Study Title: Whey Protein Support to Metabolic and Performance Adaptations in Response to High Intensity Interval Training in Young Adult Men

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Detailed study protocol 7867835

Baseline screening

Prior to experimental testing, participants will attend the lab to complete baseline screening including an incremental cycle ergometer test to exhaustion to determine maximal oxygen update (VO_2max) via indirect calorimetry. A body composition assessment via bioelectrical impedance analysis (BIA) will also characterise participants for inclusion in the study (BMI <30 kg.m^{-2}). The protocol to determine VO_2max comprises a 5 minute warm-up stage at 50 W with subsequent 1 minute incremental stages (30 W) until exhaustion. The following criteria are used to establish if VO_2max has been achieved: Volitional fatigue, Rate of perceived exertion (RPE) ≥18, respiratory exchange ratio (RER) ≥1.10, and heart rate (HR) ≥85% age-predicted max.

Pre-intervention performance testing

Following baseline screening, included participants attend the lab 2 h after a standardised meal and having refrained from caffeine on the day of testing, to complete pre-intervention performance testing. This comprises in order a baseline i) Wingate test; ii) Submaximal incremental cycling test; iii) 20 minute cycling performance test.

Wingate Test

After recording height and weight, participants complete a standardised 5 minute warm-up on a Wingate cycle ergometer, followed by 30 s maximal cycle sprinting against a resistance equivalent to 7.5% of their body mass (BM). A 3 minute cool down period follows this test.

Submaximal incremental cycling test

After 15 minutes seated rest, participants begin a submaximal incremental cycle test consisting of a 2 minute standardised warm-up followed by 4 minute stages starting at 50 W and incrementing by 50 W after each completed stage. The test ends after the final stage is completed, corresponding to a power output of 250 W, or when participants can no longer maintain a cadence of ≥ 80 rpm during exercise. Participants must also maintain their cadence within a range of 80-85 rpm throughout the test. RPE and HR are used as markers of exercise intensity during this test. Capillary lactate is sampled at rest and during the final 30s of each stage and concentrations are determined using a handheld meter. Power output at the lactate threshold (W_{LT}) is calculated from this data. Cycling economy (CE) is calculated by determining the ratio of oxygen uptake to power output during the last 30s of each stage.

20 minute cycling performance test

After 15 minutes of seated rest, participants complete a 20 minute cycling performance test where they are instructed to exert maximal effort throughout the 20 minute period. RPE and HR are monitored at 5 minute intervals throughout the test. Mean power output and total work are calculated and used as performance markers for this test.

Dietary controls

A 7 day weighed food diary is administered before the beginning of the HIIT intervention in order to characterise habitual dietary intake and analyse each participant's energy and macronutrient intake. Participants are instructed to maintain their habitual dietary intake throughout the HIIT intervention. A 24 h dietary recall is conducted upon each visit to the lab for participants, and dietary analysis using this information is compared with the 7 day food diary analysis to assess maintenance of habitual dietary intake for the duration of the HIIT intervention.

Nutritional intervention

All 3 supplement/placebo drinks are matched for volume, flavour (Synergy® Flavours, Carbery Food Ingredients Ltd) and appearance while the whey protein concentrate (WPC) and whey protein hydrolysate (WPH) drinks are matched for protein content. For each exercise session, participants attend the lab the morning after a ≥10 h overnight fast, and ingest $0.33g.kg^{-1}$ BM protein of WPC or WPH, or $0.33 g.kg^{-1}$ BM of a flavoured placebo. All drinks are ingested 45 minutes pre-exercise, and are all food grade and safe for human consumption. Participants are also provided with a standardised post-exercise snack ($2.43kcal.kg^{-1}$ BM, $0.25g.kg^{-1}$ BM protein) which they were instructed to consume 30 minutes after each exercise session (*excluding exercise session 1)*.

Experimental design

This study will be conducted in a randomised, double-blind (participants and outcomes assessor blind) parallel groups design. Participants are randomly allocated into one of 3 nutritional intervention groups; fasted placebo, WPC fed or WPH fed. The exercise intervention comprises 9 HIIT sessions completed over 3 weeks with 48h rest in between exercise sessions. HIIT sessions will consist of 4-6 (4 sprints in the initial 3 sessions, 5 sprints in the middle 3 sessions, and 6 sprints in the final 3 sessions) X 30s maximal cycle sprints on a Wingate cycle ergometer, pedalling at a resistance equivalent to 7.5% BM. Each sprint is interspersed with 4 minutes active recovery. Standardised 5 minute warm-up and 3 minute cool-down periods precede and follow each exercise session, respectively. On the morning of each exercise session, participants arrive to the lab after a ≥10h overnight fast, having abstained from caffeine for 12h, alcohol intake for 24h and vigorous exercise 48h beforehand. On the morning of exercise session 1, a resting micro-needle muscle biopsy will be obtained under local anaesthetic (1% lidocaine) from m. vastus lateralis, as well as a venous blood sample. Following this, participants ingest the allocated pre-exercise drink and rest quietly in the lab for 45 minutes before beginning exercise. Further venous blood samples are obtained immediately pre and post-exercise. For exercise session 1, HIIT comprises 4 X 30s "all out" Wingate sprints, interspersed with 4 minutes active recovery. A fourth venous blood sample is obtained 1 h post-exercise, as well as a second muscle biopsy, which is obtained 3 h post-exercise using the same procedures as before. Between the post-exercise recovery period and second muscle biopsy, participants are not permitted any food or drinks except for consuming water *ad libitum*. For the remaining exercise sessions, participants arrive the lab under the same conditions, ingest their allocated pre-exercise drink and rest quietly for 45 minutes before completing HIIT. They are also

provided with a standardised snack (2.43kcal.kg^{-1} BM, 0.25g.kg^{-1} BM protein) to consume 30 minutes following post-HIIT. Following HIIT session 9, a final muscle biopsy and venous blood sample are obtained 48-72h post-exercise under the same conditions (overnight fasted) and using the same procedures. Anaerobic exercise performance markers are obtained for each HIIT session (exercise sessions 1-9) as well as for individual sprints within-session (sprints 1-6) including peak power, mean power, total work and fatigue index.

Post-intervention performance testing

The performance testing completed pre-intervention (pre-intervention performance testing) is repeated by participants in an identical manner 72-96 h following completion of the final HIIT session. 24 h dietary recall is repeated for this lab visit, and participants refrain from caffeine intake and attend the lab 2 h following a standardised meal.

Statistical Analysis Plan

Normality and homogeneity of variance are tested prior to statistical tests. Paired sample t-tests will be used to assess within group changes. Between group treatment effects will be evaluated using a One-way ANOVA to examine the main effects of treatment based on delta scores from pre to post intervention. If the ANOVA yields a significant effect, post hoc comparisons will be performed using a bonferroni correction. A probability level $P < 0.05$ will be considered statistically significant and *cohen's d* effect sizes will be calculated. All normal data will be expressed as Mean ± Standard Deviation/Standard Error, or non-parametric as median (Interquartile Range).