**STUDY DESIGN: THE EFFECTS OF HYPOXIA ON MUSCLE GROWTH AND INFLAMMATORY RESPONSES TO RESISTANCE EXERCISE.**

**1. AIMS**

1) Determine the acute skeletal muscle and inflammatory responses of older adults to resistance exercise in hypoxia.

2a) Determine the chronic skeletal muscle and inflammatory adaptations of older adults to resistance training in hypoxia.

2b) Determine the effects of resistance training in hypoxia on cardiometabolic risk factors of older adults.

**2. STUDY DESIGN**

A randomised control trial will be used to address these aims. 60 healthy adults aged 18-35 and 60 healthy adults aged 60-80 will be recruited to participate in an eight week resistance training intervention in either normoxia or hypoxia.

Healthy adults aged 18-35

N=60

Healthy adults aged 60-80

N=60

Randomised

Post intervention assessment:

* Day 1 (4 days after intervention): Anthropometrics, body composition (DXA & pQCT), resting biopsy and blood collection.

Baseline assessment:

* Day 1 (2wks before intervention): Health screening, anthropometrics, body composition (DXA & pQCT), exercise test familiarisation, training program familiarisation.
* Day 2 (1wk before intervention): Strength, muscular endurance, aerobic fitness assessment.

8wk Hypoxia resistance training

 n=15

8wk Normoxia resistance training

 n=15

8wk No exercise controls (normoxia)

n=15

8wk No exercise controls (hypoxia)

n=15

Muscle biopsy & blood collection during first training session: for baseline assessment and acute response to exercise

15th session: venous blood collection.
16th session: physical fitness reassessment.

**3. PRIMARY OUTCOME MEASURES**

Aim 1: Acute immune and muscle response to a single bout of resistance exercise in hypoxia:

* Muscle growth markers
* Inflammatory markers (blood)

Aim 2: Chronic muscle and immune response to resistance training in hypoxia:

* Muscle growth markers
* Inflammatory markers (blood)

Aim 3: Functional muscle outcomes from resistance training in hypoxia:

* Muscle hypertrophy & strength
* Skeletal muscle angiogenesis

Subjects will be blinded to the oxygen concentration.

**4. STUDY POPULATION**

Participants aged 18-35 or 60-80 years will be recruited from the Geelong community. Requirements for recruitment include:

* Participants should be untrained or recreationally active, having not partaken in resistance training in ≥ 6 months. This is to ensure that participants are unaccustomed to the initial exercise bout, to elicit a moderate muscle/inflammatory response.
* Participants must have a BMI <30.
* Participants should not be exposed to altitude above 3000m within 3 months of commencing the exercise intervention.
* No history of heart disease, stroke, cancer, smoking.
* No history of diabetes.
* No history of blood disorders including anaemia, histologic, cytotoxic and stagnant conditions.
* No history of respiratory diseases
* Not taking anabolic hormones/protein supplements.

**5. POWER ANALYSIS**

Using the primary outcomes of muscle strength, muscle hypertrophy and inflammation, means and standard deviations were sourced from relevant literature on resistance training in young and older adults. The required sample size was computed using a priori power analysis of the differences between two means. Power was set at 0.8 and significance level (α) was set at 0.05. For measurement of muscle strength, a sample size of 11 is required for an 81.3 % chance of correctly detecting a large effect size. For measurement of type II muscle fibre hypertrophy, a sample size of 8 is required for an 80.7 % chance of correctly detecting a large effect size. For measurement of myeloid cell populations (B cells and natural killer cells), a sample size of 14 is required for an 81.5 % chance of correctly detecting a moderate effect size.

**6a. STUDY PROCEDURES**

Following recruitment, the 60 young and 60 older participants will be randomly allocated to one of four groups:

* 8wk hypoxia resistance training intervention (n=15)
* 8wk normoxia resistance training intervention (n=15)
* 8wk passive exposure to hypoxia intervention (n=15)
	+ This group will allow us to compare the effects of resistance training alone to resistance training in hypoxia.
* 8wk passive exposure to normoxia intervention (n=15)
	+ This group will serve as a time control.

Hypoxic stimulus: Participants being exposed to normoxia will breathe 20.93 % O₂ in an environmental tent. Participants being exposed to normobaric hypoxia will breathe 14.4 % O₂. This level of hypoxia (< 16 %) has been successfully used to elicit muscle hypertrophy with 4-8wk resistance training programs in young healthy males. Selecting an O₂ concentration below this could be too severe, as a 4wk passive exposure to hypoxia 1hr per day, 5 days per week (12 % O₂) reduced antioxidant capacity and suppressed vascular endothelial function (Wang et al. 2007).

**6b. PHYSICAL ACTIVITY AND DIET**

Participants will be instructed to maintain their current activities of daily living and physical activity for the duration of the intervention, and refrain from commencing any new exercise programs. Participants should not partake in any additional resistance training during the intervention. The dietary behaviours of participants will be assessed using a food and physical activity survey before, during and after the intervention. For the duration of the study, participants will be recommended a standard breakfast prior to each training session.

**6c. BASELINE TESTING**

Participants will report to Deakin University for two pre-assessment sessions in the fortnight prior to the training intervention. Participants will be instructed to abstain from alcohol or caffeine consumption 48 before testing, and to abstain from moderate/vigorous physical activity in the 48 hours prior to baseline testing. On day 2, participants will arrive in the morning after an overnight fast. Participants will undergo assessment of:

* Day 1 (two weeks before training commencement):
	+ Anthropometric measurements (height, weight, blood pressure, cholesterol, glucose)
	+ APSS health questionnaire
	+ DXA scan (lean and fat mass)
	+ pQCT scan (muscle cross-sectional area)
	+ Exercise familiarisation: subjects will be taught the exercises used in the training program
	+ Exercise test familiarisation: subjects will be taught the exercise test protocols for the muscle strength, muscle endurance and physical fitness tests
* Day 2 (one week before training commencement):
	+ Strength/fitness measurements:
		- Strength: 5RM leg press, leg extension, bench press and seated row
		- Muscular endurance: rate of force development on BioDEX MVC (30 leg extensions)
		- Cardiovascular fitness: incremental oxygen consumption exercise test (VO₂max)

**6d. FREQUENCY & INTENSITY OF INTERVENTION**

The intervention will consist of resistance training two times weekly for 8 weeks. Participants will complete 16 sessions in total over the 8 week period. Recovery between training sessions will be at least 48hrs. Previous studies with young healthy men used 8wk resistance training programs in hypoxia 2-3x per week and found significant improvements in muscle size compared to training in normoxia (Nishimura et al., 20009).

Training days: All participants will report to the gym at the same time of morning. Participants will enter the environmental tent, and acclimatise to the randomly allocated oxygen concentration for 15 minutes. Participants will then perform a warm up protocol, which consists of 5 minutes of low resistance cycling. Participants will then complete a series of lower body exercises (leg press, leg extension, bench press, seated row). 4 sets and 10 repetitions of each exercise will be performed at 70 % of participants 1 repetition maximum. Exercises will be performed with 60 seconds rest between sets, and 2 minutes rest between exercises. Once the exercise protocol is finished, participants will rest for two minutes, then exit the tent. 5RM will be re-assessed at the end of week 2, 4 and 6 of the program and load will be adjusted accordingly (progressive overload). The non-exercising groups will be seated for the duration of the passive normoxic and hypoxic exposure. The duration of the passive exposure will be identical to the duration of the exercise training protocol (~45-60 minutes).

**6e. MUSCLE BIOPSY & BLOOD COLLECTION**

To address aim 1 (acute muscular and inflammatory responses to a single bout of resistance exercise), muscle biopsy and blood samples will be taken during the first session of the 8 week training intervention. All participants will report to the gym at 7.30am after an overnight fast. After 30 minutes of resting in the supine position, a resting venous blood sample (15 mL) will be collected from the antecubital vein, before a muscle biopsy of the vastus lateralis is collected. Participants will rest for a further 15 minutes, enter the environmental tent, then acclimatise to the randomly allocated oxygen concentration for 15 minutes (either 14.4 % or 20.93 % O₂). Participants will then complete the warm up and exercise protocol outlined above, which will last approximately 45 minutes. Participants will rest for two minutes after the cessation of the exercise protocol, then exit the tent. Venous Blood will be collected at 0, 30, 60, 90, 120 and 180 minutes following exercise, and a muscle biopsy will be taken 180 minutes following the completion of exercise. Participants will be provided a standard meal after the last venous blood collection, and return 24 hours after the exercise protocol for a final blood and muscle biopsy sample collection. When participants return 48 hours later for session two of the program, one more blood sample will be collected.

**6f. REASSESSMENT OF BASELINE MEASURES**

To address aim 2 and 3 (chronic muscular and inflammatory adaptations to resistance training), the initial resting muscle biopsy and venous blood sample (described above) will serve as the baseline assessment for the 8 week training intervention. To track any changes in inflammatory profile throughout the resistance training intervention, participants will have a small blood samples taken before the 15th (second last) session, and 0, 30, 60, 90, 120 and 180 minutes following the session. Participants will return 24 and 48 hours later for 2 further blood samples. Participants will be asked to arrive at the 15th and 16th training session in an overnight fasted state. On the 16th session, participants’ strength, muscular endurance and physical fitness will be re-assessed. Approximately four days after the cessation of the 8 week training program, participants will return for reassessment of body composition (DXA and pQCT scan), anthropometrics and collection of a resting muscle biopsy and blood sample.

**7. STATISTICAL ANALYSIS**

A two-way repeated measures ANOVA will be used to examine change in muscle growth (post-pre) as the outcome and treatment (resistance training or passive exposure to normoxia/hypoxia) considered as main effect. All data will be checked to ensure they meet the criteria for an ANOVA, namely that the data are normally distributed and groups have similar standard deviations. A Bonferroni or Tukey’s post hoc analysis will be used if the ANOVA reveals a significant time\*treatment interaction.