

**PROTOCOL**

Mussel with fucoidan as supplemented superfood – product development and clinical benefits

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# PROJECT FUNDING

High-Value Nutrition – National Science Challenge

Beyond Capital MedTech Management Ltd as the commercial partner

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# OVERVIEW

## TITLE

Mussel with fucoidan as a supplemented superfood – product development and clinical benefits

## INVESTIGATORS AND STUDY CENTRES

Professor Jun Lu, School of Science and School of Interprofessional Health, Auckland University of Technology (study centre).

Associate Professor Rinki Murphy, School of Medicine, University of Auckland.

## STUDY PERIOD

1 March 2020 to 31 July 2022

## STUDY OBJECTIVES

The main objectives of the study are to:

1. Develop a novel value-added mussel food product supplemented with fucoidan
2. Demonstrate the health benefits of the new supplemented product in comparison with the original mussel product in a clinical trial in the targeted population

## STUDY DESIGN AND METHODOLOGY

We will evaluate the product in a parallel two-arm, double-blind, randomised, placebo-controlled clinical trial.

## STUDY POPULATION

The main study population are ethnic Chinese males and females, aged over 30 years with prediabetes (defined as HbA1c between 39-49 mmol/mol) and who have self-reported joint pain.

## UNIVERSAL TRIAL NUMBER

U1111-1254-4149

# BACKGROUND

## NEW ZEALAND GREEN LIP MUSSEL AND JOINT PAIN

The green-lipped mussel (*Perna canaliculus)* is native to the New Zealand coast. Interest in the health benefits of green-lipped mussel arose from the observation that coastal Māori have lower incidence of arthritis compared to Europeans or inland Māori (1). Since then, the anti-inflammatory effects of green-lipped mussel have been studied extensively (2).

## FUCOIDAN

Studies shown that fucoidan may have dual health benefits of anti-inflammatory and anti-diabetes (3, 4). Fucoidan is a sulphated polysaccharide extracted from the seaweed *U. pinnatifida*, which is an invasive species infesting long lines of mussel farms in New Zealand (5). *U. pinnatifida* is a waste product which interferes with the mussel harvest machinery. Recent research suggests that fucoidan extracted from *U. pinnatifida* grown in New Zealand possesses various bioactivities (6-13). Globally, fucoidan from *U. pinnatifida* has been shown to have anti-inflammatory properties that may ameliorate joint pain. It is also a widely accepted food and health supplement in East Asia (14). Even with its existing wide usage, fucoidan cannot be imported into China alone, as it is not a recognized food item by itself but rather a food additive.

Fucoidan from *U. pinnatifida* may have anti-diabetic effects by improving insulin-stimulation glucose uptake and inhibiting basal lipolysis in adipocytes without inducing adipogenesis based on an *in vitro* model (15). Furthermore, fucoidan from New Zealand *U. pinnatifida* is a strong inhibitor of starch hydrolase, which may be used in diabetes management (3).

## MUSSEL-FUCOIDAN

Mussel and fucoidan have never been formulated together before, even though mussel and seaweed are often consumed together as food items (i.e. in seafood restaurants). Therefore, we will create new knowledge about the bioactivities of mussel-fucoidan combination.

There is also no current clinical data on whether the mussel-fucoidan combination has any benefit towards glycaemic control and insulin resistance. Recently, a clinical trial has been conducted in Australia using fucoidan in obese but non-diabetic population, where improvement in insulin resistance is not detectable (16). Higher baseline insulin resistance may be required to show any effect; thus, we have chosen to recruit individuals with pre-diabetes.

## STUDY OBJECTIVES AND MILESTONES

The main objectives of the study are to:

1. Develop a novel value-added mussel food product supplemented with fucoidan
2. Demonstrate the health benefits of the new supplemented product in comparison with the original mussel product in a clinical trial in the targeted population

Milestones and deliverables are as follows:

**Within Objective 1:**

Milestone 1: With the project starting date to be 1 March 2020, by 30 September 2020, we will produce a batch of around 25 kg of food grade fucoidan from seaweed sporophylls.

The deliverables in this period will be a validated industrial method to manufacture food grade fucoidan and resource (25 kg of fucoidan) for the product formulation and clinical trial.

Milestone 2: By 30 November 2020, complete the supplemented mussel food product development, and produce the prototype product ready for the clinical trial. The deliverables at this date will be the prototype product which has gained food certification.

Food product development for the Chinese market: Although the actual food product development for the market is not included in this project, it is an integrated part of the overall aim of this research and business development.

**Within Objective 2:**

Milestone 1: By 1 November 2020, gain human ethics approval and start to recruit patients for the trial. The deliverables at this date will be the ethics approval from Health and Disability Ethics Committee.

Milestone 2: By 31 December 2020, publish in vitro and ex vivo results of the anti-inflammatory, immune-stimulation, glycaemic control activities of mussel-fucoidan combination in comparison with mussel alone.

The deliverables at this date will be peer-reviewed publication(s) of our laboratory cell-based testing results of new product and/or trial study protocol.

Milestone 3: By 30 November 2021, complete the clinical part of the trial. The deliverables at this date will be a report of the trial progression and completion.

Milestone 4: By 30 March 2022, complete all the data collection, including laboratory biochemical data, and start data analysis. The deliverables at this date will be a scientific conference abstract and presentation of the preliminary findings of the trial.

Milestone 5: By 31 July 2022, complete all the data analysis, complete writing manuscripts for publication, and complete a clinical trial report to the industrial partner. The deliverables at this date will be a manuscript or manuscripts submitted to scientific journals and an industrial report to the funder about the outcome of the trial.

# ROLES AND RESPONSIBILITIES

|  |  |
| --- | --- |
| **Principal Investigator (PI)** | * Supervise and oversee all study procedures and documentation
* Co-lead clinical trial
 |
| **Co-Principal Investigator** | * Co-lead clinical trial
* Assist with patient recruitment
* Assist with trial design
* Assist with clinical assessments
 |
| **Research Physiotherapists** | * Provide practical clinical expertise
* Responsible for patients’ clinical assessment of joint pain
* Assist with trial design
* Assist with sample collection
* Assessment with data analysis in relation to pain relief and management
 |
| **Biostatistician** | * Assist with clinical study design
* Develop the statistical analysis plan
* Assist with final statistical analysis and reports
 |
| **International Collaborator** | * Assist with the mussel-fucoidan product development
* Provide advice on product health claim and regulatory approval application overseas
 |
|  |  |

# TIMELINE



# STUDY DESIGN

This is a parallel two-arm, double-blind, randomised, placebo-controlled trial. We aim to recruit 150 participants (75 for each arm). Each participant will take a daily dose before dinner of either treatment regimen. Assessment visits will occur at baseline and at 100 days post-randomisation. Screening data will be reviewed to determine eligibility. Participants who meet all inclusion criteria and none of the exclusion criteria will be recruited into the study and randomised to one of the two treatment groups.

The following treatment regimens will be used:

* Experimental treatment – Chinese baked product (e.g. mung bean or green bean cake) containing 1000 mg mussel and 1000 mg fucoidan
* Placebo – Chinese baked product (e.g. mung bean or green bean cake) containing an inert substance (e.g. inert starch)

Total duration of the study is expected to be 100 days.

## STUDY OUTCOMES

The primary outcomes measured at the end of treatment are:

* Change in insulin resistance, defined by the homeostasis model of assessment (HOMA) values
* Patient reported pain, measured by the Western Ontario and McMasters OA Index (WOMAC) pain subscale – B

Secondary outcomes include:

* Fasting glucose
* HbA1c
* Lipids
* 2-hour glucose during OGTT
* Blood pressure
* Inflammatory markers
* VAS pain scale
* Change of analgesic medication use

# PARTICIPANT SELECTION

## RECRUITMENT

We aim to recruit approximately 150 adults who identify as Chinese and live in the Auckland region. Only Chinese adults will be recruited as we hope to develop a food product that will be suitable for the Chinese market. The funder High Value Nutrition requires to specifically target this particular group. Further information justifying our sample size is detailed in Chapter 18.

We will recruit participants through a multi-pronged recruitment approach:

* Primary healthcare organisations
* General practitioners
* Practice nurses
* Asian health organisations (e.g. Asian Family Health, Asian Health Network)
* Advertising in Chinese print media, Chinese community radio and TV

Participants who meet the inclusion and exclusion criteria will be eligible for participation in this study.

### INCLUSION CRITERIA

* Of Chinese ethnicity (both parents are ethnically Chinese)
* Lives in Auckland
* Aged over 30 years
* Able to speak and understand English and/or Mandarin
* Recent HbA1c between 39-49 mmol/mol
* Has lasting joint pain (e.g. hip or knee pain) for the last 3 months or longer
* Owns a smartphone or tablet and access to Wi-fi or a broadband connection

### EXCLUSION CRITERIA

* Diabetes diagnosis
* Has a known or suspected seafood (fish and shellfish) allergy
* Currently taking a green lipped mussel shell supplement
* Has had joint replacement surgery, or planning to have this in the next 6 months
* Is pregnant or breastfeeding, or intends to become pregnant in the next 6 months
* Previous bariatric surgery
* Has any conditions that may influence body weight regulation (e.g. malabsorption, thyroid disorders)
* Has thalassemia (or other blood disorders which may affect accuracy of HbA1c testing)
* Has asthma, gout, liver disease, kidney disease, or any medical condition which the researchers believe may influence the results of the study
* Taking any glucose-lowering medications (e.g. metformin, gliclazide, vildagliptin, insulin)
* Taking any systemic steroids (e.g. prednisone)
* Currently taking medication to thin the blood (e.g. aspirin or warfarin)
* Planning major changes to physical activity (frequency, duration, intensity) during the study that may interfere with the study outcome
* Blood donation within 2 months prior to screening visit or the final follow-up visit
* Significant weight loss or weight gain within 6 months prior to screening visit
* Has a fear of needles or giving blood
* No access to a smartphone, tablet, or internet

# RANDOMISATION AND BLINDING

## RANDOMISATION

Eligible participants will be randomly assigned to either the mussel-fucoidan (prototype product) or placebo group in a 1:1 ratio. The biostatistician will generate two separate random lists: one for study participants and one for drug labelling. The participant randomisation list will indicate which group the participant is randomised to, e.g. A or B. Once randomised, a pack number will be linked to A or B with the correct treatment (either intervention or placebo) and sent to the participant. This pack number will be generated in the drug labelling list and pre-packed in identical boxes by the food company. Once a participant is ready to be randomised, the database will link the allocated group to the correct pack number at the backend. It will only show the pack number to be used by the participant. The box pre-packed with that pack number will then be distributed to the participant. To maintain blinding, an independent person will allocate the participant and treatment pack with no information on actual group allocation.

## BLINDING

This is a double-blind trial. Treatment allocation will be blinded to study investigators, research staff, and participants throughout the trial period. The following study procedures will be in place to ensure double-blind administration of study treatments:

* Access to the randomisation code will be strictly controlled (as mentioned in Section 13.1)
* Prototype product and placebo will be compared in sensory tests to ensure they are indistinguishable
* Packaging and labelling of prototype product and placebo will be identical

During the study, the blind may be broken only in emergencies when knowledge of the participant’s treatment group is necessary for further participant management. When possible, the PI should discuss the emergency with the Independent Data Safety Monitoring Committee prior to unblinding.

# FORMULATION OF TEST AND CONTROL PRODUCTS

Seaweed sporophylls will be purchased from a New Zealand South Island supplier. This will then be processed by NZ Extract Ltd, Callaghan Innovation, and Alaron Products Ltd (all in New Zealand) according to previously published procedures to produce 25 kg of fucoidan. Beyond Capital Ltd will acquire the food product certification for this batch. The fucoidan produced will be used to formulate a mussel food product in the food laboratory of AUT (Auckland, New Zealand) and test its flavour and Chinese customer acceptance. Alaron Products Ltd will manufacture the mussel-fucoidan prototype food product according to our formulation. Beyond Capital Ltd will acquire the food product certification for this product.

The intervention treatment will consist of 1000 mg mussel powder, 1000 mg fucoidan, as well as vanilla powder to neutralise the smell. The same amount of inert starch will be added with vanilla powder and stored in a container previously used to store mussel powder and fucoidan extract to produce an identical smell to the mussel-fucoidan powder. Both of these will then be incorporated in the same test food (e.g. Chinese mung bean or green bean cake) to form the prototype products. Sea salt may be added in the placebo to create a similar taste to the intervention product.

# ETHICAL APPROVAL & CONSENT

## ETHICAL APPROVAL

Ethical approval will be sought from the Health and Disability Ethics Committee (HDEC). All participants will receive a Participant Information Sheet written in either English or Mandarin and a copy of their consent details prior to taking part in the study. All consent form hard copies will be treated as confidential and stored securely at Auckland University of Technology for a period of six years.

## INFORMED CONSENT

Participation in the study is entirely voluntary. Potential participants will be provided with a Participant Information Sheet, written in English or Mandarin, as well as verbal information as appropriate. Research officer is proficient in English and Mandarin. Potential participants will be advised to consider the information and to ask the research officer any questions by email or telephone or in person. Written informed consent will be obtained at the screening visit.

# STUDY PROCEDURES

## SCREENING VISIT

The Research Officer Kelvin Wang will distribute recruitment flyer in various Chinese communities. He will contact those who are interested to answer questions and find out their eligibility, either via phone or in person. Individuals who are interested in study participation will then be invited to attend a screening visit at AE108, 90 Akoranga Drive, AUT North Campus. The Research Officer or Research Assistant will provide participants with a Participant Information Sheet and Informed Consent Form, written in English or Mandarin, as well as verbal information as appropriate. After informed consent has been obtained, individuals will provide information on demographics, and medical history including history of joint pain and prior or current treatments.

A finger prick blood sample will be also collected to measure HbA1c. If the HbA1c result is 39-49 mmol/mol, the participant will be assigned a unique study ID code and a baseline clinical assessment will be scheduled. Participants will also be provided a 7-day food diary to complete in the seven days prior to the scheduled baseline visit.

If the HbA1c result is below 39 mmol/mol, individuals will be informed that their finger prick blood test indicated that they are not within the pre-diabetic range and therefore not eligible for our study. They will be thanked for their interest and time.

If the HbA1c result is above 49 mmol/mol, individuals will be informed that their finger prick blood test indicated that they are above the pre-diabetic range and therefore not eligible for our study. They will be encouraged to discuss this further with their General Practitioner who can provide further follow-up, and thanked for their interest and time.

## CLINICAL ASSESSMENTS

This study requires two clinic visits – one at Baseline and one at Day 100. Case record forms will be used to record data for all participants. Data will be recoded directly into an electronic form. Data will also be recorded on coded paper-based forms, which will be kept securely by the Research Officer.

### Medical History

A questionnaire will be used to collect data on medical history, including presence of chronic diseases, and regular medication. There will also be questions regarding smoking status, and alcohol consumption. This will be completed both at baseline and at Day 100.

### Dietary Habits

Dietary habits will be assessed using a 7-day food diary at both baseline (Day -7 to Day 0) and at the end of the study (Day 93 to Day 100). This is to confirm that there were no significant changes to dietary habits during the study, which may affect metabolic outcomes. The 7-day food diary will be delivered through the WeChat Mini Application (mini-app). *For further information on the WeChat mini-app , please refer to Section 15.3.* It will be reviewed by the Research Officer to ensure that all pertinent information is included, such as brand names and specific amounts (e.g. grams, millilitres, cups, tablespoons).

### Anthropometry

Height, body weight, and hip and waist circumference will be measured by either the Research Assistant, Research Officer, or Research Physiotherapists for all participants at both baseline and at Day 100. Participants will be measured in light clothing, without shoes. Height will be measured, without shoes, to the nearest 0.1 cm, using a calibrated stadiometer. A minimum of two measurements will be taken and the average will be used. A third measurement will be taken if the second is not within 10mm of the first. Weight will be measured to the nearest 0.1 kg using a calibrated portable electronic scale. A minimum of two measurements will be taken and the average will be used. A third measurement will be taken if the second is not within 0.1kg of the first. Waist circumference at the midpoint between the lowest rib and the top of the iliac crest using an anthropometric tape measure (Lufkin) to the nearest 0.1cm. Hip circumference will be measured as a horizontal plane at the level of greater trochanter using an anthropometric tape measure (Lufkin) to the nearest 0.1cm. A minimum of two measurements will be taken and the mean will be used. A third measurement will be taken if the second is not within 10mm of the first.

A mean will be used if only two measurements are taken; if three measurements are required, the median value will be used. Body mass index (BMI) centiles will be determined using the recorded height and weight.

### Blood pressure

Blood pressure will be measured before blood sampling using an OMRON automatic blood pressure monitor and using an appropriately sized cuff. Measurements will be taken from participants in a seated position, after ten minutes rest, with the participant’s right arm resting on a table at the level of the heart. Participants will be asked to sit as still as possible and avoid coughing or talking. The average of three measures will be recorded for analysis.

### Oral Glucose Tolerance Test (OGTT)

Participants will complete an OGTT at baseline and at Day 100. They will be asked not to eat or drink anything (except water) 12 hours before coming for their visit. It is important to confirm that the participant has fasted for 12 hours prior to starting the OGTT. A cannula is inserted by a trainer research nurse or phlebotomist in an antecubital vein for blood sampling. Fasting blood samples are first drawn and collected into heparinized tubes. The participant will then be asked to consume the 75g oral glucose drink (Carbotest, Thermo Fisher Scientific) within 5 minutes. Venous samples are drawn at 15 minutes, 30 minutes, 1 hour, and 2 hours. Each blood sample will be labelled with the participant’s study ID code and sample number. The labelling is cross-checked between the phlebotomist and research assistant. Samples will be stored on ice and refrigerated. The samples are then immediately transported to the AUT Roche lab for centrifugation. Plasma is then extracted and will be stored in a -80 °C freezer in a PC1 lab located on the 4th floor of WN building, AUT, until required for analysis.

#### Blood sample analysis

Blood samples will be analysed for glucose, insulin, bile acids, FGF-19 and inflammatory markers including C-reactive protein (hs-CRP), tumour necrosis factor alpha (TNF–α), interleukin (IL)-6, IL-2, IL-8, IL-1beta, IL-10 and IL-4. Homeostatic model assessment of insulin resistance (HOMA-IR) will be calculated as previously described (15).

#### Incidental findings

Incidental findings may occur from collecting participants’ blood samples. These include abnormalities such as an elevated C-reactive protein or HbA1c. These abnormalities may indicate an underlying medical condition but are not diagnostic. In the case of such findings, participants will be contacted and informed. Relevant information will also be forwarded to the participant’s General Practitioner who will follow this up with the participant and complete further investigations as required.

### WOMAC Index

The Western Ontario and McMaster Universities Arthritis Index (WOMAC) is widely used to evaluate hip and knee osteoarthritis (16). It is a self-administered questionnaire, which has been validated both paper and electronic formats (17). The index consists of 24 items, divided into 3 subscales:

1. Pain (5 items): during walking, using stairs, in bed, sitting or lying, and standing upright
2. Stiffness (2 items): after first waking and later in the day
3. Physical function (17 items): using stairs, rising from sitting, standing, bending, walking, getting in/out of a car, shopping, putting on/taking off socks, rising from bed, getting in/out of bath, sitting, getting on/off toilet, heavy domestic duties, light domestic duties

Participants will complete the WOMAC index at baseline and at Day 100. The index will be incorporated into the WeChat mini-app. *For further information on the WeChat mini-app, please refer to Section 15.3.*

**15.2.7 Visual analogue scale (VAS) Questionnaires**

Two VAS questionnaires will be included in this study, one for measuring pain and another for measuring satiety. The VAS will take the form of a straight line with two extreme states anchored at either end. For example, a scale with a question “How much pain are you currently experiencing?” would be anchored with ‘no pain at all’ at one end and ‘the most extreme pain imaginable’ at the other end.

The pain questionnaire will be based on the 4-item pain intensity measure (P4) and the Musculoskeletal Health Questionnaire (MSK-HQ). The questionnaire will be incorporated into the WeChat mini-app and completed during the 2-hour OGTT. *For further information on the WeChat mini-app, please refer to Section 15.3.*

### Physical activity questionnaire

The New Zealand Physical Activity Questionnaire Short Form (NZPAQ-SF) will be used to assess three dimensions of physical activity (frequency, duration, and intensity). NQPAQ-SF is a modified version of the International Physical Activity Questionnaire – Short (IPAQ-Short) and is relevant and culturally appropriate to the New Zealand context. This is needed to make sure physical activity habits did not change during the clinical trial period, which may influence the outcome. Participants will complete this questionnaire independently both at baseline and at Day 100 during the 2-hour OGTT. The questionnaire will be incorporated into the WeChat mini-app. *For further information on the WeChat mini-app, please refer to Section 15.3.*

## WECHAT MINI APPLICATION (mini-app)

A WeChat mini-app will be specifically designed and developed for this study by the National Institute for Health Innovation, University of Auckland. This mini-app will be accessible via the WeChat social network platform, which is regarded as one of the most used social network platform in the world with over 1.2 billion monthly active users (18). Participants who own a smartphone and use WeChat will be able to have access to the project mini-app.

The following questionnaires will be delivered via the WeChat mini-app:

* WOMAC index – At baseline and at Day 100
* Physical activity questionnaire – At baseline and Day 100
* 7-day food diary – At baseline (Day -7 to Day 0) and at the end of the study (Day 93 to Day 100)
* Questionnaire on adherence, acceptability of food product, and any experienced side effects – At Day 30 and Day 50
* 4-item pain intensity measure (P4) – At Day 30 and Day 50
* Musculoskeletal Health Questionnaire (MSK-HQ) – At Day 30 and Day 50

Participants of the trial will be provided with a WeChat account or a QR-code to subscribe to a WeChat Official account that is created for the study. Links to surveys and reminders to complete questionnaires will be sent by the project WeChat account to individual participants. Participants can simply tap on the link to have access to the project WeChat mini-app and to complete different questionnaires.

Participants’ engagement with the WeChat platform will be completely anonymous. Any data in relation to participant’s personal identity will not be collected. An IP address check will be implemented to ensure no multiple identities participate in the study (i.e. one participant participating in the study with different WeChat accounts).

The mini-app will be maintained by Research Assistants at the National Institute of Health and Innovation (University of Auckland) who will send links to surveys and reminders in a timely manner. Participants will be given 7 days from the initial prompt to complete questionnaires.

Data will be stored in the WeChat mini-app database. Participants’ compliance to answer questionnaires will be recorded by the WeChat mini-app database. These data will be stored at the same place as data collected from online questionnaires. These data will be exported to Microsoft Excel spreadsheets (Microsoft Office) for data analysis by the end of the study. The data collected via the App will only be accessible to core members of the research team.

## EVALUATIONS BY VISIT

### Screening Visit: 1 hour

1. Review the study with the participant and obtain written informed consent
2. Record demographics data
3. Record medical history, including history of joint pain and prior treatments
4. Record concomitant medications
5. Measure HbA1c

### Visit 1 (Baseline/Day 0): 2.5 hours

1. Record any changes to concomitant medications
2. Review 7-day diet record and check for completion and accuracy
3. Perform and record anthropometric measurements (height, weight, waist and hip circumferences)
4. Perform and record results of blood pressure testing
5. Collect fasting blood sample for clinical laboratory tests
6. Complete 2-hour oral glucose tolerance test
7. Complete visual analogue scales for pain and satiety
8. Complete physical activity questionnaire

### Visit 2 (Day 100): 2.5 hours

1. Record any adverse events
2. Review treatment compliance
3. Review 7-day diet record and check for completion and accuracy
4. Perform and record anthropometric measurements (height, weight, waist and hip circumferences)
5. Perform and record results of blood pressure testing
6. Collect fasting blood sample for clinical laboratory tests
7. Complete 2-hour oral glucose tolerance test
8. Complete visual analogue scales for pain and satiety
9. Complete physical activity questionnaire

## EXAMPLE SCHEDULE OF EVALUATIONS AT EACH TIME-POINT

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Visit 1(Baseline)** | **Survey(Day 30)** | **Survey(Day 50)** | **Visit 2(Day 100)** |
| Informed Consent | **x** |  |  |  |
| Medical History | **x** |  |  |  |
| 7-day food record | **X** |  |  | **X** |
| Height | **x** |  |  | **x** |
| Weight | **X** |  |  | **X** |
| Waist circumference | **x** |  |  | **X** |
| Hip circumference | **x** |  |  | **x** |
| 2-hr OGTT | **x** |  |  | **x** |
| VAS - Pain | **x** |  |  | **x** |
| VAS - Satiety | **x** |  |  | **x** |
| Physical activity questionnaire | **X** |  |  | **X** |
| Concomitant Medication review | **x** |  |  | **X** |
| Adverse events review | **X** | **X** | **X** | **X** |
| Adherence review |  | **X** | **X** | **X** |
| Acceptability review |  | **X** | **X** | **X** |

# DATA MANAGEMENT PLAN

## OVERVIEW

Data will be collected to understand the effect of the mussel-fucoidan supplement on self-reported joint pain and insulin resistance. Data will be collected from participants at the clinic visits (as outlined in Section 15.2). The Research Assistants, PhD student, Research Officer, and Co-investigators will be involved in the data collection process. All required training pertaining to data collection will be completed prior to recruitment.

## CONFIDENTIALITY

The PI is responsible for ensuring that participant anonymity is protected and maintained. They must also ensure that their identities are protected from any unauthorised parties. All information related to study participants will be kept confidential and managed in accordance with the Data Protection Act and HDEC approval.

## IDENTIFIABLE INFORMATION

At the screening visit, each participant will be assigned a unique screening number, under which their identifiable information will be held. Personal identifiable data is only accessible to personnel with training in data protection who require this information to perform their study role. Personal data to be collected will include: name, date of birth, gender, and contact details. Identifiable information will be stored in a separate but linked database to enable the research team to undertake the study. Only those members of the research team whose role requires access to personal identifiers will have access. All computers used in this study will be password protected. The following groups may also have access to identifiable information during the study:

* The sponsor (Beyond Capital Ltd) and its representatives, if a participant makes a compensation claim for study-related injury
* The sponsor, ethics committees, or government agencies from New Zealand or overseas, if the study or site is audited. Audits are done to make sure that participants are protected, the study is run properly, and the data collected is correct.
* A participant’s usual doctor, if a study test gives an unexpected result that could be important for their health. This allows appropriate follow-up to be arranged.

No identifiable data will be included in research publications or progress reports.

## DE-IDENTIFIED (CODED) INFORMATION

Once written informed consent is obtained from eligible participants, a unique study ID code will be allocated. From this point, all study data and samples will be link-anonymised. All results from study assessments will be entered into password protected electronic case report forms labelled with each participant’s unique study ID code. These forms are accessible only to the study researchers. All paper copies of study data will be stored under participants’ study ID codes and kept in locked cabinets at the School of Science, AUT. Researchers involved in data and sample analysis will not have access to personal identifiable data, only the anonymised research data.

The following groups may have access to coded information:

* The sponsor for the purposes of this study
* People and companies working with or for the sponsor, for the purposes of this study
* Regulatory or other governmental agencies worldwide

## RETENTION OF INFORMATION

During the study, all records are the responsibility of the PI and must be kept in secure conditions. Electronic records will be kept in a secure database which is owned and managed by the National Institute of Health and Innovation (University of Auckland). Only the study management team who are involved in daily data collection, data entry, and quality checks will have access to this database. When the study is complete, it is a requirement of the HDEC that the records are transferred to a secure archiving site and stored for at least 10 years, then destroyed. Coded information which has been entered into electronic case report forms are sent through a secure server to the sponsor. Coded study information will be kept by the sponsor in secure, cloud-based storage indefinitely. All storage will comply with local and/or international data security guidelines.

## PARTICIPANTS’ RIGHTS TO INFORMATION

Participants have the right to request access to their information held by the research team. They also have the right to request that any information they disagree with is corrected. Participants may request to access the results of their screening and clinic visits during the study. All questions about the collection and use of information should be directed to the PI.

Participants may withdraw their consent for the collection and use of their information at any time, by informing the PI. If consent is withdrawn, their study participation will end, and the study team will stop collecting information from the participant. Information collected up until their withdrawal from the study will continue to be used and included in the study. This is to protect the quality of the study.

## DATA OWNERSHIP

Individual study data will remain the property of the individual participants. Information from this study may lead to the development of a commercial product. Project intellectual property (IP) will be owned by AUT and managed by its commercialisation and technology transfer office, AUT Ventures. AUT Ventures will meet every six months with the project team during the latter half of the project to evaluate opportunities for IP protection. Expected areas of IP protection are the exact formulation and production method of the new product. AUT Ventures will exclusively licence the product formulation to the commercial partner, Beyond Capital, on a royalty-free basis for its primary market of Asia.

Participants and their families will not receive any financial benefits or compensation, nor have any rights in any developments, inventions, or other discoveries that might come from collected information.

# ADVERSE EVENTS

An adverse event is any untoward medical occurrence in a clinical investigation of a participant administered a study treatment and that does not necessarily have a causal relationship with the treatment (20). Participants may experience unwanted side effects as a result of the mussel-fucoidan supplement, such as feeling unwell. Such event may be very rare because there has not been any report in the literature regarding adverse effects in either mussel or fucoidan trials. Participants are encouraged to contact the PI if they do experience any side effects, as well as discuss it with their General Practitioner.

All adverse events will be recorded in the participant case record form. Adverse events will be described by duration (start and stop dates), severity, outcome, treatment, and relation to study treatment or cause. Since mussel and seaweed are commonly consumed in restaurants around the world, it is unlikely any adverse reaction will occur. Therefore, we do not think a safety monitoring committee is necessary.

Participants will be asked about their compliance, pain and medication usage, and any symptoms or recent adverse events. The research assistants will also contact each participant by telephone if questionnaires are not returned. These arrangements are appropriate to ensure the safety of participants even though the side effects of mussel-fucoidan supplements are unlikely.

### Serious adverse events

The only serious adverse effect is likely to be food allergy. Since we screen out anyone with seafood allergy, it is unlikely to happen. Nevertheless, all serious adverse events that occur (whether or not related to study treatment) will be documented. The collection period for all serious adverse events will begin after informed consent is obtained and end after procedures for the final study visit at Day 100 have been completed. All serious adverse events will reported to the Health and Disability Ethics Committee. The PI and co-investigators will meet on a monthly basis to review any cases of adverse events and are responsible for making a judgement as to whether there is a significant difference in adverse events between the two groups. The US Department of Health and Human Services Common Terminology Criteria for Adverse Events: version 4: National Institutes of Health 2010 will be used to judge the severity and relatedness of adverse events (20). If the investigators deem that the adverse events are of a level that warrants terminating the study, all participants will be informed immediately and the study will be terminated.

# DISCONTINUATION OF PARTICIPANTS

## EARLY DISCONTINUATION OF STUDY TREATMENT

A participant may be discontinued from study treatment at any time if the participant, the PI, or the Sponsor feels that it is not in the participant’s best interest to continue. The following is a list of possible reasons for study treatment discontinuation:

* Participant withdrawal of consent
* Participant is not compliant with study procedures (indicated by unused product packages etc)
* Adverse event that in the opinion of the PI would be in the best interest of the participant to discontinue study treatment
* Protocol violation requiring discontinuation of study treatment
* Lost to follow-up
* Sponsor request for early termination of study
* Positive pregnancy test (females)
* Joint replacement surgery

## WITHDRAWAL OF PARTICIPANTS FROM THE STUDY

Participants may withdraw from the study at any time, for any reason, specified or unspecified, and without judgement. Reasonable attempts will be made by the PI to provide a reason for participant withdrawals. The reason will be specified in the participant case record form. Participants who withdraw from the study will not be replaced.

## PROTOCOL DEVIATIONS

A protocol deviation occurs when the subject, PI, or Sponsor fails to adhere to significant protocol requirements affecting the inclusion, exclusion, participant safety, and primary outcome criteria. Protocol deviations for this study include, but are not limited to, the following:

* Use of a prohibited concomitant medication
* Non-compliance with daily treatment regimen

Failure to comply with Good Clinical Practice guidelines will also result in a protocol violation. The PI will determine if a protocol deviation will result in withdrawal of a participant. When a protocol deviation occurs, it will be discussed with the PI and a Protocol Deviation Form providing details will be generated. This form will be signed by the PI and will be filed in the site’s regulatory folder.

# STATISTICAL METHODS

## SAMPLE SIZE

A total sample size of 150 participants (75 per arm), will provide 90% power at an overall significance level of 5% (two-sided) to detect a standardised effect size of 0.625 on either of the two co-primary outcomes, with the Bonferroni correction (α=0.025 for each outcome) and allowing for 10% loss to follow-up. This effect size is equivalent to a group difference of 1 cm on patient reported pain, and a 0.5-unit difference in change in HOMA at the end of the intervention, both of which are considered clinically significant. The standard deviation (SD) for change in HOMA is assumed to be 0.8 as reported in a previous study (22). For pain reduction, calculations were based on a recent published trial (23) in which a SD of 1.6 cm was reported for WOMAC-B.

## STATISTICAL ANALYSIS

Statistical analysis will be performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). Treatment evaluation will follow the principle of intention to treat (ITT), including all randomised participants in the group they were allocated to. Statistical tests will be two-sided and maintained at 5% significance level.

Baseline demographics and clinical characteristics of all trial participants will be summarised using descriptive statistics by treatment group. Continuous variables will be presented as mean, standard deviation (SD), median and interquartile range (IQR) as appropriate. Categorical variables will be presented as frequencies and percentages. No formal statistical tests will be conducted at baseline, as recommended by the CONSORT 2010 guidelines for reporting parallel group randomised trials.

Primary and secondary outcomes measured at the end of the intervention will be summarised descriptively by treatment group, together with their baseline measures. Adverse and serious adverse events will be summarised separately. Generalised linear regression model will be used to estimate the effect of the intervention on each outcome, using a link function appropriate to the distribution of the outcome variable. The model will adjust for baseline outcome value and important baseline confounders as pre-specified. Missing data on the primary outcomes will be imputed using multiple imputations in the main ITT analysis, and explored in sensitivity analysis under different assumptions to test the robustness of main trial results. Per-protocol (PP) analysis will include those participants without major protocol deviations. No data imputation will be considered on secondary outcomes. No interim analysis will be performed during the trial.

# REIMBURSEMENT

Each participant will receive a $100 MTA voucher as reimbursement for their time and travel costs.

Participants who were randomised to the control group will also be offered 100 days’ worth of the mussel-fucoidan supplement at the end of the study.

# DISSEMINATION OF RESULTS

## STUDY PARTICIPANTS

At the end of the study, participants will be provided with a one-page lay summary outlining the findings of the study via email. Participants will also be emailed a copy of the formal study report once it has been published in a scientific journal.

## INDUSTRIAL PARTNER AND ACADEMIC/ PROFESSIONAL COLLEAGUES

Results will be written as an industrial report first to the industrial partner (Beyond Capital) and then we aim to submit the result to peer-reviewed international journals for publication. Academic publications will be sought in a high impact medical, diabetes-related, osteoarthritis-related or nutrition journal and a presentation will be given at a national or international medical or physiotherapy conference. No restrictions will be placed by the study sponsor on the publication of results.

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