control group be given the opportunity to undertake the intervention if it is found to be effective (e.g. separate to the study)?* Not applicable

11.0 Reporting of findings
Are there any limitations or restrictions on the reporting or publication of results by researchers (e.g. of negative results or adverse events)? NO