Effect of protein supplementation during a 6-mo strength and conditioning program on insulin-like growth factor I and markers of bone turnover in young adults¹–³

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ABSTRACT

Background: Exercise is beneficial for bone when adequate nutrition is provided. The role of protein consumption in bone health, however, is controversial.

Objective: The objective was to ascertain the effect of high protein intake on insulin-like growth factor I (IGF-I) and markers of bone turnover during 6 mo of exercise training.

Design: Fifty-one subjects aged 18–25 y (28 men, 23 women) received a protein supplement (42 g protein, 24 g carbohydrate, 2 g fat) or a carbohydrate supplement (70 g carbohydrate) twice daily. Exercise consisted of alternating resistance training and running 5 times/wk. Plasma concentrations of IGF-I, insulin-like growth factor–binding protein 3, serum bone alkaline phosphatase, and urinary N-telopeptide collagen crosslink (NTx) concentrations were measured at 0, 3, and 6 mo after 24 h without exercise and a 12-h fast.

Results: Three-day diet records indicated no difference in energy intake between the groups. Average protein intakes after supplementation began in the protein and carbohydrate groups were 2.2 ± 0.1 and 1.1 ± 0.2 g/kg, respectively (P < 0.001). The increase in plasma IGF-I was greater in the protein group than in the carbohydrate group (time × supplement interaction, P = 0.01). There were no significant changes over time or significant differences by supplement in plasma insulin-like growth factor–binding protein 3 (44 and 40 kDa). Serum bone alkaline phosphatase increased significantly over time (P = 0.04) and tended to be higher in the protein group than in the carbohydrate group (P = 0.06). NTx concentrations changed over time (time and time squared; P < 0.01 for both) and were greater in the protein group than in the carbohydrate group (P = 0.04). Men had higher NTx concentrations than did women (74.6 ± 3.4 and 60.0 ± 3.8 nmol/mmol creatinine; P = 0.005).

Conclusion: Protein supplementation during a strength and conditioning program resulted in changes in IGF-I concentrations.

Key Words: Protein, bone alkaline phosphatase, collagen crosslinks, exercise, insulin-like growth factor–binding proteins

INTRODUCTION

Dietary protein has been suggested to have deleterious effects on bone. On the basis of the proposed role of bone in acid-base balance, theorists suggested that the greater acid load generated by high-protein diets may require neutralization by calcium salts of the bone (1). Over time, the expected result would be increased bone resorption and, ultimately, bone loss. This theory has been both supported (2) and refuted (3–7) in the literature in the past 10 y. However, despite the continued debate over the proposed action of protein in bone health, most research in humans implies that protein has a favorable, rather than a detrimental, effect on the skeleton (3, 5, 6, 8–10).

Although nutritional status is an important contributor to bone balance, exercise-induced mechanical loading also has a significant and positive effect on bone. In athletes, observed increases in bone stress (or, possibly, enhanced eccentric tendinous force applied to the bone) are thought to be responsible for the increased bone mineral density than is seen in athletes who do not apply weight-bearing stress (eg, swimmers) (11). Furthermore, in these populations, blood profiles have been used to identify the fluctuations in modeling that occurred at the skeletal sites (11, 12).

Insulin-like growth factor I (IGF-I) is an anabolic peptide that has been correlated with bone mineral density (13–16). Studies that examined the effects of exercise on IGF-I reported increases (16–18), decreases (19–21), or no change (19, 22–24) in plasma concentrations of IGF-I. Some reports of decreases in plasma or serum concentrations of IGF-I with exercise indicated the possibility that it was an energy deficit during the study duration, and not exercise per se, that was responsible for the decrease (19, 25). Energy or protein balance (or both) may influence the endocrine response to exercise by increasing growth hormone, which stimulates IGF-I release. Inadequate energy or protein may be a factor distinguishing between a normal or blunted response to growth hormone and its action on IGF-I (26, 27). Furthermore, variations in study duration and subject populations warrant more research. IGF-I is often found bound in a ternary complex with an acidic subunit and insulin-like growth factor–binding protein 3 (IGFBP-3), a transport protein that binds 95% of the circulating IGF-I. IGFBP-3 has been suggested to potentiate the actions of IGF-I and has been positively associated with bone mineral...
density, exercise, and nutritional status (4, 14, 22, 25, 26, 28). However, the effects of IGF-I on bone are often reported as being independent of this influential transport protein. Accordingly, the primary purpose of this study was to ascertain whether markers of bone formation and resorption in the blood and urine favor bone formation during 6 mo of exercise with or without a protein supplementation.

SUBJECTS AND METHODS

Subjects

Sixty-eight persons (33 men and 35 women) aged 18–25 y with no known history of diabetes; liver, kidney, or cardiovascular disease; thyroid or parathyroid disorder; or gastrointestinal disorder were enrolled to participate. All subjects were untrained (ie, they exercised < 2 times/wk) and were not taking any medications that would influence the study outcomes. Fifty-two persons completed the study. The 11 who did not complete the study either withdrew on their own or were asked to withdraw because of their noncompliance with the exercise or supplementation protocol (or both).

Written informed consent was obtained from all participants. The protocol was approved by the Human Subjects Committee at South Dakota State University, as well as the Human Subjects Review Board of the US Army Medical Research and Materiel Command.

Experimental design

Testing was performed at baseline (before intervention) and at 3 and 6 mo (after intervention). After a 12-h overnight fast, blood samples were obtained via venipuncture, and the second urine void of the morning was collected. Individual weight was measured by using a balance scale, and height was measured with a stadiometer. After the baseline measurements, study participants were randomly assigned to receive and consume a protein (n = 17 M, 12 F) or carbohydrate (n = 12 M, 11 F) supplement during the 6-mo exercise intervention.

Exercise testing and strength assessment

A one-repetition maximum (1-RM) exercise test was performed each month to determine maximal dynamic strength. 1-RM was defined as the maximal resistance against which the subject could move through the full range of motion for one repetition. The process usually took 3–4 attempts. The 1-RM was ascertained after 2 wk of light lifting so as to avoid muscle damage and soreness.

Cardiovascular assessment

At baseline and 3 and 6 mo, all subjects participated in a graded treadmill exercise test (chosen constant speed and 2% increase in grade every 2 min) to exhaustion. Expired gases were collected and analyzed (Max II; Physiodyne, Quogue, NY) to determine maximal aerobic capacity. Maximal exercise heart rates were monitored with heart rate monitors (Polar USA, Lake Success, NY).

Exercise training protocol

Subjects participated in a 5 times/wk supervised strength and conditioning program. Strength training and running were performed on alternate days so that, in 1 wk, subjects performed 3 d of strength training and 2 d of running, and, in the next week, they performed 3 d of running and 2 d of strength training. Endurance training consisted primarily of running at 70% of maximum heart rate. During each running session, the participant used a heart rate monitor (Polar USA) that was programmed for the subject’s heart rate range (70–80% maximum heart rate). The heart rate monitor kept track of time spent in and out of range. The watch emitted a tone when the subject was outside of his or her range. During the initial 3 wk of the training, the duration of running was increased progressively until subjects could exercise continuously for 45 min.

Strength training consisted of 2 sets of 10 repetitions and a final set to failure or fatigue, all at 70% of each subject’s 1-RM. If the subject was able to reach 12 repetitions during the last set, weight was added. The following exercises were performed: bench press, incline bench press, shoulder press, latisimus pull-down, cable rows, arm curls and extension, hip sled, squats, and calf raises. All exercises were performed on Magnum Fitness Systems plate-loaded machines (Magnum, South Milwaukee, WI).

Exercise compliance

Attendance was recorded at all exercise sessions. Subjects were allowed weekend makeup sessions if a session was missed during the week. Subjects missing >3 total sessions without making them up were dropped from the study. Participants were provided incentives to promote attendance. These incentives (eg, tee-shirts or water bottles) were awarded each month for good compliance. Subjects were also reimbursed monetarily for their time and effort.

Diet and dietary supplements

All subjects maintained their normal dietary intake during the study. Three-day food records were analyzed with FOOD PROCESSOR software (version 8.1; ESHA Research, Salem OR). Each serving of the protein supplement (Myoplex; EAS Inc, Golden, CO) contained 280 kcal, 42 g protein, 21 g carbohydrate, and 1.5 g fat, as well as a blend of vitamins and minerals. The carbohydrate supplement was an isocaloric supplement (70 g carbohydrate) with an equivalent vitamin and mineral blend. The supplements were consumed twice a day: one supplement dose was taken immediately after the participant’s workout under supervision of study personnel, and the other supplement dose was taken in the afternoon if the subject exercised in the morning or in the morning if the subject exercised in the afternoon or evening.

Blood and urine analysis

Formation marker

Serum bone alkaline phosphatase (BAP) concentrations were measured with the Alkphase-B assay (Metra Biosystems, Mountain View, CA). Intraassay and interassay precision CVs were 3.9–5.8% and 5.0–7.6%, respectively.

Resorption marker

The second urine void of the morning was obtained for the measurement of collagen X-link N-telopeptide (NTx). The NTx molecule is liberated from the bone by way of osteoclast-mediated bone breakdown, and it is found as a stable end product in the urine (29). Urinary NTx was measured with the Osteomark assay (OsteX International, Seattle, WA). NTx concentrations
were corrected for differences in urine concentration and output by dividing by creatinine (in mmol). Because of the possible effects of exercise, protein supplementation, or both on creatinine excretion, single-sample urine collections were obtained on each of the 3 testing dates. The final creatinine-corrected results are expressed as nanomoles of bone collagen equivalents per liter per millimole creatinine per liter (nmol bone collagen equivalents · mmol creatinine⁻¹ · L⁻¹). Of the diurnal variation of NTx excretion, the reference intervals are appropriate for use only with first or second morning urine samples collected before 1000. Intraassay and interassay precision CVs were 7.6% and 4.0%, respectively.

Concentrations of plasma IGF-I were measured in duplicate by radioimmunoassay (30, 31). IGF-I–binding proteins were extracted from serum with a 1:17 ratio of sample to acidified ethanol (87.5% absolute ethanol; 32). Reombinant human IGF-I (GF-050; Austral Biological, San Ramon, CA) was used as the radioiodinated antigen, and standard antisera to nant human IGF-I (GF-050; Austral Biological, San Ramon, CA). Each sample was divided by the same control to normalize each blot for comparison.

Statistical analysis
Mixed model repeated-measures analyses were performed with the subject, the random effect, nested within the supplement group (JMP IN software, version 5.1; SAS Institute Inc, Cary, NC). The supplement × time interaction was tested for significance, with time as a continuous variable. Sex was included in all analyses as a covariate. If the interaction term was not significant, the main effects of time, supplement group, and sex were tested for significance. Baseline values were included in models predicting BAP and NTx concentrations. Time was included as a polynomial term in the model predicting NTx. Data are mean (±SEM) unless stated otherwise.

RESULTS
Baseline characteristics are given in Table 1. Age and height at baseline did not differ significantly by group (age: 20.9 ± 2.4 and 21.1 ± 2.2 y in the protein and carbohydrate groups, respectively; height: 175 ± 8.1 and 176.9 ± 8.4 cm, respectively). Weight and BMI were significantly greater in the carbohydrate group than in the protein group (P = 0.004 and 0.02, respectively), and they increased significantly over the course of the study (P < 0.001 for both). Body-composition and strength changes are reported elsewhere (MD Vukovich, S Tausz, TLP Ballard, T Binkley, BL Specker, unpublished observations, 2004). Briefly, at 3 mo, the protein group had significantly greater increases in FFM and decreases in body fat than did the carbohydrate group. However, by 6 mo, these differences were no longer evident. Bench-press strength increased significantly more in the protein group than in the carbohydrate group (51% and 35%, respectively) from baseline to 6 mo.

Table 1: Subject characteristics of and dietary information on participants in protein and carbohydrate groups at baseline, 3 mo, and 6 mo of a strength and conditioning program

| Group     | Baseline | 3 Mo     | 6 Mo     | Significance
|-----------|----------|----------|----------|--------------
| Weight (kg) | 2004     | 2004     | 2004     | 2004         | 2004         |
| BMI (kg/m²) | 2004     | 2004     | 2004     | 2004         | 2004         |
| Carbohydrate + S (g) | 2004     | 2004     | 2004     | 2004         | 2004         |
| Fat (g) | 2004     | 2004     | 2004     | 2004         | 2004         |
| Protein + S (g) | 2004     | 2004     | 2004     | 2004         | 2004         |
| Calcium + S (mg) | 2004     | 2004     | 2004     | 2004         | 2004         |

1 Data were least-squares x ± SEM adjusted for sex, supplement use, and time by using mixed models. +S, plus intake from the supplement.
2 Significance for the main effects of group and time as determined without the interaction term if it was not significant.
course of the study, nor did it differ significantly by group. Carbohydrate intake increased significantly over the course of the study in the carbohydrate group, whereas protein intake increased significantly more in the protein group (Table 1). Average protein intake in the protein and carbohydrate groups was 2.2 ± 0.1 and 1.1 ± 0.1 g/kg, respectively. Both calcium and vitamin D intakes also increased significantly over the study period. However, the increase in vitamin D intake was significantly greater in the carbohydrate group than in the protein group (time × supplement interaction, \( P = 0.001 \)) because of the lower vitamin D intake at baseline in the former group.

There were no significant group differences in plasma IGF-I concentrations at baseline (protein group: 135.9 ± 7.5 ng/mL; carbohydrate group: 131.8 ± 8.6 ng/mL; \( P = 0.7 \)). Least-squares mean plasma IGF-I concentrations tended to be slightly higher in the men than in the women (142.0 ± 5.5 and 126.6 ± 6.1 ng/mL, respectively; \( P = 0.06 \)). Changes in plasma IGF-I concentrations over time differed according to the supplement group; there was a significantly greater increase in the protein group than in the carbohydrate group (time × supplement group interaction, \( P = 0.01 \)) (Figure 1).

Least-squares mean 44-kDa IGFBP3 concentrations were not significantly different between the protein and the carbohydrate groups throughout the study period (1.61 ± 0.1 and 1.52 ± 0.1 ng/mL, respectively; \( P = 0.5 \)) (Figure 1). Similar results were observed for 40-kDa IGFBP3 (Figure 1).

Baseline serum BAP concentrations did not differ significantly between the protein and carbohydrate groups (14.1 ± 1.2 and 11.6 ± 1.3 U/L, respectively; \( P = 0.13 \)). In the mixed-model analyses, serum BAP increased significantly over time (\( P = 0.04 \)), and BAP tended to be consistently higher in the protein group than in the carbohydrate group (\( P = 0.06 \), Figure 2).

NTx concentrations did not differ significantly at baseline between the protein and carbohydrate groups (68.8 ± 6.6 and 57.8 ± 7.3 nmol/mmol creatinine, respectively; \( P = 0.27 \)). In the mixed-model analyses, NTx changed significantly over time and time squared (\( P < 0.01 \) for both) and was significantly greater in the protein group than in the carbohydrate group (\( P = 0.04 \)) (Figure 2). Men also had significantly higher least-squares mean NTx concentrations than did women (74.6 ± 3.4 and 60.0 ± 3.8 nmol/mmol creatinine, respectively; \( P = 0.005 \)).

**DISCUSSION**

The current study is unique in that it is, to our knowledge, the first study to investigate the effect of protein supplementation in conjunction with strength and conditioning training on IGF-I, IGFBP-3, and biomarkers of bone turnover over a 6-mo period. The results of this study indicate that a protein supplement consumed during a strength and conditioning program led to an increase in plasma concentrations of IGF-I in those subjects compared with the concentrations in a group of persons who also trained but consumed a calorically equivalent carbohydrate supplement. Serum BAP concentrations increased over time and tended to be higher in the protein group than in the carbohydrate group, which indicated increased bone formation. Urinary NTx concentrations changed significantly with time and tended to be higher in the protein group than in the carbohydrate group. However, at 6 mo, NTx concentrations had decreased in both groups, whereas BAP continued to increase. It can be speculated that, if the duration of the study was increased, BAP may continue to
for either variable. The protein group had slightly (P = 0.06) higher BAP concentrations and significantly (P = 0.04) higher NTx concentrations than did the carbohydrate group. BAP concentrations increased with time (P = 0.04), and changes in NTx over time were significant (P < 0.01). There were no group differences in serum BAP or urinary NTx concentrations at baseline. Data for both variables were obtained from mixed-model analyses after control for baseline concentrations and sex.

These results are in agreement with some (16, 28) but not all (22, 35) previous reports including exercise training in their protocols that were designed to specifically measure changes in IGF-I. Research in adolescent males and females has consistently reported significant decreases in circulating IGF-I and its binding complexes with exercise (20, 36, 37). However, caution should be used when comparing endocrine responses in adolescent or prepubertal boys and girls, who are most likely in a rapid period of growth. Furthermore, despite the decreases in circulating IGF-I that have been observed in those populations with exercise, some authors reported anabolic activity such as increased muscle mass (36, 38, 39). Kraemer et al (22) reported no change in IGF-I in either young or older men after exercise training. However, IGFBP-3 concentrations increased significantly in the younger men after exercise training, but not in the older men. It should be noted that the training protocol in their study was only 3 times/wk for 10 wk, in contrast to our training protocol of 5 times/wk for 6 mo. It is possible that the frequency of exercise, the duration of the study, and the low subject numbers (n = 8 younger men, n = 9 older men) in the study of Kraemer et al were inadequate to detect any changes in IGF-I that may occur. It is interesting that Rosendal et al (35) reported decreases in IGF-I after 11 wk of intense physical training. However, their subjects were subjected to a training program that was more exhaustive than the program in the current study, and they did not include any nutritional information. This difference is important, because nutritional deprivation with or without exercise training has a negative influence on IGF-I concentrations (12, 19, 40, 41). It is well established that restriction of energy or protein (or both) can affect hormonal responses by reducing IGF-I gene expression and circulating IGF-I and IGFBP concentrations (19, 25, 26, 42). Furthermore, when nutritional deficiency is concurrent with enhanced stressors such as sleep deprivation and excessive physical demand, declines in IGF-I and IGFBP-3 result (25, 43). It should be noted, however, that most persons are not subject to intense training programs as described by Nindl et al (25) and Friedl et al (43), and even sedentary persons in the fasted state have been documented as having low IGF-I concentrations (41). In contrast, when a protein or carbohydrate supplement is added to the normal diet and consumed in conjunction with a resistance training program, the IGF-I concentration rises (44).

Insufficient dietary consumption also is reflected in bone markers and indexes of bone health. Specifically, endurance runners, who have a large training volume and tend to have inadequate nutrition, were reported to have low bone collagen synthesis and low bone mass (12, 45). In addition, in patients who are energy deficient, protein supplementation increased serum IGF-I concentrations (46). Moreover, patients who were recovering from hip fractures and received protein supplementation had higher serum concentrations of IGF-I, shorter hospital stays, and improved (ie, shorter) recovery times than did equivalent patients who did not receive protein (4). Thus, it is important to consider nutritional status when identifying changes in IGF-I and biomarkers of bone turnover.

It has been reported that dietary protein can adversely affect bone because of the acidogenic effects of protein (1, 2, 47). Although we did not measure urinary pH, we used the methods of Remer et al (48) to estimate the potential renal acid load (PRAL) of the supplements provided. In accordance with these methods, the PRAL of the protein supplement was between 1.0 and 8.0 mEq/100 g, whereas the PRAL of the carbohydrate supplement was approximately –0.1 mEq/100 g. Sources of protein such as processed and cheddar cheese have a PRAL of 28.7 and 8.0 mEq/100 g, respectively. Thus, the PRAL of our protein supplement, which consisted of milk proteins, remained in the lower 30% of the PRALs reported by Remer et al (48). Despite the higher renal acid load delivered by the protein supplement, the results presented in the current study, in combination with
those previously mentioned, refute the acidogenic hypothesis and suggest that protein intake may favorably affect bone. A recent report also stated that diets with protein at or below the current recommendation (0.8 g/kg) may be detrimental to bone because of impaired intestinal calcium absorption (49). It should be noted that a difference between previously published works and the current study is that subjects in the current study were taking a protein supplement daily for 6 mo, which is substantially longer than the schedule in much of the research available to date. To our knowledge, this is also the first study to examine both exercise and protein supplementation over such a long period. Furthermore, in our study population, it is useful to note that the carbohydrate group was not protein deficient and, in fact, was receiving adequate protein in their diet (1.0 g/kg), and that both groups were receiving adequate daily calcium, which has been suggested to work synergistically with dietary protein to negate the acidogenic effect of dietary protein (5, 7, 50).

Although the biomarkers used in the current study are consistent with an increase in bone formation, their data are not without limitation. Bone marker data are limited because they are not specific to the type of bone that may be affected. It is difficult to delineate the local site of resorption or formation and to ascertain whether the turnover reflected in the biomarkers is occurring at the same site. This also holds true for IGF-I because of its autocrine and paracrine effects. For instance, IGF-I is found in almost all tissues throughout the body, so the results for total IGF-I should be viewed with caution. Although we can allude to the anabolic potential of IGF-I on bone and muscle, without biopsy samples of the tissues of interest, we are forced to recognize underlying assumptions when interpreting the data.

It is worthwhile to consider that the IGF-I-induced anabolism at the muscle and the resulting increase in tendinous force on the bone over time (MD Vukovich, ST Tausz, TL Ballard, TBinkley, BL Specker, unpublished observations, 2004) may be mediating the relation between lean mass and bone. Finally, although the current study provided sufficient numbers to address the supplement groups, larger groups would be preferable to more clearly elucidate possible sex differences in response to exercise and protein intake.

In summary, protein supplementation during a 6-mo endurance and resistance training program resulted in changes in serum IGF-I concentrations. Because the exercise training and energy intake were similar in the 2 groups, we feel the observed results were due to the protein supplementation. Future studies should include larger numbers of subjects and a nonexercise group so that the interactions between protein intake and exercise can be examined in addition to the main effect for protein.

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