Study Protocol

#### Title: Investigation of the effect of eight weeks whole body high intensity resistance exercise training on immune measures in healthy males.

Short title: Resistance exercise and immune health

Universal Trial Number:

ANCTRN:

Investigators

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# Background

It is widely recognised that high intensity resistance exercise is of great value for increasing athletic performance, but also improving health and longevity. The health benefits associated with resistance training include: a reduction in resting blood pressure [1, 2]; decreased lower back pain [3, 4]; a reduction in pain and discomfort for those suffering from arthritis [5]; a decrease in gastrointestinal transit time [6]; an increase in resting metabolic rate [7]; and improvement in blood lipid profiles [8]

In recent years there have been an increased interest in the study of the effect of high volume endurance activities on immune function. However, the full health related implications of high intensity exercise are not yet fully understood, especially with regard to chronic adaptations to resistance exercise. Successfully analysing immune and myokine (skeletal muscle related cytokine) markers pre- and post-training, with comparison to strength and body compositional measures, will allow us to gain a deeper understanding of how the body adapts to exercise. This will have a potential application in altering the response to exercise with targeted nutritional intervention.

Overall aim

To evaluate the effect of 8 weeks of whole body high intensity resistance exercise training on body composition, skeletal muscle function and immune measures.

References

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6. Koffler KH, Menkes A, Redmond RA, Whitehead WE, Pratley RE, Hurley BF. Strength training accelerates gastrointestinal transit in middle-aged and older men. Med Sci Sports Exerc. 1992 Apr;24(4):415-9. PMID: 1560736.
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# Regulatory statement

This study will be conducted in accordance with the protocol, Good Clinical Practice and the appropriate ethical and regulatory guidelines. It will also comply with guidelines/regulations outlined by the Biosafety Advisory and Health & Safety Committees at The New Zealand Institute for Plant & Food Research Limited.

# Outcome measures

## Primary outcome measures

Physical performance

1. Skeletal muscle strength: Isometric strength measures (knee flexion & extension), estimated 1 rep max testing for each of the 10 prescribed resistance exercises and isometric mid-thigh pull.

Immune parameters

1. Cell phenotype of circulating granulocytes, monocytes and lymphocytes, specifically neutrophils, inflammatory macrophages, T cells and NK cells, by flow cytometry
2. Cytokine production ((IL-4, IL-2, CXCL10 (IP-10), IL-1β, TNFα, CCL2 (MCP-1), IL-17A, IL-6, IL-10, IFN-γ, IL-12p70, CXCL8 (IL-8), TGF-β1) following T cell stimulation with phorbol myristate acetate (PMA).

## Secondary outcome measures

1. Saliva Cortisol levels
2. Plasma myokine concentrations (IL-6, IL-15, TGF-β1, IL-4, IL-8, IL-7, Irisin, apelin, FABP-3, FGF-21, MGF, IGF-1).
3. Profile of Mood States (POMS) Questionnaire
4. Cardiovascular measures: resting heart rate and blood pressure.
5. Body composition: body weight, body fat %, muscle distribution, muscle mass. Limb circumference and bone mineral content

# Study details, visit times, and protocols

This is a single-arm, repeated measures study where eight healthy male volunteers (18 – 40 years), who are not undergoing any resistance training programme, will be recruited from the general population to participate in this research. An overview of the trial design in illustrated in Figure 1.

Pre study protocol

Individuals will be asked to complete a health screening questionnaire which will be checked against the study’s inclusion/exclusion criteria. When a participant’s eligibility has been confirmed and they have provided written consent, the individual will be accepted into the study. The Trial Co-ordinator will then arrange a date for the participants to attend a familiarisation session at Massey University School of Sport, Exercise and Nutrition Human Performance Laboratory.



Figure 1: Overview of exercise trial design.

Familiarisation session:

Study participants will be asked to attend a familiarisation session at The Human Performance Laboratory, Massey University. The purpose of this session is to familiarise each participant with the nature of the exercises they will be performing on the training and pre/post training assessment days. Participants will also be introduced to the equipment used to collect biological samples (saliva and venous blood) as well as body composition, heart rate and blood pressure measures and baecke questionaire.

Following a five-minute warmup on a bicycle ergometer at an intensity of 70 watts, participants will be familiarised to each of the HIT training exercises they will be required to perform during the training days. They will also be familiarised to the measures of muscle function that will be conducted on pre and post training days.

**HIT training exercise protocols**

Participants will be introduced to the lower body (squat, leg extension, leg curl, standing calf raise) and upper body (pull down, bench press, seated row, shoulder press, bicep curl, and tricep extension) exercises that they will be required to complete during the training and pre/post training assessment days. During each exercise, the Trial Co-ordinator, Study Investigator or Co-investigator will lead each participant through the movement of each exercise to ensure correct technique and to ascertain the correct position settings of exercise machines. The weight resistance during each exercise will be kept at a minimum to ensure no exercise-induced muscle damage occurs during the familiarisation session.

**Quadriceps muscle function**

Participant’s quadriceps muscle function will be measured using a Biodex isokinetic dynamometer and the seating position determined for each participant will be recorded and used for subsequent measurements. Participants will be seated on the machine and the position adjusted so that the femoral epicondyle of the participant’s test leg is aligned with the dynamometer’s axis of rotation and the ankle strap is positioned 5 cm proximal to the participant’s medial malleolus. The ankle and thigh of the test leg will be strapped firmly to the dynamometer at the optimal position to isolate the movement to the quadriceps. The range of motion of the test leg will be set at 110° and 75°C for isometric contractions. Using these settings, participants will be required to perform three maximal contractions each of the 2 angles. Muscle function measures on quadriceps of both legs will be conducted.

**Isometric mid thigh pull**

Participant’s isometric mid-thigh pull strength will be assessed using a custom build dynamometer consisting of a dynamometer with a chain and metal bar attached to a wooden platform. Participants will be instructed to stand directly above the dynamometer, their feet shoulder width apart and gripping each side of the bar. The length of the metal chain linking the bar to the dynamometer is then adjusted so that participant’s back is in a neutral position, the knees are slightly bent and the bar is positioned just above the knees. Participants will then be instructed to pull the metal bar upwards as hard and fast as possible for 3 seconds to elicit the greatest peak force. Participants will be required to complete 3 maximal pulls with each pull separated by 30 seconds rest.

At the conclusion of the familiarisation session, the Trial Co-ordinator will arrange a date with the participants to return to the lab to complete their pre-training assessments.

Pre-training assessment

Participants will be asked to refrain from any strenuous activity 48 hours prior to their scheduled pre-training assessment session. After completing a Profile of Mood States (POMS) questionnaire, participant’s bodyweight and height measurements will be measured. Body compositional analysis (body fat percentage, muscle distribution and muscle mass) will be assessed using an InBody S10 body composition analyser. Limb circumference (mid upper arm, mid-thigh and calf) and waist measurements will be measured by tape measure. A chewing-stimulated saliva sample (3 mL) and venous blood sample (9 mL) will then be collected from each volunteer.

Following the collected anthropometric measurements and biological samples, participant’s pre-training muscle function will be assessed on the Biodex and isometric mid-thigh dynamometers, as described in the Familiarisation session section.

Finally, the estimated 1-rep max (1RM) for each of the lower and upper body exercises will be determined by requiring participants to perform each of the exercise set at a resistance to induce volitional fatigue within 10 repetitions; each exercise having a cadence of 4 seconds per repetition (i.e. 2 seconds lifting, followed by 2 seconds lowering). The 1RM will be estimated using the equatio*n*

1 RM = weight lifted/ (1.0278-(reps to fatigue x 0.0278)) *.* (Brzycki, M. (1993). Strength Testing: Predicting a One-Rep Max from Reps-to-Fatigue. Journal of Health, Physical Education, Recreation, and Dance. 64: 88-90).

The exercises will be carried out in the following order with a description of each exercise is briefly detailed below:

1. Squat:
2. Leg extension:
3. Leg curl:
4. Standing calf raise:
5. Pull down
6. Bench press:
7. Seated row:
8. Shoulder press:
9. Bicep curl:
10. Tricep extension:

At the conclusion of their pre-training assessments, the Trial Co-ordinator will arrange a date with the participants on when they are able to begin their eight-week training session.

Eight-week HIT training

All participants will be required to complete an eight-week HIT programme consisting of two HIT training sessions a week with at least 72 hours recovery between sessions.

Prior to starting each session, participants are required to complete a 5 minute warm-up cycle on a cycle ergometer set at an intensity of 70 watts.

Participants will then be asked to complete a full body high intensity weight circuit, training with maximal intensity on each given exercise, with a rest of 60-90 seconds between each exercise.

Squat

Participants will begin on the smith machine for the squatting exercise. The bar will be set to shoulder height and then the participant will stand underneath it with a shoulder-width stance so that the barbell is resting on the upper trap muscles. The participant will hold the bar with a shoulder-width grip and then bring their hands in a little closer while squeezing the upper back muscles (helping maintain proper core tightness). The bar is then unracked by extending yhe hips and then flare the feet out around 15-30 degrees. The spine should be in a neutral position with eyes gazing straight ahead. Elbows should point slightly back rather than straight down, as this prevents the resistance from travelling directly through the joints. The participant will squat as deeply as is comfortably possible while maintaining a neutral spine and grounded heels for as many repetitions as possible in a slow speed (2 seconds up and 2 seconds down). When the point of the point of momentary muscular failure (mmf) is reached, whereby the bar is re-racked.

Leg extension

The participant will sit on the chair with their back straight and against the back of the seat and hold the side handles. The shins will be behind the padded bar (roller) and position feet so that they’re facing forward. The padded bar should be sat on top of the shins, just above the feet (adjust settings. The participant will then be asked to raise the padded bar by extending the quads to straighten their legs followed by slowly lowering the padded bar back down towards the starting position without letting the weight stop. This will be continued for as many repetitions as possible in a slow speed (2 seconds up and 2 seconds down). When the point of the point of mmf is reached, the bar is slowly lowered.

Leg curl

The participant will lie prone on the machine. The shins will be behind the padded bar (roller) and position feet so that they’re facing down. The padded bar should be sat on top of the achiles tendon, just above the feet (adjust settings. The participant will then be asked to raise the padded bar by contracting the hamstrings to flex their legs followed by slowly lowering the padded bar back down towards the starting position without letting the weight stop. This will be continued for as many repititions as possible in a slow speed (2 seconds up and 2 seconds down). When the point of the point of momentary muscular failure (mmf) is reached, the bar is slowly lowered.

Standing calf raise

Participants will begin on the smith machine for the standing calf raise exercise. The bar will be set to shoulder height and then the participant will stand underneath it with a slightly narrower than shoulder-width stance on the ball of the feet on a slightly elevated platform so that the heel is off the floor. that the barbell is resting on the upper traps. The participant will grab the bar with a shoulder-width grip and then bring their hands in a little closer while squeezing the upper back muscles (helping maintain proper core tightness). The bar is then unracked by extending the hips. The spine should be in a neutral position with eyes gazing straight ahead. Elbows should point slightly back rather than straight down, as this prevents the resistance from travelling directly through the joints. The participant will flex at the ankle, extending as to go on tip-toes as high as is comfortably possible while maintaining a neutral spine for as many repetitions as possible in a slow speed (2 seconds up and 2 seconds down). When the point of the point of (mmf) is reached, whereby the bar is re-racked.

Pull down

The participant will sit on the pulldown seat, feet flat on the floor. The bar should be at a height that the outstretched arms can comfortably grasp the bar without having to fully stand up, but it should also be possible to still extend the arms to achieve full range of motion. Adjust the knee pad so that the upper thighs are tucked firmly under the pad. This will assist when applying apply effort to the bar. The participant will grasp the bar with a shoulder wide grip with an overhand, knuckles up grip. The participant will be instructed to pull the bar down until it's approximately level with the chin. While shifting just slightly backward is OK, the participant should aim to keep their upper torso stationary. The bottom of the motion should be where the elbows can't move downward any more without moving backward. Squeeze the shoulder blades together while maintaining square shoulders. From the bottom position with the bar close to the chin, the participant will slowly return the bar to the starting position while controlling its gradual ascent. This will be continued for as many repetitions as possible in a slow speed (2 seconds up and 2 seconds down). When the point of the point of momentary muscular failure (mmf) is reached, the weight is slowly lowered.

Bench press

The participant will sit on the chair with their back straight and against the back of the seat (adjust so the handle is chest height). The participant will then be asked to push the bar by extending their arms, pushing to straighten their arms followed by slowly lowering the bar back down towards the starting position without letting the weight stop. This will be continued for as many repetitions as possible in a slow speed (2 seconds up and 2 seconds down). When the point of the point of mmf is reached, the bar is slowly lowered.

Seated row

The participant will sit on the platform with their knees bent and grasp the bar Position with the knees slightly bent and so that the participant has to reach to grab the handle with outstretched arms yet without curling the lower back over. The participant will pull the handle and weight back toward the lower abdomen while trying not to use the momentum of the row too much by moving the torso backward with the arms. Targeting the middle to upper back by keeping the back straight and squeezing the shoulder blades together as the participant rows, chest out. This will be continued for as many repetitions as possible in a slow speed (2 seconds up and 2 seconds down). When the point of the point of mmf is reached, the bar is slowly lowered.

Shoulder press

The participant will sit on the chair with their back straight and against the back of the seat (adjust so the handle is shoulder height). The participant will then be asked to push the bar by extending their arms, pushing to straighten their arms followed by slowly lowering the bar back down towards the starting position without letting the weight stop. This will be continued for as many repetitions as possible in a slow speed (2 seconds up and 2 seconds down). When the point of the point of mmf is reached, the bar is slowly lowered.

Bicep curl

Adjust the cable machine at one end so that the cable is attached at the bottom with the sliding adjustment. The cable metal grip should extend so that the participant can grasp it comfortably in their hands with arms outstretched and palms up. Standing comfortably with feet firmly placed on the floor. Brace the abdominal muscles, straighten the back, keep the head steady. Curling the cable weight upward toward the chest. Only the forearms should move, rising up from the elbow. At peak contraction then unbend the arms at the elbow to let the cable weight return the arms to the lower resting position. Stop before the weights return to the stack, keeping the cable under tension. This will be continued for as many repetitions as possible in a slow speed (2 seconds up and 2 seconds down). When the point of the point of mmf is reached, the bar is slowly lowered.

Tricep extension

Adjust the cable machine at one end so that the cable is attached at the top with the sliding adjustment. The cable metal grip should extend so that the participant can grasp it comfortably in their hands with arms outstretched and palms down. Standing comfortably with feet firmly placed on the floor. Brace the abdominal muscles, straighten the back, keep the head steady and keep shoulders tensed by the side, extending the cable weight downward toward the floor. Only the forearms should move, lowering up from the elbow. At peak contraction then unbend the arms at the elbow to let the cable weight return the arms to the upper resting position. Stop before the weights return to the stack, keeping the cable under tension. This will be continued for as many repetitions as possible in a slow speed (2 seconds up and 2 seconds down). When the point of the point of mmf is reached, the bar is slowly lowered.

As the participants develop strength throughout the training program, the measure of intensity will remain maximal, as measured by reaching mmf in each exercise. Therefore, as the participant reaches the upper end of the repetition range (10 repetitions (~75% 1RM) for upper body exercises, and 16 repetitions (~65% 1RM) for lower body exercises) the resistance will be adjusted to provide a greater challenge the following training session. This will ensure adaptation occurs during the training duration. Based on the number of repetitions completed in each exercise, in a given training session, load will be adjusted so that it remains greater than 75% 1RM and 65% 1RM for upper and lower body exercises, respectively. Through the duration of their eight-week training period, participants will be encouraged to maintain their physical activity and diet as per pre-training.

Post-training assessment

The week following their final training session, participant’s post-training POMS, anthropometric, muscle function and 1RM for each of the upper and lower body exercises will be assessed as previously described in the Pre-training assessment section. A saliva and venous blood sample will also be collected for subsequent biochemical analysis.

Biochemical analysis

Chewing stimulated saliva samples collected into tubes and spun to remove any food debris. Plasma, leukocyte and peripheral blood mononuclear cells (PBMC) will be separated from whole blood by centrifugation immediately after collection. Aliquots of saliva, plasma, leukocyte and PBMC samples will be prepared for the following assays:

* Salivary cortisol: Salivary cortisol concentrations will be measured using commercial ELISA kits.
* Plasma myokine: Myokine concentrations (*list these*) will be assayed using a bead based multiplex panel and measured using flow cytometry.
* Cell phenotyping: White blood cells will be stained with fluorophore-conjugated antibodies to identify granulocytes, monocytes and lymphocytes by flow cytometry.
* Immune response: Cytokine (IL‐12p70, IFN‐γ, IL‐17A, IL‐2, IL‐10, IL‐9, IL‐22, IL‐6, IL‐13, IL‐4, IL‐5, IL‐1β, and TNF‐α) concentration following T-cell activation with PMA will be measured using a bead-based multiplex panel and measured using flow cytometry.

# Participant criteria

The following study inclusion and exclusion criteria will be applied:

**Inclusion criteria:**

Healthy individuals (male) 18-40 years, who are not following any specialised diets and not currently undertaking regular resistance exercise. Participants will be required to complete a health questionnaire and provide written consent for this study.

**Exclusion criteria:**

Participants will be excluded if they are unwilling or unable to provide informed written consent or comply with study procedures. All participants will be in good physical health and. Participants will also be excluded if they have (i) blood borne diseases (e.g., hepatitis), (ii) clinically diagnosed high/low blood pressure, (iii) recent bacterial or viral illness, (iv) are taking any mediation that affects the properties of blood (e.g. blood clotting) (iv) any recent musculoskeletal injuries within the past 3 months).

#### Withdrawal criteria

It will be emphasized to participants that involvement in the study is voluntary and that individuals may withdraw at any time. It will also be emphasized to Plant and Food Research/ Massey University employees that their participation is voluntary and they will not be penalised for not taking part in this research.

If a participant withdraws from the study, any biological samples (and data generated) they have provided will be removed from the study and destroyed. Biological samples cannot be handed back to the individual due to health and safety regulations, but we will assure the individual that they have been destroyed, in writing if required.

In addition, subjects may be removed from the trial for the following reasons;

* Staying in the study would be harmful (i.e., an injury occurs during the intervention period). In these scenarios, the participant will be strongly advised to visit their local health practitioner.
* Failure to follow trial instructions (i.e. not training with sufficient intensity for the duration of the trial).
* Develop a health condition or sustain an injury that would affect results.
* Failing to attend three of the exercise training sessions will result in withdrawl from the study

In these unlikely scenarios, participants will be strongly advised to visit their local health practitioner. The principal investigator will inform the Biosafety Advisory and Health & Safety Committees within Plant and Food Research.

# Research location

The study will be carried out at Massey University’s School of Sport, Exercise and Nutrition Human Performance Laboratory in Palmerston North

# Recruitment:

Participants will be recruited from within Plant and Food Research, Massey University, and the wider community by (a) displaying flyers on the appropriate notice boards or (b) verbal communication at the beginning of selective seminars within Plant and Food Research and Massey University.

All interested individuals will have an initial discussion with Trial Co-ordinator (Ms Nayer Ngametua) and will be provided with an information sheet, health and fitness questionnaires, and a consent form to take home. Potential participants will have 2 weeks to decide if they would like to participate in the study.

Study investigators will not take part in this study or will have no relationship with the study participants in any way that could influence their decisions.

# Subject numbers

8 participants

Power analysis from a previous exercise study was used to calculate the number of volunteers appropriate for this study (Minitab Version 18.1). Our analysis reveal that 3 participants is sufficient to detect a difference in strength between time points with a power of 80% (p = 0.05). To account for the possibility of participant withdrawal, we will enrol 8 participants to this study. Additionally, as previous HIIT studies assessing immune markers have used a similar number of participants, this number should also be suitable for secondary outcome measures (Alizadeh H, Safarzade A. High intensity intermittent training induces anti-inflammatory cytokine responses and improves body composition in overweight adolescent boys. Horm Mol Biol Clin Investig. 2019 Aug 1;39(3):/j/hmbci.2019.39.issue-3/hmbci-2019-0004/hmbci-2019-0004.xml. doi: 10.1515/hmbci-2019-0004. PMID: 31369392.).

# Minimise Risks and Adverse Effects

For this study, we have identified the following risks and have implemented procedures to minimise any potential risk associated:

*General*: If any participant displays an adverse reaction due to the treatment consumed as part of the study, the principal investigator will inform Biosafety Advisory and Health & Safety Committees at Plant and Food Research. Participants will also be advised to talk to their general medical practitioner before embarking on any part of the study.

*Participant’s health and confidentiality*: Potential trial participants will be required to complete a health questionnaire relevant to this study that will help the principal investigator evaluate whether individuals (using the inclusion and exclusion criteria) meet the requirement outlined in this study. Once assessed, to maintain confidentiality the health questionnaires will be securely locked away by the principal investigator and will either be returned to the participant or destroyed at the end of the study period. Only the principal investigator and trial co-ordinator will have access to a participant’s health questionnaire.

*High intensity exercise*: The exercise prescription used in the trial is high intensity exercise. The exercises participants will not exceed normal recommendations, and has been used in a number of previous exercise intervention studies in the past without any adverse reaction. The trial co-ordinators will monitor all trial participants and report any adverse reaction from the exercise to the principal investigator and the Plant and Food Research Biosafety Advisory and Health & Safety Committees. Participants will also be advised to contact their medical practitioner if they feel ill during the study.

*Blood sampling:* Two venous blood samples (~ 9 mL) will be collected from each participant, one on pre-training and one at post training measures. Needle pricks may present a minor risk to the participant. The participants may feel slight discomfort (or even feel light-headed) during the needle prick procedure. Participants will also be advised to eat and drink something, as well as report to the First Aider if they feel light headed or dizzy. The Trial Co-ordinator will check the participant’s well-being throughout the study. Use of a phlebotomist who holds a current certificate (MedLab NZ) will minimise any physical risks. The participants will also be assured and advised of what is happening throughout the procedure to alleviate any psychological risk

*Emergency facilities:* Qualified phlebotomists (MedLab NZ) and First Aiders (who have taken a refresher course [NZ Red Cross] within the last 2 yrs and are trained to use resuscitation equipment) will be on hand within Massey ubiversity to deal will any possible emergencies. The study will be performed in a room with an emergency telephone, access to a bed to lie down and a defibrrillator.

# Funding

This work is funded internally by The New Zealand Institute for Plant and Food Research Ltd.

# Ethics

Ethical approval will be sought from the New Zealand Health and Disability Ethics Committees. Plant and Food Research will comply with any trial-related monitoring, audits, and HDEC/regulatory inspection, providing direct access to source data/documents when required. In addition, this study has been assessed by the Biosafety Advisory Committee at Plant and Food Research and registered with ANZCTR.