

The Impact of Varying Dietary Protein on Serum IGF-I, IGFBP-1, and IGFBP-3 During 6 Days of Physical Activity

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This study was designed to investigate the impact of dietary protein intake on serum concentrations of IGF-I and IGFBP-1 and relative amounts of serum IGFBP-3 during 6 d of physical activity. Ten men (23.8 ± 2.0 y of age) were assigned to 1 of 3 trials in a random crossover design. Each trial was isocaloric but with varying amounts of dietary protein: 50 g, 100 g, or 200 g. Subjects expended 500 kcal through treadmill running or weightlifting on alternate days for 6 d. Fasting blood samples were obtained for measurement of IGF-I, IGFBP-1, and IGFBP-3. Pre-post 24-h urine was measured for urea nitrogen. 50 g/d of protein resulted in a negative nitrogen balance, whereas 100 g/d and 200 g/d resulted in a positive nitrogen balance—200 g greater ($P < 0.05$) than 50 g and 100 g. Baseline IGF-I, BP-1, and BP-3 were not different among treatments. IGF-I decreased ($P = 0.002$) during the 6 d. Postintervention IGFBP-I was greater ($P = 0.03$) than at baseline. Postintervention IGFBP-3 values were not different from baseline or between trials. A 6-d modification of protein intake, while in energy balance, during a strength and conditioning program does not appear to modify serum concentrations of IGF-I or IGFBP-1 or relative amounts of IGFBP-3.

Key Words: exercise, protein intake, diet, growth factors, nitrogen

Insulin-like growth factor-I (IGF-I) is a protein hormone that mediates the effects of growth hormone and is reported to have numerous anabolic effects on skeletal muscles and other tissues (1, 2, 22, 27). Insulin-like growth-factor binding proteins (IGFBP) control the activity of IGF-I by regulating the amount of IGF available to bind to IGF receptors (23). Because IGF-I is anabolic by nature and mediates growth in many tissues, including muscle, it is of interest to the scientific community and to the general public to examine these hormones. Therefore, the responses of IGF-I and 2 of its 6 known binding proteins, IGFBP-1 and IGFBP-3, have received considerable attention in regard to the effect that exercise and nutrition have on them (4, 5, 6, 14).

Endurance and resistance exercise have shown varied effects on serum concentrations of IGF-I and IGFBP-3. After their 16-wk training intervention,

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Manetta et al. (22) reported an increase in serum concentrations of IGF-I (~12%) and IGFBP-3 (~20%) in trained cyclists but not in their sedentary counterparts. Similarly, Koziris et al. (17) demonstrated increased serum concentrations of total IGF-I of as much as 76% and of IGFBP-3 from 30% to 97% in college swimmers after 4 mo of endurance training. Likewise, both acute (5) and long-term (6) resistance training have been shown to increase serum IGF-I levels. Bermon et al. (5) studied 32 healthy elderly subjects and found a significant increase in serum IGF-I immediately (18%) and 6 h (7.5%) after strength training. In addition, Borst et al. (6) reported a 20% increase in serum concentrations of IGF-I after 13 and 25 wk of resistance training.

In contrast to the aforementioned studies, numerous researchers have been unable to show an increase in serum IGF-I and IGFBP-3 with either endurance (10, 26, 30) or resistance (6, 17, 18, 28, 31) training. These studies, however, varied with respect to subject demographics and the protocol used. Moreover, most exercise studies relating to IGF-I do not control for energy or macronutrient intake, and because energy intake is known to affect IGF-I and IGFBPs, it must be considered when discussing this hormone (37).

An adequate nutritional intake of calories and protein appears to be necessary to maintain normal serum concentrations or circulating levels of IGF-I and IGFBP (37). Research has shown that serum concentrations of IGF-I are diminished after fasting (8, 14, 16, 37), and a diet adequate in protein and carbohydrates is needed to return IGF-I levels back to prefasting values (14). Recently, we reported that the addition of a dietary protein supplement (84 g/d) to a diet already providing 1.0 g·kg⁻¹·d⁻¹ of protein during a 6-mo strength and conditioning program resulted in a significant increase in serum concentrations of IGF-I (4). Although a change in energy balance (primarily energy restriction) has a rather sudden effect on the IGF system, the effect of changes in protein balance has not been determined.

The purpose of this study was to determine the impact of varying amounts of protein intake on serum concentrations of IGF-I and IGFBP-1 and relative amounts of IGFBP-3 during 6 d of strictly monitored physical activity in healthy college-age men. Accordingly, the central hypothesis of this study was that a low protein intake (50 g/d of protein) would result in diminished serum concentrations of IGF-I, whereas moderate protein intake (100 g/d of protein) would not alter serum concentrations of IGF-I and high protein intake (200 g/d of protein) would increase serum concentrations of IGF-I.

Methods

Subjects

A preparticipation health-history questionnaire was used to screen all individuals for inclusion in this study. Volunteers consisted of 10 healthy men (21–28 y of age) who were physically active (exercised more than 3 d/wk for 45–60 min) and free of any liver, kidney, or metabolic disease. All subjects gave written consent to participate, and the protocol was approved by the human subjects committee at South Dakota State University. Subject data are presented in Table 1.

Table 1 Subject Characteristics, N = 10

Characteristic	Mean \pm SD
Age (y)	23.8 \pm 2.0
Height (cm)	181.7 \pm 7.3
Body weight (kg)	87.4 \pm 11.0
% Body fat	18.1 \pm 8.3
Fat mass (kg)	16.3 \pm 9.6
Fat-free mass (kg)	71.4 \pm 8.9
VO _{2peak} (mL·kg ⁻¹ ·min ⁻¹)	56.3 \pm 4.3
Resting metabolic rate (kcal/d)	2076.0 \pm 113

Experimental Protocol

Baseline testing included measuring resting energy expenditure, body composition via air-displacement plethysmography, maximal oxygen uptake (VO_{2max}), and 1-repetition-maximum (1-RM) lifts. All testing was completed in the morning (6:30 to 9:30 AM) after a 10- to 12-h fast. Subjects abstained from vigorous exercise and caffeine and alcohol intake during the 48 h before the initial visit.

Before each trial, study participants consumed an isocaloric diet (based on a dietary recall to ensure palatability) and the current daily recommended intake for protein (0.8 g·kg⁻¹·d⁻¹) for 3 d while abstaining from any vigorous physical activity or planned exercise. After 3 d of an isocaloric nutrient intake and 0.8 g protein·kg⁻¹·d⁻¹, subjects were randomly assigned to consume a low-protein diet (50 g protein per day), moderate-protein diet (100 g protein per day), or high-protein diet (200 g protein per day) for 1 wk in a crossover study design. Subjects ran on a treadmill at 70% of their VO_{2max} for approximately 30 min, the time needed to expend 500 kcal, and lifted weights in a circuit fashion to expend ~500 kcal on alternate days for 6 d. Each morning, after a 12-h overnight fast, during the 6-d intervention and on the first posttest day (Day 7), subjects reported to the human-performance laboratory to have blood samples taken from the antecubital vein. On the day immediately before starting the trial diet and on the last day of each protocol, subjects completed a 24-h urine collection. All trial periods were separated by a minimum of 5–7 d.

Trial 1: Subjects consumed 50 g of protein per day for 6 d (50 g) and an isocaloric diet (enough calories to account for resting energy expenditure, as well as daily activities and the calories expended during exercise).

Trial 2: Subjects consumed 100 g of protein per day for 6 d (100 g) and an isocaloric diet.

Trial 3: Subjects consumed 200 g of protein per day for 6 d (200 g) and an isocaloric diet.

Testing and Training Protocol

Resting energy expenditure was determined using indirect calorimetry (ParvoMedics, TrueOne 2400, Sandy, UT) by the method of Weir (39). Subjects were instructed to arrive at the laboratory in the morning after a 10- to 12-h fast and with minimal previous activity. They rested for 10 min before beginning the test, and then energy expenditure was measured for 20 min.

Estimated energy expenditure for daily activities was determined by an activity questionnaire according to method of Ainsworth et al. (3). By adding the resting energy expenditure, daily physical activity, and the predetermined caloric cost of the exercise conducted in this study (500 kcal), we determined the appropriate caloric intake needed for each subject.

Maximal oxygen consumption ($\text{VO}_{2\text{max}}$) was determined during a graded treadmill (Trackmaster, Pensacola, FL) exercise test using a metabolic cart (ParvoMedics, TrueOne 2400, Sandy, UT) so that exercise intensity during each training day would be established as a percentage of $\text{VO}_{2\text{max}}$. The test protocol began with a 3-min warm-up at 4.0 miles/h, 0% grade. This was followed by 2 min each at 5, 6, and 7 miles/h, 0% grade. The speed was held constant at 7 miles/h, and the grade was increased 2% every 2 min until the subject could no longer keep pace.

Body volume was measured via air-displacement plethysmography (Bod Pod Life Measurement Instruments, Concord, CA) using the procedure recommended by the manufacturer. Percentage body fat was calculated using the equation of Siri (32).

During the study, subjects ran on a treadmill (Trackmaster, Pensacola, FL) and weight-trained in the applied physiology laboratory's weight room for all 3 trials. All exercise was supervised by a certified strength and conditioning specialist (CSCS, National Strength and Conditioning Association). Running intensity corresponded to 70% of subjects' maximal oxygen consumption. The duration of exercise varied for each individual, but it took approximately 30–40 min for participants to expend 500 kcal. All individuals were accustomed to the exercise—only those who exercised on a treadmill more than 3 d per week for 30–60 min per session were recruited. Weight training corresponded to 60% of subjects' 1-RM, and 3 circuits were completed. One circuit was completed in the following order: leg press, seated calf raise, bench press, isolateral pull-down, squat, seated shoulder press, biceps preacher curl, triceps extension, inclined bench press, lat pull-down, seated cable row, and 3 abdominal exercises (1 set of 12–15 repetitions of each exercise; Magnum Fitness, South Milwaukee, WI). A pilot study was conducted to determine how many sets were required to expend 500 kcal (data not presented).

All individuals were supplied with a detailed dietary plan for each day of the 3 trials during this study. All diets were analyzed with Food Processor software (v. 8.1, ESHA Research, Salem OR) and consisted of an isocaloric diet with varying amounts of protein intake. Each diet plan provided 50 g of protein per day. Similar diet plans were provided during each of the 3 trials, except that additional protein for the 100-g/d and 200-g/d trials was provided by Myoplex CarbSense Ready-to-Drink shakes (EAS, Golden, CO). Each serving of the shake provided 150 kcal, 3.5 g fat, 5 g carbohydrate, and 25 g protein. Compliance was determined through daily contact, specific questioning of the participants by the research staff, and collection of daily food logs.

Serum and Urine Measurements

Blood samples were analyzed for serum concentrations of IGF-I and IGFBP-1 and for relative amounts of IGFBP-3. All blood samples were collected in Vacutainer collection tubes, allowed to clot, and centrifuged. Serum was aliquotted and immediately stored at -40°C until analysis. Serum concentrations of IGF-I were measured in duplicate by radioimmunoassay using the methods of Daughaday et al. (9). Recovery of $[125\text{I}]\text{IGF-I}$ added to human serum before acidified ethanol extraction was 89%. The IGF-I interassay coefficient of variation (CV) was 7.1%, and intra-assay CV was 4.8%. Assay sensitivity was 5.96 pg/tube. Serum concentrations of total IGFBP-1 were determined by enzyme-linked immunosorbent assay (ELISA; DSL-10-7800, DSL, Inc, Webster, TX). Assay sensitivity was 0.25 ng/mL, the interassay CV was 6.1%, and the intra-assay CV was 4.3%. Relative amounts of serum IGFBP-3 were analyzed by 1-dimensional SDS-PAGE and Western ligand blot analysis (13). Relative abundance of the 40- and 44-kDa forms of IGFBP-3 was determined by phosphorimagery (Bio Rad, Hercules, CA). Urine samples were measured for total volume, and an aliquot was frozen at -40°C until analysis for urinary urea nitrogen by the urease method (21, 29).

Calculations and Statistics

Nitrogen balance was calculated as described by Isley et al. (14). A 2-way repeated-measures analysis of variance with time and treatment as factors was used to determine the main effect of protein on serum concentrations of IGF-I and IGFBP-1, relative abundance of IGFBP-3, urinary urea nitrogen, nitrogen balance, and dietary variables. A Tukey post hoc test was used to identify significant differences when a significant *F*-ratio was obtained. Significance is reported at $P < 0.05$, and all values are reported as least-square means \pm SEM unless noted otherwise in tables or figures.

Results

Nutritional Information

During the 3 d before the beginning of the exercise program, subjects were prescribed an isocaloric diet with a daily protein content of $0.8 \text{ g}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$ (Table 2). Beginning with the first day of exercise, the energy intake of the diet remained isocaloric but increased by 500 kcal to account for the increase in energy expenditure for the exercise. Energy intake was not different among trials. Dietary protein intake was altered as described previously and was different among the 3 trials (Table 2).

Urinary Urea Nitrogen and Nitrogen Balance

Baseline urinary urea-nitrogen concentrations were not different between trials (pre: 50 g, 578.7 ± 128.6 ; 100 g, 581.0 ± 128.6 ; 200 g, 450.0 ± 128.6 mg/dL; Figure 1). A Time \times Treatment interaction ($P = 0.0004$) was found for urinary urea nitrogen, with postintervention levels for the 200-g trial being different from

Table 2 Nutritional Composition for Subjects Participating in Three 6-d Exercise Trials to Expend 500 Calories While Consuming an Energy-Balanced Diet With Varying Protein Intake

	Prediet	50-g Diet	100-g Diet	200-g Diet
Calories, kcal	2304 ± 288	2780 ± 314	2802 ± 291	2825 ± 301
Carbohydrate, g	420 ± 70	544 ± 59 ^a	504 ± 72 ^a	390 ± 61 ^a
% of kcal	72 ± 6	78 ± 2 ^a	72 ± 6 ^b	55 ± 4 ^c
Fat, g	43 ± 10	50 ± 8 ^a	53 ± 9 ^{ab}	61 ± 9 ^b
% of kcal	17 ± 4	7 ± 1	8 ± 1	8 ± 1
Protein, g	73 ± 10	57 ± 3 ^a	105 ± 3 ^b	199 ± 4 ^c
Protein, g/kg	0.8 ± 0.1	0.6 ± 0.1 ^a	1.2 ± 0.16 ^b	2.3 ± 0.3 ^c
% of kcal	12 ± 1	8 ± 1 ^a	15 ± 2 ^b	28 ± 3 ^c

Data are mean ± SD. Treatments with different superscript letters were significantly different ($P < 0.05$) from one another.

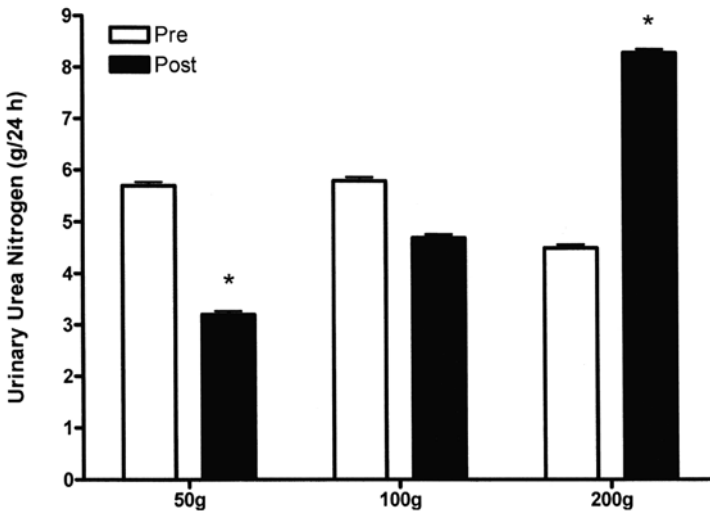


Figure 1 — Relationship between protein intake and 24-h urinary urea-nitrogen excretion measured before and on Day 6 of the exercise program. Values are least-squares mean ± SEM. * $P < 0.05$ compared with preintervention value of same trial.

the baseline (pre) 200-g value, as well as from postintervention values obtained during the 100-g and 50-g trials (post: 50 g, 322.1 ± 72.1; 100 g, 471.9 ± 72.9; 200 g, 825.9 ± 72.1 mg/dL).

Nitrogen balance was negative at baseline for all trials before the initiation of the exercise program and nutritional intervention, despite the subjects' consuming the recommended 0.8 g·kg⁻¹·d⁻¹ of protein (pre: 50 g, -4.3 ± 1.3; 100 g, -6.6 ± 1.3;

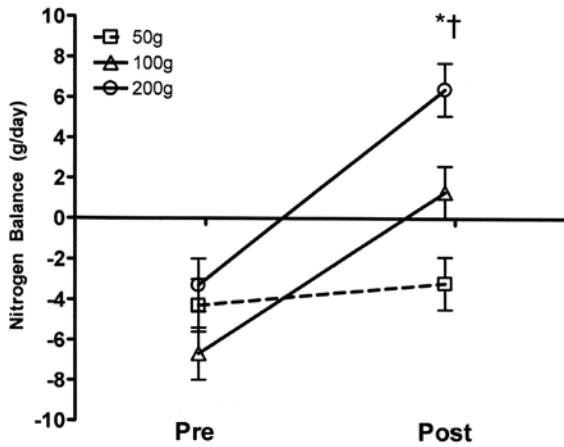


Figure 2 — Nitrogen balance during the 24 h before the initiation of and on Day 6 of the exercise program. Values are least-squares mean \pm SEM. * $P < 0.05$, different from preintervention for 100-g and 200-g trials. † $P < 0.05$, postintervention 200 g compared with 100 g and 50 g.

200 g, -3.3 ± 1.3 g/d; Figure 2). Nitrogen balance remained negative in the 50-g trial but significantly increased in the 100-g and 200-g trials (post: 50 g, -3.2 ± 1.3 ; 100 g, 1.3 ± 1.3 ; 200 g, 6.3 ± 1.3 g/d; Time \times Treatment $P = 0.007$). The postintervention measurement of nitrogen balance for the 200-g trial was significantly greater than the postintervention measurements for the 50-g and 100-g trials.

IGF-I System Response

Baseline serum concentrations of IGF-I were not different between treatments (pre: 50 g, 223.9 ± 16.9 ; 100 g, 210.4 ± 15.1 ; 200 g, 214.9 ± 15.9 ng/mL; Figure 3). There was a significant effect of time, with serum concentrations of IGF-I decreasing ($P = 0.002$) during the intervention (post: 50 g, 173.7 ± 15.9 ; 100 g, 185.9 ± 15.1 ; 200 g, 178.2 ± 15.9 ng/mL; Figure 3). There was, however, no time-by-treatment interaction. The effect of varying protein intake during the exercise did not have an effect on serum concentrations of IGF-I.

Serum concentrations of IGFBP-1 were not different among treatments at baseline (pre: 50 g, 17.5 ± 2.3 ; 100 g, 19.9 ± 2.4 ; 200 g, 18.8 ± 2.4 ng/mL; Figure 4[A]). The 6-d intervention did result in a significant effect of time ($P = 0.03$), with serum concentrations of IGFBP-1 being greater than baseline samples (post: 50 g, 24.4 ± 2.3 ; 100 g, 23.6 ± 2.4 ; 200 g, 21.8 ± 2.4 ng/mL). There was no difference between trials.

Because of the significant decline in serum IGF-I and increase in IGFBP-1 during the intervention, the ratio of IGF-I to IGFBP-1, an indirect measure of IGF-I availability, was analyzed to determine whether the intervention altered the availability of free IGF-I. There was, however, no effect of time or treatment on this variable.

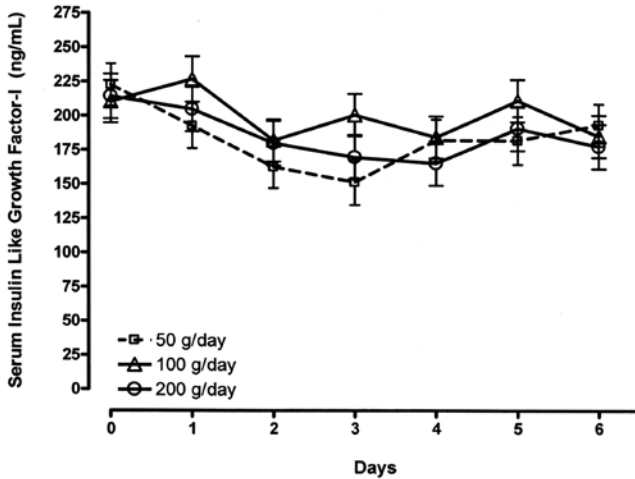


Figure 3 — Serum IGF-I concentrations (ng/mL) during 6 d of a strength and conditioning program while subjects consumed protein intakes of 50, 100, or 200 g/d. Values are least-squares mean \pm SEM.

Relative amounts of serum IGFBP-3 at baseline were not different between treatments for the 40-kDa IGFBP-3 isoform (pre: 50 g, 1.03 ± 0.07 ; 100 g, 0.88 ± 0.07 ; 200 g, 0.83 ± 0.07 arbitrary densitometric units [ADU]; Figure 4[B]) or for the 44-kDa IGFBP-3 isoform (pre: 50 g, 1.08 ± 0.06 ; 100 g, 1.08 ± 0.06 ; 200 g, 0.95 ± 0.06 ADU; Figure 4[C]). After 6 d of intervention, the relative amounts of IGFBP-3 were not different from baseline or between trials for the 40-kDa IGFBP-3 isoform (post: 50 g, 0.89 ± 0.07 ; 100 g, 0.86 ± 0.07 ; 200 g, 0.80 ± 0.07 ADU; Figure 4[B]) or for the 44-kDa IGFBP-3 isoform (post: 50 g, 1.08 ± 0.06 ; 100 g, 0.97 ± 0.06 ; 200 g, 0.92 ± 0.06 ADU; Figure 4[C]).

Discussion

An improvement in nitrogen balance has been reported to be associated with an increase in serum concentrations of IGF-I (14, 24). It has not been determined, however, whether changes in protein balance while in energy balance affect changes in IGF-I. For this reason we wanted to investigate the result of short-term alterations in protein intake during 6 d of physical activity, both of which would influence nitrogen balance and in theory affect the IGF system. The primary finding from this investigation was that varying the protein intake between 50 g/d and 200 g/d during 6 d of physical activity in trained individuals did not elicit changes in the IGF system. Although nitrogen balance was negative during the trial in which the subjects consumed only 50 g of protein per day and was positive during the trials in which they consumed 100 g and 200 g per day, the change from negative to positive nitrogen balance did not appear to affect serum concentrations of IGF-I or IGFBP-1 or relative amounts of IGFBP-3. This is in contrast to the results of

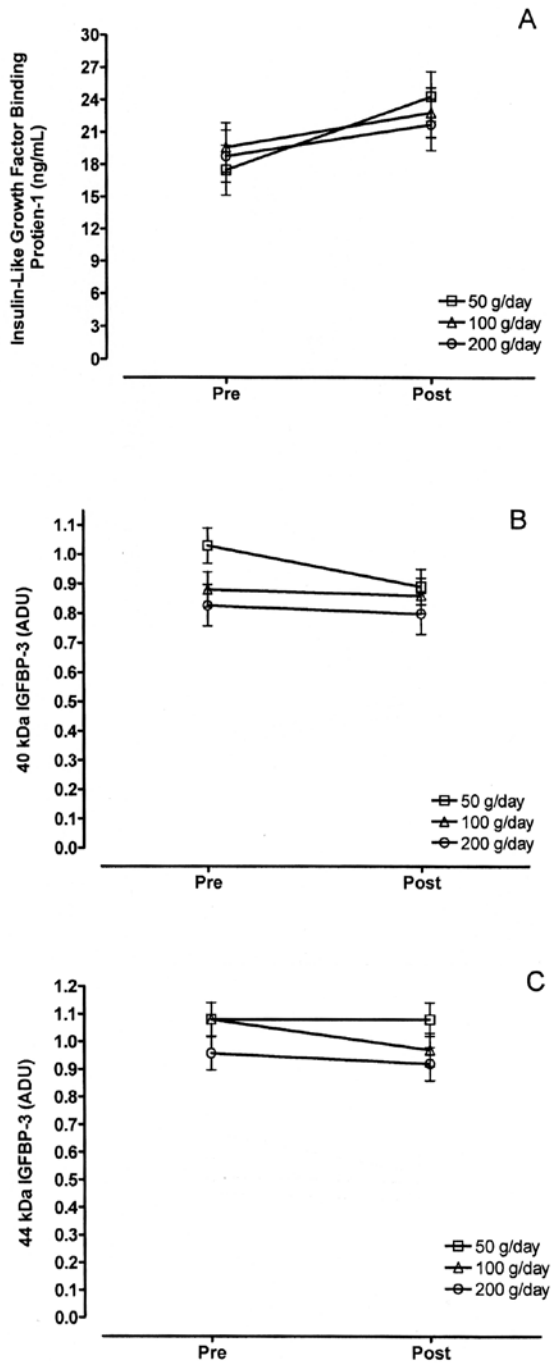


Figure 4 — Serum concentrations of IGFBP-1 (A) and relative abundance of IGFBP-3 (40 kDa and 44 kDa, [B] and [C], respectively) before and after a 6-d strength and conditioning program while subjects consumed protein intakes of 50, 100, or 200 g/d. Values are least-squares mean \pm SEM.

others who have reported that nitrogen balance was significantly related to serum IGF-I concentrations (11, 14, 24, 33). The reason for the discrepancy might be that our subjects were receiving adequate energy, whereas the other studies induced a negative energy balance through fasting or caloric restriction (11, 14, 24, 33).

IGF-I plays a considerable role in mediating a number of metabolic and anabolic processes in the cells (see Jones and Clemmons [15] for a review). The action of IGF-I is even more complex, considering that it is regulated by 6 binding proteins that either stimulate or inhibit IGF-I action (15, 37).

Both endurance and resistance training increase one's protein requirements (20, 35, 36). In the present study, the 3-d diet before the initiation of the exercise intervention did not provide enough protein for the subjects, as evidenced by the negative nitrogen balance during baseline (pre) data collection. This negative nitrogen balance occurred despite the intake of $0.8 \text{ g}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$ of protein and no exercise for 48 h. During the 50-g trial, subjects remained in negative nitrogen balance. During the 100-g and 200-g trials, however, subjects were in positive nitrogen balance during the sixth day (post). Dietary intakes of protein, as used in the present study, have been previously shown to result in positive nitrogen balances, in agreement with the present findings (35, 36).

Despite the differences in nitrogen balance among the 3 trials, serum concentrations of IGF-I and IGFBP-1, as well as relative abundance of IGFBP-3, were unaffected by the 3 dietary treatments in the present study. Serum concentrations of IGF-I exhibited a similar decline in all 3 trials, $\sim 10\%$, despite the positive nitrogen balance observed in the 100-g and 200-g trials. Smith et al. (34) demonstrated that protein restriction ($0.66 \text{ g}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$) in sedentary adults in an amount similar to our 50-g trial ($0.6 \text{ g}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$) also resulted in an $\sim 10\%$ decline in plasma concentrations of IGF-I.

Whereas the 6 d of physical activity used in the present study were enough of a stress to affect protein metabolism, the additional protein was unable to influence serum concentrations of IGF-I and IGFBP-1 or relative abundance of IGFBP-3. The reason for the decline in serum concentrations of IGF-I might be an underestimation of the subjects' energy requirements. Although care was taken to determine the energy requirements of daily activities, it is possible that the participants underreported daily activities, leading to a negative energy balance, which has been shown to reduce serum concentrations of IGF-I (14, 25, 33, 34).

An alternative explanation for our results is that a combination of protein and energy (specifically carbohydrate) must be provided in surplus in order to elicit an increase in serum concentrations of IGF-I (37) during exercise training. Research from Forbes et al. (12) and Kraemer et al. (18) supports our conjecture. Forbes et al. (12) observed an increase in plasma IGF-I concentrations while overfeeding subjects 1200 to 1600 kcal/d. Kraemer et al. (18) reported an increase in serum concentrations of IGF-I during 3 d of resistance training when subjects consumed an additional 1800 kcal and 150 g of protein per day. Our subjects were consuming an isocaloric diet, with the increase in dietary protein being offset by a decrease in dietary carbohydrate. Thus, the subjects might not have been consuming enough carbohydrate to support the elevated protein intake.

Despite the lack of an effect of protein on serum concentrations of IGF-I and IGFBP-1, as well as relative abundance of IGFBP-3, during the 6 d of intervention, there is concern that protein restriction results in an attenuation of the biological

actions of IGF-I (8, 19). Thissen et al. (38) reported that the administration of recombinant human IGF-I to protein-restricted rats did not restore growth rates in rats despite the normalization of serum concentrations of IGF-I. Bourrin et al. (7) concur in reporting the failure of recombinant human IGF-I to restore bone formation in protein-restricted rats. Although it is possible that resistance to IGF-I occurs during suboptimal protein intake, we are unable to determine whether IGF-I resistance occurred in the present study.

In conclusion, the results of the present study indicate that short-term (6-d) alterations in protein intake might not affect serum concentrations of IGF-I or IGFBP-1 or relative abundance of IGFBP-3. The reasons for this could be insufficient energy intake to match the increased protein intake or an underreporting of daily physical activity by the participants, leading to a negative energy balance.

Acknowledgments

Michael Ormsbee was supported by a grant from the Department of Defense, DAMD1701-1-0814. This project was also supported, in part, by a grant from EAS, Inc.

References

1. Adams, G.R. Exercise effects on muscle insulin signaling and action invited review: autocrine/paracrine IGF-I and skeletal muscle adaptation. *J. Appl. Physiol.* 93:159-1167, 2002.
2. Adams, G.R., and F. Haddad. The relationship among IGF-I, DNA content, and protein accumulation during skeletal muscle hypertrophy. *J. Appl. Physiol.* 81:2509-2516, 1996.
3. Ainsworth, B.F., W.L. Haskell, M.C. Whitt, A. Swartz, S.J. Strath, W.L. O'Brien, D. Bassett, K.H. Schmit, P. Emplaincourt, D.R. Jacobs, and A.S. Leon. Compendium of physical activities: an update of activity codes and MET intensities. *Med. Sci. Sports Exerc.* 32:S498-S516, 2000.
4. Ballard, T.L.P., J.A. Clapper, B.L. Specker, T.L. Binkley, and M.D. Vukovich. Effect of protein supplementation during a 6-month strength and conditioning program on IGF-I and markers of bone turnover in young adults. *Am. J. Clin. Nutr.* 88:1442-1448, 2005.
5. Borst, S., P. Ferrari, P. Bernard, S. Altare, and C. Dolisi. Responses of total and free insulin-like growth factor-I and insulin-like growth factor binding protein-3 after resistance exercise and training in elderly subjects. *Acta Physiol. Scand.* 165:51-56, 1999.
6. Borst, S.E., D.V. De Hoyos, L. Garzarella, K. Vincent, B.H. Pollock, D.T. Lowenthal, and M.L. Pollock. Effects of resistance training on insulin-like growth factor-I and IGF binding proteins. *Med. Sci. Sports Exerc.* 33:648-653, 2001.
7. Bourrin, S., P. Ammann, J.P. BonJour, and R. Rizzoli. Dietary protein restriction lowers plasma insulin-like growth factor-I (IGF-I), impairs cortical bone formation, and induces osteoblastic resistance to IGF-I in adult female rats. *Endocrinology.* 141:3149-3155, 2000.
8. Clemmons, D.R., A. Klibanski, L.E. Underwood, J.W. McArthur, E.C. Ridgway, I.Z. Beitins, and J.J. Van Wyk. Reduction of plasma immunoreactive somatomedin C during fasting in humans. *J. Clin. Endocrinol. Metab.* 53:1247-1250, 1981.
9. Daughaday, W.H., I.K. Mariz, and S.L. Blethen. Inhibition of access of bound somatomedin to membrane receptor and immunobinding sites: a comparison of radioreceptor and

- RIA of somatomedin in native and acid-ethanol-extracted serum. *J. Clin. Endocrinol. Metab.* 51:781-788, 1980.
10. Deuschle, M., W.F. Blum, J. Frystyk, H. Orskov, U. Schweiger, B. Weber, A. Korner, U. Gotthardt, J. Schmider, H. Standhardt, and I. Heuser. Endurance training and its effect upon the activity of the GH-IGFs system in the elderly. *Int. J. Sports Med.* 19:250-254, 1998.
 11. Donahue, S.P., and L.S. Phillips. Response of IGF-I to nutritional support in malnourished hospital patients: a possible indicator of short-term changes in nutritional status. *Am. J. Clin. Nutr.* 50:962-969, 1989.
 12. Forbes, G.B., M.R. Brown, S.L. Welle, and L.E. Underwood. Hormonal response to overfeeding. *Am. J. Clin. Nutr.* 49:608-611, 1989.
 13. Hossenlopp, P., D. Seurin, B. Segovia-Quinson, S. Hardouin, and M. Binoux. Analysis of serum insulin-like growth factor binding proteins using Western blotting: use of the method for titration of the binding proteins and competitive binding studies. *Anal. Biochem.* 154:138-143, 1986.
 14. Isley, W.L., L.E. Underwood, and D.R. Clemmons. Dietary components that regulate serum somatomedin-C concentrations in humans. *J. Clin. Invest.* 71:175-182, 1983.
 15. Jones, J.I., and D.R. Clemmons. Insulin-like growth factors and their binding proteins: biological actions. *Endocr. Rev.* 16: 3-34, 1995.
 16. Katz, L., D.D. DeLeon, H. Zhao, and A.F. Jawad. Free and total insulin-like growth factor (IGF)-I levels decline during fasting: relationship with insulin and IGF-binding protein-1. *J. Clin. Endocrinol. Metab.* 87:2978-2983, 2002.
 17. Koziris, L.P., R.C. Hickson, R.T. Chatterton, Jr., R.T. Groseth, J.M. Christie, D.G. Goldflies, and T.G. Unterman. Serum levels of total and free IGF-I and IGFBP-3 are increased and maintained in long-term training. *J. Appl. Physiol.* 86:1436-1442, 1999.
 18. Kraemer, W.J., K. Hakkinen, R.U. Newton, B.C. Nindl, J.S. Volek, M. McCormick, L.A. Gotshalk, S.E. Gordon, S.J. Fleck, W.W. Campbell, M. Putukian, and W.J. Evans. Effects of heavy-resistance training on hormonal response patterns in younger vs. older men. *J. Appl. Physiol.* 87:982-992, 1999.
 19. Kraemer, W.J., J.S. Volek, J.A. Bush, M. Putukian, and W.J. Sebastianelli. Hormonal responses to consecutive days of heavy-resistance exercise with or without nutritional supplementation. *J. Appl. Physiol.* 85:1544-1555, 1998.
 20. Lemon, P.W.R., D.G. Dolny, and K.E. Yarasheski. Moderate physical activity can increase dietary protein needs. *Can. J. Appl. Physiol.* 22:494-503, 1997.
 21. Lionel, P., M. Lynch, J. Savory, and D.M. Haverstick. Urine total protein measurement with the Vitros dry reagent technology: modification of the diluent to resolve positive bias of diluted samples. *Clin. Chem.* 44:674-675, 1998.
 22. Manetta, J., J. Brun, L. Maimoun, C. Fedou, C. Prefaut, and J. Mercier. The effects of intensive training on insulin-like growth factor I (IGF-I) and IGF binding proteins 1 and 3 in competitive cyclists: relationship with glucose disposal. *J. Sports Sci.* 21:147-154, 2003.
 23. Mohan, S., and D.J. Baylink. IGF-binding proteins are multifunctional and act via IGF-dependent and -independent mechanisms. *J. Endocrinol.* 175:19-31, 2002.
 24. Musey, V.C., S. Goldstein, P.K. Farmer, P.B. Moore, and L.S. Phillips. Differential regulation of IGF-1 and IGF-binding protein-1 by dietary composition in humans. *Am. J. Med. Sci.* 305:131-138, 1993.
 25. Nemet, D., P.H. Connolly, A.M. Pontello-Pescatello, C. Rose-Gottron, J.K. Larson, P. Galassetti, and D.M. Cooper. Negative energy balance plays a major role in IGF-I response to exercise training. *J. Appl. Physiol.* 96:276-282, 2004.
 26. Nemet, D., Y. Oh, H.S. Kim, M. Hill, and D.M. Cooper. Effect of intense exercise on inflammatory cytokines and growth mediators in adolescent boys. *Pediatrics.* 110:681-689, 2002.

27. Nindl, B.C., J.W. Catellani, A.J. Young, J.F. Patton, M.J. Khosravi, A. Diamandi, and S.J. Montain. Differential responses of IGF-I molecular complexes to military operational field training. *J. Appl. Physiol.* 95:1083-1089, 2003.
28. Nindl, B.C., W.J. Kraemer, J.O. Marx, P.J. Arciero, K. Dohi, M.D. Kellogg, and G.A. Loomis. Overnight responses of the circulating IGF-I system after acute, heavy-resistance exercise. *J. Appl. Physiol.* 90:1319-1326, 2001.
29. Raby, N., C. Bonneau, S. Gillier, J. Le, R. Granouillet, J. Frey, and A. Chamson. Single dilution for urine assays on the Vitros 250 or 700 analyzers. *Clin. Chem.* 44:1746-1748, 1998.
30. Rosendal, L., H. Langberg, A. Flyvbjerg, J. Frystyk, H. Orskov, and M. Kjaer. Physical capacity influences the response of insulin-like growth factor and its binding proteins to training. *J. Appl. Physiol.* 93:1669-1675, 2002.
31. Schmitz, K.H., R.L. Ahmed, and D. Yee. Effects of a 9-month strength training intervention on insulin, insulin like growth factor (IGF)-I, IGF-binding protein (IGFBP)-1, and IGFBP-3 in 30-50-year-old women. *Cancer Epidemiol. Biomarkers Prev.* 11:1597-1604, 2002.
32. Siri, W.E. Body composition from fluid spaces and density: analysis of methods. In: *Techniques for Measuring Body Composition*, J. Brozek and A. Henschel (Eds.). Washington DC: National Academy of Sciences, National Research Council, pp. 223-234, 1961.
33. Smith, A.T., D.R. Clemmons, L.E. Underwood, V. Ben-Ezra, and R. McMurray. The effect of exercise on plasma somatomedin-C/insulinlike growth factor I concentrations. *Metabolism.* 36:533-537, 1987.
34. Smith, W.J., L.E. Underwood, and D.R. Clemmons. Effects of caloric or protein restriction on insulin-like growth factor-I (IGF-I) and IGF-binding proteins in children and adults. *J. Clin. Endocrinol. Metab.* 80:443-449, 1995.
35. Tarnopolsky, M.A., S.A. Atkinson, J.D. MacDougall, A. Chesley, S. Phillips, and H.P. Swarcz. Evaluation of protein requirements for trained strength athletes. *J. Appl. Physiol.* 73:1986-1995, 1992.
36. Tarnopolsky, M.A., J.D. MacDougall, and S.A. Atkinson. Influence of protein intake and training status on nitrogen balance and lean body mass. *J. Appl. Physiol.* 64:187-193, 1988.
37. Thissen, J.P., J.M. Ketelslegers, and L.E. Underwood. Nutritional regulation of the insulin-like growth factors. *Endocr. Rev.* 15:80-101, 1994.
38. Thissen, J.P., L.E. Underwood, D. Maiter, M. Maes, D.R. Clemmons, and J.M. Ketelslegers. Failure of insulin-like growth factor-I (IGF-I) infusion to promote growth in protein-restricted rats despite normalization of serum IGF-I concentrations. *Endocrinology.* 128:885-890, 1991.
39. Weir, J.B. New methods for calculating metabolic rate with special reference to protein metabolism. *J. Physiol.* 109:1-9, 1949.