**STUDY PROTOCOL**

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| **Study N°** | 1 |
| **Massey RMS Code N°** |  |
| **Massey RM** | **PR96444 RNIEL** |

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| **How does the Digestible Indispensable Amino Acid Score (DIAAS) influence Protein Turnover? The Efficacy Potential of Combinatorial Proteins in Humans**  Short title: **Combining Dietary Protein Sources to Improve Amino-Acid Digestibility and Net Protein Balance - The EPiC Study** |

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|  |  |
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**TABLE OF CONTENTS**

[1 INVESTIGATOR(S) SIGNATURE(S) PAGE 5](#_Toc138152717)

[2 SPONSOR TEAM SIGNATURES PAGE – Not Applicable for Current Application 7](#_Toc138152718)

[3 PROTOCOL AMENDMENT 9](#_Toc138152719)

[4 STUDY SITES 10](#_Toc138152720)

[5 STUDY CONTACT INFORMATION 10](#_Toc138152721)

[6 ABBREVIATIONS - DEFINITIONS 11](#_Toc138152722)

[7 SYNOPSIS 12](#_Toc138152723)

[8 STUDY PLAN OVERVIEW 13](#_Toc138152724)

[9 INTRODUCTION 14](#_Toc138152725)

[10 OBJECTIVES OF THE STUDY 15](#_Toc138152726)

[10.1 Primary objective 15](#_Toc138152727)

[10.2 Primary outcome 15](#_Toc138152728)

[10.3 Secondary objectives 15](#_Toc138152729)

[10.4 Secondary outcomes 15](#_Toc138152730)

[11 STUDY DESIGN 16](#_Toc138152731)

[11.1 Type of Study 16](#_Toc138152732)

[11.2 Subjects, groups, and centers 16](#_Toc138152733)

[11.3 Expected study duration and milestones 16](#_Toc138152734)

[12 STUDY POPULATION 16](#_Toc138152735)

[12.1 Description 16](#_Toc138152736)

[12.2 Subject screening 16](#_Toc138152737)

[12.3 Subject inclusion criteria 16](#_Toc138152738)

[12.4 Subject exclusion criteria 17](#_Toc138152739)

[12.5 Subject withdrawal criteria 17](#_Toc138152740)

[13 STUDY INTERVENTION 18](#_Toc138152741)

[13.1 Study intervention description 18](#_Toc138152742)

[13.1.1 Composition 19](#_Toc138152743)

[13.1.2 Form and dosage 19](#_Toc138152744)

[13.1.3 Quality control 20](#_Toc138152745)

[13.1.4 Packaging and labeling 20](#_Toc138152746)

[13.1.5 Randomization technique and coding 20](#_Toc138152747)

[13.2 Study product administration 20](#_Toc138152748)

[13.2.1 Amount, composition, and preparation 20](#_Toc138152749)

[13.2.2 Familiarization trials 21](#_Toc138152750)

[13.2.3 Route of administration 21](#_Toc138152751)

[13.2.4 Subject compliance 21](#_Toc138152752)

[13.3 Study product handling 21](#_Toc138152753)

[13.3.1 Storage and distribution 21](#_Toc138152754)

[13.3.2 Study product accountability and reconciliation 21](#_Toc138152755)

[14 ASSESSMENT OF EFFICACY 21](#_Toc138152756)

[15 ASSESSMENT OF SAFETY 21](#_Toc138152757)

[16 CONDUCT OF THE STUDY 21](#_Toc138152758)

[16.1 Subject recruitment 21](#_Toc138152759)

[16.2 Visit 0 (day -40 to 0) 22](#_Toc138152760)

[16.3 Visit 1 (day 1) 22](#_Toc138152761)

[16.4 Visits 2-5 (days approx. 8 to 29-40) 22](#_Toc138152762)

[16.5 Biological samples 22](#_Toc138152763)

[16.6 Follow-up 23](#_Toc138152764)

[17 DATA MANAGEMENT 23](#_Toc138152765)

[17.1 Electronic data storage 23](#_Toc138152766)

[17.1.2 Access rights 23](#_Toc138152767)

[17.2 Audit trail 23](#_Toc138152768)

[18 AUDIT OF ACTIONS PERFORMED BY USERS STATISTICS 24](#_Toc138152769)

[18.1 Effects to be estimated 24](#_Toc138152770)

[18.2 Sample size calculations 24](#_Toc138152771)

[18.3 Randomization 24](#_Toc138152772)

[18.4 Interim Analysis 24](#_Toc138152773)

[18.5 Datasets to be analyzed 24](#_Toc138152774)

[18.5.1 Full analysis dataset 24](#_Toc138152775)

[18.5.2 PP analysis dataset 24](#_Toc138152776)

[18.5.3 Missing values and outliers 24](#_Toc138152777)

[18.6 Statistical analysis 24](#_Toc138152778)

[18.6.1 Primary analysis 24](#_Toc138152779)

[18.6.2 Secondary analyses 24](#_Toc138152780)

[19 HANDLING OF ADVERSE EVENTS 25](#_Toc138152781)

[19.1 Definition: Adverse event 25](#_Toc138152782)

[19.2 Intensity 25](#_Toc138152783)

[*19.3* Seriousness 25](#_Toc138152784)

[19.4 Unexpected or expected SAE 25](#_Toc138152785)

[19.5 Relation to study 26](#_Toc138152786)

[19.6 Reporting and Documentation 26](#_Toc138152787)

[19.7 Follow up 26](#_Toc138152788)

[19.8 Notification 27](#_Toc138152789)

[20 CONCOMITANT DIET AND TREATMENT 27](#_Toc138152790)

[20.1 Permitted concomitant diets/treatments/medications 27](#_Toc138152791)

[20.2 Unauthorized concomitant diets/treatments/medications 27](#_Toc138152792)

[20.3 Concomitant diets/treatments/medications record 27](#_Toc138152793)

[21 REGULATORY AND ETHICAL PREREQUISITES 27](#_Toc138152794)

[21.1 Competent Authority requirements 27](#_Toc138152795)

[21.2 IRB / IEC requirements 27](#_Toc138152796)

[21.2.1 Ethics Committee and Registered Clinical Trial Approval 27](#_Toc138152797)

[21.2.2 Protection of the subject's confidentiality 27](#_Toc138152798)

[21.2.3 Written Informed Consent 27](#_Toc138152799)

[21.2.4 Declaration of Helsinki 28](#_Toc138152800)

[22 QUALITY CONTROL AND QUALITY ASSURANCE 28](#_Toc138152801)

[22.1 Monitoring – No Monitoring is Applicable for the current study 28](#_Toc138152802)

[22.2 Source documents 28](#_Toc138152803)

[22.3 Quality Control 29](#_Toc138152804)

[22.3.1 Quality control of essential documents 29](#_Toc138152805)

[22.4 Audits and inspections 29](#_Toc138152806)

[22.5 Responsibilities of Investigator 29](#_Toc138152807)

[23 STUDY END PROCEDURES 29](#_Toc138152808)

[23.1 Premature termination of the study 29](#_Toc138152809)

[23.2 Termination of study 29](#_Toc138152810)

[REFERENCES 30](#_Toc138152811)

[APPENDIX 32](#_Toc138152812)

# INVESTIGATOR(S) SIGNATURE(S) PAGE

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I have read this Protocol and agree that it contains all the necessary details for this study. I will conduct the study as outlined herein and will complete the study within the time designated. By my signature, I agree to conduct this study in compliance with the Protocol, Written Informed Consent, IRB / IEC procedures, the Declaration of Helsinki, ICH Good Clinical Practices guidelines, and the applicable local regulations governing the conduct of clinical studies.

I will provide copies of the Protocol and all pertinent information to all individuals responsible to me who assist in conducting this study. I will discuss this material with them to ensure they are fully informed regarding the study product and the conduct of the study.

I will use only the Written Informed Consent form approved by the Independent Ethics Committee (IEC) ethics committee. I will fulfill all responsibilities for submitting pertinent information to the IEC responsible for this study.

# SPONSOR TEAM SIGNATURES PAGE – Not Applicable for Current Application

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| **Contract Research Organization** | |
| Name  Address  Tel:  Email: | Date:    Signature |

# PROTOCOL AMENDMENT

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| **Applicable Section(s) Page(s)** | **Original text** | **New / revised text** | **Reason for change** |
| **Protocol version N° 1 –** | | | |
| **Protocol Amendment N° 1 –** | | | |
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# STUDY SITES

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| Monitors | Not applicable |  |
| Clinical Research Organization | Not applicable |  |
| Central storage | Not applicable |  |
| Medical Safety Officer | Not applicable |  |
| Clinical Safety Manager | Not applicable |  |
| Product Manager | Not applicable |  |

# ABBREVIATIONS - DEFINITIONS

**AE** Adverse Event

**AUC** Area Under the Curve

**CRA** Clinical Research Associate (synonym: monitor)

**CRF** Case Report Form

**CPM** Clinical Project Manager

**DIAAS** Digestible Indispensable Amino Acids Score

**GCP** Good Clinical Practice

**IAA** Indispensable Amino Acids

**IEC** Independent Ethics Committee

**IRB** Institutional Review Board

**ITT** Intent-To-Treat

**MP** Method and Procedure: providing all the information necessary to carry out activities efficiently, safely, and in an authorized manner.

**NB** Net Protein Balance (protein synthesis minus protein degradation)

**PD** Protein Degradation (i.e., protein breakdown)

**PP** Per-Protocol

**PS** Protein Synthesis

**SAE** Serious Adverse Event

**SOP** Standard Operating Procedure:

(Providing all the information necessary to use equipment efficiently, safely, and in an authorized manner)

**SUSAR** Serious Unexpected Suspected Adverse Reaction   
 (see definition in chapter 19.4.)

**SMF** Study Master File

# SYNOPSIS

An innovative development in nutrition involves assessing dietary protein quality through the application of the Digestible Indispensable Amino Acid Score (DIAAS). Compared to the traditional Protein Digestibility Corrected Amino Acid Score (PDCAAS), the DIAAS offers a more accurate approach to assessing dietary protein quality (FAO, 2013). The DIAAS is based on measuring the true ileal digestibility of each amino acid using growing pig digesta, which has been confirmed to predict human digestibility (Hodgkinson et al., 2022). It eliminates truncation of the protein quality score, allowing for a more accurate ranking system to identify dietary protein quality and complementarity (Moughan, 2019; Bailey et al., 2019). The ratio between the first limiting IAA in a test protein with the corresponding IAA in a reference protein derives from the DIAAS. The DIAAS score can be classified into one of three quality categories: <75 (no quality), 75-99 (high-quality), and ≥100 (excellent quality). A DIAAS score of ≥100 implies that the dietary protein source is effectively utilized (bioavailable) and satisfies the estimated average requirement for protein (EAR: 0.66 g kg−1 d−1). However, the effect of DIAAS on whole-body protein turnover has yet to be verified in humans.

To answer the question of the influence of protein quality measured by the DIAAS on whole-body protein turnover and the efficacy potential of combinatorial proteins in a whole-foods matrix, we will conduct a nutritional crossover study to compare how incremental increases in protein quality, as defined by the FAO DIAAS criteria: 50% (no quality), 75% (high-quality), and ≥100% (excellent quality) affects protein kinetics relative to the controlled condition in normal-category men and eumenorrheic women aged 18 to 45 years.

The study will compare 5 dietary protein interventions increasing in the DIAAS. The control condition will be a DIAAS of ~100% (beef and bread), ~50%, ~75%, ~100% (vegetarian combinatorial protein sources, matched for bioavailable IAA, leucine, and lysine), and a ~50% (matched to meet the per-meal IAA requirement, in digestible/bioavailable values, for the first limiting amino acid: lysine). The protein-rich meals will be ingested orally on-site and be isocaloric (see Section 13.1). To measure splanchnic extraction, each meal will include oral ingestion of 84 mg of L-[15N]-Phenylalanine. Administration and sampling will occur in an air-conditioned clinic room after an overnight fast between 7:00 and 9:00 am. The participants will follow a controlled dinner and snack the evening before the interventional days. Infusion of stable isotopes L-[Ring-2H5]-Phenylalanine (Phe) and L-[Ring-2H2]-Tyrosine (Tyr) will be administrated over 5.5 hours to measure protein synthesis and breakdown from post-absorption after meal ingestion at 4.5 h to 4-h post-prandial. Arterialized-venous blood will be collected from a hand vein at specified intervals for 5.5 hours (post-absorption and post-prandial) based on prior infusion studies. Participants will remain rested during sampling and may do computer work, watch TV, or relax. There will be a minimum 1-week washout between trials in men, while women will be tested on days 3-11 following the onset of menstruation. The 4-hour post-prandial response will be calculated as the area under the curve (AUC) using the post-absorptive concentration as the baseline reference. The AUC will then be divided by time to determine the average increase in post-prandial plasma concentration. We will calculate the whole-body PS, PB, and NB (NB = PS - PB) using the standard steady-state isotope dilution equations (Jonker et al., 2019). Outcomes will be analyzed using mixed model analysis of variance (SAS, Cary, NC). Fixed effects will be meal condition and time (where relevant). Random effects will be subject to an unstructured covariance matrix to account for correlated data within the crossover. Data will be log-transform to improve linearity and model fit and to express outcomes as percent differences. Primary outcomes (PS, PB, and NB) will be referenced to our estimate of the smallest important clinical changes. Data will be presented as the least-squares mean and uncertainty (confidence interval) and, where useful, the effect size as a standardized mean difference with modified Cohen d effect size descriptors for the mean and breadth of the confidence interval.

Results will be used to determine the influence of protein quality in a whole-food matrix defined by DIAAS and the effect of combining proteins to modify DIAAS on the primary health outcome of whole-body protein turnover governing lean mass homeostasis.

# A picture containing text, font, screenshot, line Description automatically generatedSTUDY PLAN OVERVIEW

*Study plan showing the five visits to the lab. Days 1 through 29 assume weekly testing for men, and days 1 through 160, the upper range expected for eumenorrheic women.*

# INTRODUCTION

In recent years, there has been a growing interest in shifting global protein consumption towards more sustainable food production based on the assumption that animal protein production impacts the environment significantly (Espinosa-Marrón et al., 2022). However, reducing dietary proteins from animal sources could potentially lower the protein quality of most diets, which is of crucial concern, given the vital role of dietary protein in human growth, maintenance, and physiological function (Wu, 2016). Unlike other macronutrients, it comprises all the nitrogen and amino acids required for synthesizing muscles and organs, the production of enzymes and hormones, and the functioning of the immune system (Wu, 2009).

Amino acids can be categorized as dispensable (DAAs) or indispensable amino acids (IAAs) based on their ability to be synthesized de novo in the body. Only DAAs can be synthesized effectively in humans, and IAAs must be obtained through dietary protein sources. In this context, assessing dietary protein quality should be based on the digestibility, absorbability, and bioavailability of IAAs. An innovative development in nutrition involves assessing dietary protein quality through the application of the Digestible Indispensable Amino Acid Score (DIAAS). Compared to the traditional Protein Digestibility Corrected Amino Acid Score (PDCAAS), the DIAAS offers a more accurate approach to assessing dietary protein quality (FAO, 2013). The DIAAS is based on measuring the true ileal digestibility of each amino acid using growing pig digesta, which has been confirmed to predict human digestibility (Hodgkinson et al., 2022). It eliminates truncation of the protein quality score, allowing for a more accurate ranking system to identify dietary protein quality and complementarity (Moughan, 2019; Bailey et al., 2019). The ratio between the first limiting IAA (LimAA) in a test protein with the corresponding IAA in a reference protein derives from the DIAAS. The DIAAS score can be classified into one of three quality categories: <75 (no quality), 75-99 (high-quality), and ≥100 (excellent quality). A DIAAS score of ≥100 implies that the dietary protein source is effectively utilized and satisfies the estimated average requirement for protein (EAR: 0.66 g kg−1 d−1).

It has been shown that higher-quality proteins (beef sirloin, pork loin, eggs) induce a greater increase in whole-body protein balance compared to lower-quality proteins (tofu, kidney beans, peanut butter, mixed nuts) due to greater IAA availability (Park et al., 2021). Even when consumed isocaloric (500 kcal) and isonitrogenous (26g protein from eggs and cereal) (Kim et al., 2018). However, these findings did not account for equal amounts of IAAs content. These differences in protein turnover can be attributed to limiting IAAs in lower-quality proteins, as indicated by their DIAAS values, which have been shown in cell cultures to decrease global rates of protein synthesis (Vaughan et al., 1971; van Venrooij et al., 1972; Pain & Henshaw, 1975). In addition, since the limiting amino acid also limits the use of all other dietary amino acids for protein synthesis, the body must oxidize excess amounts of these amino acids (Bos et al., 2003; Tujioka et al., 2011; Luiking et al., 2005). In contrast, if one increases the dietary amount of the first limiting amino acid, protein synthesis will increase, and so will the utilization of the other dietary amino acids, reducing their oxidation (Gorissen et al., 2016). These findings align with recent research indicating that elevated peripheral levels of IAAs are linked to enhanced muscle and whole-body protein synthesis, especially following meals (Church et al., 2020). Once the limiting amino acid requirement is reached, further increases in dietary intake will cause no further increase in protein synthesis nor a decrease in the oxidation of the other IAAs (Gaudichon & Calvez, 2021; FAO, 2007).

Despite our growing understanding of protein quality and advancements in measuring dietary protein quality through the DIAAS, the relationship between the DIAAS and protein turnover has yet to be verified in humans. This knowledge gap is significant as dietary protein is crucial for maintaining health, preventing disease, and mitigating protein malnutrition (Manary et al., 2016; Layman et al., 2008).

Integrating the DIAAS is paramount, as most developing countries fall below the recommended average daily protein intake of 50g/day (62 kg individual multiplied by RDA 0.8 equals 50g) when considering the DIAAS (Moughan et al., 2021). This is important as the RDA and EAR are based on the minimum daily requirements for dietary protein intake. Hence, the primary objective of this study is to examine the relationship between stepwise increases in dietary protein quality (i.e., the DIAAS) on whole-body protein turnover while taking advantage of complementary dietary proteins to increase protein quality.

Based on current research investigating whole-body protein kinetics, we hypothesize that higher-quality proteins (DIAAS ≥100, limiting IAA: none) elicit larger changes in whole-body protein balance compared to stepwise lower-quality proteins (i.e., DIAAS: 75 and 50, limiting IAA: lysine) and that supplementing with free lysine to meet the IAA requirements for the limiting amino acid (lysine) within lower quality proteins elicits similar responses in whole-body protein balance to that of high-quality proteins (DIAAS: 100) in a whole-foods matrix.

# OBJECTIVES OF THE STUDY

## Primary objective

To determine the relationship between stepwise increases in DIAAS (protein quality) derived from modifications to combinatorial proteins within a whole-foods matrix and whole-body protein kinetics (PS + PB = NB) as a lean tissue mass homeostasis metric.

## Primary outcome

Post-prandial PS, PB, and NB responses.

## Secondary objectives

To validate DIAAS as a metric of bioavailability of key and limiting amino acids in the plasma following meal ingestion from analysis of total plasma IAA, leucine, and lysine concentrations and total 4-h post-prandial AUC. To study the glycemic response to meals as a measure of health index and the insulin response as part of the mechanisms driving PS.

## Secondary outcomes

Post-prandial plasma:

1. AUC for total IAA concentration
2. AUC leucine (anabolic trigger for protein synthesis)
3. AUC lysine (first limiting amino acid)
4. Insulin
5. Glucose

Gastrointestinal comfort in response to the meals using a linear Likert scale. This will be measured every 15 min for the first hour and every 30 min for the last three hours postpradial.

# STUDY DESIGN

## Type of Study

A randomized controlled trial with a crossover design (cRCT) comprising five arms in a clinical laboratory setting.

## Subjects, groups, and centers

This study will recruit 10 healthy male and eumenorrheic (mid-follicular phase) female participants (18-45 years of age; BMI ≥18 to ≤25 kg/m2. Participants will be recruited from the local Auckland community via general advertisement at the University, University participant databases, social media, community notice boards, web pages, and word of mouth.

The study will be a single-center, lab-based study.

Dropouts, that is, participants who start and do not complete, are predicted from several levels:

1. Illness
2. Unexpected life events

With the estimated sample size of 10, the conservative target recruitment into the study (working backward) should be 13 (25% dropout) based on recent experience. Participants who withdraw will be replaced with two analyses available: full dataset (includes any partially completed participants) and per Protocol.

## Expected study duration and milestones

Participant recruitment and commencement of the study will be approximately 30th July 2023 and will proceed until complete. All data collection is anticipated to be completed by 15th April 2024. Sample analysis is anticipated to be completed by Nov 2024. Statistical analysis and write up early 2025. The analysis components will continue (stated Feb 2023 with the preparation of plasma amino acid assay) and continue during data collection through total and stable-isotopically labeled amino acid assay, with samples run in batches to cluster between-run variability.

* February to April 2023 – study protocol development and organization.
* May to June 2023 – study protocol submission to the ethical committee.
* July-January 2023-24 - recruitment period.
* 30th July 2023 to mid-2024 - testing/data collection period.
* 1st October to late 2024 - data analysis, manuscript/thesis chapter preparation, and reporting.

# STUDY POPULATION

## Description

10 healthy male and eumenorrheic (mid-follicular phase) female participants (18-45 years of age; BMI ≥18 to ≤25 kg/m2. Further definition is provided within the inclusion/exclusion criteria sections *12.3 and 12.4*.

## Subject screening

Participants recruited will be individually screened via interview to establish availability and electability.

## Subject inclusion criteria

All participants must comply with the following inclusion criteria:

* Men and eumenorrheic women aged 18 to 45.
* BMI: ≥18 and ≤25
* HbA1c within the non-diabetic or pre-diabetic range of <40 mmol/mol.
* Physical activity level (PAL) is within the range of 1.60-1.99, defined as light to moderate by the FAO (FAO, 2001).
* Obtained his/her (or his/her legal representative's) informed consent.

## Subject exclusion criteria

Participants representing one or more of the following criteria are excluded from participation in the study:

* Planning on leaving the city or proximity to participate in all 5 study arms for the entire study duration, or any other foreseen factor that may prevent completion of the study.
* Other foreseen factors that may prevent the completion of the study.
* Criteria-defined sedentary due to a precluding disability.
* Missing hands (for arterialized-venous blood sampling)
* Active malignancy (cancer) within the past six months.
* Current pregnancy.
* Unwilling to ingest animal proteins.
* Allergy to experimental foods (i.e., gluten, lectin, and allergens).
* Any gastrointestinal disease or disorder that may affect the study outcomes.
* Gastrointestinal bypass surgery or congenital gastrointestinal issues.
* Chronic inflammatory disease (rheumatoid arthritis, psoriasis, psoriatic arthritis, Crohn's disease, ulcerative colitis, and ankylosing spondylitis).
* Taking supplements or medications that are thought to interfere with the study outcomes.
* Currently participating or having participated in another clinical study during the last four weeks prior to the beginning of this study that may affect results.

## Subject withdrawal criteria

The study participants are entitled to withdraw from the study at any time voluntarily and for any other reason without affecting their access to the treatment or their future treatment by the Investigator. In addition, a participant may be withdrawn from the study at any time for reasons including, but not limited to, the following:

* Investigator's medical decision when continuing the study would compromise the safety of the participant.
* The participant is unwilling or unable to adhere to protocol requirements.
* In the event of injury.
* Non-compliance with the dietary intervention protocols (pre-intervention control diet and interventional diet).
* Observation of adverse effects.
* Absence from more than two testing sessions.

Participants will be notified by the Investigator prior to withdrawal from the study/investigational product in writing with a full explanation for their withdrawal. Depending on the time of withdrawal from the study protocol, participants will be followed up as follows:

* Prior to or following participation in any trials, participants will be provided standard notification of reasons for withdrawal.
* During the trials, participants will be withdrawn following non-compliance to the interventional diets, inability to maintain a normal diet and allowed physical activity patterns, or any observed adverse effect. Participants' condition will be monitored for 24 hours following withdrawal if due to adverse effects.

Participants withdrawn during the study will be replaced in randomized sequence order until n=10 is complete or a maximum dropout replacement of 3 is met or exceeded.

# STUDY INTERVENTION

## Study intervention description

The combinatorial protein (CP) interventional meals will comprise five isocaloric (~810 kcal) and isonitrogenous (~22g protein) with stepwise increases in DIAAS (DIAAS: 50, 75, 100, 105, and 105). The interventional foods will be commercially bought and stored at the lab location. An in-depth overview of the macronutrient and caloric composition of the five meals is shown in Table 1.

* CP1. Topside steak and Bread (DIAAS 105) (reference protein)
* CP2. Chickpeas, Quinoa, Quorn, and Bread (DIAAS 105) (matched to meet the per-meal bioavailability of the reference protein, CP1)
* CP3. Chickpeas, Quinoa, Tofu, and Bread (DIAAS 75)
* CP4. Chickpeas, Quinoa, and Bread (DIAAS 50)
* CP5. Chickpeas, Quinoa, Bread, and L-Lysine supplement (DIAAS 100) (matched to meet the per-meal IAA requirement, in digestible/bioavailable values, for the first limiting amino acid: lysine to increase the quality to match CP1)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **CP1** | **CP2** | **CP3** | **CP4** | **CP5 + L-Lysine** |
| DIAAS, % | 105 | 105 | 75 | 50 | 100 |
| First limiting amino acid | Lysine | Lysine | Lysine | Lysine | Lysine |
| Protein, g | ~22 | ~22 | ~22 | ~22 | ~22 |
| Protein, kcal | 85 | 89 | 84 | 88 | 89 |
| Ingested IAA, g | 9 | 9 | 9 | 9 | 9 |
| Bioavailable IAA, g | 8 | 8 | 8 | 8 | 8 |
| Ingested Leu, g | 1.6 | 1.6 | 1.6 | 1.6 | 1.6 |
| Bioavailable Leu, g | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 |
| Ingested Lys, g | 1.2 | 1.2 | 0.9 | 0.7 | 1.2 |
| Bioavailable Lys, g | 1.1 | 1.1 | 0.8 | 0.5 | 1.1 |
| Carbohydrates, g | 132 | 131 | 132 | 131 | 131 |
| Carbohydrates, kcal | 528 | 524 | 528 | 524 | 524 |
| Fats, g | 22 | 22 | 22 | 22 | 22 |
| Fats, kcal | 198 | 198 | 198 | 198 | 198 |
| Fiber, g | 6 | 13 | 7 | 9 | 9 |
| Total kcal | ~810 | ~810 | ~810 | ~810 | ~810 |

*Table 1: An overview of the macronutrient composition for the experimental meals with an overview of ingested and bioavailable IAAs, Leu (leucine), and Lys (lysine).*

### Composition

The macronutrient composition of each meal will consist of ~810 kcal using fruits, juice (carbohydrates and fiber), and olive oil (fats) to balance the macronutrient profiles (amounts need is shown in Table 2). The macronutrient profile will be ~11% protein, ~65% carbohydrates, and ~24% fats, as recommended by the Institute of Medicine (Manore, 2005). All interventional meals will contain 6-13g of fiber which is within normal per-meal fiber range (Table 1).

|  |  |  |
| --- | --- | --- |
| **CP1:** Topside steak and Bread (DIAAS 105) (reference protein) | | |
| Baker's Mango and Passionfruit juice | 240 | ml |
| Banana, raw | 90 | g |
| Olive oil | 11 | ml |
| **CP2:** Chickpeas, Quinoa, Quorn, and Bread (DIAAS 105) | | |
| Baker's Mango and Passionfruit juice | 218 | ml |
| Banana, raw | 50 | g |
| Olive oil | 16 | ml |
| **CP3:** Chickpeas, Quinoa, Tofu, and Bread | | |
| Baker's Mango and Passionfruit juice | 113 | ml |
| Banana, raw | 50 | g |
| Olive oil | 13 | ml |
| **CP4:** Chickpeas, Quinoa, and Bread | | |
| Baker's Mango and Passionfruit juice | 0 | ml |
| Banana, raw | 0 | g |
| Olive oil | 13 | ml |
| **CP5:** Chickpeas, Quinoa, Bread, and L-Lysine supplement (DIAAS 100) | | |
| Baker's Mango and Passionfruit juice | 0 | ml |
| Banana, raw | 0 | g |
| Olive oil | 13 | ml |

*Table 2: An overview of the amount of fruits, juice (carbohydrates and fiber), and olive oil (fats) to balance the macronutrient profiles for every experimental meal to reach ~810 kcal.*

### Form and dosage

The meals will be ingested orally on-site and calibrated to meet the per-meal protein requirement (0.27 g /kg) for light to moderate physically active individuals (FAO, 2001). As 80% of total energy is assumed to be derived from larger meals (snacks subtracted), the calculated per-meal protein requirement is: 1.0 g/kg/d x 0.80 (80%) = 0.80 g/kg/d. Assuming that three larger meals are consumed daily, 0.8 g/kg/d is divided by three = 0.27 g/kg/meal, resulting in 22g protein/meal for the average weight of the NZ population (80.5 kg) ([MoH, 2017-2020](https://minhealthnz.shinyapps.io/nz-health-survey-2017-20-regional-update/_w_7421f894/#!/compare-indicators): body size). The 1.0 g/kg/d to calculate the per-meal requirements was derived from reviewed literature for light to moderate physically active individuals (Park et al., 2021; Luiking et al., 2005; Tang et al., 2009; Volek et al., 2013; Pinckaers et al., 2022). The calculation of the per-meal IAA requirements was adjusted with a correction factor of 1.52 (1.0 g/kg/d divided by 0.66 g/kg/d = 1.52) (Table 2) as the FAO 2013 IAA requirements (mg/kg/d) is based on the 0.66 g/kg/d model, and a 1.0 g/kg/d model is used in this study.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| IAAs | FAO (2013) IAA requirements, mg/kg/day | FAO (2013) IAA requirements, mg/day | FAO (2013) IAA requirements, mg/meal | IAA requirements (CF), mg/meal |
| Threonine | 15 | 1208 | 322 | 488 |
| Valine | 26 | 2093 | 558 | 846 |
| Isoleucine | 20 | 1610 | 429 | 651 |
| Leucine | 39 | 3140 | 837 | 1268 |
| Phenylalanine (AAA) | 25 | 2013 | 537 | 813 |
| Tyrosine (AAA) |  |  |  |  |
| Histidine | 10 | 805 | 215 | 325 |
| Lysine | 30 | 2415 | 644 | 976 |
| Methionine (SAA) | 15 | 1208 | 322 | 488 |
| Cysteine (SAA) |  |  |  |  |
| Tryptophan | 4 | 322 | 86 | 130 |

*Table 2: The numbers are based on an 80.5 kg individual. The corrected IAA requirements (mg/meal) are the FAO IAA requirements per meal multiplied by the correction factor (CF) 1.52. AAA: aromatic amino acids, SAA: sulfur amino acids, IAAs: indispensable amino acids.*

### Quality control

The investigators will perform quality control, ensuring that the food items are within the expiry dates and in good condition (no breaches). All foods will be commercially bought from the same brand and supplier throughout the study.

### Packaging and labeling

The food items will be packed with the identity of treatment and frozen on-site or dry-stored depending on the food item need. Unique codes are placed on each food item according to the sequence code (*13.1.5*).

### Randomization technique and coding

The participants and investigators will be aware of the treatment assignments. Five abbreviation codes will be applied. The codes will be (CP1) Topside steak and bread, (CP2) Quinoa, Quorn, chickpeas, and bread, (CP3) Chickpeas, quinoa, tofu, and bread, (CP4) Chickpeas, quinoa, and bread, (CP5) Chickpeas, quinoa, and bread. The abbreviations are CP1, CP2, CP3, CP4, and CP5. Participants will be allocated to sequences based on the Latin Square design. The randomization list with participant number and the code sequence will be shared with the study investigators to select the abbreviation label for each participant on each intervention day.

## Study product administration

### Amount, composition, and preparation

As mentioned above, one of five meal conditions will be ingested once within 15 min, along with 300 ml of water, followed by blood sample collection. The investigators will prepare the meals on-site in a research kitchen under hygienic conditions. The amount of food being ingested will be ~22g of protein, ~132g of carbohydrates, and ~22g of fats to meet the macronutrient composition stated in *13.1.1*.

CP1 containing beef will be prepared pan-fried to a minimum internal temperature of 63°C. CP2, CP3, CP4, and CP5 contain chickpeas and tofu (only CP3) will also be pan-fried. Quinoa and Quorn will be simmered in water until cooked (approx. 10 min). All pan-fried foods will be seasoned with salt and paper in small quantities. The bread included in all interventional meals will be heated in an oven at 200°C for 10-12 minutes the olive oil will be added to the bread with garlic.

To CP5 will be added 0.5g of L-Lysine supplement to the fruit juice to add up for the first limiting amino acid to reach per-meal IAA requirements. Other interventional foods that makeup the isocaloric cut-off of ~810 kcal will be served cold as a snack and fluid (uncooked).

The controlled diet, consisting of dinner (pre-packed), snack (muesli bar), and fluid (300 mL water) will be commercially bought from the same supplier throughout the study. The macronutrient composition will be ~25g of protein, ~70g of carbohydrates, and ~30g of fats translating to ~560 kcal.

### Familiarization trials

N/A.

### Route of administration

Meals will be ingested orally on-site under investigator supervision.

### Subject compliance

Arrival on time or within reasonable limits. Ingesting meal within 10 min. Maintaining a normal diet and physical activity pattern within the intervention period with no strenuous or vigorous physical activity two days before interventions and comply with the controlled diet the day before intervention.

## Study product handling

### Storage and distribution

The interventional food items will be stored in a freezer and dry-stored on-site with limited access.

### Study product accountability and reconciliation

The Investigator agrees not to supply the test food items to anyone except the subjects participating in this study.

# ASSESSMENT OF EFFICACY

Efficacy will be assessed upon completion of the data collection and full statistical report and will be addressed according to the primary and secondary objectives listed above.

# ASSESSMENT OF SAFETY

Safety will be continuously assessed based on the AE/SAE reporting and medical supervision ensured by the Principal Investigator or a designated person.

# CONDUCT OF THE STUDY

## Subject recruitment

A total of 10 men and eumenorrheic women (18-45 years of age) will be required to complete the study, with contingency for another 3 if required due to dropout. The target is 5 in each gender, with contingency to complete 6 and 4 of either gender combination, depending on recruitment. Participants will be recruited from the local Auckland community via general advertisement at the University, University participant databases, social media, community notice boards, web pages, and word of mouth. Each participant will have the study outline and requirements explained in detail. At this point, they will be allowed to ask questions regarding the study and answer a health questionnaire. An inclusion checklist will be completed after the subject, or the subject's legal representative has agreed to participate by signing the consent. Subjects will be enrolled after fulfilling all inclusion criteria and presenting none of the exclusion criteria.

## Visit 0 (day -40 to 0)

In-person screening will be conducted during the visit (V0), and consent will be obtained. Afterward, baseline parameters (personal data, history, anthropometric measures, and blood) will be recorded with instructions. In addition, participants will undergo blood sample collection, which will be carefully placed in EDTA-containing tubes to prevent coagulation. This process enables the measurement of HbA1c levels, ensuring they fall within the designated inclusion range. The blood samples are centrifuged with plasma aspirated and stored frozen at -80°C until further analysis. All evaluations will take place after the signing of informed consent.

## Visit 1 (day 1)

It will comprise the completion of the first arm of the test protocol.

* Participants will report to the lab/clinic after an overnight fast between 7-9:00h.
* The lab environment will be air-conditioned at 21-22°C.
* Each participant will report at a consistent time across the study to account for circadian variability.
* Upon arrival, anthropometric measurements (bioelectrical impedance scales) will be taken to establish baseline data.
* The stable isotope infusion protocol will last for 5.5 hours, during which participants will remain in a semi-supine or seated position. A calibrated syringe infusion pump will administer a primed-constant continuous infusion of stable isotopes via a catheter inserted into the antecubital vein in one arm. The primary outcome measure for this study will be the determination of net whole-body protein turnover, which will be assessed using the stable isotopes L-[Ring-2H5]-Phenylalanine and L-[Ring-2H2]-Tyrosine. Infusion rates for L-[Ring-2H5]-Phenylalanine and L-[Ring-2H2]-Tyrosine will be 270 µmol/h-1 and 85.5 µmol/h-1, respectively, with priming doses of 270 µmol and 85.5 µmol. To prime the phenylalanine-derived plasma tyrosine pool, a bolus dose of L-[Ring-2H4]-tyrosine will be administered (prime = 23.25 µmol) (Jonker et al., 2014; Jonker et al., 2019). To measure splanchnic extraction, each meal will include oral ingestion of 84 mg of L-[15N]-Phenylalanine. A second catheter will be inserted in a collateral dorsal hand vein to obtain arterialized-venous blood samples following the positioning of the hand in a customized heated-hand box (overview 16.5).

## Visits 2-5 (days approx. 8 to 29-40)

* A repeat of Visit 1 but with another randomized order of treatment, allowing for a minimum period of 7 days (men) or 3-11 days following onset of menstruation (women) of washout between visits to minimize any carry-over effects.

## Biological samples

Arterialized venous blood will be collected from a dorsal hand vein heated hand method in a Perspex chamber (~55°C air temperature) ([Gallen and Macdonald 1990](#_ENREF_2)). Blood samples will be collected from a superficial dorsal vein of the hand and placed in a thermostatically controlled box to mimic arterial samples (time points for blood sample collection can be viewed in Figure 1). Triplicate arterialized-venous blood samples will be collected at -20, -10 and 0 minutes after the infusion begins to measure pre-prandial enrichment of amino acids and glucose and insulin plasma concentrations. Post-prandial measurements will be collected between time 0-240 minutes. Each intervention will involve a protein meal given 90 minutes after the start of the infusion, and approximately 70 mL of blood will be collected per intervention. An overview of the infusion protocol is provided below (Figure 1).

A screenshot of a computer

Description automatically generated with medium confidence *Figure 1. Overview of the study protocol. In randomized order, participants receive one of the five meals at time=0 (x). A primed continuous infusion of Phe and Tyr stable isotopic tracers is used to calculate whole-body protein synthesis, breakdown, and net balance of the 4-h post-prandial period.*

Blood samples will be collected and placed in tubes with EDTA to prevent clotting. To minimize potential reactions, the samples will be immediately placed on ice, followed by centrifugation, and half of the resulting plasma deproteinized using trichloroacetic acid matrices, with the other half divided into two samples. The deproteinized and untreated plasma will be frozen and stored at -80°C until further analysis. To analyze the samples, we will use a liquid chromatography-electrospray ionization-tandem mass spectrometry quadrupole-time of flight (LC/MS-QTOF) system to determine isotope tracer enrichments and concentrations. The amino acid enrichment will be expressed as a tracer:tracee ratio corrected for natural background abundance. A COBAS c111 semiautomatic analyzer and enzyme-linked immunosorbent assay will determine plasma glucose and insulin concentrations. We analyze the samples in batches to minimize potential variability, and all procedures follow established protocols and guidelines. Quality control measures will be taken throughout the analysis to ensure the accuracy and precision of the results.

## Follow-up

Following the completion of the study, each participant will be given a contact number at Massey University, where they can contact a member of the experimental team should any adverse effects develop.

# DATA MANAGEMENT

## Electronic data storage

Data collected are listed in sections 16.2 to 16.5.

The researchers will capture all data required in the Protocol into a secure web-based password-protected repository only accessible by the researchers. Hard copies of laboratory data sheets and mass spec output feed will be scanned and saved, and the hard copies will be saved in swipe-card access or locked office areas.

## 17.1.2 Access rights

Designated researchers will be provided with a username and password to access the

study database. This username/password pair may be used by a single individual only; passwords

must not be shared with anyone else.

## Audit trail

The clinical data management systems developed for this study comply with Good Clinical Practice (GCP) predicate rule requirements, laws, and regulations (Personal data protection) and allows an audit of actions performed by users.

# AUDIT OF ACTIONS PERFORMED BY USERS STATISTICS

## Effects to be estimated

Protein balance parameters, amino acid concentration AUC, gastrointestinal comfort scale.

## Sample size calculations

The sample size was determined from the only relevant available NB data of Kim et al., 2018 where the egg vs. cereal difference (expected effect size between no and excellent quality) was 7 g net protein accretion in 165 min of infusion time, and the standard error derived from the p-value of 1.53 g (Kim et al., 2018). With the smallest important change of 2.2 g protein, using traditional null hypothesis significance testing with 5% type-1 and 20% type-2 error rates, a sample size of 8 was required (Hopkins, 2006), which we felt provided sufficient precision for the 30% of the anticipated difference between high and excellent protein quality, relative to the no quality contrast. The meet the full Latin Square matrix for 5 treatment arms, a final sample size of 10 is required.

## Randomization

Treatment sequences will be randomly assigned to participants in the Latin-square sequence for n=5 treatments.

## Interim Analysis

Not applicable

## Datasets to be analyzed

### Full analysis dataset

All participants provided at least 3 complete treatments.

### PP analysis dataset

All participants providing a full set of 5 treatments finished.

### Missing values and outliers

No imputations are foreseen. The mixed model will consider incomplete sequences as missing at random.

## Statistical analysis

### Primary analysis

Outcomes will be analyzed using a mixed model analysis of variance. Fixed effects will be meal condition and time (where relevant). The random effect will be subject to an unstructured covariance matrix to account for correlated data within the crossover. Data will be log-transformed to improve linearity and model fit and to express outcomes as percent differences. Primary outcomes (PS, PD, and NB) will be referenced to our estimate of the smallest important clinical changes. Data will be presented as the least-squares mean and uncertainty (95% confidence interval) and, where useful, the effect size as a standardized mean difference.

### Secondary analyses

The statistical analysis on secondary outcomes will be as for the primary, except psychometric scale data will not be log-transformed.

# HANDLING OF ADVERSE EVENTS

## Definition: Adverse event

An adverse event is defined as any untoward occurrence in a patient or clinical investigation subject administered an investigational product and which does not necessarily have to have a causal relationship with this treatment.

Adverse events are illnesses, signs, or symptoms (including an abnormal laboratory finding) occurring or worsening during the study. Adverse events can be serious or non-serious. They may or may not lead to the withdrawal of the subject/patient from the study. All reported adverse events must be documented and assessed for a relationship to the study.

Investigators must know and record the following information about adverse events:

* Subject and date
* Description of event
* Reporting source
* Suspect product
* Duration
* Frequency
* Intensity
* Seriousness
* Action taken
* Outcome and sequel
* Relationship to test product

## Intensity

Mild: Symptoms hardly perceived, only slight impairment of general well-being.

Moderate: Clearly noticeable symptom, but tolerable without immediate relief.

Severe: Overwhelming discomfort.

## Seriousness

A serious adverse event is any untoward medical occurrence at any dose:

* results in death,
* is life-threatening,
* requires inpatient hospitalization or prolongation of existing hospitalization,
* results in persistent or significant disability/incapacity,
* is a congenital anomaly/congenital disability, or
* is otherwise medically significant.

A non-serious event is all other adverse events not corresponding to the definition of a serious adverse event.

## Unexpected or expected SAE

The evaluation of expectedness is based on current knowledge and applicable product information and will be assessed by the researchers. An unexpected AE is an AE in which the nature, severity, or frequency is inconsistent with information about the condition under study and/or inconsistent with information on the investigational product.

All adverse events suspected to be related to an investigational product, both unexpected and serious, are considered SUSARs (Suspected Unexpected Serious Adverse Reactions). A SUSAR is to be reported to the regulatory authority on short notice.

## Relation to study

The reporting healthcare professional will assess the possibility of a link between the study and an adverse event based on the following criteria:

*Unrelated:*

* An adverse event that is not related to interventional administration.

*Unlikely:*

* The temporal relationship to product administration makes a causal relationship improbable.
* Other drugs, chemicals, or underlying diseases explain the event more plausibly.

*Probable:*

* There is a reasonable time relationship to product administration.
* The event is unlikely to be attributed to concurrent disease, drugs, or chemicals.
* The event should follow a clinically plausible response on withdrawal (dechallenge).
* Rechallenge information is not required to fulfill this definition.

*Related:*

* There is a plausible time relationship to product administration.
* It cannot be explained by concurrent disease or other drugs or chemicals.
* The response to the withdrawal of the product (dechallenge) should be clinically plausible.
* Confirmation must include a satisfactory rechallenge procedure.

## Reporting and Documentation

*Serious adverse event*

In the scope of this study, the PI or designee should enter any Serious Adverse Events (SAE) in the EDC system within 24 hours of knowledge. Once an AE is considered serious and the corresponding SAE form has been captured, an email will be sent to the ethics committee and any appropriate University Committee.

Notification does not depend on whether there is a causal relationship with the study.

*Non-serious adverse event*

Adverse events must be documented for inclusion in study reporting and records.

## Follow up

All SAEs must be followed up until the SAE outcome is known.

A follow-up visit may be required if an SAE(s) persist beyond study termination. Further analyses are required to evaluate a potential cause-effect relationship between the study and the adverse event. In that case, all examinations, laboratory analyses, and their results will be documented in the case report forms or an attached file. Any SAE occurring within 30 days after the last study product intake will be similarly reported within 24 hours.

## Notification

The University, under the Principal Investigator, is responsible for the ongoing safety evaluation of the investigational product(s).

The Principal Investigator should promptly notify all concerned and the regulatory authority(ies) of findings that could adversely affect subjects' safety, impact the study's conduct, or alter the IRB/IEC's approval/favorable opinion to continue the study.

# CONCOMITANT DIET AND TREATMENT

## Permitted concomitant diets/treatments/medications

Participants will be allowed to consume habitual foods, if they avoid taking food other than their standard diet. However, they must comply with a control diet the evening before the test days.

## Unauthorized concomitant diets/treatments/medications

Any special diet affecting nutrient metabolism (nutritional supplements, multivitamins, etc.) will not be permitted in the 15 days preceding and during the study. Further, avoidance of pregnancy is of priority as it would lead to exclusion from the study.

No concomitant treatments or prescribed medications that may affect gastrointestinal function, kidney, skeletal muscle, or liver function that are thought to affect study outcomes are allowed other than painkillers.

## Concomitant diets/treatments/medications record

All concomitant special diets, treatments, or medication will be recorded in the source documents and transposed into the database.

# REGULATORY AND ETHICAL PREREQUISITES

## Competent Authority requirements

The study will be conducted according to the relevant legal requirements.

## IRB / IEC requirements

### Ethics Committee and Registered Clinical Trial Approval

The Investigator will submit the study protocol for examination to the Institutional Review Board (IRB) / Independent Ethics Committee (IEC). The trial will be registered with the Australian New Zealand Clinical Trials Registry (ANZCTR). Commencement of the clinical study is not permitted without the written approval of the ethics committee and ANZCTR.

The IRB / IEC must be notified of all subsequent additions or changes in the study protocol.

### Protection of the subject's confidentiality

Confidentiality of all study participants will be maintained; codes for subject identification will be utilized.

### Written Informed Consent

Written Informed Consent will be obtained from the potential subject prior to any study-related activities and in accordance with all applicable regulatory requirements.

The Investigator and/or his/her designee will inform the subject, in addition to the Written Informed Consent, about all aspects of the subject's study participation. The written Informed Consent must be approved by the competent Ethic Committee and competent regulatory authority if applicable. Any amendments to these documents must be approved by the competent Ethic Committee and competent regulatory authority if applicable.

The Investigator and/or his/her designee and the subject and/or the subject's legally authorized representative (guardian, next of kin, or other authorized individuals) must sign and date the Written Informed Consent before any study-related activities are performed. The subject or the authorized representative must complete the printed name and enter the date of signature themselves. If an authorized representative signs the ICF, all efforts should be made to obtain an additional signature from the subject himself/herself.

The ICF will be signed in double, and the subject and/or the authorized representative will obtain one original of the signed Written Informed Consent. The second original is filed with the study documents at the investigational site.

The decision to participate in the study is entirely voluntary by the subject and/or by the authorized representative. The Investigator and/or his/her designee must emphasize to the subject and/or the authorized representative that the consent to participate can be withdrawn at any time without penalty or loss of benefits to which the subject is otherwise entitled.

### Declaration of Helsinki

This study will be conducted according to the principles and rules laid down in the Declaration of Helsinki (Appendix) and its subsequent amendments.

# QUALITY CONTROL AND QUALITY ASSURANCE

## Monitoring – No Monitoring is Applicable for the current study

An appropriate monitoring visit by sponsor representatives will be made during the study. All detailed monitoring information is described in the related Monitoring Plan.

Monitoring will begin with an initiation visit prior to study commencement to clarify all aspects of the Protocol and documentation. The purpose of later visits during the implementation period will be to evaluate the study's progress and adherence to Protocol. The monitor will check CRFs for completeness, clarity, and consistency with the information in the subject's file (source data checking). At the end of the study, the monitor will make a study closing visit to all sites to ensure that all documentation is complete. In all cases, it is the responsibility of the CPM / monitors to maintain subject confidentiality.

Protocol deviations will be reported in the monitoring report, and the corresponding corrective action plan will be implemented. The main protocol deviations that will be looked for are:

* Written Informed Consent process not adequately performed;
* Violation of Inclusion / Exclusion criteria;
* Non-compliance with IP storage, dispensation, allocation, use, or return requirements;
* Sampling procedures incorrectly performed;
* Intake of unauthorized concomitant diets/treatments/medications;
* SAE and AE reporting requirements not followed;
* Study visit schedule not followed;
* Any other GCP non-compliance

The monitor will communicate any detected protocol deviation to the Investigator reported in a protocol deviation form. The signed form will be sent to the Clinical Project Manager.

## Source documents

N/A

## Quality Control

### Quality control of essential documents

The research study team will ensure quality control of essential documents.

## Audits and inspections

In addition to the routine monitoring procedures, IRB/IEC and other University audits and inspections may occur.

## Responsibilities of Investigator

The investigators are responsible for the following:

* Obtaining the written and dated approval of the local ethics committee (and other local regulatory agencies, if any) prior to the beginning of the study.
* Selection of participants in accordance with the inclusion and exclusion criteria; obtaining the Written Informed Consent of the subject or legal guardian.
* Maintain confidentiality of subjects and potential subjects in accordance with the Declaration of Helsinki.
* Adherence to the study protocol and the spirit of Good Clinical Practice. If a modification becomes necessary, the rationale will be provided in a protocol amendment signed by the Investigator and sponsor for submission to the ethics committee.
* Accurate, complete, and timely data reported to the Riddet Institute.
* During the study, provide subjects with any information that may be relevant to them.
* Identification of adverse events with notification to the University, ethics committee, and health authorities, as applicable.
* Co-operation with monitoring visits, audits, and regulatory inspections. Providing direct access to source data and documents.
* Investigators may select a second contact at their study center to assist in implementing the study. However, in all cases, the main responsibility for all aspects of this implementation rests with the principal and co-investigators.
* Archiving of the Investigator's file (including all subjects' original signed Written Informed Consent forms) for at least ten years after the study's end or termination.

# STUDY END PROCEDURES

## Premature termination of the study

Should it be necessary to discontinue the study permanently before completion, the investigators will notify the IRB / IEC / ANZCTR of the rationale.

## Termination of study

After the study's completion or termination, the Investigator will inform the Ethical Committee and Clinical Trials Register of the end of the study. A certificate of study closure will be established.

The study products will be destroyed after the last subject's last visit and in agreement with the Product Manager. Certificates of destruction will be issued and filed in the Study Master File.

The remaining biological samples will be destroyed after the final statistical report or publication in agreement with the Project Manager.

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# APPENDIX

**Declaration of Helsinki**

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| |  |  | | --- | --- | | **WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI  Ethical Principles for Medical Research Involving Human Subjects** |  | |
| Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the: 29th WMA General Assembly, Tokyo, Japan, October 1975 35th WMA General Assembly, Venice, Italy, October 1983 41st WMA General Assembly, Hong Kong, September 1989 48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996 52nd WMA General Assembly, Edinburgh, Scotland, October 2000  53th WMA General Assembly, Washington 2002 (Note of Clarification on paragraph 29 added) 55th WMA General Assembly, Tokyo 2004 (Note of Clarification on Paragraph 30 added) 59th WMA General Assembly, Seoul, October 2008   1. INTRODUCTION    1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.   The Declaration is intended to be read as a whole and each of its constituent paragraphs should not be applied without consideration of all other relevant paragraphs.   * 1. Although the Declaration is addressed primarily to physicians, the WMA encourages other participants in medical research involving human subjects to adopt these principles.   2. It is the duty of the physician to promote and safeguard the health of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfillment of this duty.   3. The Declaration of Geneva of the WMA binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act in the patient's best interest when providing medical care."   4. Medical progress is based on research that ultimately must include studies involving human subjects. Populations that are underrepresented in medical research should be provided appropriate access to participation in research.   5. In medical research involving human subjects, the well-being of the individual research subject must take precedence over all other interests.   6. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best current interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.   7. In medical practice and in medical research, most interventions involve risks and burdens.   8. Medical research is subject to ethical standards that promote respect for all human subjects and protect their health and rights. Some research populations are particularly vulnerable and need special protection. These include those who cannot give or refuse consent for themselves and those who may be vulnerable to coercion or undue influence.   9. Physicians should consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.  1. BASIC PRINCIPLES FOR ALL MEDICAL RESEARCH    1. It is the duty of physicians who participate in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects.    2. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.    3. Appropriate caution must be exercised in the conduct of medical research that may harm the environment.    4. The design and performance of each research study involving human subjects must be clearly described in a research protocol. The Protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The Protocol should include information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest, incentives for subjects and provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study. The Protocol should describe arrangements for post-study access by study subjects to interventions identified as beneficial in the study or access to other appropriate care or benefits.    5. The research protocol must be submitted for consideration, comment, guidance and approval to a research ethics committee before the study begins. This committee must be independent of the researcher, the sponsor and any other undue influence. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration. The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No change to the Protocol may be made without consideration and approval by the committee.    6. Medical research involving human subjects must be conducted only by individuals with the appropriate scientific training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional. The responsibility for the protection of research subjects must always rest with the physician or other health care professional and never the research subjects, even though they have given consent.    7. Medical research involving a disadvantaged or vulnerable population or community is only justified if the research is responsive to the health needs and priorities of this population or community and if there is a reasonable likelihood that this population or community stands to benefit from the results of the research.    8. Every medical research study involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and communities involved in the research in comparison with foreseeable benefits to them and to other individuals or communities affected by the condition under investigation.    9. Every clinical trial must be registered in a publicly accessible database before recruitment of the first subject.    10. Physicians may not participate in a research study involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians must immediately stop a study when the risks are found to outweigh the potential benefits or when there is conclusive proof of positive and beneficial results.    11. Medical research involving human subjects may only be conducted if the importance of the objective outweighs the inherent risks and burdens to the research subjects.    12. Participation by competent individuals as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no competent individual may be enrolled in a research study unless he or she freely agrees.    13. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information and to minimize the impact of the study on their physical, mental and social integrity.    14. In medical research involving competent human subjects, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information. After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.    15. For medical research using identifiable human material or data, physicians must normally seek consent for the collection, analysis, storage and/or reuse. There may be situations where consent would be impossible or impractical to obtain for such research or would pose a threat to the validity of the research. In such situations the research may be done only after consideration and approval of a research ethics committee.    16. When seeking informed consent for participation in a research study the physician should be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent should be sought by an appropriately qualified individual who is completely independent of this relationship.    17. For a potential research subject who is incompetent, the physician must seek informed consent from the legally authorized representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the population represented by the potential subject, the research cannot instead be performed with competent persons, and the research entails only minimal risk and minimal burden.    18. When a potential research subject who is deemed incompetent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorized representative. The potential subject's dissent should be respected.    19. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research population. In such circumstances the physician should seek informed consent from the legally authorized representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research should be obtained as soon as possible from the subject or a legally authorized representative.    20. Authors, editors and publishers all have ethical obligations with regard to the publication of the results of research. Authors have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. They should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results should be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest should be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication. 2. ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE    1. The physician may combine medical research with medical care only to the extent that the research is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.    2. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best current proven intervention, except in the following circumstances:  * The use of placebo, or no treatment, is acceptable in studies where no current proven intervention exists; or * Where for compelling and scientifically sound methodological reasons the use of placebo is necessary to determine the efficacy or safety of an intervention and the patients who receive placebo or no treatment will not be subject to any risk of serious or irreversible harm. Extreme care must be taken to avoid abuse of this option.   1. At the conclusion of the study, patients entered into the study are entitled to be informed about the outcome of the study and to share any benefits that result from it, for example, access to interventions identified as beneficial in the study or to other appropriate care or benefits.   2. The physician must fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never interfere with the patient-physician relationship.   3. In the treatment of a patient, where proven interventions do not exist or have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorized representative, may use an unproven intervention if in the physician's judgment it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, this intervention should be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information should be recorded and, where appropriate, made publicly available.   22.10.2008 |