Study Protocol and Statistical Analysis Plan

Subjects

The present study will recruit 17 male volunteers aged 18–35 years who will have no resistance training experience in the past 6 months, no arm injuries in the past 2 years, no illnesses in the month prior to data collection, and no known allergies to broccoli or other cruciferous vegetables. Because no effect-size data from comparable crossover trials will be available (only a parallel-group pilot study), we will recruit to a feasibility target of at least ~15 completers and will use a within-subject crossover design to limit inter-individual EIMD variability, with post hoc sensitivity analysis ($\alpha = 0.05$, $1–\beta = 0.80$) showing we will be able to detect medium Time × Supplement effects (~partial $\eta^2 \geq 0.06$). Participants will maintain their regular diet, will abstain from exercise for one week before the experiment, and will not be allowed to engage in any additional physical activities during the experimental period. Supplement adherence will be quantified via daily logs and will be confirmed by sachet count, with compliance expected to exceed 95% for all participants. Participants will be surveyed about the consumption of various cruciferous vegetables (cabbage, turnips, mustard greens, broccoli, horseradish, arugula, radishes), indicating frequency and approximate amounts, which will reveal no significant additional intake. Each participant will read and sign a written informed consent form.

Study Design

Participants will undergo two separate sessions of muscle-damaging exercise targeting the elbow flexors: (1) following seven days of broccoli powder consumption (condition X), and (2) following an equivalent placebo regimen (condition Y). The broccoli supplementation will consist of 10 g of broccoli powder [a freeze-dried glucoraphanin-rich broccoli powder (99.5% broccoli and 0.5% mustard seed)] dissolved in 125 mL of boiling water, providing 320 μg of glucoraphanin per serving. It will be consumed when still hot/warm once daily for seven days before and for four days following the eccentric exercise protocol. The placebo powder, matched in appearance and preparation, will contain no broccoli-derived bioactives. The 7-day, ~320 μg/day glucoraphanin dose (manufacturer standard serving) will be selected for feasibility. The selected dose will be higher than those used in most studies, as reviewed by Fahey et al. (2015). Data on the complete elimination of sulforaphane and its metabolites will range from 12–24 hours to as long as 72 hours.

Intervention order will be randomized in a double-blind, crossover design with a 14-day washout period, during which participants will switch to the alternate supplement and will repeat the same protocol using the contralateral arm. Simple randomization will be performed using a random number generator for the interventions, with all participants starting the first intervention with their dominant arm to ensure equal dominant arm use between the broccoli powder and placebo conditions. Both participants and investigators conducting the assessments will be blinded to supplement allocation. Supplements will be provided in identical sealed sachets.

Markers of muscle damage will include reductions in isometric and isokinetic peak torque, restricted elbow range of motion (ROM), increased arm girth, soreness, and elevated plasma creatine kinase (CK) activity and will serve as dependent variables. On the test day, a fingertip blood sample will be collected for CK analysis, followed by assessments of elbow ROM, arm girth, biceps muscle soreness, and muscle strength using an isokinetic dynamometer (Biodex Medical Systems, System 4, Inc., Shirley, New York, USA). Prior to testing, participants will complete a standardized warm-up on an arm ergometer (6 min, workload ≈ body mass in watts). All assessments will be repeated immediately post-exercise, and at 48 and 96 h post-exercise.

In Muscle-damaging Exercise Load

The subjects will be seated on a Biodex isokinetic dynamometer with the backrest fixed at an angle of 90°, and the trunk, pelvis, and upper arm will be stabilized with Velcro straps. The axis of rotation will be aligned with the elbow joint, and the lever arm pad will be attached at the wrist. Subjects will perform six sets of ten maximal voluntary eccentric contractions of the elbow flexors with an angular velocity of 60°/s, starting from 120° elbow flexion to full extension (the full elbow extension angle will be considered as 0°), with a 1-min rest between sets. Each subject will use the Biodex dynamometer to induce muscle damage in a randomly selected arm, either dominant or non-dominant. After 14 days, the same exercise will be repeated with the alternative intervention on the other arm. Verbal encouragement will be provided to maintain maximal effort throughout each stretch. Work done during each eccentric action will be recorded.

Strength Assessment

To evaluate voluntary muscle strength, isometric (PTISOM) and concentric isokinetic peak torque (PTISOK) of the elbow flexors will be measured using the same dynamometer (Biodex System 4) and the same setup as during the muscle-damaging exercise load. The elbow will be positioned and

fixed at 90° of flexion, with full elbow extension defined as 0°. PTISOM will be assessed first through two maximal voluntary contractions, each lasting approximately 1–2 seconds, with a 1-min rest between trials. After a 1-min rest, PTISOK will be measured at an angular velocity of 60°/s through a 120° ROM (0°–120°), using two continuous contractions in the same setup. The highest torque value from the two trials in each condition will be recorded for analysis. Verbal encouragement and real-time visual torque feedback will be provided to promote maximal effort. A decline in voluntary contraction force and prolonged recovery will be considered indirect markers of EIMD.

Range of Motion and Arm Girths

Arm circumference and elbow joint ROM will be assessed according to standardized protocols. Arm girths will be measured using a flexible, non-elastic 1-meter tape to the nearest 0.1 cm, with the participant standing upright. Measurements will be taken at the midpoint between the acromion and olecranon processes, first with the arm relaxed and then in a fully flexed, contracted state, following the recommendations of the American College of Sports Medicine. Elbow ROM will be assessed using a digital goniometer (Jamar Plus+ Digital Goniometer, Performance Health, Warrenville, Illinois, USA) first with the arm in a neutral resting position. Then, participants will actively move their elbow into full flexion and extension, sustaining each position for approximately five seconds while the angle will be recorded.

Musculoskeletal Ultrasound

Biceps brachii muscle thickness (MT), cross-sectional area (CSA), and distal tendon thickness (TT) will be measured using B-mode grayscale ultrasound (10–15 MHz linear-array probe, Echoblaster 128, Telemed, Vilnius, Lithuania). Imaging procedures will follow the guidelines of the European Society of Musculoskeletal Radiology and prior protocols. Participants will sit with the arm supported on an armrest and a water-based transmission gel will be applied to minimize transducer pressure. MT and CSA will be acquired at two-thirds of the distance between the acromion and antecubital crease. Three images per muscle will be captured and analyzed in ImageJ (v.1.46; NIH, USA). For TT, the transducer will be aligned with the long axis of the bicep tendon using an oblique ulnar approach at the elbow joint. To reduce anisotropy artifacts, participants will slightly bend the arm in full supination, and TT will be calculated as the average of measurements taken at 5, 10, and 15 mm proximal to the radial tuberosity. Echo intensity (EI) will be analyzed from the same ultrasound images using grayscale histogram analysis in ImageJ, with values ranging from 0 (black) to 255 (white), following similar previous muscle and tendon approaches. Specifically, a region of interest

will be manually selected to encompass the largest possible portion of the muscle, excluding the superficial and deep aponeuroses, and for the tendon, the central region bounded by hyperechoic borders. Both assessments will be performed using longitudinal views to ensure consistent anatomical alignment across time points. All ultrasound measures will be performed by a single experienced operator. Intrarater reliability was assessed prior to data collection on four separate occasions in two male participants, showing excellent consistency (MT: ICC = 0.97, CV = 3.46%; TT: ICC = 0.98, CV = 2.73%).

Muscle Soreness

Muscle soreness will be assessed subjectively using a 10-point visual analogue scale during active movement. Each point on the scale will have a written description of soreness: 0 (none), 1 (very slight), 2 (slight), 3 (mild), 4 (less than moderate), 5 (moderate), 6 (more than moderate), 7 (intense), 8 (very intense), 9 (barely tolerable), and 10 (intolerably intense). Subjects will be required to evaluate the severity of soreness in their biceps brachii during 2–3 full-range eccentric–concentric contractions. Muscle soreness will be widely accepted as an indirect indicator of EIMD.

Plasma CK Activity

Approximately 0.25 mL of capillary blood will be drawn from the finger and immediately will be centrifuged. The plasma will then be used to measure CK activity using a Spotchem™ biochemical analyzer (EZ SP-4430, Menarini Diagnostics, Winnersh-Wokingham, UK) with soft reagent strips (Arkray Factory, Inc., Shiga, Japan) reported as micro-Katalytic units per L (μkat·L–1). According to the analyzer manual, the normal reference range for human plasma CK using this method will be 0.9–4.1 μkat·L–1. Elevated plasma CK activity will be considered an indirect biochemical marker of muscle membrane damage.

Statistical Analysis

Data will be analyzed using SPSS (version 30.0.0; IBM Corp., Armonk, NY, USA) and will be presented as mean ± SD. Data normality will be assessed using the Shapiro–Wilk test and visual inspection of Q–Q plots, with no clear outliers expected to be identified. A repeated-measures design will be used to assess the effects of eccentric EIMD and supplementation on multiple physiological outcomes. For all dependent variables except CK and muscle soreness, a three-way repeated-measures ANOVA will be conducted with Time (Pre, Post, 48 h, 96 h), Supplement (X vs. Y), and

Arm Condition (eccentrically exercised [E] vs. control [C]) as within-subject factors. For CK and muscle soreness, which will only be measured in the exercised arm, a two-way repeated-measures ANOVA (Time × Supplement) will be used. Mauchly's test will be used to assess sphericity, and when violated, Greenhouse–Geisser corrections will be applied. Effect sizes will be reported using partial eta squared (η^2), with values of 0.01, 0.06, and 0.14 interpreted as small, medium, and large effects, respectively. Post-hoc pairwise comparisons will be Bonferroni-adjusted. Statistical significance will be set at $p < 0.05$.