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Multi-modal exercise training and protein-pacing enhances physical performance adaptations independent of growth hormone and BDNF but may be dependent on IGF-1 in exercise-trained men☆

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ABSTRACT

Objective: Protein-pacing (P; 5–6 meals/day @ 2.0 g/kg BW/day) and multi-mode exercise (RISE; resistance, interval, stretching, endurance) training (PRISE) improves muscular endurance, strength, power and arterial health in exercise-trained women. The current study extends these findings by examining PRISE on fitness, growth hormone (GH), insulin-like growth factor-1 (IGF-1), and brain-derived neurotrophic factor (BDNF) response, cardiometabolic health, and body composition in exercise-trained men.

Design: Twenty active males (>4 days exercise/week) completed either: PRISE (n = 11) or RISE (5–6 meals/day @ 1.0 g/kg BW/day; n = 9) for 12 weeks. Muscular strength (1-repetition maximum bench and leg press, 1-RM BP, and 1-RM LP), endurance (sit-ups, SU; push-ups, PU), power (squat jump, SJ, and bench throw, BT), flexibility (sit-and-reach, SR), aerobic performance (5 km cycling time-trial, TT), GH, IGF-1, BDNF, augmentation index, (AIx), and body composition, were assessed at weeks 0 (pre) and 13 (post).

Results: At baseline, no differences existed between groups except for GH (RISE, 230 ± 13 vs. PRISE, 382 ± 59 pg/ml, $p < 0.05$). The exercise intervention improved 1-RM, SJ, BT, PU, SU, SR, 5 km-TT, GH, AIx, BP, and body composition in both groups (time, $p < 0.05$). However, PRISE elicited greater improvements in 1-RM BP (21 vs. 10 Δlbs), SJ (171 vs. 13 ΔW), 5 km-TT (−37 vs. −11 Δs), and sit-and-reach (5.3 vs. 1.2 Δcm) over RISE alone ($p < 0.05$) including increased IGF-1 (12%, $p < 0.05$).

Conclusions: Exercise-trained men consuming a P diet combined with multi-component exercise training (PRISE) enhance muscular power, strength, aerobic performance, and flexibility which are not likely related to GH or BDNF but possibly to IGF-1 response.

1. Introduction

The use of protein supplementation is commonly used to enhance muscle recovery and/or improve satiety. Recently, we demonstrated that a protein-pacing diet alone (P; 5–6 meals/day @ >1.4 g/kg body weight (BW) protein/day) [1] and when combined with a multi-mode (RISE; resistance, interval, stretching, endurance) exercise intervention (PRISE) results in greater reductions in total and regional (abdominal/visceral) fat mass, greater gains in lean mass, and enhanced cardiometabolic health compared to a combined protein-pacing and traditional resistance training intervention in obese/overweight women [2]. Following on this work, we recruited healthy, normal weight, exercise-trained women who were randomized to either a control (1.0 g/kg BW protein/day) or protein-pacing group (2.0 g/kg BW protein/day), and both groups completed 12 weeks of RISE exercise training [3]. Women consuming the protein-pacing diet (PRISE, 2.0 g/kg BW protein/day) exhibited significantly greater gains in muscular strength, endurance, power, and improvements in markers of cardiovascular health [3] compared to the RISE only (1.0 g/kg BW protein/day) intervention. Thus, in exercise-trained healthy, normal weight women, protein-pacing improves the adaptations to multi-modal exercise training.

Indeed, the majority of studies investigating the potential physical or performance benefit of protein supplementation have focused on men, specifically in acute and/or mono-modal exercise paradigms (e.g. resistance training or running), which have shown protein ingestion improves muscle recovery [4,5], enhances improvements in muscle mass [6] and/or exercise performance [7–9]. However, the most recent recommendations by the American College of Sports Medicine, suggest a comprehensive approach to exercise training, by including not only endurance exercise, but also resistance, flexibility, and neuro-motor

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training [10]. Taken together with the progression of the fitness field towards a more multi-modal paradigm, understanding the potential benefit of elevated protein intake in this context is paramount. Additionally, while protein-pacing has been demonstrated to increase muscle protein synthesis [11,12], the purported mechanisms responsible for the enhanced response to multi-modal training with elevated protein intake, such as enhanced anabolic hormonal milieu (i.e. growth factors), remain relatively unexplored.

It is well recognized that growth factors such as growth hormone (GH), insulin-like growth factor-1 (IGF-1), and brain derived neurotrophic factor (BDNF) and their receptors are key regulators of neuromuscular development [13]. Indeed, recent work has found that both acute and chronic exercise increases anterior pituitary activity and GH release, suggestive of a prominent role of the endocrine system in muscular adaptations [14]. However, recent work has suggested that circulating levels of growth factors might not reflect functional changes in muscle (i.e. strength) [15], perhaps depending more upon neurological development [16] or the biological compartment being explored [17]. As much of the previous work has focused on the response to monomodal resistance exercise training, coupled with mounting controversy over whether circulating hormones reflect functional changes (i.e. strength, power, etc.), further work is needed to determine if circulating anabolic factors might explain the adaptations to training.

Accordingly, the primary aim of the present study was to compare the response to the multi-modal RISE training in healthy active men consuming either a normal protein (RISE, control group) intake versus a higher protein intake (PRISE) on fitness-related performance, cardiovascular, metabolic, and hunger/satiety outcomes, as well as blood levels of GH, IGF-1, and BDNF. We hypothesized that 1.) RISE would improve fitness, cardiovascular health, metabolic markers, and hunger/satiety, and 2.) Such improvements would be enhanced in the protein supplemented (PRISE) group, perhaps mediated by greater anabolic signaling, as measured by GH, IGF-1, and BDNF.

2. Methods

2.1. Participants

A total of 63 men from the Saratoga Springs, NY area, responded to emails, flyers and local newspapers to advertisements and were initially screened, of which 30 were eligible for participation (Fig. 1). Participants were nonsmoking, healthy, physically active men with no known cardiovascular or metabolic diseases as assessed by a medical history and a comprehensive medical examination. Specifically, all participants were highly active (minimum of >30 min, 4 day/week of structured physical activity), normal weight (BMI < 25 kg/m^2; % body fat ≤ 30%), middle aged (25–55 years), and weight stable (±2 kg) for at least 6 months prior to the beginning of the study. All participants provided informed written consent prior to participation, and the study was approved by the Institutional Review Board of Skidmore College (IRB#: 1401-382). All experimental procedures were performed in accordance with the Federal Wide Assurance and related New York State regulations, which are consistent with the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research and in agreement with the Helsinki Declaration as revised in 1983. This study was registered with ClinicalTrials.gov Identifier: NCT02593656.

2.2. Experimental design

2.2.1. Study timeline

Participants were randomly assigned to one of two groups: (1) protein pacing and multi-mode exercise training (PRISE; n = 12; 5–6 meals/day @ 2.0 g/kg BW/day) or (2) normal protein and multi-mode exercise training (RISE; n = 14; 5–6 meals/day @ 1.0 g/kg BW/day). All participants performed the same RISE exercise training program consisting of 4 days/week of closely supervised and monitored progressive exercise training for 12 weeks (see previous references [2,3]). All testing procedures (see below) were administered pre-intervention (week 0) and post intervention (week 13) unless noted otherwise. Upon arrival at the laboratory, anthropometric and body composition measurements and blood sampling for subsequent analysis were performed.

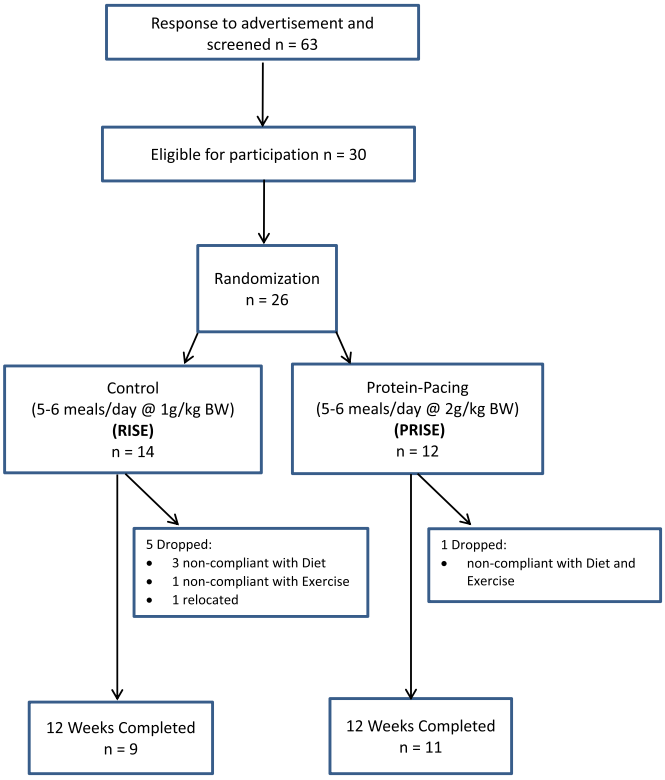

Fig. 1. Subject recruitment, enrollment, and assignment procedures.

2.3. Nutrition intervention

Meal plans were identically matched in terms of total kcals, meal frequency and timing and dietary support. By design, the only differences between the two groups was the amount of protein (1.0 vs. 2.0 g/kg BW per day). Additional supplementation (daily multi-vitamin/minerals, and caffeine and electrolytes on workout days) was also provided to participants and differed only by the type of product manufacturer. Participants in both groups were provided detailed meal plans designed by a registered dietitian and instructed to follow the meal plans throughout the 12-week intervention (Table A1). The registered dietitian met with participants weekly for the first two weeks and thereafter on an "as needed" basis. In addition, investigators met with participants a minimum of 4 day/week to answer questions and reinforce compliance to meal plans. To facilitate adherence to the meal plans, food was provided to both groups. It's important to note that the protein dosing was equivalent to >0.25 g/kg BW per meal which has been shown to be the optimal intake for muscle protein synthesis [18]. By study design, the only macronutrient that was intentionally different between groups was the protein per kg BW. Participants in both groups were given a 1-week. supply of the supplements and asked to return empty packets before they received the next week's supply as a means of assessing their compliance. Both groups were provided equivalent nutritional support and similar caloric intakes throughout the 12 week intervention.

The timing of meals was an important component of the current study and both groups consumed meals using an identical meal pattern schedule. On resistance and interval exercise days (see below), participants consumed a small snack (~250 kcals) prior and on stretching and endurance days arrived a minimum of 2–3 h fasted but well-hydrated and were allowed to consume the electrolyte beverage as needed on all exercise days. Breakfast was consumed after the exercise and remaining meals were consumed at 3 h intervals throughout the remainder of the day. On non-exercise days, participants consumed breakfast within an hour of waking in the morning and remaining meals at 3 h intervals thereafter (Table A1).

2.4. RISE exercise training protocol

Subjects in both groups underwent the same closely supervised/monitored progressive multiple exercise training regimen as described previously [2]. Briefly, the training program consisted of four specific types of exercise training: 1) resistance exercise; 2) interval sprints; 3) stretching/yoga/pilates, and 4) endurance exercise (RISE training, [2,3]. Subjects underwent four exercise sessions per week and the sessions rotated through the four types of exercise, such that each of the four exercises were performed 1 day per week. To familiarize participants with the individual exercises and to ensure compliance, all training sessions were performed in the Skidmore College Sports Center under the close supervision of the research team. Intensity level was monitored at every exercise sessions with heart rate monitors (Polar H7, Polar Electro, Lake Success, NY, USA) to ensure subject safety and proper compliance with the exercise program.

Specific details of the 4 types of exercises that comprise the RISE training have been previously published [2,19]. Briefly, the resistance training sessions were completed within 60 min and consisted of a dynamic warm-up, footwork and agility, lower and upper body resistance, and core exercises all performed at a resistance to induce muscular fatigue in 10–15 repetitions and for 2–3 sets. A 30-s recovery was provided between sets and a 60-s recovery was allowed between different exercises. The sprint interval training sessions were completed within 35 min and consisted of either 7 sets of 30-s "all-out" with 4 min recovery or 10 sets of 60-s "almost all-out" with 2 min of rest after each interval. Participants were allowed to perform the sprints using any mode of exercise. The stretching routine incorporated traditional yoga poses with additional stretches and Pilates movements providing a total body stretching, flexibility and strengthening workout. All sessions were completed within 60 min and were led by a certified yoga instructor. Finally, endurance exercise training was performed for 60 min at a moderate pace (60% of maximal effort). Participants were allowed to choose from a variety of aerobic activities, including running, cycling, rowing, swimming, etc.

2.5. Laboratory testing procedures

All testing was performed between 0600 and 0900, following a 12-h fast and 48-h abstinence from caffeine and alcohol intake, and 48–72 h after the last exercise session to eliminate the acute effects of the last bout of exercise. These tests were performed at week 0 and 13.

2.6. Cardiovascular health

2.6.1. Heart rate and blood pressure

Resting heart rate and systolic and diastolic blood pressure (BP) were obtained in the supine position as previously described [2]. Heart rate and BP were obtained following a minimum of 10 min of quiet resting.

2.6.2. Vascular function

Vascular function was assessed using pulse contour analysis (augmentation index) and pulse wave velocity (Arteriograph, version 1.10.0.1, TensioMed Kft., Budapest, Hungary). Augmentation index was determined by the following formula:

$$AIx(\%) = (P2-P1)/PP \times 100$$

where P_1 is the early (direct) wave's amplitude; P_2 is the late (reflected) systolic wave's amplitude; and PP equals the pulse pressure.

The aortic pulse wave velocity (PWVao) was determined by the wave reflection generated from the early direct pulse wave as it is reflected back from the aortic bifurcation. Return time (RT) is determined by measuring the time interval between peaks from the early direct (P_1) and reflected late (P_2) systolic waves. The PWVao calculations were measured using the distance from the upper edge of the pubic bone to the sternal notch (Jugulum-Symphisis1/4), as this provides the closest approximation of the actual aortic length. PWVao was calculated with the following formula:

$$PWVao(m/s) = [Jug\text{-}Sy(m)]/[(RT/2)(s)]$$

where, RT is return time; Jug-Sy is the aortic distance (Jugulum-Symphisis).

2.7. Blood assays

2.7.1. Blood lipids and C-reactive protein

A 12-hour fasted venous blood sample (~20 ml) was obtained at baseline (week 0) and post-intervention (weeks 13). Blood was collected into EDTA-coated vacutainer tubes and centrifuged (Hettich Rotina 46R5) for 15 min at 2500 rpm at 4 °C. Upon separation, plasma was stored at −80 °C in aliquots until analyzed. Plasma C-reactive protein and insulin concentrations were determined using commercially available ELISA kits (Millipore, Billerica MA). Total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TRG) were assessed using the Cholestech LDX blood analysis system (Hayward, CA). Test-retest intraclass correlation (r) and coefficient of variation (CV) in our laboratory with n = 15 is: TC, and HDL-C (mg/dl) r = 0.95, CV = 3.2%, and r = 0.97, CV = 5.3%, respectively.

2.7.2. Growth hormone, BDNF, and IGF-1

Growth hormone (GH), brain derived neurotrophic factor (BDNF), and insulin-like growth factor-1 (IGF-1) concentrations were determined using commercially available ELISA kits (R&D Systems, Minneapolis, MN). The intra- and inter-assay coefficient of variation (CV) is 3.1, and 8.0%, respectively for GH. The intra- and inter-assay coefficient of variation (CV) is 5.0, and 9.0%, respectively for BDNF. The intra- and inter-assay coefficient of variation (CV) is 4.0, and 8.0%, respectively for IGF-1.The linearity was $r^2 = 0.997$, $r^2 = 1.000$, and $r^2 = 0.995$ for GH, BDNF, and IGF-1, respectively.

2.8. Resting metabolic rate (RMR)

Resting metabolic rate (RMR; kcal/min) was measured via indirect calorimetry at weeks 0 and 13 using the ventilated hood technique (ParvoMedic; analyzed via True One software, Salt Lake City UT). Participants arrived at the Human Nutrition and Metabolism Laboratory immediately upon waking (between 0600 and 0800). Following 20 min of quiet lying, REE was measured for 30 min while subjects lay supine in a darkened, temperature controlled room. Test-retest intraclass correlation (r) and coefficient of variation (CV) in our laboratory with n = 14 is: RMR r = 0.92, 4.2%, respectively.

2.9. Total and regional body composition

Anthropometric measurements were obtained at baseline and 13 weeks. Body weight was obtained during each visit with a standard digital scale. Height was measured without shoes using a stadiometer.

Waist circumferences were obtained in centimeters with a standard tape measure. Waist measurement was obtained at the area with the smallest circumference between the rib cage and the iliac crest. Body Composition was assessed by Dual Energy X-ray Absorptiometry (iDXA; Lunar iDXA; GE Healthcare, Madison, WI; analyzed using encore software version 13.6). Total body adiposity, % body fat, lean body mass, visceral adipose tissue (VAT), and regional abdominal adiposity were all analyzed from iDXA scans as previously described [2]. Test-retest intraclass correlation (r) and coefficient of variation (CV) for body composition analysis using iDXA in our laboratory with n = 12 is: LBM and FM r = 0.99, CV = 0.64%, and r = 0.98, CV = 2.2% respectively and for regional abdominal body composition analysis is: %fat r = 0.99, CV = 2.4%.

2.10. Dietary intake and feelings of hunger and satiety

Throughout the intervention, subjects maintained a daily food log that included all food and beverages consumed each day, including meal timing. To further verify compliance, food intake was analyzed from a representative 3-day period at weeks 0 and 12 using Food Processor SQL Edition (version 10.12.0, 2012; ESHA Research, Salem, OR) as previously described [2]. All dietary analyses were performed by the same technician. Visual analog scales (VAS) were administered at baseline and week 13 to evaluate the effects of the lifestyle interventions on hunger, satiation, and desire-to-eat [2].

2.11. Physical performance assessments

Physical performance outcomes were assessed at weeks 0 and 13. Following a familiarization session of all testing procedures, physical performance measures were obtained at the same time of day and completed over a two day period. For example, aerobic power (5 km TT), muscular endurance (sit-ups/push-ups), flexibility (sit and reach), and balance (standing stork balance) were completed on day one, whereas, upper and lower body strength (bench press/leg press) and power tests (squat jumps/bench throws) and vertical jump were completed on day two (see below).

2.11.1. Upper body muscular endurance

Upper body muscular endurance was assessed with timed push-ups in 1 min. Women started in the plank position balancing on the knees with arms extended and hands placed under the shoulders. A successful push-up was defined as lowering the body so elbows reached 90° followed by a return to the starting plank position. Participants were asked to perform as many push-ups as possible within 60 s in a continuous pattern with no more than 2 s of rest between repetitions.

2.11.2. Core muscular endurance

Timed sit-ups were performed in the supine position with arms folded across the chest, knees bent at 90° and feet flat on the ground and supported by a research team member. A successful sit-up required participants to curl up to a 90° position (vertical) to the floor and then return to the starting position. The sit-up action was continuous, with a rest duration of no >2 s allowed between repetitions. Participants were instructed to perform as many sit-ups as possible in 60 s.

2.11.3. Standing balance

Postural balance was assessed with the stork balance test. While in the standing position participants were instructed to balance on the dominant leg with the heel lifted off the ground and the non-dominant knee flexed to 90° with the foot placed gently against the inside of the dominant knee. Hands were placed on the hips at the level of the iliac crests. The trial ended when the heel of the dominant leg touched the floor, the hands came off of the hips, or the non-dominant foot was removed from the dominant standing leg. Participants were provided three attempts and the best time was recorded for analysis.

2.11.4. Flexibility

Lower back and hamstring flexibility were assessed with the sit and reach test. This was administered using a standard sit and reach box (Lafayette Instrument Company, Lafayette, IN), following standard technique. The maximal distance reached of 3 trials was recorded.

2.11.5. 5 km cycle ergometer time trial

Subjects arrived to the laboratory for performance testing sessions having consumed a standardized meal (PRISE, IsaLean bar; RISE, granola bar) 1 h prior. Before the time trial began, seat and handle bar length, height and tilt were adjusted according to each subject's preferences. Each adjustment was recorded and used for the post test (week 13). Following a 5–7 min warm-up at 60% of heart rate reserve (HRR) on the Velotron Dynafit Pro cycle ergometer (Racermate, CompuTrainer 3D Software, Version 1, Seattle, WA, USA) participants completed a 5 km time trial (5 km TT) as fast as possible. Pedaling cadence and gear ratio were selected freely by the participant during each ride (week 0 and 13). Subjects were permitted to drink water, if needed (ad libitum). Total time to complete the time trial, mean and max watts were all recorded. HR and blood pressure were monitored every 5 min during the time trial, immediately upon finishing, and 5 and 10 min after completion.

2.11.6. Upper and lower body maximal strength

Measures of one repetition maximal strength (1RM) of the upper and lower body were assessed via the bench (barbell) and leg press, respectively as previously described [20]. Test-retest intraclass correlation (r) and coefficient of variation (CV) in our laboratory with n = 15 is: chest 1-RM and leg 1-RM r = 0.99, CV = 1.6% and r = 0.99, CV = 2.7%, respectively.

2.11.7. Upper and lower body maximal force and power

Following 1RM's of the bench and leg press, dynamic maximal force and power of the upper and lower body were assessed with bench throws (BT) and jump squats (JS), respectively using the Ballistic Measurement System (Innervations Inc., Muncie, IN) interfaced with a commercial smith rack. Prior to performing the tests participants were provided instructions on how to perform the tests safely and with proper technique. During the familiarization process subjects performed 3–5 un-weighted practice trials for the BT and JS. For the JS, participants performed 3 consecutive repetitions with the barbell loaded to 30% of their predetermined IRM for the leg press. Participants began the JS in the standing position with feet slightly wider than hip width apart and the loaded barbell across the upper trapezius muscles. When instructed, they lowered into the squat position until 90° of knee flexion was achieved then jumped as high as possible and landed with bent knees. Immediately upon landing, without pause, participants repeated the same upward jumping movement for a total of three maximal JS's in succession.

For the bench throws (BT), participants followed identical familiarization procedures as the JS by performing 3–5 un-weighted practice trials lying supine on a bench with hands positioned on the barbell slightly wider than shoulder width apart and arms fully extended. The bar was then loaded with 20% of the 1 RM of the bench press. To initiate the BT's, subjects lowered the barbell to the chest just above the distal end of the sternum and were instructed to explosively push and then release the barbell with the intent to project the barbell as high as possible. Participants caught the bar on its descent and immediately, without pause, initiated another maximal BT until 3 successive repetitions were completed. Throughout both the JS and BT tests, spotters were present on both sides of the barbell to provide verbal encouragement and ensure safety of the participants. Peak power (watts) was taken as an average of the three repetitions.

2.12. Statistical analysis

Statistical analysis was performed using SPSS software (Ver. 23; IBM). A 2 × 2 repeated measures ANOVA was performed to assess for interactions between groups (RISE vs. PRISE) in response to training and to determine main effects of time (pre vs. post) and group. Post hoc comparisons (Tukey's HSD) were performed if there was an interaction with the addition of between-group independent samples *t*-tests at the pre and post time points. One-tailed tests were utilized for this study based upon our hypotheses and previous investigation showing improved body composition metrics following PRISE training [2] and the significance was set at $p < 0.05$. All values are reported as means ± SE unless stated otherwise.

3. Results

3.1. Participant characteristics

The participant characteristics are presented in Table 1. Prior to the intervention, all variables in each outcome domain (physical performance, cardiovascular health, body composition, diet, and metabolic profile) were not different between groups, with the exception of basal growth hormone levels which were lower in RISE. Any participants who were unavailable for post-testing or non-compliant to the diet and/or exercise routine were excluded from analysis, resulting in an adherence rate > 70% for both the nutrition and exercise components.

3.2. Muscular fitness and exercise performance

By design, each of the fitness and performance outcomes was improved following the interventions. Specifically, core (abdominal situps) and upper body muscular endurance (push-ups) were improved (training effect, $p < 0.01$, Fig. 2A, B) but no group differences were found (interaction, $p > 0.05$). Upper and lower body maximal strength, assessed via 1RM bench press and leg press, respectively, were significantly improved ($p < 0.01$, Fig. 2C, D), and upper body maximal strength was improved to a significantly greater extent in the PRISE group (interaction, $p < 0.01$, Fig. 2C). Likewise, upper (bench throws) and lower (squat jumps) body muscular power were significantly improved as a result of the training ($p < 0.05$, Fig. 2E, F); however, lower body power increased to a greater extent in the PRISE group (interaction, $p < 0.05$, Fig. 2F).

Flexibility, as assessed by the sit reach test, was significantly ($p < 0.05$) improved following the intervention (Fig. 2G), though the PRISE group increased flexibility to a greater extent (interaction, $p < 0.05$). Balance, assessed with the stork stand test, was unchanged following the intervention and no differences were found between groups (data not shown). Lastly, aerobic power, as assessed by time to complete a 5 km cycling time trial was significantly ($p < 0.05$) improved following the training, with the PRISE group exhibiting a greater improvement ($p < 0.05$) (Fig. 2H).

Table 1
Baseline subject characteristics (N = 20).

	RISE (n = 9)	PRISE (n = 11)
Age (year)	45 ± 9	45 ± 6
Height (cm)	179 ± 7	179 ± 10
Weight (kg)	81 ± 11	83 ± 14
Body mass index (kg/m^2)	25 ± 2	26 ± 3
Systolic blood pressure (mm Hg)	119 ± 5	119 ± 10
Diastolic blood pressure (mm Hg)	75 ± 6	75 ± 7
Pulse pressure (mm Hg)	44 ± 9	44 ± 9
Heart rate (beats/min)	54 ± 6	57 ± 6
Total cholesterol (mg/dl)	176 ± 18	173 ± 28
HDL cholesterol (mg/dl)	45 ± 10	55 ± 19
LDL cholesterol (mg/dl)	106 ± 15	106 ± 25
Triglycerides (mg/dl)	125 ± 37	82 ± 43
Glucose (mg/dl)	81 ± 6	83 ± 9

RISE, normal protein (5–6 meals/day @ 1.0 g/kg BW/day) and RISE training; PRISE, protein-pacing (5–6 meals/day @ 2.0 g/kg BW/day) and RISE training; HDL, high density lipoprotein; LDL, low density lipoprotein. Data are means ± standard deviation.

3.3. Cardiovascular health

Systolic blood pressure (RISE: 119 ± 2 vs. 112 ± 3 mm Hg; PRISE: 121 ± 3 vs. 109 ± 3 mm Hg, pre- vs. post-intervention, respectively) were significantly improved following the exercise intervention ($p < 0.05$), though no group differences were found ($p > 0.05$). However, diastolic blood pressures were unchanged in response to the training protocol (RISE: 75 ± 2 vs. 74 ± 2 mm Hg; PRISE: 75 ± 2 vs. 73 ± 2 mm Hg, pre- vs. post-intervention, respectively), and no differences were observed between groups ($p > 0.05$). Resting heart rate was significantly reduced by the exercise intervention (RISE: 54 ± 2 vs. 51 ± 2 beats/min; PRISE: 57 ± 2 vs. 55 ± 2 beats/min, pre- vs. post-intervention, respectively, training effect $p < 0.05$).

Augmentation index was significantly improved following the training ($p < 0.05$) in both the brachial artery (RISE: −6 ± 11 vs. −17 ± 8%; PRISE: −16 ± 10 vs. −20 ± 8%, pre- vs. post-intervention, respectively), and the aorta (RISE: 35 ± 6 vs. 29 ± 4%; PRISE: 30 ± 5 vs. 27 ± 4%, pre- vs. post-intervention, respectively), but no differences were found between groups (interaction, $p > 0.05$). Aortic pulse wave velocity and return time were not significantly impacted by the intervention in either group ($p > 0.05$). Assessment of circulating C-reactive protein, was unaffected by training in either group (RISE: 0.41 ± 0.29 vs. 0.70 ± 0.39 μg/ml; PRISE: 0.70 ± 0.37 vs. 0.38 ± 0.22 μg/ml, $p > 0.05$, pre- vs. post-intervention, respectively), and no differences were found between groups.

3.4. Body composition

Body composition was significantly improved in both groups following the training protocol, though no interactions were observed between groups. Independent of changes in body weight, significant improvements were observed in body composition (% body fat, Table 2). Specifically, significant reductions in total body, abdominal and hip fat were observed following the intervention in both groups (Table 2).

3.5. Diet, satiety, and hunger

At baseline, all participants met recommended daily intakes and were not different between groups (Table 3). By design, the PRISE group consumed significantly more protein in absolute (grams) and relative (grams/kg body weight) terms (interaction, $p < 0.05$). Both RISE and PRISE groups exhibited a reduction in the self-reported VAS question "How hungry do you feel right now?" (training effect, $p < 0.05$). All other dietary factors remained constant across the intervention and were similar between groups (Table 3).

3.6. Metabolic profile

The exercise training protocol had no effect on resting metabolic rate ($p > 0.05$), with no group effect (Table 4). However, RER and carbohydrate utilization (%) were reduced ($p < 0.05$) while fat utilization (%) was increased in response to training ($p < 0.05$), but no differences were found between groups. High density cholesterol levels were increased in both groups ($p < 0.05$) in response to training (Table 4), though no group differences were evident. Accordingly, the total cholesterol/HDL ratio was also significantly improved with training ($p < 0.05$), though again no group differences were found ($p > 0.05$).

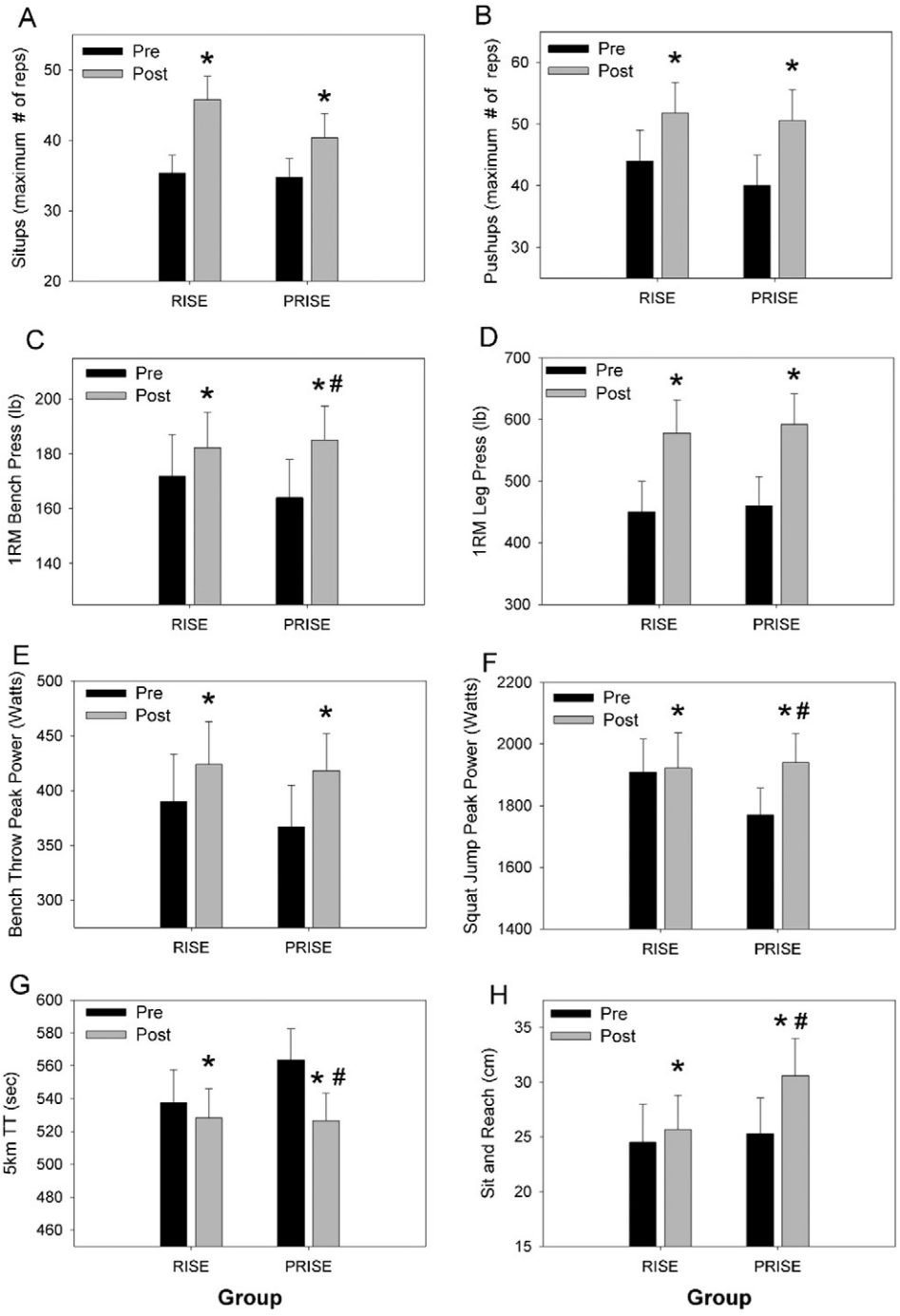

Fig. 2. Exercise performance parameters at baseline (pre) and following 12 weeks (post) between RISE and PRISE. Abdominal strength and endurance (Panel A); upper body strength and endurance (Panel B); upper and lower body maximal strength (Panels C and D); upper and lower body peak power (Panels E and F); endurance exercise (5 km cycling time trial) performance and lower body flexibility (Panels G and H). *$p < 0.05$ pre vs. post training, #$p < 0.05$ group difference in training response. RISE, normal protein + RISE training; PRISE, protein-pacing + RISE training. Mean ± SE.

3.7. Growth hormone, brain derived neurotrophic factor, and IGF-1

At baseline, the RISE group had significantly lower basal growth hormone (Fig. 3A), but basal levels of BDNF and IGF-1 were not different between groups (Fig. 3B and C). In terms of the GH response, the RISE group exhibited a significant increase, and the PRISE group had a tendency for increase basal GH levels ($p = 0.10$). BDNF levels tended to decline in response to the intervention, but did not approach significance ($p = 0.48$). In the RISE group IGF-1 exhibited a non-significant decline (126.9 ± 13.8 vs. 116.9 ± 9.3 ng/ml; $p > 0.05$), whereas, the PRISE group had a significant (98.6 ± 7.8 vs. 110.5 ± 8.3 ng/ml; $p < 0.05$) increase in IGF-1 (Fig. 3C), however, the interaction did not reach significance ($p = 0.09$).

4. Discussion

The aim of this study was to determine the effect of a 12 week multimodal RISE training program (resistance, interval, stretch and endurance) with a normal protein intake (RISE, 5–6 meals/day @ 1.0 g/kg BW/day) or in combination with a protein-pacing (P) diet (PRISE, 5–6

Table 2
Changes in body composition pre- and post-intervention.

		Pre	Post
Body weight (kg)	RISE	81 ± 11	79 ± 10
	PRISE	83 ± 14	83 ± 14
Body fat (%)	RISE	23.0 ± 6.0	22.1 ± 5.9[a]
	PRISE	23.8 ± 6.5	22.6 ± 6.4[a]
Fat mass (kg)	RISE	17.9 ± 5.6	17.2 ± 5.6[a]
	PRISE	19.8 ± 5.4	18.6 ± 8.0[a]
Fat free mass (kg)	RISE	62.0 ± 7.7	62.4 ± 7.6
	PRISE	63.6 ± 7.0	64.3 ± 7.0
Lean body mass (kg)	RISE	58.8 ± 7.4	59.2 ± 7.3
	PRISE	60.5 ± 6.6	61.1 ± 6.6
Abdominal fat (%)	RISE	26.8 ± 9.0	25.4 ± 10.0[a]
	PRISE	28.3 ± 12.5	26.3 ± 12.3[a]
Hip fat (%)	RISE	21.5 ± 5.6	20.2 ± 5.4[a]
	PRISE	23.8 ± 5.7	22.0 ± 5.3[a]

RISE, normal protein (5–6 meals/day @ 1.0 g/kg BW/day) and RISE training; PRISE, protein-pacing (5–6 meals/day @ 2.0 g/kg BW/day) and RISE training; data are means ± standard deviation.
[a] Denotes significant effect of intervention (pre vs. post).

meals/day @ 2.0 g/kg BW/day) on exercise performance, growth hormone (GH), insulin-like growth factor-1 (IGF-1), brain-derived neurotrophic factor (BDNF) responses, cardiovascular health, body composition, and metabolism in healthy active men. The main findings of the current study are that the RISE protocol: 1) elicited significant improvements in performance (5 km TT, upper and lower body maximal strength and peak power, flexibility), and several of these improvements were enhanced in the PRISE group, specifically upper body maximal strength, lower body muscular power, flexibility and aerobic power; 2) improved cardiovascular outcomes (systolic blood pressure as well as both aortic and brachial augmentation index), shifted substrate utilization to reduce carbohydrate and increase fat oxidation, improved body composition (%fat, total body, abdominal, and hip fat loss, and increased lean body mass) were improved with training, and

Table 3
Diet, satiety, and hunger ratings pre- and post-intervention.

		Pre	Post
Caloric intake (kcal/day)	RISE	1970 ± 430	1898 ± 294
	PRISE	2148 ± 435	2286 ± 213
Fat intake (g/day)	RISE	66 ± 25	59 ± 28
	PRISE	77 ± 39	76 ± 10
Carbohydrate intake (g/day)	RISE	206 ± 23	217 ± 19
	PRISE	230 ± 38	234 ± 33
Protein intake (g/day)	RISE	116 ± 59	90 ± 18
	PRISE	119 ± 36	182 ± 34[a]
Protein intake (g/kg BW/day)	RISE	1.5 ± 0.6	1.1 ± 0.1
	PRISE	1.4 ± 0.4	2.2 ± 0.7[a]
Cholesterol intake (mg/day)	RISE	406 ± 424	221 ± 64
	PRISE	463 ± 300	604 ± 237[a]
Sodium intake (mg/day)	RISE	2356 ± 917	2221 ± 1275
	PRISE	2608 ± 1494	2505 ± 403
Fiber intake (g/day)	RISE	24 ± 7	23 ± 10
	PRISE	33 ± 14	30 ± 7
How hungry are you feeling (0 − 100)	RISE	46 ± 21	32 ± 18[b]
	PRISE	47 ± 25	42 ± 27[b]
How full do you feel (0–100)	RISE	33 ± 18	42 ± 16
	PRISE	27 ± 23	24 ± 12
How much food could you eat (0–100)	RISE	55 ± 14	53 ± 8
	PRISE	53 ± 22	55 ± 29
What is your desire to eat (0–100)	RISE	46 ± 22	37 ± 20
	PRISE	46 ± 28	45 ± 28

RISE, normal protein (5–6 meals/day @ 1.0 g/kg BW/day) and RISE training; PRISE, protein-pacing (5–6 meals/day @ 2.0 g/kg BW/day) and RISE training; data are means ± standard deviation.
[a] Denotes significant interaction of group (RISE; 1 g/kg of body weight) vs. (PRISE; 2 g/kg of body weight).
[b] Denotes significant effect of intervention.

Table 4
Metabolic profile pre- and post-intervention.

		Pre	Post
Resting metabolic rate (kcal/day)	RISE	1776 ± 280	1804 ± 243
	PRISE	1843 ± 280	1864 ± 380
Respiratory exchange ratio	RISE	0.84 ± 0.07	0.81 ± 0.04[a]
	PRISE	0.83 ± 0.04	0.80 ± 0.03[a]
CHOox (%)	RISE	47 ± 24	38 ± 16[a]
	PRISE	43 ± 14	33 ± 11[a]
FATox (%)	RISE	53 ± 24	62 ± 16[a]
	PRISE	57 ± 14	67 ± 12[a]
Fasting blood glucose (mg/dl)	RISE	81 ± 6	81 ± 8
	PRISE	83 ± 9	86 ± 10
Insulin (uU/ml)	RISE	2.7 ± 0.5	2.5 ± 0.3
	PRISE	2.8 ± 0.7	2.9 ± 0.8
Total cholesterol (mg/dl)	RISE	176 ± 18	180 ± 27
	PRISE	173 ± 28	171 ± 27
HDL cholesterol (mg/dl)	RISE	45 ± 10	49 ± 12[a]
	PRISE	51 ± 15	53 ± 13[a]
LDL cholesterol (mg/dl)	RISE	106 ± 15	111 ± 22
	PRISE	114 ± 27	109 ± 24
Total cholesterol/HDL	RISE	4.0 ± 0.7	3.8 ± 0.8[a]
	PRISE	3.5 ± 1.0	3.3 ± 1.0[a]
Triglycerides (mg/dl)	RISE	125 ± 37	109 ± 42
	PRISE	92 ± 46	90 ± 60

RISE, normal protein (5–6 meals/day @ 1.0 g/kg BW/day) and RISE training; PRISE, protein-pacing (5–6 meals/day @ 2.0 g/kg BW/day) and RISE training; CHOox, relative contribution of carbohydrate to energy expenditure; FATox, relative contribution of fat to energy expenditure; HDL, high density lipoprotein; LDL, low density lipoprotein. Data are means ± standard deviation.
[a] Denotes significant effect of intervention (pre vs. post).

decreased sensations of hunger; however, there were no group differences in these responses; and 3) the RISE training increased GH levels, which was not as pronounced in the PRISE group, likely, in part, due to different baseline levels, while BDNF was unchanged in both groups, and IGF-1 was increased in the PRISE group only.

In summary, we provide novel insight into the impact of a multimodal (RISE) exercise training protocol which improves multiple aspects of performance (muscle strength, power, flexibility, and aerobic power) in healthy active males, which might be, in part, due to increased GH levels. These benefits can be augmented, through adding a protein-pacing dietary approach (5–6 meals/day @ 2.0 g/kg BW/day) which acts synergistically to enhance the increases in upper body muscle strength, lower body power, flexibility and aerobic power performance associated with multi-modal exercise training, which might occur independent of changes in circulating GH, IGF-1, or BDNF.

4.1. Fitness, and performance outcomes

Our previous investigation using the multimodal RISE training protocol in overweight/obese men and women, targeted and observed improvements in body composition and cardiometabolic risk reduction [2]. However, it remained unanswered whether RISE may enhance physical performance outcomes. To this aim, we recently determined the RISE protocol does, in fact, improve performance, in healthy exercise trained women [3]. Previous work on concurrent training, where strength and endurance training are combined, has revealed that either endurance capacity [21] or muscle strength [22], may be compromised, which might be due to competing signals for adaptation or perhaps the decreased emphasis on training for either or possibly due to overtraining. Herein the current study, we observed significant improvements in endurance performance (5 km TT), muscular strength (1RM), power (jump squat or bench throw), flexibility (sit and reach), and muscle endurance (maximum # of pushups and sit-ups), which is in agreement with our prior work [3]. Thus, in both men and women, we contend that multimodal training paradigms are not antithetical to fitness-related gains in performance and might reduce over-training or "burn out".

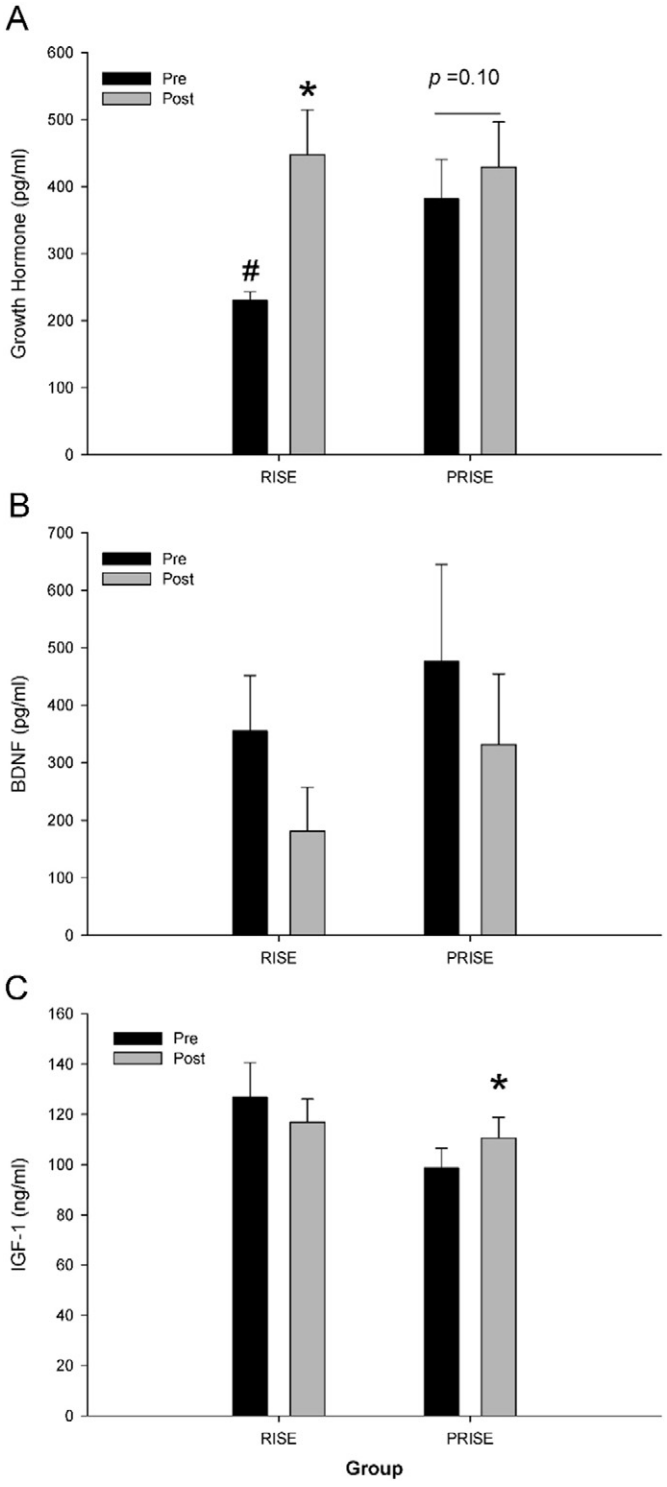

Fig. 3. Blood growth factor parameters at baseline (pre) and following 12 weeks (post) between RISE and PRISE. Growth hormone (Panel A); brain derived neurotrophic factor (BDNF) (Panel B), and insulin-like growth factor-1 (Panel C). *p < 0.05 pre vs. post training, #p < 0.05 group difference at baseline. RISE, normal protein + RISE training; PRISE, protein-pacing + RISE training. Mean ± SE.

Given the heightened emphasis on increasing dietary protein among athletes and the general population [23–25], it's important to evaluate its efficacy within the context of a comprehensive exercise training intervention. Indeed, our data suggests a P diet augments the training-induced improvement in performance outcomes [6,8,9]. Further, our protein intake per meal in the current study was 0.41 g/kg/meal which has been shown to optimally stimulate muscle protein synthesis [18] and may be partly responsible for the enhanced performance outcomes in the PRISE group. The finding of increased aerobic power (5 km TT) in PRISE men corroborates previous data showing a high dietary protein (whey) intake increases muscle strength and endurance, even during intense training [8].

4.2. Cardiovascular health

Acute ingestion of milk and/or whey proteins alone has been demonstrated to improve vascular health or factors contributing to CVD risk [26–28]. In the current study, we demonstrate that 12 weeks of a multi-mode training program targeting multiple aspects of fitness (muscular endurance, strength and power, flexibility, aerobic power, and balance), results in significant reductions in systolic blood pressure (Δ10 vs. Δ6 mm Hg, PRISE vs. RISE). Such changes are known to significantly reduce risk of coronary heart disease events and stroke, by approximately 25% [29]. Similarly, the observed improvement in augmentation index (AIx) also translates into a reduction in CV risk [30]. Taken together, these findings suggest that the multimodal RISE training improves vascular health, independent of protein intake.

Although previous investigations report resistance training may elevate vascular stiffness [31], the current study, in agreement with recent work in women [3], shows that a multimodal training protocol reduces peripheral and central augmentation index (along with reduced systolic blood pressure), suggestive of a training-induced reduction in peripheral resistance. This is particularly relevant in light of the growing emphasis, via guidelines and popularity, on multi-modal exercise paradigms and how each fitness component may influence vascular health.

4.3. Body composition

Similarly to our previous finding with exercise trained women [3], both groups experienced significant improvement in body composition, highlighting the independent effect of the RISE exercise protocol in stimulating fat loss and increasing lean body mass regardless of dietary protein intake. However, this finding also suggests that further body composition changes may be delayed compared to physical performance changes that respond most favorably to the higher protein per meal regimen in PRISE (Supplemental Table 1). It is plausible that a higher amount of protein may be needed to elicit enhanced body composition changes during PRISE training in exercise trained individuals. This contention is supported by others showing protein intakes as high as 3.4 g/kg body weight/day in combination with heavy resistance training induced additional reductions in fat mass [32]. Whether this level of protein intake is necessary beyond the current 2.0 g/kg BW/day warrants further investigation.

4.4. Satiation and hunger ratings and dietary intake

In the current study, self-reported feelings of hunger ("*how hungry are you feeling right now*?") were significantly lower following the 12 week intervention in both groups (Table 3). There is limited evidence supporting the hunger suppressing effects of exercise training [33]. Most of the available evidence showing a reduction in hunger ratings is due to increased dietary protein [34–36]. In the current study, protein intake was intentionally different between groups and matched the protein goals for each group (RISE, 1.0 g/kg BW/day; PRISE, 2.0 g/kg BW/day) (Table 3). Therefore, the likely explanation for a similar reduction in hunger ratings may be attributed to the RISE exercise intervention and not necessarily the protein intake.

4.5. Metabolic profile

Increased dietary protein intake, beyond the recommended daily intake, has been suggested to acutely [27] and/or chronically improve cardiometabolic profile [28]. In agreement, the PRISE protocol has been demonstrated to improve metabolic profile in overweight/obese [2], and recently in active women [3]. However, the magnitude and degree of protein pacing (g/kg BW/day) as well as the population studied (healthy vs. disease) likely play a role in whether PRISE alters metabolic profile, and the extent to which it is improved. The current study demonstrated a shift in resting substrate utilization, specifically RER and CHO oxidation (% of total energy expenditure, EE) were decreased, while fat oxidation (% of total EE) was increased (Table 4), which might have contributed to the reduction in total body, abdominal, and hip fat. Additionally, the increase in HDL cholesterol, and corresponding improvement in TC/HDL ratio (Table 4), is in agreement with previous researchers who have demonstrated that exercise training improves lipid profile in response to exercise training [37,38]. However, there may be a sex specific lipid profile response to the RISE training as women do not seem to exhibit any change in lipid profile [3].

4.6. Growth hormone, brain derived neurotrophic factor, and insulin-like growth factor-1

Resistance exercise is well recognized to stimulate release of growth factors, such as human growth hormone and insulin-like growth factors [39,40]. While less attention has been paid to the impact of endurance exercise on growth hormone release, previous researchers have documented acute increases in response to aerobic exercise [41,42], particularly repeated bouts [43], or exercise performed at an intensity at or above lactate threshold or of long duration [44,45], which when performed chronically might increase GH response [46]. Thus, in the current exercise paradigm, utilizing multiple modalities such as resistance, interval, and even the endurance components of RISE have the potential to increase GH release, which might help explain the body composition changes as well as changes in muscle performance. However, given the group differences at baseline, which might confound any group difference in the response to the training protocol (Fig. 3A), suggests that differences in circulating GH are unlikely to explain the group-related differences in performance.

Given the lineage and nomenclature of the anabolic factor, BDNF, perception often dictates BDNF plays a minimal role in musculoskeletal adaptations to exercise. However, relatively recent work has indicated that BDNF, plays a significant role in activation of satellite cells and growth of new muscle fibers [47], suggesting a role in muscle recovery. Other work has also suggested that endurance exercise increases expression of BDNF in muscle [48], but resistance exercise does not appear to increase circulating BDNF [49]. In the current study, we observed no significant change in basal circulating BDNF in response to training (Fig. 3B), which is perhaps unsurprising, given the BDNF response to training appears to depend upon exercise intensity, and is likely fiber type specific [50]. However, based upon the current study, group related differences in fitness do not appear to depend upon training-induced differences in BDNF.

In the current study, IGF-1 increased (12%) in the PRISE group only, whereas the RISE group had no significant change (−10%) (Fig. 3C). Thus, it is tempting to speculate that greater IGF-1 levels might have contributed to the greater increase in performance in those receiving protein-pacing (PRISE). Though, in contrast, recent work by Morton et al. [15] found that the acute response of circulating levels of growth factors did not correlate well with functional changes in muscle (i.e. strength) [15] following resistance training. Thus, other factors such as neurological development [16] or neuromuscular coordination also contribute to the training-induced adaptations. Alternatively, it is important to note that the temporal patterns of the growth factor signaling pathways are likely to peak well before the cessation of the training intervention. Additionally, the limitation of measuring circulating anabolic hormones must also be acknowledged, in that alterations in the receptor availability and/or post-receptor signaling could play a role independent of blood levels of GH, BDNF, or IGF-1. Finally, the additive effect of protein pacing with RISE training on fitness-related outcomes could be wholly independent of hormonal signaling and rely more upon a simple improvement in nitrogen balance.

5. Conclusion

In conclusion, the RISE training protocol improved multiple aspects of exercise performance, cardiovascular health, shifted metabolism towards increased fat utilization, and improved body composition. Inclusion of protein-pacing (P, 2.0 g/kg BW/day) in conjunction with RISE (PRISE) enhanced the training-induced adaptations in upper body strength and lower body muscle power, and aerobic power performance in exercise trained men. This study provides evidence that the RISE exercise training is capable of eliciting further adaptations in those currently performing exercise training, and that increasing dietary protein intake beyond the recommended daily intake may augment the training-induced adaptations to multimodal exercise training programs.

Competing interests

This study was funded by Isagenix International, LLC through an unrestricted research grant to Skidmore College and PJA. PJA received an honoraria for travel to present preliminary data from Isagenix International LLC. All authors have no financial interests regarding the outcomes of this investigation. All other authors declare no conflict of interests.

Author contributions

SJI prepared the manuscript, and assisted in data collection and analysis; VM, OM, JR, GO, DE, MP, CS, KC, KR, NR and FH assisted in data collection and analysis; PJA (senior corresponding author) assisted in the design of the study, subject recruitment, exercise training, data collection and analysis, manuscript preparation, obtained the grant, and served as the study PI. All authors read and approved the final manuscript.

Conflict of interest

The authors declare a potential conflict of interest and state it below.

Isagenix International is a multilevel marketing company that markets the products tested in this research study. Isagenix International funded this study to PJA. Isagenix International was provided a summary report of the initial findings as well as a summary report of the data contained in the current manuscript. However, Isagenix International did not have access to the data throughout the study. PJA is president and founder of PRISE LLC, a health and wellness consulting company not related to any Isagenix products used in this study. There are no patents, products in development or marketed products to declare. This does not alter our adherence to all the policies on sharing data and materials.

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Appendix A

Table A1
Sample menus from the RISE and PRISE nutritional intervention diet plans during the 12 week intervention. Menus were isocaloric and similar in meal timing.

	RISE (1.0 g/kg BW/day)	PRISE (2.0 g/kg BW/day)
Breakfast	Steel cut oats, eggs, honey, nut/seed butter, fruit, caffeine beverage, One-A-Day Multivitamins®; 15 g protein; 30 g carbohydrate; 15 g fat	Eggs/egg whites, blueberries, coconut butter/milk, e+® caffeine beverage, Ageless Essentials®; 25 g protein; 15 g carbohydrate; 15 g fat
Mid-morning snack	Nature Valley Protein Chewy Bars® 12 g protein, 14 g carbohydrate, 12 g fat	IsaPro®, fresh fruit, 30 g protein; 3 g carbohydrate; 1.5 g fat
Lunch	Whole grain pita, tuna/turkey/chicken, baked chips, fresh fruit; 20 g protein; 30 g carbohydrate; 15 g fat	IsaLean Pro®; 36 g protein; 21 g carbohydrate; 6 g fat
Mid-Afternoon snack	Nature Valley Sweet and Salty Nut Granola Bars®, Horizon Organic Milk®; 12 g protein, 42 g carbohydrate, 10 g fat	IsaLean Bars®, 1/2 cup of Greek yogurt or fruit 25 g protein; 30 g carbohydrate; 5 g fat
Dinner	Fish/poultry/beef, whole grain rice/pasta or legumes, fresh vegetables, dried fruit, olive oil, water; 20 g protein; 30 g carbohydrate; 15 g fat	Fish/poultry/beef, fresh vegetables, chopped nuts, dried fruit, olive oil, milk; 25 g protein; 20 g carbohydrate; 15 g fat
Evening snack	Fresh fruit, nuts; 2–3 g protein; 20 g carbohydrate; 9 g fat	Greek yogurt, fruit, Ionix Supreme®; 20 g protein; 20 g carbohydrate; 5 g fat;
Exercise days*	Gatorade G2®, electrolyte beverage	Replenish®, electrolyte beverage

RISE, protein based on 1.0 g/kg BW/day for an 80 kg man; PRISE, protein based on 2.0 g/kg BW/day for an 80 kg man. Meals were consumed ~3 h apart throughout the day.

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