|  |  |
| --- | --- |
| **DEAKIN UNIVERSITY HUMAN RESEARCH ETHICS COMMITTEE****PROJECT DESCRIPTION/PROTOCOL** |  |

**Instructions for preparing the project description/protocol**

1. The purpose of the Project Description is to provide the scientific and academic background and context of a research project.
2. A Project Description is a **mandatory** component of a submission using the Human Research Ethics Application (HREA).
3. The section headings in this Project Description template represent a structure for presentation of information about a research project that meets the needs of an ethics review body.
4. Not all headings or sub-headings in this template are relevant for each research project. Where a question is not relevant please enter NA into the response box. Please do not delete the question.
5. Researchers may use visual aids embedded in the project description/protocol to assist in describing their project where appropriate (e.g. images, videos etc.).
6. Submissions of clinical trial proposals may use alternative protocol templates, such as the [SPIRIT statement](http://www.spirit-statement.org/).
7. Researchers may choose to submit an existing document (such as a protocol or project description that has already been developed) instead of developing a new document.
8. If researchers choose to submit an existing document instead of using one of the templates provided, they may need to provide indications to the ethics review body of where in the submitted document the content corresponding to the relevant fields in the template are located.
9. There is no need to duplicate information in the HREA into the Project Description or vice versa.
10. Language that is understandable to non-technical reviewers should be used.

**COVID-19**

All research must comply with current COVID-19 restrictions, as well as with Deakin’s [COVIDSafe Management Plan](https://deakin365.sharepoint.com/sites/CampusReactivation/SitePages/Being-COVIDSafe.aspx%22%20%5Co%20%22https%3A//deakin365.sharepoint.com/sites/CampusReactivation/SitePages/Being-COVIDSafe.aspx). Any activities considered as having high COVID-19 risk (e.g. requiring safety measures over and above the COVIDSafe Management Plan and risks covered by the general requirements of entry to campus) must have an approved [COVIDSafe Activity Plan](https://deakin365.sharepoint.com/sites/CampusReactivation/SitePages/Being-COVIDSafe.aspx%22%20%5Co%20%22https%3A//deakin365.sharepoint.com/sites/CampusReactivation/SitePages/Being-COVIDSafe.aspx) in place. This includes any on-campus research involving a face-to-face element, as well as off-site research (e.g., site visits, fieldwork etc).

**1. Project details:**

1.1 Please provide the project title ***Effect of foods rich in omega-3 fats on muscle microvascular blood flow.***

1.2 Please provide an acronym for the project (if appropriate)N/A

1.3 Please provide the project description/protocol version number- 1

**2. Project Team Roles & Responsibilities:**

2.1 Please provide the names, affiliations, positions and responsibilities of individuals involved in the project beyond those outlined in the HREA (e.g. technical or support staff).

***Dr. Gunveen Kaur*** *(Principal Investigator): Study conceptualisation, participant recruitment, participant screening and consent, data collection, data analysis, research dissemination, student supervision.*

***Prof. Michelle Keske*** *(Associate Investigator): Study conceptualisation, data collection, data analysis, research dissemination, student supervision.*

***Dr Lewan Paker*** *(Associate Investigator): Study conceptualisation, data collection, data analysis, research dissemination, student supervision.*

***Ms. Elmira Karimi*** *(Student Investigator): Participant recruitment, participant screening and consent, data collection, data analysis, and research dissemination.*

***Dr Barbara Brayner*** *(Associate Investigator): Data collection, data analysis and research dissemination.*

**3. Resources:**

3.1 Please provide details of the resources necessary for the project to be conducted, and the funding or support being sought or secured.

***Resources:*** *metabolic cart, two ultrasounds, SphygmoCor, contrast agent, lab consumables, blood sampling equipment, access to foodworks and online ASA tool for dietary data analysis.*

***Funding:*** *This project is being funded by a combination of IPAN Seed Funds ($9,000) and Deakin HDR funds ($2,000 p.a.).*

**4. Background:**

Please provide:

4.1 A lay summary of the literature review (approximately 1 A4 page)

*Poor dietary patterns such as a high-calorie, high-fat (HCHF) diet can contribute to insulin resistance (IR), which is a condition where body does not respond to insulin as it should, and eventually lead to type 2 diabetes (T2D)* (1, 2)*. Several human studies have confirmed that even 3-7 days of a HCHF diet impairs glucose metabolism, that is it impairs the ability of the body to remove glucose from the blood stream for energy storage and/or energy production (2, 3). Our team has recently completed a human study (funded by Diabetes Australia, Ethics No: 2019-014) that showed 7-days of a HCHF diet in 14 healthy individuals led to increased meal-induced insulin concentrations within 3 days, followed by reduced meal-induced blood flow in the smallest capillaries of the muscle, also known as muscle microvascular blood flow after 7 days, compared to pre-intervention (4). However, the above-mentioned studies focused on saturated fat as their main source of fat. Whether the incorporation of high omega-3 polyunsaturated fats into a HCHF diet may protect against HCHF diet-induced hyperinsulinemia and impairments in skeletal muscle microvascular blood flow in healthy humans is not known and is the aim of the current study.*

4.2 A rationale/justification (i.e. how the research will fill any gaps, contribute to the field of research or contribute to existing or improved practice)

*This research will provide new knowledge on how diet with high caloric intake and high omega-3 fat impact vascular insulin sensitivity in healthy individuals. Findings may have implications for the development of dietary strategies to prevent insulin resistance.*

4.3 The research questions/aims/objectives/hypothesis

*The aim of this project is to determine the impact of a 7-day dietary intervention that is high in calories and fat (HCHF), particularly omega-3 fat, on meal-induced skeletal muscle microvascular blood flow and metabolic responses in healthy adults.*

4.4 The expected outcomes

*We anticipate that omega-3 fats are protective and will mitigate the impact of a high calorie diet on meal-induced insulin concentrations and muscle microvascular blood flow.*

*1) The insulin response to a mixed meal drink (chocolate milkshake) will be simillar to baseline after 3 days and 7 days of high calorie diet containing omega-3 fats in healthy people.*

*2) Microvascular blood flow in skeletal muscle and adipose tissue will be not be impaired after 3 days and 7 days of high calorie diet containing omega-3 fats in healthy people.*

**5. Project** **Design:**

Please provide details of:

**The research project setting**

5.1 This may include physical sites, online forums and alternatives

*This study will be conducted at the Institute for Physical Activity and Nutrition (IPAN), within the School of Exercise and Nutrition Sciences research facility at Deakin University, Burwood.*

**6. Methodology:**

6.1 The methodological approach

*This is a single arm (pre/post) interventional study.*

6.2 The rationale for choices of method/s (tied to project aims/objectives)

*We aim to compare the participant’s metabolic and vascular responses to a mixed meal drink at pre, mid and post-intervention. Therefore, a single arm study design is appropriate to meet the objectives.*

**7. The participants including:**

7.1 A description and the number of participants

*This study will recruit 20 healthy adults to achieve a final sample size of 14 to allow for 15-20% dropout rate (7 females and 7 males).*

7.2 The inclusion and exclusion criteria

***Inclusion criteria:***

*• Healthy males and females aged 18-45 years with a normal body mass index (BMI) [between 18 and 24.9 kg/m2, considered normal weight according to the World Health Organization].*

*• Normotensive [seated brachial blood pressure less than 140/90 mmHg].*

*• Have no dietary allergies or food intolerances.*

*• Have given signed informed consent to participate in the study.*

***Exclusion criteria***

*• People who cannot read and communicate in English.*

*• People with any known food allergies.*

*• Regular smokers, or who have smoked in the past 10 years or/and smoked for more than 10 years will be excluded.*

*• History of severe liver disease, any vascular diseases or cardiovascular disease (previous stroke or heart attack), or T2D or who have a family history of T2D (parents with T2D).*

*• Pregnant or lactating women.*

*• People on a high fat diet (e.g. ketogenic diet) or a vegan/vegetarian diet.*

*• People consuming fish>2 times a week or take omega-3 supplements (supplementation for 2 or more days a week for more than a week, within the past 3 months).*

*• People exercising more than the current guidelines for physical activity (>5 hours of moderate intensity physical activity or > 2.5 hours of vigorous intensity physical activity per week).*

7.3 The sample size and statistical or power issues

*The previous study conducted by our team Brayner et. al. (4) demonstrated that changes in skeletal muscle microvascular blood flow 60min after the mixed meal drink in healthy individuals was significantly impaired 7 days after HCHF feeding (day 0 ∆ muscle microvascular blood flow 0.96±1.41 versus post 7 days HCHF ∆ muscle microvascular blood flow -1.28±1.36 AI/sec; n=14, mean ± SD, p=0.013). We anticipate the omega-3 fat rich diet (similarly matched for total fat and caloric intake as the Brayner study) will not cause a reduction in post prandial muscle microvascular blood flow after 7 days. We will recruit 20 participants to achieve a final sample size of 14 to allow for 15-20% dropout rate. We are well powered (90%) to see a significant difference in ∆ muscle microvascular blood flow between the two dietary interventions (if a comparison is made) with an alpha of 0.01 to allow for multiple comparisons.*

7.4 Your participant recruitment strategies and timeframes (as required in addition to that outlined in the HREA)

*Participants will be recruited through official IPAN social media accounts (e.g., Facebook, Twitter (X) advertisements) and flyers located around Deakin University, Burwood Campus (see attached advert). Researchers personal/academic social media accounts will not be used for recruitment. Students will not be directly targeted and recruited and therefore an organisational consent form is not required. Volunteers responding to the advertisements will be invited to contact the investigators by phone or email to undergo initial screening. Initial screening will be based on basic health and medical history questions to rule out major exclusion criteria (e.g., age, BMI, physical activity levels, history of cardiometabolic disease) and some dietary questions. Initial screening forms will be deleted/destroyed for all volunteers that are not eligible for study participation.*

7.5 Your approach/es to provision of information to participants and/or consent (as required in addition to that outlined in the HREA)

*Only named investigators on the approved ethics application will be involved in obtaining informed written consent, which will occur at Deakin University in a private consultancy room. No information or data will be collected before consent except for questions on major exclusion criteria which will be asked over the phone, email, or in person. All people will be treated with the same level of respect and dignity. Potential participants will be informed that involvement is entirely voluntary and that if they choose not to partake in the study, this will not disadvantage them in any way. Participants will be excluded if they cannot understand their involvement in the project (including risks and benefits) or cannot provide their own consent. For those individuals whose primary language is other than English, if there is a question that there could be any misunderstanding due to inadequate English, participants will not be included.*

7.6 If necessary, the type of consent provided to different participant groups, when and where, and any arrangements to confirm that consent

N/A

7.7 If necessary, details of who will be confirming or re-negotiating consent with participants and the process/es that will be undertaken

N/A

**8. Research Activities:**

What you are going to do? Please include:

8.1 The participant commitment

***Pre-visit General, health, dietary and physical activity questionnaires***

*Prior to enrolment in the study the participants will be asked to complete a general health and medical history questionnaire to identify conditions that may exclude them from participating.*

*They will also be asked to complete diet and physical activity questionnaires****.***

***Visit 1: Pre-intervention body composition assessment and metabolic and vascular responses to meal ingestion***

*The participants will arrive in the laboratory after an overnight fast.*

*Resting heart rate, echocardiograph, blood pressure and body composition analysis (height, weight and a DEXA scan) will be measured. They will provide a one-day food diary for the day before the testing visit. Intravenous catheter will be inserted into participants’ arm for collection of blood samples and for infusions. They will then undergo a mixed meal challenge (ingestion of a liquid meal; providing 1306.4kJ (39.6g of carbohydrate, 20.6g of protein and 5.3g of fat). Blood samples will be collected at 0, 15, 30, 45, 60, 75, 90, 105 and 120 minutes.*

*Resting metabolism (carbohydrate and fat utilisation) will be measured using a metabolic cart (Quark RMR Gas Analyzer, Cosmed, Italy) before the meal and 1 hour after the meal.*

*The participants will also undergo blood flow assessment which will include large vessel blood flow (femoral and brachial artery blood flow and flow-mediated dilatation) and microvascular blood flow (using contrast ultrasound imaging during microbubble infusion) in the skeletal muscle and adipose tissue using an ultrasound machine. Blood pressure and vascular stiffness will be measured using a cuff placed around the arm (Mobil-0-graph). These vascular measures will be performed at 0 min (before meal ingestion), 60 min, and 120 min during the mixed meal challenge.*

***Dietary intervention***

*After visit 1 is completed, participants will be instructed to consume a high calorie, high fat diet intervention for a total of 7 days. This involves participants consuming additional snacks (i.e. fish, nuts, sesame snaps, omega-3 enriched eggs, etc.) on top of their normal diet and by changing their eating habits to increase fat intake (e.g., cooking using olive oil). After completing 3 and 7 days of feeding intervention, participants will replicate the foods they consumed the day before Visit 1 and again return to the laboratory on day 4 (Visit 2) and day 8 (Visit 3) after an overnight fast.*

***Visit 2 and Visit 3: Post-intervention body composition assessment and metabolic and vascular responses to meal ingestion***

*• Participants will undergo the same metabolic and vascular testing as outlined in Visit 1, both 3 days (visit 2) and 7 days (visit 3) after the dietary intervention. The body composition scan will only be done on visit 1 and visit 3.*

8.2 The project duration

3 years

8.3 Any participant follow-up

*There will be no participant follow up. However, participants will be provided their body composition values, blood pressure and blood clinical chemistries (glucose, insulin, and lipid levels) results after the third visit.*

Please ensure your responses to Sections 9-12 comply with Deakin’s [Research Data Management procedure](https://policy.deakin.edu.au/document/view-current.php?id=23) and accurately reflect the details you have included in your [Research Data Management Plan](https://research-data.deakin.edu.au/footprints/dashboard/login?fromUrl=) (a compulsory document for all Deakin research).

**9. Data Collection/Gathering:**

9.1 What information are you going to collect/gather/generate? (as required in addition to that outlined in the HREA)

Please see section 13 Outcome measures.

9.2 Data collection/gathering techniques: How will you collect/gather the information? Will any third parties be involved in any aspects of recruitment or data collection?

***Anthropometrics*** *– height and weight by standard scale and measuring tape.*

***Body Composition*** *– by Dual-energy X-ray absorptiometry (DEXA) scan. A total body scan using a dual-energy X-ray absorptiometry machine will be used to assess body composition (fat and lean tissue mass) and bone mineral density. This is important, as body composition is an outcome that may be influenced by dietary intervention. The participants will be asked to lie-down on their back on the DEXA table in a gown for approximately 10-15 minutes while the machine scans their body. All DEXA measurements will be conducted by trained and accredited staff at Deakin University. Dr Kaur and Dr Parker are approved to perform DEXA (both hold valid licences). DEXA is a fast, simple and safe technique to evaluate body composition. The DXA method is well established at Deakin University (previous ethics no- DUHREC 2019-426; DUHREC 2019-014) and the radiation exposure for this study is considered very small (refer to Medical Physicist Report).*

***Physical activity*** *– self-reported physical activity using IPAQ questionnaire (attached).*

***Dietary intake****- self-reported dietary intake using online ASA24 form and three one day food diaries (day 0, day 3 and day 7).*

***Echocardiography*** *- A resting echocardiography using an ultrasound machine (Philips Ultrasound) with a variable-frequency phased array transducer for all two-dimensional, M-mode, Doppler and tissue Doppler examinations. A standard resting echo will assess systolic and diastolic function, cardiac output, ejection fraction, heart rate and LV filling pressure. This will require the researchers placing an ultrasound probe on the participants’ chest. The participants will be given a lab gown to wear at and the bed will be surrounded by curtains to protect their privacy.*

***Blood flow assessment.*** *Brachial and femoral arterial diameter and blood velocity will be measured non-invasively using a high frequency L12-5 linear array transducer interfaced to a commercial ultrasound machine (Philips Ultrasound). Diameter is assessed using 2D ultrasound and velocity assessed by Doppler ultrasound. Brachial artery blood flow (ml/min) is calculated as Πr 2 x mean velocity x 60 min. Where radius (r) is cm and mean velocity is cm/s.*

*Microvascular blood flow will be measured via contrast enhanced ultrasound imaging during microbubble infusion, previously approved at Deakin University (DUHREC 2017-172, DUHREC 2019-014). The technique involves intra-venous infusion of a commercially available contrast agent (Definity, Lantheus Medical Imaging) composed of haemodynamically inert, perflutren lipid microbubbles sufficiently small in size to perfuse capillaries. Definity is indicated in diagnostic ultrasound imaging – both liver/kidney assessment (lesion characterisation) and echocardiography (chamber opacification, endocardial border definition and regional wall motion assessment) (Therapeutic Goods Administration approved - ARTG ID 124808). Prof Keske and her collaborators at the University of Virginia have used Definity for assessment of skeletal muscle perfusion in healthy, insulin resistant and type 2 diabetes humans to assess microvascular responses in skeletal muscle and adipose tissue. In past seven years Prof Keske has been using this technique successfully at Deakin. Prof Keske has 20 years’ experience with this technique and has 18 years’ experience with particular brand of contrast agent (i.e. Definity). Professor Keske’s research team has completed >500 infusions at Deakin University since 2017. A standard ultrasound machine (Philips, Philips Healthcare, Australia) will be used to image the thigh muscle in cross-section. Depth, gain and focus will be optimised for each participant and maintained during repeated imaging sequences. A microbubble suspension [Definity (1.0ml) will be added to 30 mL of saline] will be infused at ≤3.8mL/min (infused rate based on body weight) using a syringe pump. Images will be acquired using ultrasound and analysed using computer software. The acoustic signal generated from the microbubbles will be measured and is directly proportional to the number of capillaries open/active and volume of blood in the microvascular system. After a high energy pulse of ultrasound, all microbubbles within the ultrasound beam are destroyed. The rate of microbubble reappearance within the ultrasound beam provides an indication of microvascular blood velocity which, combined with microvascular blood volume measurements, is used to determine total microvascular blood flow (i.e., microvascular function). After imaging the thigh muscle, the ultrasound probe will be placed on the abdomen (just right of the umbilicus) to image subcutaneous adipose tissue. Imaging is identical to that described above for skeletal muscle.*

***Venous Blood Sampling.*** *For visit 1, 2 and 3, venous blood samples (20 ml) will be taken before (baseline) and 10 ml at 15, 30, 45, 60, 75, 90, 105 and 120 minutes after ingestion of the mixed meal (Visit 2 and 3 total blood draw: 100 ml each). The volume of blood collected at each visit (maximum 100 ml) is substantially less than the amount provided during a single blood donation ~500 ml. Research staff qualified to perform cannulation and venepuncture will collect blood samples via intravenous catheter and venepuncture. Catheters are used when several blood samples are needed from one site over a brief duration such as to be used here. Once the catheter is in place, it is a simple and painless procedure to remove further blood samples. In between each sample, the catheter will be flushed and left filled with a small volume (~1ml) of isotonic saline solution to maintain catheter patency. Catheterisation and blood sampling is a well-established and accepted technique routinely performed at Deakin University. Venous blood samples will be collected and transported on ice to the local pathology lab (Dorevitch labs) for glucose, insulin and lipid analysis. A venous sample will also be sent to OmegaQuant for omega-3 level analysis. The samples sent will be coded and will not contain the name of the participants. Additional blood that is sampled throughout the 2 hour mixed meal challenge will be immediately tested for glucose levels. The blood will then be centrifuged and plasma, serum and buffy coats kept at -80°C for further analysis.*

***Resting metabolism.*** *Resting metabolism (oxygen consumption) will be measured before the meal using a metabolic cart (Quark RMR Gas Analyzer, Cosmed, Italy) and 1 hour after the meal. This involves participant laying on the clinical bed for 20 mins, breathing normally into a mask that is connected to the oxygen and carbon dioxide sensors.*

***Blood Pressure (Brachial and central) and Vascular Stiffness.*** *Brachial blood pressure, central blood pressure and aortic stiffness will be recorded non- invasively with a validated Mobil-O-Graph device (from I.E.M.; http://www.iem.de/en/products/mobil-o-graph.html). Measures will be taken with a correct sized cuff. This is an automated device making data collection easy and non-invasive.*

***Responsibilities:*** *All trials will be attended by the student and by one of the experienced members of the research team (Dr Kaur, Dr Brayner, Dr Parker or Prof Keske). Venepuncture will be done by Ms Elmira, Dr Kaur, Dr Brayner or Dr Parker. The Definity microbubble infusion will be conducted by Dr Kaur, Dr Brayner, Dr Parker and/or Prof Keske. The blood collection from the cannula will be done by Ms Elmira, Dr Kaur, Dr Brayner or Dr Parker. DEXA measurements will be performed by Dr Parker or Dr Kaur. Metabolic cart measures will be performed by Ms Elmira, Dr Barbara, Dr Parker or Dr Kaur. The student investigator will be trained in imaging using the ultrasound by Prof Keske, Dr Kaur and Dr Brayner and the imaging will be done by the student investigator and Dr Kaur.*

9.3 Impact of and response to participant withdrawal

*Participants are informed (in the Plain Language Statement and Consent form) they can freely withdraw at any time without consequence or repercussion. It is unlikely to see any negative impacts of stopping consumption of the snacks. Participants can just resume their usual diet.*

**10. Data Management:**

10.1 How will you store, provide access to, disclose, use/re-use, transfer, destroy or archive the information that you collect/gather? (as required in addition to that outlined in the HREA)

*A central database will be located and managed at the Institute for Physical Activity and Nutrition (managed by Dr Gunveen Kaur) and accessed from Deakin Computers only. All data will be retained for a minimum of 15 years from the date of final publication. Digital data will be stored on a RSD drive connected to Deakin’s secure network and password protected. Paper files will be stored in locked filing cabinets in the PI Dr Kaur’s office. Each participant is given a unique code to de-identify them. All samples and data collected will use this unique code. Statistical analysis will be performed on de-identified data and material. However, we will have the ability to re-identify during circumstances where participants have abnormal test results that require the study team to contact the participant and recommend they contact their GP/doctor for follow-up. The identification codes will be held on password protected databases. Only the investigator team will have access to them. We will publish the results in a peer-reviewed medical journal. Data will be presented as individual data points or group statistics and there will be no identification of participants or their personal results.*

Include a Research Data Management Plan in accordance with National Statement [3.1.45 and 3.1.56](https://www.nhmrc.gov.au/about-us/publications/national-statement-ethical-conduct-human-research-2007-updated-2018#toc__556) and Deakin’s [Research Data Management procedure](https://policy.deakin.edu.au/document/view-current.php?id=23).

**11. Data Analysis:**

11.1 How will you measure, manipulate and/or analyse the information that you collect/gather?

*Clinical chemistries – will involve a combination of in-house techniques and a certified pathology laboratory (Dorevitch labs) and OmegaQuant. In-house techniques include a glucose analyser, ELISA kits, and calorimetric kits for measuring glucose, insulin, gut-derived hormones, free fatty acids and inflammatory cytokines. Values will be plotted versus time and areas under the time curve calculated and this will be used to compare pre, mid and post intervention values.*

*Blood flow, vascular stiffness and blood pressure – cardiovascular outcomes are calculated from the measured values as outlined above in 9.2. Cardiovascular values per participant will be analysed individually at baseline, 60 and 120min after the meal as well as within each participant a relative index (change from baseline); these will be compared between pre, mid and post-intervention.*

*Body composition: Body weight will be assessed pre, mid and post intervention. Body fat mass and fat free mass will be assessed pre and post intervention.*

*Resting metabolism: Resting energy expenditure will be assessed by metabolic cart and assessed pre, mid and post intervention.*

*Statistical analyses: Student paired t-test will be used to compare endpoint measurements of fat mass and fat free mass at pre- and post-intervention. Two-way repeated-measures ANOVA followed by a suitable post hoc test will be used for all other continuous variables. Significance will be set at P < 0.05.*

11.2 Please describe your matching and sampling strategies

*We only have one group in the study and we will be assessing pre, mid and post-intervention values. Therefore all participants will serve as their own control.*

11.3 Please outline how you will account for potential bias, confounding factors and missing information

*Confounding factors and missing information will be handled in the statistical analysis if necessary.*

*We will attempt to avoid potential bias by using non-identifiable coding for all data analysis so as to be blinded to the participant as much as possible. All blood flow analysis occurs after the testing date.*

11.4 Please include your statistical power calculation

*The previous study from Brayner et al. demonstrated that changes in skeletal muscle microvascular blood flow 60min after the MMC in healthy individuals was significantly impaired 7 days after HCHF feeding (baseline D muscle microvascular blood flow 0.96±1.41 versus post 7 days HCHF D muscle microvascular blood flow -1.28±1.36 AI/sec; n=14, mean ± SD, p=0.013). We anticipate the omega-3 fats rich diet (similarly matched for total fat and caloric intake as the Brayner study) will not cause a reduction in post prandial muscle microvascular blood flow after 7 days. We will recruit 20 participants to achieve final sample size of 14 to allow for 15-20% dropout rate. We are well powered (90%) to see a significant difference in D muscle microvascular blood flow between the two dietary interventions (if a comparison is made) with an alpha of 0.01 to allow for multiple comparisons.*

**12. Data Linkage:**

12.1 What linkages are planned or anticipated?

N/A

**13. Outcome measures:**

13.1 Please describe your outcome measures

***Whole body insulin sensitivity and blood glucose area under the curve following a mixed meal.*** *These measures are calculated from the plasma glucose and insulin levels that are measured at rest (fasted state) and in 30 min intervals for 2 hours following the meal. The premise for these measures is that following a meal the blood glucose levels raise and then insulin is secreted from the pancreas into the bloodstream to facilitate glucose uptake in the tissues and thereby reducing blood glucose back to resting levels. The degree in how elevated these values are in the blood and the length of time they are elevated gives an indication of a participant’s insulin sensitivity.*

***Microvascular blood flow following a mixed meal drink****. Microvascular perfusion is measured using contrast-enhanced ultrasound imaging during intravenous infusion of a commercially available contrast agent (Definity, Lantheus Medical Imaging), previously approved at Deakin University. This will be done before ingesting a mixed meal drink (baseline), and at 60 and 120 min after the meal. The premise for this time point is that the meal normally increases microvascular perfusion at 60 min and declines by 120 min so this is an ideal window of opportunity to assess differences in muscle microvascular perfusion.*

***Brachial and femoral artery blood flow****: Brachial and femoral artery diameter and blood velocity will be measured using a commercial ultrasound (Epiq 7, Philips Medical System, North Ryde, NSW, Australia). Diameter (two-dimensional ultrasound) will be recorded at the peak of the QRS complex (determined via three-lead electrocardiography). Blood velocity (pulsed-wave Doppler ultrasound) will be averaged over approximately ten cardiac cycles. Artery mean blood flow (mL/min) is calculated as: πr2 (cm) x mean velocity (cm/s) x 60. Probe location and ultrasound settings will be recorded for repeat measures within and between visits.*

***Echocardiography****: Left ventricle function will be assessed non-invasively using echocardiography (Epiq 7; Philips Medical System, North Ryde, NSW, Australia) and three-lead electrocardiography. Two-dimensional and Doppler images will be obtained from the parasternal long axis view and apical four- and five-chamber views. Cardiac output will be calculated by multiplying LV outflow tract (LVOT) area (during DUHREC Project Description/Protocol (version 4, dated June 2022), peak systole in the parasternal long axis view), velocity time integral (pulsed-wave Doppler; apical five-chamber view), and heart rate, indexed to body surface area. Ejection fraction will be calculated as end-diastolic minus end-systolic volumes (apical four-chamber view) using the Simpson method. LV early (E) and late (A) diastolic flow velocity and deceleration time of early diastolic flow wave (DT) will be assessed via pulsed-wave Doppler (apical four-chamber view). LV filling pressure (E/e’) will be calculated as the ratio of early diastolic mitral inflow and annular velocities (pulsed-wave Doppler; apical four-chamber view).*

***Pulse wave analysis and pulse wave velocity****: will be measured non-invasively using a SphygmoCor XCEL device (Version 1.3; AtCor Medical, Australia). The SphygmoCor uses a standard blood pressure cuff placed around the upper arm to measure central systolic and diastolic pressure, pulse pressure, augmentation index (AIx) AIx normalised to a heart rate of 75bpm (Aix@75bpm), heart rate and brachial systolic and diastolic pressure. A pressure cuff is then placed as superior as possible on the upper left thigh and a tonometer on the left carotid pulse to measure carotid-femoral pulse wave velocity (PWV). PWV will be calculated from measurements of: (i) left carotid pulse to sternal notch; (ii) sternal notch to top of femoral pressure cuff; and (iii) left inguinal fold to top of femoral pressure cuff. The SphygmoCor device includes built-in quality checks for both pulse wave analysis and PWV to ensure the accuracy and reliability of the measurements. In instances where research staff are unable to obtain measurements on the left side, the right side will be used.*

***Blood sampling****: Researchers qualified to perform cannulation and venepuncture will collect blood samples using an intravenous catheter. Intravenous catheters are used when multiple blood samples are required from a single site over a short duration, as is the case in this study. Once the catheter is in position, the process of obtaining additional blood samples is straightforward and painless. Following each sample collection, the catheter will be flushed with a small volume (~3ml) of isotonic saline solution to maintain patency of the line. Catheterisation and blood sampling is a well-established and widely accepted technique routinely conducted at Deakin University.*

*Venous blood collected from an antecubital vein (at rest, and during mixed meal challenge) will be analysed immediately for blood glucose (ABL800 Flex; Radiometer Medical, Denmark). Blood samples will sent to the Dorevitch path lab and OmegaQuant for analysis of fasting glucose and insulin, HbA1c, total cholesterol, high- and low-density lipoprotein, triglycerides and omega-3 levels. Blood samples will also be stored at -80°C for later analysis of relevant markers.*

***Dual-energy x-ray absorptiometry (DEXA)****: will be used to assess total body fat and lean body mass. Participants will lie supine on the DEXA table wearing light clothing (free of metal). The scanning process takes approximately 10–15 minutes and will be conducted by trained and accredited staff at Deakin University. DEXA is a simple, accurate, and safe method to assess body composition. The radiation dose is considered very low (see attached Medical Physicist Report). Before undergoing the procedure, participants will receive thorough explanations regarding the very low radiation dose used in the DEXA scan and how it compares to typical daily background radiation. Participants will also be screened for contraindications (e.g., pregnancy status, history of radiation exposure, and known sensitivity/adverse reactions to radiation). Participants who provide any response other than “No” to the inquiry about pregnancy status will be excluded from the study.*

***Risks associated with participation***

*Please refer to HREA Q2.3.1. Please also note all procedures and questionnaires used in this project are identical to the previously approved study (DUHREC #2019-014) with the exception of the dietary intervention which will contain higher amounts of omega 3 fats instead of saturated fats.*

***Blood sampling****: Potential risks include some pain and discomfort upon cannula insertion, bleeding, bruising, vasovagal episodes, thrombophlebitis, and infection.*

***DEXA scan****: Risks include radiation exposure.*

***Definity contrast agent infusion****: A small percentage of individuals (8.4%) may experience side effects during the infusion of the contrast agent (Definity) in ultrasound imaging. Common side effects include back pain (1.2% of individuals), chest pain (0.8%), headache (2.3%), dizziness (0.6%), nausea (1.0%), and flushing (1.1%). Serious cardiopulmonary or allergic reactions are exceedingly rare and typically occur within 30 minutes of administration. The risk of infection is minimal given the suitability of the contrast agent for intravenous infusion and the use of sterile equipment and aseptic techniques. Professor Keske has >18 years of experience working with Definity and the research team has completed >500 infusions at Deakin University since 2017.*

***Mixed Meal Challenge****: It is possible that participants may experience hypoglycaemia or hyperglycaemia during or following the mixed meal challenge.*

***Participant privacy during ultrasound assessment of the heart****: In some cases, participants (predominantly females) will be asked to wear a loose research gown instead of a shirt to assist with access to the chest and obtaining a clear image of the heart. This is common practice and often necessary when imaging the heart.*

***Mitigation of risks***

*Please refer to HREA Q2.3.2*

***Screening process:*** *Screening before participation will exclude individuals with disease or risk of allergies etc. Participants indicating medical conditions or risk factors will undergo further assessment by the PI to determine eligibility. Depending on the contraindication, participants may be excluded before commencing the study.*

***Blood sampling:*** *Sterile and disposable (single-use) equipment will be used to minimise the risk of infection. Venepuncture and cannulation will only be performed by qualified and experienced researchers to reduce the likelihood of bruising and infection. Aseptic techniques and infection control procedures will be used at all times.*

***DEXA:*** *This study involves a single exposure to a very small amount of radiation (~0.02mSv). At this dose level, no harmful effects of radiation have been demonstrated, as any effect is too small to measure. Therefore, the risk is believed to be minimal, as confirmed by the attached Medical Physicist Risk Assessment report. The procedure will be performed by an experienced and licenced researchers at Deakin University.*

***Definity contrast agent infusion:*** *All contrast agent infusions will be supervised by research staff with advanced cardiopulmonary resuscitation training and resuscitation equipment readily available prior to administration. To further minimise the risk of adverse events, all substances to be infused (contrast agent and saline) will be recorded for their expiration date and cross-checked by a minimum of two researchers prior to infusion; a dedicated syringe pump will be used to set the contrast agent infusion rate at a constant, predetermined rate; only named research staff will make up the contrast agent solution; and, a maximum of 3ml will be infused in any one day as per previous studies. All participants are monitored for acute reactions for at least 30 minutes post-infusion. In the unlikely event of a serious reaction, emergency services will be contacted.*

***Mixed Meal Challenge:*** *Diabetes is an exclusion criterion and therefore the risk of hypoglycaemic or hyperglycemia in response to MMC is low. Participant’s blood glucose will be monitored to ensure that blood glucose levels return to near baseline (safe levels) prior to leaving the laboratory. A supply of sugar (jelly beans) will be readily available and supplied to participants to increase blood sugar levels in the event of a hypoglycaemic excursion*

***Participant privacy during ultrasound assessment of the heart.*** *The research laboratory has curtains and dividers to ensure privacy for the participant. The process behind imaging the heart will be comprehensively discussed with the participant prior to enrolment in the project (i.e., during the informed consent phase). All efforts are made to ensure privacy is maintained for the participant at all times. This procedure, and what is involved, is discussed with participants during the informed consent and screening phase, and during the familiarisation sessions.*

***References***

*1. Bakker LE, van Schinkel LD, Guigas B, Streefland TC, Jonker JT, van Klinken JB, van der Zon GC, Lamb HJ, Smit JW, Pijl H, Meinders AE, Jazet IM. A 5-day high-fat, high-calorie diet impairs insulin sensitivity in healthy, young South Asian men but not in Caucasian men. Diabetes. 2014;63(1):248-58.10.2337/db13-0696*

*2. Parry SA, Turner MC, Woods RM, James LJ, Ferguson RA, Cocks M, Whytock KL, Strauss JA, Shepherd SO, Wagenmakers AJM, van Hall G, Hulston CJ. High-Fat Overfeeding Impairs Peripheral Glucose Metabolism and Muscle Microvascular eNOS Ser1177 Phosphorylation. J Clin Endocrinol Metab. 2020;105(1).10.1210/clinem/dgz018*

*3. Parry SA, Smith JR, Corbett TR, Woods RM, Hulston CJ. Short-term, high-fat overfeeding impairs glycaemic control but does not alter gut hormone responses to a mixed meal tolerance test in healthy, normal-weight individuals. Br J Nutr. 2017;117(1):48-55.10.1017/S0007114516004475*

*4. Brayner B, Keske MA, Roberts-Thomson KM, Parker L, Betik AC, Thomas HJ, Mason S, Way KL, Livingstone KM, Hamilton DL, Kaur G. Short-term high-calorie high-fat feeding induces hyperinsulinemia and blunts skeletal muscle microvascular blood flow in healthy humans. Am J Physiol Endocrinol Metab. 2024;327(1):E42-E54.10.1152/ajpendo.00070.2024*

**14. For research involving an unapproved therapeutic good (such as a drug, device or biological):**

14.1 Does this project involve an unapproved therapeutic good requiring a Clinical Trial Notification (CTN)? (See the [Clinical Trials webpage](https://www.deakin.edu.au/students/research/research-support-and-scholarships/integrity-secure/clinical-trials) for more information about CTNs)

[x]  Yes – go to the next question.

[ ]  No – skip to Section 15 (results, outcomes and future plans)

14.2 Is Deakin intended to be the Sponsor?

[x]  Yes – go to the next question

[ ]  No – skip to Section 15 (results, outcomes and future plans)

14.3 If Deakin is intended to be the Sponsor and the research requires a Clinical Trial Notification (CTN), has the CTN, Clinical Trial Sponsorship Request Form and Protocol been submitted to research-integrity@deakin.edu.au for assessment?

[x]  Yes – assessment completed and the CTN must now be submitted to the Therapeutic Goods Administration (TGA) by Deakin (as Sponsor). Please attach evidence of assessment and the CTN form. You will be contacted by the Human Research Ethics Office regarding submission of the CTN to the TGA.

If not, please submit the draft CTN, Clinical Trial Sponsorship Request Form and Protocol to research-integrity@deakin.edu.au for assessment before submitting this application to DUHREC. See the [Clinical Trials webpage](https://www.deakin.edu.au/students/research/research-support-and-scholarships/integrity-secure/clinical-trials) for further information. The Clinical Trial Sponsorship Request Form can be requested by contacting research-integrity@deaknin.edu.au.

14.4 What is/are the drug(s) and/or device(s):

* Approved name Definity
* Trade name (if any) Definity
* Manufacturer Lantheus Medical Imaging, Massachusetts, USA,
* Supplier of drug/device (e.g. manufacturer/pharmacy) Global Medical Solutions
* Approved therapeutic indication, dosage/duration in Australia- TGA approved for intravenous infusion for ultrasound enhancement of microvessels in heart, liver and kidney (ARTG ID 124808). CTN will cover for imaging skeletal muscle and adipose tissue.
* Believed mode of action N/A
* Dosage regimen Intravenous infusion 3ml total; infused 1.0 ml infused 3 times over 1.5 hours
* Mode of excretion N/A
* Known adverse events - small number of people (8.4% of people) have side-effects during the infusion of Definity during ultrasound imaging. The most common of these side-effects include: back pain (1.2% of people), chest pain (0.8%), headache (2.3%), dizziness (0.6%), nausea (1.0%), flushing (1.1%). These symptoms disappear within 30 minutes after the infusion is stopped. Serious reactions (e.g. allergic or anaphylactic reaction) during this infusion have been reported to occur, however, these are incredibly rare (incidence is estimated to be at 0.009% and 0.004%, respectively).
* Known contra-indications or warnings - Do not administer Definity to patients with known or suspected hypersensitivity to perflutren lipid microsphere or its components (i.e. people who have had an allergic reaction to a previous infusion on Definity).
* If arrangements have been made for a Pharmacy Department to receive or dispense the drugs involved in this project, explain how the drugs will be received and dispensed for the purposes of the research project – This contrast agent is supplied by Global Medical Solutions, Cardiff, NSW, Australia and securely stored in a locked temperature-controlled refrigerator in the clinic of the School of Exercise and Nutrition Sciences. Infusions for each participant (including Definity batch number, expiration date, dose, and infusion rate) are recorded into a database.

**15. Results, Outcomes and Future Plans:**

15.1 Please outline your plans for return of results of research to participants – include an ethically defensible plan in accordance with National Statement [3.1.65](https://nhmrc.gov.au/about-us/publications/national-statement-ethical-conduct-human-research-2007-updated-2018#toc__438) or [3.2.15](https://nhmrc.gov.au/about-us/publications/national-statement-ethical-conduct-human-research-2007-updated-2018#toc__725) or [3.3.36-3.3.61](https://nhmrc.gov.au/about-us/publications/national-statement-ethical-conduct-human-research-2007-updated-2018#toc__826), as appropriate.

*Participants will be provided with all personal results at the end of the participation. Participants will be provided with the overall findings of the study upon request and after study completion.*

15.2 Please describe your plans for dissemination and publication of project outcomes

*Results will be submitted to medical journals for publication for dissemination purposes. It is expected that study results will also be communicated in seminars and conferences.*

15.3 Please list other potential uses of the data at the end of the project

*Data may be used for grants or establishing rationale for future research.*

15.4 Please detail the project closure processes

*Upon project completion, proper procedures for notifying Deakin Ethics will be followed.*

15.5 Please outline your plans for sharing and/or future use of data and/or follow-up research

*There are no immediate plans for sharing data or follow-up. However, as per the Consent form, we have informed participants that samples and/or data may be used for other studies.*

15.6 Please describe any anticipated secondary use of data

N/A

**DECLARATION AND SIGNATURES**

 I/We, the undersigned declare that the information supplied in this application (including the attached original application) is true and accurate to the best of my/our knowledge.

I/We the undersigned have read the *National Statement on Ethical Conduct in Human Research* and accept responsibility for the conduct of the project detailed in this application in accordance with the principles contained in the Statement and any other conditions laid down by Deakin University Human Research Ethics Committee.

I/We the undersigned, declare that where the research project may involve contact with a child or young person under the age of 18, I/we have a current Working with Children Check.

**Principal investigator**

Name: **Gunveen Kaur**

Human Ethics Quiz (please complete the appropriate box below):

[ ]  successfully completed the Human Ethics Quiz (compulsory for Deakin staff and students)

[x]  exempt from completion of the Quiz due to prior inclusion on an ethics application at Deakin. *Please indicate HEAG or DUHREC Project ID: 2019-014*

Signature: **** Date: **18.6.24**

**Associate investigator\***

Name: **Prof Michelle Keske**

Affiliation (please select from the drop-down list by clicking on ‘Choose an item’): Choose an item.

Human Ethics Quiz (please complete the appropriate box below):

[ ]  successfully completed the Human Ethics Quiz (compulsory for Deakin staff and students)

[ ]  exempt from completion of the Quiz due to prior inclusion on an ethics application at Deakin. *Please indicate HEAG or DUHREC Project ID:* Click or tap here to enter text.

[ ]  external researcher (exempt from completing the Quiz)

Signature:  Date: **03/07/2024**

**Associate investigator\***

Name: **Dr Lewan Parker**

Affiliation (please select from the drop-down list by clicking on ‘Choose an item’): Choose an item.

Human Ethics Quiz (please complete the appropriate box below):

[x]  successfully completed the Human Ethics Quiz (compulsory for Deakin staff and students)

[ ]  exempt from completion of the Quiz due to prior inclusion on an ethics application at Deakin. *Please indicate HEAG or DUHREC Project ID:* Click or tap here to enter text.

[ ]  external researcher (exempt from completing the Quiz)

Signature:  Date: **04/07/2024**

**Associate investigator\***

Name: **Dr Barbara Brayner**

Affiliation (please select from the drop-down list by clicking on ‘Choose an item’): Choose an item.

Human Ethics Quiz (please complete the appropriate box below):

[ ]  successfully completed the Human Ethics Quiz (compulsory for Deakin staff and students)

[x]  exempt from completion of the Quiz due to prior inclusion on an ethics application at Deakin. *Please indicate HEAG or DUHREC Project ID: 2019-014*

[ ]  external researcher (exempt from completing the Quiz)

Signature:  Date: **25/06/2024**

**Please copy and paste the above for each additional associate investigator.**

**Student investigator\***

Name: **Ms Elmira Karimi**

Affiliation (please select from the drop-down list by clicking on ‘Choose an item’): Choose an item.

Human Ethics Quiz (please complete the appropriate box below):

[ ]  successfully completed the Human Ethics Quiz (compulsory for Deakin staff and students)

[x]  exempt from completion of the Quiz due to prior inclusion on an ethics application at Deakin. *Please indicate HEAG or DUHREC Project ID: 2019-014*

[ ]  external researcher (exempt from completing the Quiz)

Signature: **** Date: **26/06/2024**

**Please copy and paste the above for each additional student investigator.**

**\***All research staff involved in the project must sign the project description/protocol. Please add additional signatures blocks as required.

**ACKNOWLEDGMENT OF HEAD OF SCHOOL/DIRECTOR OF RESEARCH\*\***

I, the undersigned, acknowledge that the School/Faculty/Institute has considered and approved the academic worth of the project described in this application.

Name: Click or tap here to enter text.

Signature: Click or tap here to enter text. Date: Click or tap here to enter text.

\*\*If the Head of School (or similar) is also a member of the research or supervisory team, a more senior member of University staff e.g. Dean or Associate Dean (Research), must sign the project as authorising officer.

A Project Description is a **mandatory** component of a submission using the

Human Research Ethics Application (HREA).

Please submit all documents via direct email to <research-ethics@deakin.edu.au>.

Deakin University is collecting your personal information on this form for the primary purpose of processing your human research ethics application. It will also use this information for monitoring your compliance with the approved protocol. For these purposes Deakin may also provide this information to potential research participants, past or current research participants, or other interested parties in your research. You are not required to provide the information requested, however if the information is not provided, Deakin may not be able to process your ethics application. Deakin manages personal information it holds, including requests by individuals for access to their personal information, in accordance with the Privacy and Data Protection Act 2014 (Vic). Deakin’s Privacy Policy may be viewed on Deakin’s [Policy Library](https://policy.deakin.edu.au/?_ga=1.41072994.1915361819.1415758364). Information on privacy at Deakin is available at <http://www.deakin.edu.au/footer/privacy>.  Questions about privacy may be directed to the Privacy Officer on (03) 5227 8524 or by email to privacy@deakin.edu.au.