Higher compared with lower dietary protein during an energy deficit combined with intense exercise promotes greater lean mass gain and fat mass loss: a randomized trial[1,2]

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ABSTRACT

Background: A dietary protein intake higher than the Recommended Dietary Allowance during an energy deficit helps to preserve lean body mass (LBM), particularly when combined with exercise.

Objective: The purpose of this study was to conduct a proof-of-principle trial to test whether manipulation of dietary protein intake during a marked energy deficit in addition to intense exercise training would affect changes in body composition.

Design: We used a single-blind, randomized, parallel-group prospective trial. During a 4-wk period, we provided hypoenergetic (~40% reduction compared with requirements) diets providing 33 ± 1 kcal/kg LBM to young men who were randomly assigned (n = 20/group) to consume either a lower-protein (1.2 g · kg^{-1} · d^{-1}) control diet (CON) or a higher-protein (2.4 g · kg^{-1} · d^{-1}) diet (PRO). All subjects performed resistance exercise training combined with high-intensity interval training for 6 d/wk. A 4-compartment model assessment of body composition was made pre- and postintervention.

Results: As a result of the intervention, LBM increased ($P < 0.05$) in the PRO group (1.2 ± 1.0 kg) and to a greater extent ($P < 0.05$) compared with the CON group (0.1 ± 1.0 kg). The PRO group had a greater loss of fat mass than did the CON group (PRO: −4.8 ± 1.6 kg; CON: −3.5 ± 1.4kg; $P < 0.05$). All measures of exercise performance improved similarly in the PRO and CON groups as a result of the intervention with no effect of protein supplementation. Changes in serum cortisol during the intervention were associated with changes in body fat (r = 0.39, P = 0.01) and LBM (r = −0.34, P = 0.03).

Conclusions: Our results showed that, during a marked energy deficit, consumption of a diet containing 2.4 g protein · kg^{-1} · d^{-1} was more effective than consumption of a diet containing 1.2 g protein · kg^{-1} · d^{-1} in promoting increases in LBM and losses of fat mass when combined with a high volume of resistance and anaerobic exercise. Changes in serum cortisol were associated with changes in body fat and LBM, but did not explain much variance in either measure. This trial was registered at clinicaltrials.gov as NCT01776359. *Am J Clin Nutr* 2016;103:738–46.

Keywords: athlete, dietary protein, leucine, skeletal muscle, resistance exercise, high-intensity interval training

INTRODUCTION

Hypoenergetic diet–induced weight loss results in ~20–30% of mass lost as lean body mass (LBM),[3] with the remaining mass lost from adipose tissue (1). Retention of LBM during weight loss may be important in maintaining physical performance while also preserving skeletal muscle. Strategies that attenuate the loss of LBM and even allow gains in LBM to occur during an energy deficit are of interest to athletes and for health in general. Consuming supplemental protein during resistance training (RT) can result in an increased accretion of LBM (2). Evidence from Areta et al. (3) showed that consuming 30 g protein after resistance exercise while in an energy deficit resulted in a greater stimulation of muscle protein synthesis (MPS) than did consumption of 15 g protein. Pasiakos et al. (4) reported that daily protein at twice the Recommended Dietary Allowance (RDA) for protein attenuated the loss of LBM during an energy deficit with both aerobic and resistance exercise. Other research suggests that ≥2 g protein · kg^{-1} · d^{-1} may be required to maintain LBM when an individual is in an energy deficit (5).

RT attenuates the loss of skeletal muscle mass during an energy deficit presumably by stimulating MPS (3, 4). Combining a higher protein intake with RT during caloric restriction would act synergistically on the rates of MPS, resulting in a greater ratio of fat to LBM lost during energy restriction (5, 6), which may be advantageous for physical performance. In addition, high-intensity interval training (HIT)/sprint interval training (SIT) during a hypoenergetic period may also aid in promoting LBM retention (7). HIT/SIT also results in rapid gains in aerobic fitness, as well as endurance capacity, thus contributing to physical performance outcomes (8, 9).

In subjects who were in energy balance (or mildly positive energy balance), exercise-induced changes in hormones such as testosterone,

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[3] Abbreviations used: CON, lower-protein (1.2 g · kg^{-1} · d^{-1}) control diet; HIT, high-intensity interval training; IGF-I, insulin-like growth factor I; LBM, lean body mass; MPS, muscle protein synthesis; PRO, higher-protein (2.4 g · kg^{-1} · d^{-1}) diet; RDA, recommended dietary allowance; RT, resistance training; SIT, sprint interval training; 1RM, 1-repetition maximum.

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growth hormone, cortisol, and/or insulin-like growth factor I (IGF-I) were not associated with changes in MPS (10, 11), muscle mass (12, 13), or strength (13). Nonetheless, there is still disagreement on whether changes in systemic hormones mediate exercise-induced changes (14). The role of hormones and their association with body composition under hypoenergetic conditions combined with high-intensity exercise has been less well studied; however, when under extreme energy deprivation combined with high energy expenditure, there have been associations observed between changes in hormones and body composition (15, 16).

Given the synergistic anabolic properties of RT and dietary protein, and potentially of HIT/SIT, we evaluated whether a higher-protein (2.4 g · kg^{-1} · d^{-1}) diet (PRO) or a lower-protein (1.2 g · kg^{-1} · d^{-1}) control diet (CON) during a marked energy deficit (40% reduction compared with requirements) would attenuate the loss or promote the gain of LBM while RT and HIT/SIT were performed. We hypothesized that, during an energy deficit of ∼40% compared with estimated energy requirements (33 ± 1 kcal · kg^{-1} LBM · d^{-1}) for 28 d, consumption of the PRO compared with the CON would allow for better maintenance and possibly augmentation of LBM, while reducing adipose tissue and enhancing physical function.

METHODS

Research participants

The trial was a single-blind, randomized, parallel prospective trial conducted between January 2013 and February 2014 (NCT01776359) at McMaster University. The trial protocol was approved by the Hamilton Integrated Research Ethics Board and complied with the standards as set out in the Canadian Tri-Council Policy statement on the use of human participants in research (http://www.pre.ethics.gc.ca/pdf/eng/tcps2/TCPS_2_FINAL_Web.pdf). Forty overweight [BMI (in kg/m^2) >25] young men (23 ± 2 y, 184 ± 8 cm, 97.4 ± 16 kg) (**Table 1**) were recruited via posters and newspaper advertisements from the local Hamilton community and volunteered to participate in the study after being informed of the procedures and potential risks involved in the investigation. All participants were recreationally active (i.e., played noncompetitive sports or engaged in some form of physical activity 1–2 times/wk); however, no participants were regularly performing resistance exercise nor were they regularly performing structured progressive aerobic or anaerobic training. Participants were assessed by medical screening questionnaires at baseline to exclude those with health conditions that might affect their response to the study protocol or compromise their safety. Participants gave informed written consent before the commencement of the study. Once consent was obtained, participants were randomly assigned (with the use of the random number generation of a code: http://www.randomization.com/) by the same investigator (SYO) to either the CON group, which consumed an energy-restricted diet with a 40 ± 3% reduction in energy intake compared with estimated requirements (33 ± 1 kcal · kg^{-1} LBM · d^{-1}; 15% protein, 50% carbohydrates, and 35% fat), with 1.2 g · kg^{-1} protein · d^{-1}, or the PRO group, which consumed an energy-restricted diet with a 40 ± 3% reduction in energy intake compared with estimated requirements (33 ± 1 kcal · kg^{-1} LBM · d^{-1}; 35% protein, 50% carbohydrates, and 15% fat), with 2.4 g · kg^{-1} protein · d^{-1}. Subject flow through the protocol is shown in **Figure 1**. Subjects' preintervention descriptive characteristics are shown in Table 1.

TABLE 1
Participants' characteristics before the intervention[1]

	PRO	CON
Age, y	23 ± 2	23 ± 2
Body mass, kg	100.1 ± 12.8	96.0 ± 14.6
Height, m	1.84 ± 0.06	1.84 ± 0.08
BMI, kg/m^2	29.7 ± 3.9	29.6 ± 2.7
Fat mass, kg	22.1 ± 7.3	22.8 ± 7.2
Body fat, %	23.6 ± 6.1	24.8 ± 6.3
LBM, kg	73.0 ± 6.8	69.2 ± 8.1

[1]Values are means ± SDs. n = 40 (20/group). See Methods for determination of LBM. CON, lower-protein (1.2 g · kg^{-1} · d^{-1}) control diet; LBM, lean body mass; PRO, higher-protein (2.4 g · kg^{-1} · d^{-1}) diet.

Experimental protocol

Participants reported to the laboratory and underwent familiarization for all exercises to be performed throughout the study period. On a separate day, participants underwent a progressive maximal aerobic capacity test ($\dot{V}O_{2max}$) on a cycle ergometer with the use of a ramp protocol as described previously (17). On a subsequent day, isometric maximal voluntary contraction of the knee extensors was completed with the use of a Biodex dynamometer as described below. Participants also performed a Wingate Anaerobic Test to determine peak anaerobic power (further description provided below). On a separate day, participants reported to the laboratory to measure voluntary isotonic strength as a 1-repetition maximum (1RM) for bench press and leg press with the use of established standard operating protocols (8, 18), which were strictly controlled and followed each time participants were tested by a single investigator (TML).

Participants were provided with a 3-d diet for weight maintenance (∼15–18% protein, 55–60% carbohydrate, and 20–25% fat) with energy requirements based on the Harris–Benedict equation, with the use of an activity factor estimated based on the subject's self-reported habitual daily activities. On day 3 of the maintenance diet, participants reported to the laboratory after a 10-h overnight fast for body composition–related measures described in detail below. A blood sample was also taken from an antecubital vein (see below for details).

Diet

Participants were provided with all meals and beverages to consume throughout the intervention period (with the exception of water and noncaloric drinks, which were ad libitum). Diets corresponded to an individually constructed energy-restricted meal plan. Participants were placed on a 3-d rotating diet with lunchtime and dinnertime meals provided as prepackaged frozen meals (Copper County Foods). Both groups received beverages containing whey protein to be consumed throughout the day, with one beverage being consumed immediately after training in the presence of the investigators on exercise days. The composition of the beverages is given in **Table 2**. Compliance with the nutritional intervention (i.e., consumption of all the provided study foods) was assessed by daily contact with participants, food consumption checklists, and daily weight monitoring, and was estimated to be 93%. Deviations from the diet were recorded and adjustments were made to the subjects' diets to ensure a consistent energy deficit. Compliance with the exercise

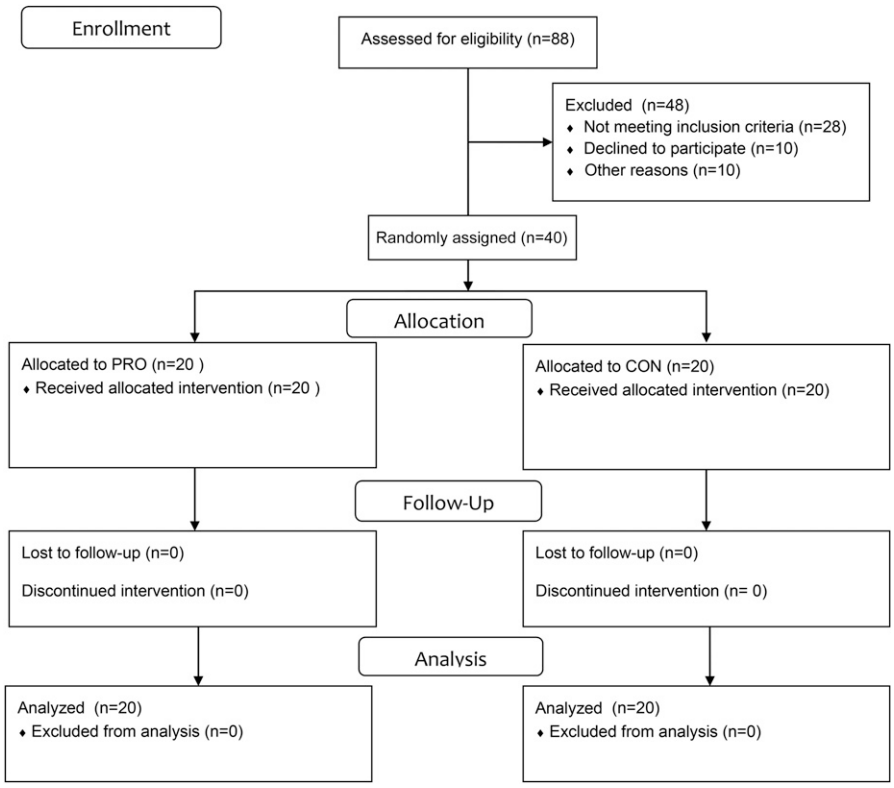

FIGURE 1 Subject recruitment and flow through the protocol. CON, lower-protein (1.2 g · kg^{-1} · d^{-1}) control diet; PRO, higher-protein (2.4 g · kg^{-1} · d^{-1}) diet.

protocol was >96% and did not differ between groups ($P = 0.89$). Dietary macronutrient breakdown and energy intake for both groups during the protocol can be found in **Table 3**.

Each participant received 3 or 4 dairy-based beverages/d (depending on their body weight) with ingredients dependent on their group assignment (Table 2). Specific meals containing higher or lower protein were consumed so that boluses of protein were spread out throughout the day. Both drinks were flavored identically, resulting in no perceptible taste differences in the drinks (based on a blinded taste test) between groups. Drink protein content was altered by adding Agropur IsoChill 9010 Instantized Whey Protein Isolate. Maltodextrin was added to each of the drinks to change their energy content, but also to keep the protein-to-carbohydrate ratios similar between groups. Blinding of the subjects to their dietary intervention group was accomplished through the subjects' assigned drinks (Table 2), which accounted for >90% of the macronutrient differences between the groups. Given that after study completion subjects guessed their nutritional assignment at rates no better than chance, we believe the blinding was reasonable.

TABLE 2
Dietary intake (including protein beverages) during the intervention[1]

	PRO	CON	*P*
Protein, g	245 ± 31	116 ± 19	<0.001
Protein, g/kg	2.4 ± 0.1	1.2 ± 0.1	<0.01
Protein, g/kg LBM	3.3 ± 0.1	1.7 ± 0.1	<0.001
Carbohydrate, g	311 ± 35	286 ± 35	0.21
Carbohydrate, g/kg	3.1 ± 0.3	3.0 ± 0.2	0.68
Fat, g	38 ± 6	86 ± 13	0.005
Fat, g/kg	0.4 ± 0.1	0.9 ± 0.1	0.012

[1]Values are means ± SDs. $n = 40$ (20/group). Comparison with the use of unpaired, 2-tailed Student's *t* test. Values were calculated with the use of preintervention body mass and LBM only. See Methods for determination of LBM. CON, lower-protein (1.2 g · kg^{-1} · d^{-1}) control diet; LBM, lean body mass; PRO, higher-protein (2.4 g · kg^{-1} · d^{-1}) diet.

Exercise training

Participants reported to the laboratory 6 d/wk for exercise training that consisted of the following: *1*) a full-body resistance exercise circuit, which was completed 2 times/wk with circuits (no rest between exercises). Circuits included 10 repetitions/set for 3 sets at 80% of 1RM, with the last set of each exercise to volitional failure, with 1 min of rest between sets; *2*) HIT/SIT, which took place 2 times/wk. Sessions consisted of one session of SIT (progressing from four to eight 30-s Wingate bouts) with a 4-min rest between bouts (protocol described in detail below), and a second session of modified HIT consisting of 10 bouts of an all-out sprint for 1 min at 90% of peak power (watts at $\dot{V}O_{2max}$), with 1-min rest intervals pedaling at 50 W; *3*) a weekly 250-kJ time trial on a cycle ergometer during which participants were instructed to complete the trial as quickly as possible while self-adjusting the ergometer resistance; and *4*) a plyometric body weight circuit with a 30-s rest between exercises.

To prevent sedentary activity at nonexercise times, all participants were provided with a hip-worn pedometer (AccuSTEP 400; ACCUSPLIT) and were instructed to accumulate at least 10,000 steps/d throughout the trial. Step counts were monitored on a daily basis and averaged 11,915 ± 2492 steps/d throughout the intervention, with no differences pre- to postintervention or

TABLE 3
Composition of study drinks[1]

	PRO	CON	*P*
Protein, g	49 ± 6	15 ± 4	<0.001
Carbohydrate, g	48 ± 7	41 ± 6	0.13
Fat, g	2 ± 0	12 ± 3	<0.01
Energy, kcal	372 ± 35	330 ± 56	0.02

[1]Values are means ± SDs. $n = 40$ (20/group). Comparison with the use of unpaired, 2-tailed Student's *t* test. CON, lower-protein (1.2 g · kg^{-1} · d^{-1}) control diet; PRO, higher-protein (2.4 g · kg^{-1} · d^{-1}) diet.

between groups. Subjects who reported >2 consecutive days of <10,000 steps/d were instructed to complete greater steps in the ensuing 2–3 d to ensure that their average number of daily steps was ≥10,000.

Body composition

Body composition was determined with the use of a 4-compartment model of body composition as described previously (19). Total body volume was determined with the use of air-displacement plethysmography (BodPod; Cosmed), total body water was determined with the use of bioelectrical impedance (Maltron Bio-Scan MPR 920-II; Maltron International), and bone mineral content was determined with the use of dual-energy X-ray absorptiometry (QDR 4500A, software version 12.31; Hologic). Calculations of body fat and LBM were made with an equation adapted from Lohman and Going (20). These measures were performed on the same day after a 10-h fast and were measured at the same time of day before and upon completion of the 28-d protocol. Subjects wore only light, form-fitting shorts for all body composition tests. Subjects were euhydrated (according to urine specific gravity) and abstained from physical activity for 48 h before their body composition testing to minimize variability. CVs for repeated measures on subsequent days were the following: BodPod, 1.2%; bioelectrical impendance, 1.9%; and dual-energy X-ray absorptiometry, 0.8%.

Strength and muscular performance

Isometric knee extensor torque was measured with the use of a Biodex dynamometer as described previously (18). Single best isotonic lift strength (1RM) testing was conducted in the exercise testing laboratory with the use of free weights and well-defined standard operating procedures. Participants were familiarized on a separate day with both the bench press and leg press exercises a minimum of 4 d before 1RM testing to reduce muscle soreness/fatigue that may have occurred as a result of the familiarization. 1RM was determined within 4 attempts with rest periods of 3–5 min between attempts.

Push-up and sit-up tests were conducted while following strict standard operating procedures. The maximum number of push-ups performed with correct form consecutively (without rest) was counted as the subjects' score (the same evaluator scored all participants). The sit-up tests were conducted (with the same evaluator, TML) so that participants performed as many sit-ups as possible with good form per protocol in 60 s.

Aerobic and anaerobic testing

Participants performed an incremental test to exhaustion on an electronically braked cycle ergometer (Excalibur Sport V2.0; Lode) to determine $\dot{V}O_{2max}$ with the use of an online gas collection system (Moxus modular oxygen uptake system; AEI technologies). On the test day, participants were instructed to warm up for 10 min on a cycle ergometer at a low resistance (70 W). Participants then completed the protocol as previously described (17), with verbal encouragement throughout the test. The measurement began with the participant cycling at a workload of 70 W with wattage increasing at 1 W/s thereafter.

A Wingate Anaerobic Test was performed on an electronically braked cycle ergometer (Wingate Velotron Racemate), as described (21), against a resistance equivalent to 0.075 kg/kg body mass. Peak and mean power were subsequently determined with the use of an online data acquisition system. During the 4-min recovery period between tests, subjects remained on the cycle ergometer and either rested or were permitted to cycle at a low cadence (50 rpm) against a light resistance (30 W) to reduce venous pooling in the lower extremities.

A time trial with the use of methods described previously (21) was completed on a day separate from all other testing before and weekly during the intervention, as well as after the intervention was completed. In brief, subjects were instructed to complete 250-kJ self-paced work laboratory time trials on an electronically braked cycle ergometer (Excalibur Sport V2.0; Lode) as quickly as possible with no temporal, verbal, or physiologic feedback. The only feedback provided during the time trials was work completed, which was presented as "distance covered" (e.g., 250 kJ was equated to 10 km such that visual feedback at any point during the time trial was presented in units of distance rather than work completed).

Blood sampling, hormonal measurements, and urinary measures

Blood was sampled by venipuncture from subjects after a 10-h overnight fast before the intervention and 48 h after the last training session at the end of the intervention. Blood was collected in evacuated tubes and allowed to clot for 15 min at room temperature before being centrifuged at 4°C for 15 min at 1500 × *g*. Serum was subsequently removed and stored at −80°C before analysis. Urine collections (24-h) were initiated after the first morning urinary void and collected into sterile urine jugs. Urine was stored at 4°C during collection and was returned to the laboratory the morning after the 24 collection period ended. Urine volume was measured and aliquots of urine (~1.5 mL each) were placed into tubes for storage at −20°C before analysis.

All analyses were carried out at the core clinical chemistry facilities at McMaster University Medical Centre with the use of the procedures used and described by our group previously (11, 13). In brief, serum samples were analyzed for cortisol, sex hormone binding globulin, total and free testosterone, growth hormone, ghrelin, and total and free IGF-I with the use of solid-phase, 2-site chemiluminescence immunometric assays (Immulite; Intermedico) or a 2-site immunoradiometric assay (Diagnostic Systems Laboratories). All intra- and interassay CVs for these hormones were <8%, with the exception of free testosterone, which was <11%. Blood urea nitrogen was measured with the

use of an automated assay system (Beckman Synchron LX20). Serum and urinary creatinine were measured with the use of an automated assay system (HumaStar 600), which is traceable to isotope dilution mass spectrometry. Intra- and interassay CVs for these metabolites were all <5%. Using the serum and urinary creatinine concentrations, we calculated creatinine clearance. Using serum creatinine, we calculated the estimated glomerular filtration rate with the use of the Chronic Kidney Disease Epidemiology Collaboration equation (22), with appropriate race- and age-specific adjustments.

Statistical analysis

Sample sizes were determined a priori powered on the primary outcome of lean mass loss (by dual-energy X-ray absorptiometry) to detect a differential lean mass loss of 1 kg, with $\alpha = 0.05$ and power at 90%, to require 16 subjects/group at a higher (2.4 g · kg^{-1} · d^{-1}) and lower (1.2 g · kg^{-1} · d^{-1}) protein intake in an equivalent energy deficit as used in the current protocol (based on pilot data collected in our laboratory). Data were assessed for normality with the use of a Kolmogorov–Smirnov test, and any non-normal data (glomerular filtration rate, testosterone, free testosterone, growth hormone, and cortisol) were corrected with the use of logarithmic transformation to ensure that kurtosis and skewedness were within normal bounds. Nonpaired pre- and postintervention data for groups were compared with the use of an unpaired Student's *t* test. All data were analyzed with the use of a 2-factor repeated measures ANCOVA with protein intake (between) and time (within) as the main variables. Covariates included age, height, weight, baseline body composition (when assessing body composition), and baseline performance measures (when assessing changes in performance). Significant interaction effects were analyzed with the use of Tukey's post hoc test to determine the location of pairwise differences within (time) and/or between (diet). Pearson's product-moment correlation coefficients were used to evaluate the relations between variables. Statistical significance was set at $\alpha \leq 0.05$. Analyses were performed with the use of SPSS (version 20.0.0). Data are presented as means ± SDs.

RESULTS

There was substantial weight loss in both groups from pre- to postintervention, but there were no differences in body weight loss between groups ($P > 0.8$) (**Figure 2**). During the intervention, LBM remained unchanged in the CON group (0.1 ± 1.0 kg; $P < 0.45$); however, LBM increased in the PRO group (1.2 ± 1.0 kg) compared with preintervention, and this increase was greater ($P < 0.05$) than in the CON group. Both PRO and CON groups showed a decrease in fat mass after the intervention ($P < 0.001$); however, fat mass losses were greater ($P < 0.05$) in the PRO group (−4.8 ± 1.6 kg) than in the CON group (−3.5 ± 1.4 kg) (Figure 2). Pre- and postintervention body composition means are shown in **Table 4**.

With the exception of isometric knee extension torque, strength increased in all exercises, as did measures of aerobic and anaerobic capacity and performance on sit-up and push-up tests (Table 4). There were no differences between groups for any performance-based variable.

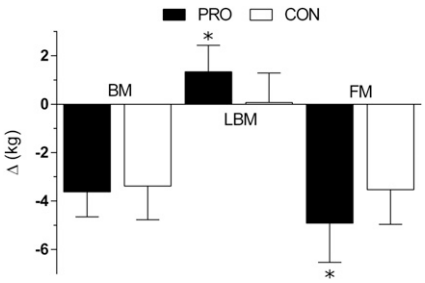

FIGURE 2 Four-compartment model-derived changes in BM, LBM, and FM during the intervention in both PRO and CON groups; data were analyzed with the use of an unpaired *t* test. Values are means ± SDs; $n = 40$ (20/group). *Significantly different from CON ($P < 0.05$). BM, body mass; CON, lower-protein (1.2 g · kg^{-1} · d^{-1}) control diet; FM, fat mass; LBM, lean body mass; PRO, higher-protein (2.4 g · kg^{-1} · d^{-1}) diet.

We observed a significant time-by-condition interaction for blood urea nitrogen, which increased in the PRO group ($P < 0.05$) and remained unchanged in the CON group (**Table 5**). Creatinine clearance remained unchanged as a result of the protocol in both groups (Table 4); however, the estimated glomerular filtration rate increased in the PRO group from pre- to postintervention, but remained unchanged in the CON group (Table 4).

Hormone and metabolite concentrations measured pre- and postintervention are shown in **Table 5**. We observed main effects for time for all hormones and no between-group differences. We performed correlational analyses between pre- and postintervention hormonal concentrations and body composition pre- and postintervention, or changes in body composition, and saw no significant relations (all $P > 0.35$) between any variables (data not shown). The correlations between the absolute changes in hormones, thought to be pertinent in determining body composition change (15, 16), are shown in **Figures 3** and **4**, as are the measured changes in fat mass (Figure 3) and LBM (Figure 4). As Figures 3 and 4 show, there was no correlation between changes in the concentration of any hormone other than cortisol, and changes in body fat or LBM. Pooling the data from the PRO and CON groups, we noted that the intervention-induced change in resting overnight-fasting cortisol was significantly correlated with the change in body fat ($r = 0.39$, $P = 0.01$) (Figure 4) and LBM ($r = -0.34$, $P = 0.03$) (Figure 5). Despite being statistically significant, the pooled changes in cortisol could explain only 16% and 11% of the variance in changes in fat mass and LBM, respectively.

DISCUSSION

We conducted a controlled feeding study in young overweight men with a protein intake that was close to habitual (CON), but still greater than the protein RDA, and at an amount 3 times the protein RDA (PRO). We included forms of exercise that would promote rapid gains in fitness and strength, as well as promotion of lean mass retention; however, we also implemented post-exercise provision of a predominantly whey protein supplement to augment lean mass preservation in the face of a marked (40%) energy deficit. The novel finding of the present study was that a higher protein–containing (2.4 g · kg^{-1} · d^{-1}) diet consumed during a period of marked energy deficit (~40% reduction in estimated energy requirement) during HIT resulted in an

TABLE 4
Participants' anthropometric, performance, and renal function variables before and after the intervention[1]

	PRO		CON	
	Pre	Post	Pre	Post
Body mass, kg	100.1 ± 12.8	94.2 ± 13.7*†	96 ± 14.6	92.5 ± 14.0*
Body fat, kg	23.6 ± 5.6	18.8 ± 6.2*†	24.8 ± 6.1	21.1 ± 6.1*
Lean mass, kg	73.1 ± 6.8	74.3 ± 6.7*†	69.2 ± 6.1	69.2 ± 6.1*
Leg press 1RM,[2] kg	171 ± 30	340 ± 77*	162 ± 30	318 ± 62*
Bench press 1RM,[2] kg	107 ± 29	146 ± 55*	99 ± 14	126 ± 36*
Isometric knee extension MVC, Nm	329 ± 59	336 ± 67	316 ± 47	328 ± 46
Push-ups,[3] count	29 ± 12	39 ± 10*	24 ± 10	31 ± 12*
Sit-ups,[3] count	36 ± 9	47 ± 8*	33 ± 12	44 ± 13*
Peak power,[4] W	1148 ± 130	1277 ± 133*	1095 ± 249	1237 ± 205*
Mean power,[4] W	768 ± 76	805 ± 76*	707 ± 83	743 ± 98*
Total work,[4] kJ	23.0 ± 2.3	24.1 ± 2.3*	20.9 ± 2.7	22.2 ± 3.0*
$\dot{V}O_{2max}$, mL · kg^{-1} · min^{-1}	41.1 ± 5.6	46.4 ± 8.4*	40.5 ± 4.9	47.4 ± 6.9*
Time trial performance,[5] min	19.15 ± 3.90	15.62 ± 2.88*	21.21 ± 3.71	17.15 ± 2.37
Creatinine clearance, mL/min	116 ± 8	121 ± 14	112 ± 11	115 ± 16
eGFR, mL · min^{-1} · 1.73 m^{-2}	109.8 ± 8.8	114.3 ± 11.1*	114.3 ± 10.9	116.8 ± 11.2

[1]Values are means ± SDs. n = 40 (20/group). All data were analyzed with the use of a 2-factor repeated measures ANCOVA with protein intake (between) and time (within) as the main variables. Covariates included age, height, weight, baseline body composition (when assessing changes in body composition), and baseline performance measures (when assessing changes in performance). No significant differences between groups at baseline. *Significantly different from Pre ($P < 0.05$); †significantly different from CON ($P < 0.05$). See Methods for details of all tests. CON, lower-protein (1.2 g · kg^{-1} · d^{-1}) control diet; eGFR, estimated glomerular filtration rate; MVC, maximal voluntary contraction force; Nm, Newton-meters; Post, postintervention; Pre, preintervention; PRO, higher-protein (2.4 g · kg^{-1} · d^{-1}) diet; 1RM, 1-repetition maximum.

[2]Maximal isotonic strength measured as single best weight lifted or 1RM.

[3]Maximum number of push-ups or sit-ups completed with form.

[4]Relevant performance variables from the Wingate test.

[5]Time trial to complete 250 kJ of work.

increase in LBM. In addition, we observed a greater loss (~1.3 kg) of fat mass in the PRO group than in the CON group. Although consumption of higher protein resulted in LBM accretion (~1.1 kg), it should be noted that LBM was unchanged during a period of high-intensity exercise training and substantial energy deficit even when the amount of protein consumed (1.2 g · kg^{-1} · d^{-1}) was lower. Despite differences in body composition changes between groups, and in contrast to our hypotheses, there were no differential responses in strength, performance, aerobic fitness, or anaerobic power between groups in response to the intervention.

Several studies have examined the impact of a higher protein intake and resistance exercise on retention of LBM during energy deficit (4–6, 23, 24). Pasiakos et al. (4) reduced the energy intake of young men by 30% from estimated requirements while they performed daily low-to-moderate–intensity (40–60% $\dot{V}O_{2max}$) treadmill and cycling as well as thrice weekly lower-intensity resistive-type exercise (3 sets of 15 repetitions). Contrary to our findings, these authors (4) reported that a higher protein diet (2.4 g · kg^{-1} · d^{-1}) still resulted in a loss of 1.2 ± 0.3 kg LBM as a result of the 21-d intervention. These authors (4) included groups that consumed protein at 3 levels—0.8, 1.6, and 2.4 g · kg^{-1} · d^{-1}—and reported a substantial retention of LBM for the 1.6 g · kg^{-1} · d^{-1} group, but not with consumption of 2.4 g · kg^{-1} · d^{-1} of protein. This finding is somewhat congruent with our observation that the 1.2 g · kg^{-1} · d^{-1} of dietary protein in the current study resulted in retention of LBM. We propose that the disparate findings of the previous study (4) and our findings may be due to the timing of our supplementation and the exercise intensity, which could be important in increasing or maintaining LBM while in a severe energy deficit (3). Previously, Mettler et al. (5) showed that during a 2-wk study, neither 1.0 nor 2.3 g · kg^{-1} · d^{-1} protein were sufficient to prevent LBM loss during a period of energy restriction similar to that which we used. We are unable to ascertain exactly why our data are different from those of the previous study (5); however, some possibilities include the fact that our intervention was longer (4 compared with 2 wk), our subjects received controlled diets, our subjects underwent individually supervised exercise sessions, and we used a 4-compartment model of body composition (considered to be of greater validity than simply dual-energy X-ray absorptiometry data) and had timed (postexercise) ingestion of protein drinks. It is also worth noting that our training program involved intense high-volume resistance exercise and HIT/SIT, which has not, to our knowledge, been studied in such a severe energy deficit previously.

In the current study, the loss of fat mass was the sole contributor to the participants' weight loss. Data from Trapp et al. (25) suggest that lipolysis increases over 20 min of HIT training gradually with each session. This research suggests that the high-intensity exercise our current participants were subjected to likely enhanced their capacity for fat oxidation and may have induced an increase in muscle mitochondrial enzyme activity (25). Evidence from the current trial suggests that high-quality weight loss (i.e., weight loss with a high fat:LBM ratio), is attainable during marked energy restriction with a higher intake of

TABLE 5
Fasting systemic blood hormone and metabolite concentrations before and after the intervention[1]

	PRO		CON	
	Pre	Post	Pre	Post
T_{total}, ng/dL	507 ± 23	126 ± 19*	586 ± 33	113 ± 18*
SHBG, nM	78 ± 13	108 ± 16*	88 ± 16	119 ± 14*
T_{free}, pg/mL	15.1 ± 2.8	6.7 ± 4.7*	17.8 ± 3.1	6.8 ± 5.1*
GH, ng/mL	8.2 ± 2.5	10.9 ± 3.7*	9.4 ± 2.8	12.8 ± 3.6*
IGF-I_{total}, ng/mL	328 ± 19	238 ± 28*	314 ± 18	276 ± 26*
IGF-I_{free}, ng/mL	3.8 ± 0.6	1.3 ± 0.5*	3.3 ± 0.6	1.3 ± 0.6*
Cortisol, nM	275 ± 21	452 ± 51*	303 ± 19	509 ± 47*
Ghrelin, pg/mL	495 ± 39	788 ± 92*	515 ± 46	833 ± 101*
Insulin, μIU/mL	12.2 ± 2.6	6.7 ± 3.2*	11.3 ± 2.9	5.9 ± 3.3*
BUN, mmol/L	5.6 ± 1.1	9.9 ± 2.2*$^{+}$	5.9 ± 1.0	6.1 ± 1.1

[1]Values are means ± SDs. n = 40 (20/group). All data were analyzed with the use of a 2-factor repeated measures ANCOVA with protein intake (between) and time (within) as the main variables. Covariates included age, height, weight, and baseline hormonal concentration. *Significantly different from Pre ($P < 0.01$); $^{+}$significantly different from CON ($P < 0.001$). BUN, blood urea nitrogen; CON, lower-protein (1.2 g · kg^{-1} · d^{-1}) control diet; GH, growth hormone; IGF-I_{free}, free insulin-like growth factor I; IGF-I_{total}, total insulin-like growth factor I; Post, postintervention; Pre, preintervention; PRO, higher-protein (2.4 g · kg^{-1} · d^{-1}) diet; SHBG, sex hormone–binding globulin; T_{free}, free testosterone; T_{total}, total testosterone.

dietary protein in overweight young men. We have previously reported a similar pattern of body composition change during a longer-duration intervention in overweight/obese premenopausal women (6). We propose that the lean mass–enhancing effect is one mediated by protein, as meta-analyses have shown (26, 27). In these same analyses, the authors noted an effect of protein in mediating a greater loss of fat mass (26, 27); however, in these studies, as in our study, the impact of changing other macronutrients needs to be recognized. We chose to lower fat intake in the PRO group and match carbohydrate intake between groups, knowing the impact that carbohydrate has on exercise performance (28). Thus, we acknowledge that a strict ascription of the phenotypic changes we observed to differential protein content of the diet per se is in light of the differing fat intake between the PRO and CON groups. Nonetheless, we are unaware of data that would suggest that a greater or lesser fat intake, at least of the magnitude seen here, would promote differential lean mass retention and/or differential fat loss when the energy deficit is identical.

Our data suggest that during a substantial energy deficit, higher protein consumption (2.4 g · kg^{-1} · d^{-1}) resulted in an increased stimulation of MPS and/or a suppression of proteolysis to a greater extent than consumption of 1.2 g · kg^{-1} · d^{-1}, as evidenced by gains in LBM in the PRO group. Current evidence suggests that the energy deficit likely reduces basal MPS (29) and may also have reduced the sensitivity of MPS to feeding (30, 31). Nonetheless, recent data have shown that lower rates of MPS can also be restored by a higher dietary protein intake (3), particularly so with whey protein (30), which was the supplemental protein source used herein. Data from our laboratory have shown that ~0.25 g protein · kg^{-1} per meal (or 0.4 g protein · kg^{-1} per meal as a safe intake amount) maximally stimulates MPS in young men when participants are in energy balance (32); however, it has not yet been established what protein dose would maximally stimulate MPS while in a period of energy deficit. The data from Areta et al. (3) do show that, while in an energy deficit, larger protein doses >0.25 g · kg^{-1} per meal continued to stimulate MPS after resistance exercise. In the current study, participants in the PRO group regularly consumed ~49 g protein/meal (~0.48 g · kg^{-1} per meal), resulting in repeated periods of maximally stimulated MPS compared with the CON group, which consumed ~22 g protein per meal (~0.23 g · kg^{-1} per meal). Importantly, the

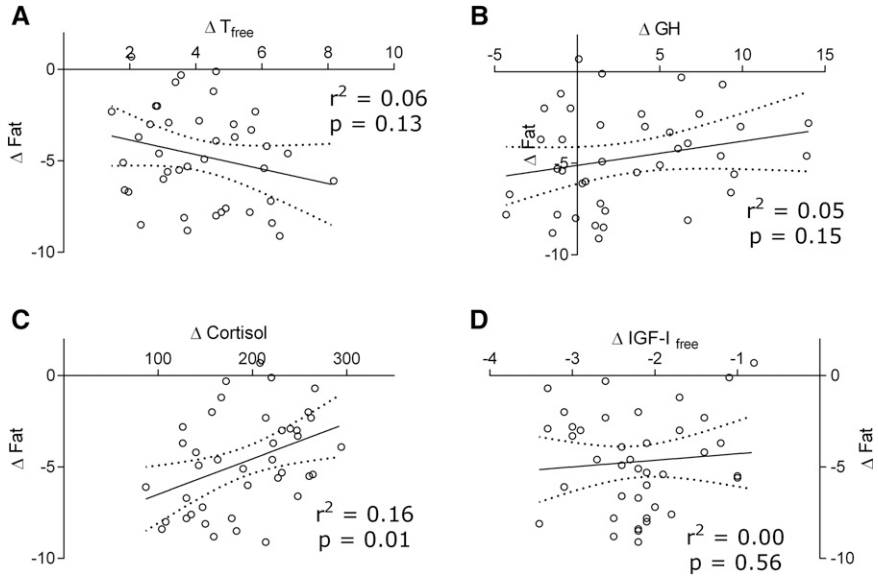

FIGURE 3 Linear relations between changes in resting fasting systemic serum hormone concentration and changes in fat mass. Changes in T_{free} and fat mass (A); changes in GH and fat mass (B); changes in cortisol and fat mass (C); and changes in IGF-I_{free} and fat mass (D). Values are individual per-subject data points; n = 40 (20/group). Solid lines indicate linear regression line of best fit ± 95% CIs (dashed lines). P values are from calculated Pearson correlation coefficients, and the proportion of variance explained is shown as r^2 values. GH, growth hormone; IGF-I_{free}, free insulin-like growth factor I; T_{free}, free testosterone.

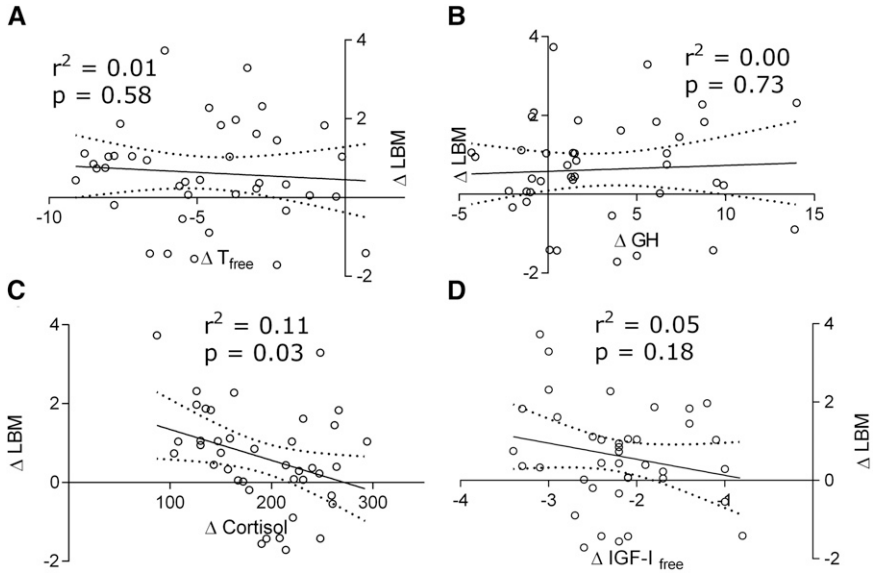

FIGURE 4 Linear relations between changes in resting fasting systemic serum hormone concentration and changes in LBM. Changes in T_{free} and lean mass (A); changes in GH and lean mass (B); changes in cortisol and lean mass (C); and changes in IGF-I_{free} and lean mass (D). Values are individual per-subject data points; $n = 40$ (20/group). Solid lines indicate linear regression line of best fit ± 95% CIs (dashed lines). *P* values are from calculated Pearson correlation coefficients, and the proportion of variance explained is shown as r^2 values. GH, growth hormone; IGF-I_{free}, free insulin-like growth factor I; LBM, lean body mass; T_{free}, free testosterone.

CON group also consumed enough protein combined with anabolic exercise throughout the intervention to retain muscle mass. We hypothesize, given our data, that protein dose per meal, protein quality, and timing of consumption relative to exercise would become more important in determining changes in LBM when in a caloric deficit because of decreases in basal rates of MPS and a reduced sensitivity of MPS to protein feeding (29–31).

Resting, overnight-fasting hormonal concentrations were made before and after the intervention (Table 5). We did not observe any interaction effects between conditions, but did observe main effects over time for all hormones (Table 5). In addition, we did not observe any independent correlations with hormones or any body composition or performance variables (data not shown). Our hormonal data align roughly with previous work in military personnel undergoing high levels of daily activity in an extreme energy deficit (15, 16). In the study described in these publications (15, 16), the degree of energy deficit was greater and protein intake was much lower than in the present study. These authors did not observe that increasing protein from 0.5 to 0.9 $g \cdot kg^{-1} \cdot d^{-1}$ aided in the retention of LBM (15, 16); however, we speculate that these levels of protein intake would not be adequate to spare LBM in such a severe energy deficit (33), so the lack of a protein-sparing effect (15, 16) is not surprising. We observed that 1.2 $g \cdot kg^{-1} \cdot d^{-1}$ ablated the usual decline in LBM seen during an energy deficit (1), and that 2.4 $g \cdot kg^{-1} \cdot d^{-1}$ allowed for an increase in LBM to occur. It is more than likely, however, that our results were due as much, if not more, to the addition of resistance exercise, which acts synergistically to stimulate MPS even in an energy deficit (3). The percentage change in free IGF-I was, in the previous work (16), shown to be correlated with the percentage change in fat mass; however, our results did not show a similar correlation (Figure 4). The only hormonal change we observed that was related to changes in body composition was the change in cortisol (Figures 4 and 5). In general, cortisol opposes fat loss and promotes loss of LBM during energy restriction (34), which is what we observed.

A potential limitation of the present trial is the free-living nature of the protocol. We did provide all food and beverages to the subjects and asked them to consume everything we provided and report any deviation from their prescribed diet. As an objective measure of compliance with the PRO diet, we measured serum urea and noted that it increased during the intervention and remained unchanged in the CON group. We had good compliance with exercise intervention and propose that subjects exerted high degrees of effort when requested. Because of the nature of the trial, it was impractical to maintain a double-blinded scheme, and yet we do not think this influenced the outcomes, because all analyses were done in a blinded manner and data were only unblinded after all analyses were complete. We opted in this trial to keep carbohydrate intake constant between the groups, given the crucial role that fuel plays in performance (28); thus, fat content (Table 2), in addition to protein, was different between the groups. Hence, given that there were differences in fat content, it cannot be stated conclusively that it was protein that was responsible for the effects we report here. However, in conducting a thorough search for manipulations in dietary fat content to the degree to which we changed it in our protocol, we could find nothing that would suggest that differing fat content would affect changes in LBM or fat mass. Thus, we propose that the effects were predominantly a protein-mediated effect.

In summary, the present study provides evidence that, in young men, consuming a higher protein diet (2.4 $g \cdot kg^{-1} \cdot d^{-1}$) during energy deficit (~40% reduction in energy intake compared with requirements) while performing intense resistance exercise training and HIT can augment LBM over a 28-d period. Furthermore, these high-intensity exercises performed during a period of energy deficit have the ability to preserve LBM despite a lower protein intake (1.2 $g \cdot kg^{-1} \cdot d^{-1}$). In conclusion, the current study provides direct evidence that a higher protein diet during substantial energy deficit and HIT not only preserves, but increases, LBM and

HIT during the energy deficit, irrespective of protein intake, and increases strength and performance in young men.

The authors' responsibilities were as follows—TML and SMP: designed the research (project conception, development of overall research plan, and study oversight), and had primary responsibility for the final content; TML, SYO, and SMP: analyzed data or performed statistical analysis; and all authors: conducted the research (hands-on conduct of the experiments and data collection), wrote and/or edited the manuscript, and read and approved the final manuscript. SMP has received research funding, travel allowances, and honoraria from the US National Dairy Council and Dairy Farmers of Canada. None of the other authors reported a conflict of interest related to the study.

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