PARTICIPANT INFORMATION LETTER

PROJECT TITLE: The effects of fish oil supplementation and resistance exercise on muscle cells
PRINCIPAL INVESTIGATOR: Dr. Orly Lacham-Kaplan
ASSOCIATE INVESTIGATORS: Dr. Donny Camera
STUDENT RESEARCHER: Mr. Bill Tachtsis
STUDENT'S DEGREE: PhD

Dear Participant,

You are invited to participate in a research project conducted by Australian Catholic University (ACU), 'Profiling the acute post-exercise biochemical environment in older females following fish oil supplementation'as you are:

i) Female aged 50-65 yo
ii) Are currently Post-Menopausal (no periods for 12 and over consecutive months)
iii) Have a Body Mass Index (BMI) of 18.5-29
iv) Not exercising more than 3 times per week
v) Not currently taking any blood thinning medication e.g. (Warfarin, Aspirin or Dabigatran), anti-inflammatory medication or supplements (anti-allergenic) and not taking fish oil supplements
vi) Do not have any known allergies to fish products

This Participant Information Sheet/Consent Form informs you about the research project. It explains the tests and research involved. Knowing what is involved will help you decide if you want to take part in the research.

Please read this information carefully and ask questions about anything that you don't understand or want to know more about. Before deciding whether or not to take part, you might want to talk about it with a relative, friend or local doctor.

Participation in this research is voluntary. If you don't wish to take part, you don't have to. You will receive the best possible care whether or not you take part. If you decide you want to take part in the research project, you will be asked to sign the consent section. By signing it you are telling us that you:

• Understand what you have read
• Consent to take part in the research project.
• Consent to the tests and research that are described
• Consent to the use of your personal and health information as described.

You will be given a copy of this Participant Information and Consent Form to keep.

What is the project about?

Exercise is well known to induce multiple physical and psychological benefits. For instance, resistance training can increase muscle strength and mass, however these benefits diminish with age. Similarly, Omega-3 fatty acids are known for their various health benefits such as improving cardiovascular health and reducing inflammation, but have also emerged as fatty acids with the potential to alter skeletal muscle metabolism and attenuate age related declines in muscle mass. Omega-3 fatty acids are abundant in deep sea fish such as salmon and tuna, but can also be consumed in capsule form without having to consume copious amounts of fish.

During and immediately after exercise, a multitude of factors are released from skeletal muscle into the circulating blood that are believed to play a role in the beneficial effects of exercise. These exercise released biochemical components are termed 'exerkines. Fish oil supplementation increases the amount of "good" fats and decreases the amount of "bad" fats in the blood circulation that subsequently alters the composition of blood. Whether fish oil supplementation and resistance exercise can work together to modulate the resting and post-exercise profile of exerkines has yet to be determined. Furthermore, no studies have investigated the effect of the fish oil supplementation on acute post-exercise exerkine response on the ability of muscle to recovery and repair following itself. Thus, the aims of this study are to:

1. Characterize the profile of circulating factors present in the serum of post-menopausal women at rest and post-exercise following fish oil supplementation.
2. Investigate the effect of resistance exercise and fish oil (omega-3) enriched serum of older women has on muscle cell recovery and repair processes *in vitro.*

Who is undertaking the project?

Mr. Bill Tachtsis is principally conducting this project with the assistance of his PhD supervisors, Dr. Orly Lacham-Kaplan and Dr. Donny Camera. Mr. Tachtsis is a PhD student at the Australian Catholic University (ACU) and this research project will form part of his PhD thesis. Mr. Tachtsis possesses 2 years co-ordinating exercise research studies and has also been a recreational powerlifter for 3 years. Dr. Camera is a Research Fellow within Centre for Exercise and Nutrition (CEN) at ACU and has ten years' research experience conducting exercise and nutrition studies. Both Dr. Camera and Mr. Tachtsis are First-Aid and Cardio-Pulmonary Resuscitation trained, and have undergone all procedures performed in this study. Dr. Evelyn Parr is also a Research Fellow in CEN and trained phlebotomist and licensed DXA technician. Dr Parr will perform all blood sample collections and DXA (body composition) scans for this study. Dr. Orly Lacham-Kaplan is the Senior Laboratory Manager and Senior Scientist of the CEN at ACU and has over 30 years' research experience. The project has been approved by the ACU Human Research Ethics Committee and is supported and funded by an ACURF project grant awarded to Dr. Lacham-Kaplan.

Are there any risks associated with participating in this project?

As a participant you will be exposed to some risks arising from blood sample collection and exercise performance. In the unlikely circumstance of an adverse event (i.e., light-headedness due to exercise, bruising, etc.), both Dr. Camera and Mr. Tachtsis are trained in first aid and basic emergency life support, and can provide care following protocols established by our First-Aid Training Provider Safety Corp™.

Should an injury be sustained during exercise, the research team will provide you the necessary care and seek further medical assistance from our research doctor, Dr. Andrew Garnham, an experience Sports Medicine Doctor, in the unlikely event this may be required.

Blood Sampling

Eight 50 mL samples of blood will be taken during this study. The total volume (300 mL) is significantly less compared to the standard 500 mL for a Red Cross donation. A trained phlebotomist (Dr Parr) will conduct all blood sampling under strict sterile conditions by using single-use sterile equipment. Should you experience any side effects (i.e.: dizziness) during this procedure, the research team will provide you the necessary care and seek further medical assistance in the unlikely event this may be required.

Physical exercise

While any type of physical exertion involves some possible risk of injury or complication, the exercise session in this study will not present any risk other than those experienced during a typical resistance. These include, but are not limited to musculoskeletal injuries (e.g. strained muscles, tendons or ligaments) and delated onset muscle soreness (DOMS). We have taken all necessary steps to minimise any discomfort by including appropriate warm up routines and selecting exercises previously used in similar research interventions.

What will I be asked to do?

If you are interested in participating in the study and meet the selection criteria (stated above), you will be required to complete and sign all relevant consent and cardiovascular risk factor forms (all attached) for clearance into preliminary strength, and the subsequent experimental intervention. You will be randomly allocated to one of the following two experimental conditions: into either group 1 (fish oil) or group 2 (placebo). After a washout period of 4 weeks, you will swap groups so that at the end of the trial you will have completed a period of fish oil supplementation and a period of placebo supplementation. Also, at the end of each supplementation period, you will undertake a short session of supervised resistance exercise. It is desirable that your local doctor be advised of your decision to participate in this research project. Factors that may exclude you from taking part in this study include: not meeting selection study inclusion criteria or an inability to consent to the study. Certain medications may also prevent inclusion in the study and the study doctor will advise of this.

Local Doctor Notification

It is desirable, however, not mandatory, that your local doctor be advised of your decision to participate in this research project. If you have a local doctor, we strongly recommend that you inform them of your participation in this research project.

What does participation in this research involve?

Exercise and Sports Science Australia's Adult Pre-exercise Screening Form: You will be required to complete this screening questionnaire to determine if you are ready to begin light/moderate intensity physical activity or if guidance from a health professional is required prior to commencing.

Consent Form: If you decide to participate in this study, a consent form will be signed prior to any study assessments being performed.

Cardiovascular Risk Factor Questionnaire: A cardiovascular risk factor questionnaire will be completed prior to any study assessments.

Body Composition:
A dual energy X-ray absorptiometry scan (DXA) is a specialised X-ray technique to provide a measure of body composition. Participants lay facing up on the scanning bed for the duration of the scan. This is just like a normal X-ray, with no pain involved and it will measure total body mass, fat mass and lean mass. The scan lasts for 7-8 minutes and participants will be scanned wearing light clothing one week prior to the exercise and nutrition intervention. Dr. Parr, a qualified technician, will co-ordinate all DXA scans.

VO_2 max test
This test will require a significant amount of exertion and therefore can result in some discomfort. A cardiovascular risk factor questionnaire will be completed beforehand to ensure that you are suitable to undertake the test. If you are not cleared to participate by the cardiovascular risk factor questionnaire you will need to obtain approval form a general practitioner to participate. In the unlikely event that you experience discomfort beyond what is expected with exercise, please notify research staff. You are able to end the test at any time. This test will be performed on a stationary bike to evaluate your aerobic fitness and personalise your exercise sessions during the experiment. The test will start with a 15 minute self-paced warm-up at a low intensity. From here, exercise intensity will increase every 150 s until you elect to end the test. During the test you will wear a mouthpiece for analysis of oxygen as well as carbon dioxide expelled. You are not required to fast for this visit.

Dietary Standardisation
For 24 hours prior to the trial, all participants will consume a standardized diet provided by our research team based on personal preferences and presence of any known allergies. The diet will consist of 15-25% energy from protein, 45-65% energy from carbohydrate and 20-35% energy

from fat. This distribution of macronutrient intake will ensure adequate energy to meet requirements outlined in the Australian Dietary Guidelines Acceptable Macronutrient Distribution Ranges (AMDR) for protein, carbohydrate and fat (expressed as a percentage contribution to total energy intake). In addition, a standardized breakfast will be served to all participants the morning of the trial. This meal will consist of similar macronutrient percentages as outlined above.

Three day food record

Participants will be asked to record all food and fluid consumed over a three day period throughout the study period to establish habitual dietary intake. To assist with compliance, and reduce the burden on participants, household measures will be recorded. A comprehensive document will be provided and this will be explained to you in detail. Participants will be asked to complete this three day record 7 times over the study period so a total of 21 days intake will be obtained (During the 2 week control period and every second week for the duration of the study thereafter).

Blood Sampling

Eight 50 mL samples of blood will be taken through venepuncture before, and after the exercise and nutrition intervention. This volume (total = 500 mL) is roughly the same as a standard 500 mL for a Red Cross donation. The 50 ml of blood collected on the 8 occasions is a volume equal to ~10% of total blood volume. Following the first blood draw, a standardized breakfast will be provided by the CEN before commencement of exercise to replenish fluid loses. In addition, light refreshments will also be provided by the CEN after the second blood sample to replenish the loss in blood volume.

Fish Oil Supplementation and Exercise Intervention

All Participants will perform a light session of resistance-based exercise following a thorough warm up. The session of resistance-based exercise consists of machine-based exercises (described below) that provide a supporting surface for balance when performing the exercise. All participants will also undertake pre-testing and measures of body composition one week prior to the trial to ensure the amount of weight lifted during the exercise session can be safely and confidently performed. All preliminary testing and exercise sessions will occur at the ACU gym under the supervision of Mr. Tachtsis and Dr. Camera. Both Mr. Tachtsis and Dr. Camera possess the appropriate Fitness Instruction qualification to safely teach through all the different exercise types.

This study is a randomised cross over trial, meaning that participants will be randomised into two groups. Either group 1 (fish oil) or group 2 (placebo). After a washout period of 4 weeks, participants will swap groups so that at the end of the trial each participant will have completed a period of fish oil supplementation and a period of placebo supplementation. Participants will be randomly allocated to one of the following two experimental:

Group 1: Fish Oil supplementation

Participants allocated to this group will be required to consume 4 fish oil capsules (6g/d containing 2.16g of EPA and 1.42g of DHA) PUFA daily for 4 weeks. We recommend that

participants space their consumption of the fish oil evenly throughout the day and advise participants to take two fish oil capsules in the morning and two capsules in the evening.

Side effects: The side effects of fish oil supplementation are rare but may include heartburn, nausea, loose stools and rash. Consumption of fish oil supplements can also leave a fishy after taste that may be unpleasant. The fish oil supplement we have chosen for this study is designed to minimise fishy after taste. If you have a known allergy or hypersensitivity to fish or other fish products (including shellfish), you should be not consuming fish oil supplements.

Group 2: Placebo

Participants allocated to this this group will be required to consume 4 placebo capsules (6g) daily for 4 weeks. We recommend that participants space their consumption of the placebo evenly throughout the day and advise participants to take 2 capsules in the morning and 2 capsules in the evening.

Side effects: There are no known side effects of the placebo

Exercise session

The duration of the exercise session, including adequate warm-ups, will be ~60 minutes. The following exercises have been selected as they are low risk and easy for untrained individuals to complete. The following exercises have been selected based on previous established protocols as they are low risk and the most basic exercises for untrained individuals to complete. The following exercises have been used in previous studies thus deemed safe for older adults to perform.

Acute Resistance Exercise Session:

1. Leg Press - 3x10 @ RPE 7-8
2. Leg Extensions - 2 x 12 @ RPE 7-8
3. Machine Chest Press - 3 x 10 @ RPE 7-8
4. Dumbbell Side Lateral Raises - 3 x 12 @ RPE 7-8

Exercise Familiarisation

Participants will be required to complete 2 exercise familiarisation sessions in the week leading up to the trial day (4 in total). The familiarisation sessions will allow participants to familiarise themselves with the exercises, decreasing the risk of exercise induced injury and increase the safety of the study. The exercises are outlined in previous section.

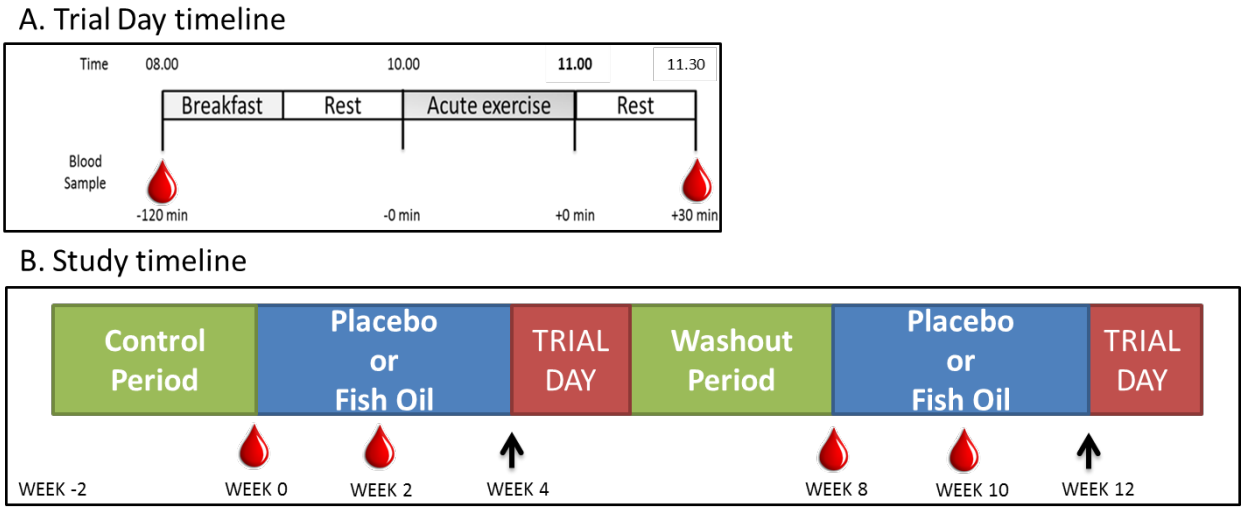

Figure 1: Overview of the trial day timeline and overall study time line

How much time will the project take?

A single experimental trial day period will be a total of ~4.5 h duration. The time commitment for the preliminary testing and exercise familiarisation will be ~1 h. Together, the total time investment for completion of the study will be ~16 h. Participants will be provided with food and drink at visits. In brief, the following 12 visits will be required:

Study week	Visit	Time	Comment	Notes
-2	1	30 min	Meet and greet	See facilities at ACU
Preliminary testing				
-1	2*	~ 1.5 h	Supplement Collection VO2/DXA/Blood collection	
Fish Oil				
1	3	~15 min	Blood collection	50 mL
2	4	~15 min	Blood collection	50 mL
3	5	~1 h	Resistance Exercise Familiarization	
4	6	~1 h	Resistance Exercise Familiarization	
4	7	~4.5 h	Trial Day	100 mL
Wash Out				
7				
Placebo				
9	8	~15 min	Blood collection Supplement collection	50 mL
11	9	~15 min	Blood collection	50 mL
11	10	~1 h	Resistance Exercise Familiarization	
12	11	~1 h	Resistance Exercise Familiarization	
12	12	~4.5 h	Trial Day	100 ml

Reimbursement and Costs

There are no costs associated with participating in this research project, nor will you be paid. However, participants will be reimbursed a total of $200, in the form of a Coles-Myer gift card, for costs incurred for travel and parking associated with participating in this study.

What are the benefits of the research project?

We cannot guarantee or promise that you will receive any benefits from this research. However, possible benefits may include an improved understanding of personal muscular strength, and important body composition measures such as muscle mass and muscle-free mass. There will be no clear benefit to you from your participation in this research.

Can I withdraw from the study?

Participation in this study is completely voluntary and you may withdraw at any time. If you do withdraw your consent during the research project, study staff will not collect additional personal information from you. Data or any samples already collected will be destroyed and disposed in accordance with laws related to data and biological waste disposal.

Will anyone else know the results of the project?

Information for each participant will be identified by a code so that your name does not appear on any data sheets. Only the principal investigators Mr. Bill Tachtsis, Dr. Orly Lacham-Kaplan and Dr. Donny Camera will have access to samples and records obtained.

Individual information (hard and soft copy where applicable) will be kept for 5 years in a locked cabinet in the office of the research laboratory manager Dr. Orly Lacham-Kaplan at ACU(The Mary MacKillop Institute for Health Research, 215 Spring Street, Level 5.36) after which all material containing confidential information will be destroyed. If you wish to gain access to your data, contact one of the principal researchers and it will be provided to you. Please note that no material that could personally identify you will be used in any reports or presentations of this project. Results from the study may be summarised and appear in publications or may be provided to other researchers in a form that does not identify the participants in any way.

Any information that you provide can be disclosed only if (1) it is to protect you or others from harm, (2) a court order is produced, or (3) you provide the researchers with written permission. The results from this study will be presented at scientific conferences and published in peer-reviewed scientific journals.

Will I be able to find out the results of the project?

You will be provided with a report detailing results of preliminary testing. If you wish to gain access to any additional data from the study please contact the principal research and it will be provided to you.

Who do I contact if I have questions about the project?

Should you have any questions relating to participation in this study, please contact Mr. Bill Tachtsis (bill.tachtsis@myacu.edu.au, ph: 0431 5994 28) or Dr. Donny Camera (donny.camera@acu.edu.au, ph: 0403 166 127).

What if I have a complaint or any concerns?

The study has been reviewed by the Human Research Ethics Committee at Australian Catholic University (2017-10H). If you have any complaints or concerns about the conduct of the project, you may write to the Manager of the Human Research Ethics Committee care of the Office of the Deputy Vice Chancellor (Research).

Manager, Ethics
C/o Office of the Deputy Vice Chancellor (Research)
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North Sydney Campus
PO Box 968
NORTH SYDNEY, NSW 2059
Ph.: 029739 2519
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Any complaint or concern will be treated in confidence and fully investigated. You will be informed of the outcome.

I want to participate! How do I sign up?

Please contact Mr. Bill Tachtsis (bill.tachtsis@myacu.edu.au, ph; 0431 599 428) or Dr. Donny Camera (donny.camera@acu.edu.au, ph: 0403 166 127).

Yours sincerely,

PRINCIPAL INVESTIGATOR
Dr Orly Lacham-Kaplan PhD

ASSOCIATE INVESTIGATOR
Dr Donny Camera PhD

STUDENT INVESTIGATOR
Mr Bill Tachtsis