Increased Protein Intake Reduces Lean Body Mass Loss during Weight Loss in Athletes

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ABSTRACT

METTLER, S., N. MITCHELL, and K. D. TIPTON. Increased Protein Intake Reduces Lean Body Mass Loss during Weight Loss in Athletes. *Med. Sci. Sports Exerc.*, Vol. 42, No. 2, pp. 326–337, 2010. **Purpose**: To examine the influence of dietary protein on lean body mass loss and performance during short-term hypoenergetic weight loss in athletes. **Methods**: In a parallel design, 20 young healthy resistance-trained athletes were examined for energy expenditure for 1 wk and fed a mixed diet (15% protein, 100% energy) in the second week followed by a hypoenergetic diet (60% of the habitual energy intake), containing either 15% ($\sim$1.0 g·kg^{-1}) protein (control group, n = 10; CP) or 35% ($\sim$2.3 g·kg^{-1}) protein (high-protein group, n = 10; HP) for 2 wk. Subjects continued their habitual training throughout the study. Total, lean body, and fat mass, performance (squat jump, maximal isometric leg extension, one-repetition maximum (1RM) bench press, muscle endurance bench press, and 30-s Wingate test) and fasting blood samples (glucose, nonesterified fatty acids (NEFA), glycerol, urea, cortisol, free testosterone, free Insulin-like growth factor–1 (IGF-1), and growth hormone), and psychologic measures were examined at the end of each of the 4 wk. **Results**: Total (-3.0 ± 0.4 and -1.5 ± 0.3 kg for the CP and HP, respectively, P = 0.036) and lean body mass loss (-1.6 ± 0.3 and -0.3 ± 0.3 kg, P = 0.006) were significantly larger in the CP compared with those in the HP. Fat loss, performance, and most blood parameters were not influenced by the diet. Urea was higher in HP, and NEFA and urea showed a group × time interaction. Fatigue ratings and "worse than normal" scores on the Daily Analysis of Life Demands for Athletes were higher in HP. **Conclusions**: These results indicate that $\sim$2.3 g·kg^{-1} or $\sim$35% protein was significantly superior to $\sim$1.0 g·kg^{-1} or $\sim$15% energy protein for maintenance of lean body mass in young healthy athletes during short-term hypoenergetic weight loss. **Key Words:** NUTRITION, EXERCISE, BODY COMPOSITION, PERFORMANCE

BASIC SCIENCES

Making weight is a significant issue in sports nutrition. Many athletes restrict energy intake to achieve a certain body mass category, aesthetic reasons or to attain a better force-to-mass ratio to improve performance. However, a hypoenergetic diet may result in a significant loss of lean body mass (18), perhaps leading to compromised performance (9).

Recently, data have accumulated suggesting that increased protein content of the diet, particularly in combination with exercise training, may increase weight loss and reduce the loss of lean body mass in overweight and obese subjects (18,19,21,24,28). This preservation of lean mass has been attributed to the increased essential amino acid levels, particularly leucine, provided by the protein (17). Leucine stimulates the initiation of translation and increases protein synthesis (1), which may help to reduce the net loss of muscle protein.

Whereas there is ample evidence for amelioration of lean body mass loss during hypoenergetic weight loss in overweight and obese populations consuming high-protein diets (18,19,21,24,28), there is little information available on athletic populations. Clearly, the metabolic and training status of athletic individuals differs from that of obese and overweight, particularly sedentary, individuals. Athletes are usually healthy and unlikely to experience metabolic diseases, or preliminary states of diseases, which are often apparent in inactive, obese subjects. Thus, the metabolic situation is different and may impact the response to high-protein hypoenergetic diets. Furthermore, initiation of a training program may influence the response to these diets, which may not be similar for already well-trained athletes. Nevertheless, in the only study to date to address this issue, Walberg et al. (40) showed that negative N balance was substantially ameliorated with a high-protein diet compared with a normal protein diet in a hypoenergetic situation in weight lifters. These data do support the notion that increased protein intake might ameliorate net protein balance and reduce lean body mass loss in athletes during hypoenergetic weight loss. However, one recent study found no

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effect of increased protein or branched-chain amino acid (BCAA) intake on lean body mass loss during hypoenergetic weight loss in athletes (26). These results do not seem to match those of the earlier study or fit with those from studies on obese individuals (18,19,21,24,28). Taken together, these limited—and apparently conflicting—data make it difficult to form solid conclusions on the effectiveness of high-protein intake during weight loss in athletes.

Whereas studies in obese subjects focused primarily on health-related parameters (18,19,21,24,28), it may be considered more relevant to focus on physical performance in an athletic population. The influence of energy restriction *per se* on different aspects of performance has been examined (9,23), but there is a paucity of information available on the influence of protein on performance during a hypoenergetic situation.

Therefore, the aim of the present study was to compare the influence of a high-protein hypoenergetic diet compared with a normal-protein hypoenergetic diet on lean body mass loss and performance in healthy, lean, resistance-trained athletes.

METHODS

Study design. The study design is summarized in Figure 1. In a parallel design, the subjects were divided into a control and a high-protein group. The first subjects were randomly allocated to the groups, whereas the latter subjects were allocated to match the groups for anthropometric values and training volume. Each subject participated in a 4-wk study. The first week was used to assess energy intake and output. In the second week, the subjects were fed 100% of their habitual energy intake. In weeks 3 and 4, energy intake was reduced to 60% of habitual intake. At the end of each week, body mass, body composition, and performance were measured in a testing session. A familiarization trial for each of the performance tests was performed before the first week. The subjects were asked to continue their habitual training throughout the study, and the 4-wk time window was scheduled in a way that no special events or stress periods in job or private life were expected during the time a subject was in the study.

Subjects. Subjects were 18- to 40-yr-old healthy (no known metabolic disorders as determined by health questionnaire) males with a body mass index >20 $kg \cdot m^{-2}$ who participated in regular resistance exercise training for at least the previous 6 months. The training had to include two or more resistance training sessions per week. Other types of training were not excluded and were recorded. Subjects were recruited from local sport facilities. Twenty-two subjects started the study. Two of them were taken out of the study in the first study week owing to lack of adherence to the study conditions. No subject pulled out after having started the study. The baseline values of the 20 subjects finishing the study are summarized in Table 1. The study was approved by the Coventry Research Ethics Committee. The purpose, potential risks, and benefits of the study were explained to each subject before written informed consent was obtained.

TABLE 1. Anthropometric values of the subjects.

	Control (n = 10)	High Protein (n = 10)	P
Age (yr)	25.8 ± 1.7	24.7 ± 1.6	0.87
Body mass[a] (kg)	78.3 ± 4.3	79.9 ± 2.9	0.26
BMI[a] ($kg \cdot m^{-2}$)	24.2 ± 0.9	23.4 ± 0.5	0.10
% body fat[a]	17.4 ± 1.5	16.1 ± 1.6	0.91
Training sessions per week[b]	4.9 ± 0.4	4.6 ± 0.4	0.96
Duration[b] ($min \cdot wk^{-1}$)	359 ± 45	334 ± 54	0.61

Values are mean ± SE.
BMI, body mass index.
[a] Before weight loss.
[b] All training including the testing sessions.

Detailed procedures. In the first week, the subjects consumed their habitual diet. For 3 d, the subjects filled in a physical activity questionnaire (4) and a food report to estimate energy expenditure and energy intake. In addition, the subjects were provided with a three-dimensional accelerometer (RT3 Research Tracker; Stayhealty.com, Monrovia, CA) and a HR monitor (Polar, Kempele, Finland). The subjects were asked to wear the accelerometer throughout the study except for sleeping and for training. All training sessions (resistance and others) were recorded with the HR monitor because the accelerometer was not thought to be ideal to measure intensities of all types of exercise, for example, resistance training. Energy expenditure of the training sessions was estimated from training duration and mean HR (14) and was added to the accelerometer data to get an energy expenditure estimation for the entire day. Every evening before going to bed, the subjects filled in a Daily Analysis of Life Demands for Athletes (DALDA) questionnaire (34), a satiety

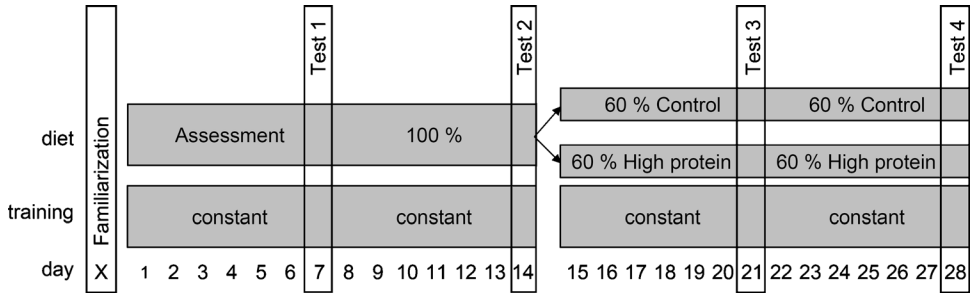

FIGURE 1—Schematic overview of the study design.

questionnaire, a training diary, and a general problem report. The satiety questionnaire consisted of two 100-mm visual analog scales anchored by the extreme values "extreme hunger" at 0 mm and "very full" at 100 mm. The middle of the scale was designated as the comfortable zone. One scale was designated to rate the present satiety in the evening, whereas the other was for estimation of the satiety during the entire day. Subjects were asked to report fatigue and muscle soreness ratings between 1 (not fatigue and not sore at all) and 10 (training not possible any more) for each training session. In the general problem report, the subjects were asked to report any problems they felt may have impacted training or habitual activities.

Diets. The energy and macronutrient intake of the two groups is given in Table 2. In week 1, all subjects consumed their habitual diet as discussed above. During week 2, all subjects were provided with food containing 100% of their habitual energy with a macronutrient composition of 50% carbohydrate, 15% protein, and 35% fat. The subjects were instructed not to eat or drink anything other than the provided food and drinks. The only exceptions were water and diet soft drinks that were allowed to be consumed *ad libitum*. In addition, they were allowed to add salt and pepper to spice up their food. The energy level for the first feeding day in week 2 was estimated from the food report, the physical activity questionnaire, the accelerometer, and Polar data, as well as population reference and the estimated physical activity level of the subjects. The four methods were basically averaged. However, advantages and disadvantages of each method were considered, and the set energy level may also have been slightly above or below the mathematical value. Food protocols are known to be prone to underreporting (22), and the physical activity questionnaire carries the risk of overreporting, particularly if training intensities are overrated (42). These intensity ratings were crosschecked, for example, with HR monitor data or training protocols, to assess their validity. In case of discrepancies between methods and detected potential weaknesses of a method with a specific subject, qualitative considerations were made as well. For example, if the physical activity questionnaire suggested a higher energy output than all other methods and it was supported by HR data that did not support reports of high-intensity training, then the average of the four methods was considered to be a slight overestimate. In such a case, the energy level would be estimated as slightly less than the average. This level of energy intake was then used as the starting point during week 2. Finally, during the first few feeding days of week 2, the subjects were advised to provide daily feedback on the dietary energy level. When a subject reported feeling hungry, we increased the energy content of the food slightly. Similarly, when a subject reported an inability to comfortably consume the provided food, subjects were advised not to eat the excess and to report the surplus food. We then reduced the energy content for the following days accordingly to ensure that the energy intake for each subject was appropriate by the end of the second week. The absolute necessary adjustments were not different between groups and amounted to 4% ± 4% (mean ± SD) of the originally estimated energy level.

TABLE 2. Energy and macronutrient composition of the provided diets per day.

	Time	Control (n = 10)	High Protein (n = 10)
Energy, kJ (kJ·kg^{-1})	Week 2	14,411 ± 977 (184 ± 6)	13,936 ± 479 (177 ± 6)
	Week 3	8649 ± 603 (113 ± 4)	8464 ± 288 (108 ± 4)
	Week 4	8583 ± 587 (114 ± 4)	8469 ± 281 (109 ± 4)
Carbohydrates, g (g·kg^{-1})	Week 2	428 ± 32 (5.4 ± 0.2)	415 ± 14 (5.3 ± 0.2)
	Week 3	259 ± 18 (3.4 ± 0.1)	257 ± 9 (3.3 ± 0.1)
	Week 4	258 ± 18 (3.4 ± 0.1)	257 ± 9 (3.3 ± 0.1)
Fat, g (g·kg^{-1})	Week 2	133 ± 9 (1.70 ± 0.05)	131 ± 5 (1.65 ± 0.06)
	Week 3	82 ± 6 (1.06 ± 0.05)	31 ± 1 (0.40 ± 0.02) *
	Week 4	81 ± 6 (1.07 ± 0.04)	31 ± 1 (0.40 ± 0.02) *
Protein, g (g·kg^{-1})	Week 2	128 ± 9 (1.64 ± 0.06)	125 ± 5 (1.58 ± 0.06)
	Week 3	74 ± 4 (0.98 ± 0.02)	180 ± 6 (2.31 ± 0.08) *
	Week 4	73 ± 4 (0.97 ± 0.02)	180 ± 6 (2.32 ± 0.08) *

Values are mean ± SE.
* Significantly different from control group ($P < 0.001$).

In weeks 3 and 4, the subjects were allocated either to the control or to the high-protein group. For both groups, the energy was dropped to 60% of the habitual energy intake. The relative macronutrient composition of the control diet remained the same (i.e., a linear reduction of all three nutrients). The high-protein diet was changed to 50% carbohydrate, 35% protein, and 15% fat. The values in Table 2 represent the nutrient composition of the subjects during weeks 2, 3, and 4 considering reported noncompliance. Protein intake was ~1.0 g protein per kilogram body mass per day (g·kg^{-1}·d^{-1}) for the control group and ~2.3 g·kg^{-1}·d^{-1} for the high-protein group. These values would be enough to maintain lean body mass in an energy balance situation for the control group (38) and approximately three times the US recommended daily allowance in the protein group. Protein was distributed among the different meals and snacks throughout the day to have a more or less steady protein supply throughout the day. However, the nutrient and protein intake was not explicitly timed with the training sessions.

During weeks 2, 3, and 4, the subjects were asked to report any foods assigned but not consumed. In addition, food items not or only partially consumed had to be returned to the laboratory by the subjects in order for them to be weighed. Every attempt was made to give the subjects the confidence to honestly report any noncompliance without any consequences. The diets were designed individually for each subject considering individual preferences and eating patterns to minimize noncompliance. The subjects were not informed of group assignment. However, given the composition of the high-protein diet, perfect blinding was not feasible. Thus, in an attempt to blind the diets as much as possible, we attempted to make the foods of both diets similar by providing similar food items whenever possible, for example, by providing "protein" shakes containing fat and hardly any protein and by providing food looking meaty or protein-rich while containing hidden fat to the control group. At the end of the

study, 19 of 20 subjects guessed to be in the high-protein group. Subjects usually reported to the laboratory to pick up food two times per week in addition to the testing sessions to allow for appropriate food variety and freshness.

Testing sessions. On the morning of the last day of every week, the subjects arrived in the laboratory after an overnight fast. Subjects were asked to have an easy evening the night before, and they were advised not to eat and drink anything after 2200 h until arriving in the laboratory the next morning.

In the morning of a testing session, the subjects had to go to the toilet first to ensure empty bladder for body weight measurement, which was measured without shoes in light sport clothing (shorts and T-shirt) on a laboratory scale (CD31; OHAUS, Pine Brook, NJ) to the nearest 0.01 kg. Subjects were asked to wear the same clothing for all tests. Body composition was assessed by dual-energy x-ray absorptiometry (DXA; QDR Discovery-C 4500; Hologic, Bedford, MA). A fasting blood sample was taken from an antecubital vein with an ethylene diamine tetra acetic acid (EDTA) vacutainer (Becton Dickinson and Co, Plymouth, United Kingdom) in a relaxed supine position. A profile of mood state (POMS-24) questionnaire was filled in at that time, and total mood disturbance was calculated by summing up scores on the negative subscale and then subtracting the score on the positive subscale (12). Afterward, the subjects were offered a breakfast consisting of *ad libitum* water and three slices of white bread (50 g per slice), butter (3 g per slice), and apricot jam (20 g per slice) consisting of 2050 kJ, 89 g of carbohydrates, 14 g of protein, and 9 g of fat in total. However, many subjects preferred to eat less than three slices to feel comfortable (2.3 slices consumed on average in both groups). In the second testing session, they were served the amount eaten in the fist testing session, and for the third and fourth testing sessions, they got 60% of previous energy according to the 40% energy cut off in these weeks.

Approximately 10 min after breakfast, performance assessment began. This assessment began with a general warm-up of 5 min on the bike. The warm-up was followed by a squat jump, a maximal isometric leg extension, a one-repetition maximum (1RM) chest press, a chest press muscle endurance test, and a Wingate test. Bike and machine settings were set in the familiarization trial and held constant for all tests. The investigator performing the laboratory testing was not blinded in this study.

The 5-min warm-up was done at a self-selected load on a cycle ergometer (Excalibur Sport; Lode, Groningen, the Netherlands). The load was kept constant for all testing sessions. The squat jump was performed on a force plate (Kistler Instrumente AG, Winterthur, Switzerland), and the vertical force component was recorded (BioWare version 3.2; Kistler Instrumente AG) to measure peak force (6). Jump height was directly measured with a jump meter (T.K.K.5406 Jump MD; Takei, Niigata, Japan). The subject supported the hands on the hip and descended slowly down to the crouched position (90° knee angle). The position was controlled before every jump by the investigator, and the subject had to hold the position for at least 2 s before jumping as high as possible without arm support. Four easy jumps without measurement were performed as warm-up and to get familiar with the crouched position before the first jump. Three serious jumps were then performed, separated by 1 min (6). In case of a visible countermovement, the jump was repeated. For jump height and peak force, the average of the two highest jumps was used. The average of all three jumps was used if no jump could be discarded.

To test the isometric quadriceps strength, the subject sat in a custom-built adjustable strength testing chair (8) with hips and knees flexed to 90°. A strap around the ankle was attached to a strain gauge at the back of the chair. The output of the strain gauge was amplified, converted to a digital signal (Power 1401; CED Limited, Cambridge, England) and sampled at 1000 Hz (Spike 2 version 5.15; CED Limited). Three maximal contractions of 4–6 s, separated by a 2-min recovery, were performed. The subjects were verbally encouraged to keep up the contraction until a maximal plateau was reached. The output signal was smoothed with a moving average of 200 ms. The highest 200-ms average of each attempt was noted, and the average of the two best attempts was determined to be maximal isometric force. To convert the strain gauge output into newtons, the strain gauge was calibrated daily. The coefficient of variation of the calibration factor was 0.9%.

The 1RM chest press was determined on a commercial chest press machine (VR3 chest press; Cybex International UK Ltd, Derbyshire, United Kingdom). A warm-up of eight repetitions at 60% 1RM and three repetitions at 80% 1RM separated by 1 min was made first (15). The warm-up loads were set according to the 1RM determined in the familiarization trial and were held constant for all tests. Two minutes after the warm-up the first 1RM attempt was made. The loads were chosen according to the perceived exertion of the subject. The first attempt was done with 2.27–4.54 kg (5–10 lb) less than the 1RM established in the previous week. The resistance was increased by 2.27–4.54 kg after a successful attempt. The 1RM was determined to the nearest 2.27 kg (5 lb). Attempts were separated by a 3-min recovery or longer until the subject felt recovered (15). In 84% of all testing sessions, three to four attempts were needed to determine the 1RM. In a few cases, it was necessary for the subject to attempt the lift only two or up to five times.

After a recovery of at least 4 min from the final 1RM, the load was set to 60% of the 1RM of the familiarization trial for the chest press muscle endurance test (15). This load was kept constant for all testing sessions even if the 1RM changed during the study. A pacing of 60 beeps per minute was used, and one repetition per two beeps was done (1 beep extension, 1 beep flexion). The subjects were asked to follow the pacing as long as possible and to continue until failure in a slower pace if unable to follow the pacing.

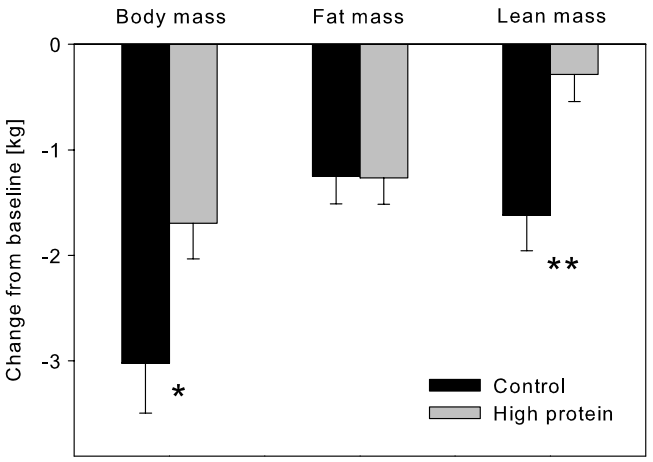

FIGURE 2—Change of body mass, fat, and lean mass from baseline (average of the two measurements before the weight loss) to the end of the 2-wk weight loss for the control (n = 10) and the high-protein (n = 10) groups. Values are mean ± SE. *Significant difference between the two groups (P = 0.036). **Significant difference between the two groups (P = 0.006).

The tests were video recorded for reliable evaluation. The tests were continuously supervised to ensure that the subject achieved the full range of motion, and immediate feedback was given when the range of motion was not complete.

The 30-s Wingate test was performed on an electronically braked ergometer (Excalibur Sport; Lode) (25) using the official Wingate software (Wingate version 1.0.13; Lode) to record power output and determine peak power and mean power. A warm-up of 4 min of easy cycling was first performed, including two 5-s full-acceleration sprints at 2 and 3 min of the warm-up. After a 3-min recovery, the subjects started pedaling at 60 W (as a minimal resistance is more convenient than real zero load). As soon as a cadence of 60 revolutions per minute was stabilized, the start command was given (31), and the subject accelerated maximally at a torque factor of 0.7 (25) and continued to keep cadence as high as possible to the end of the 30-s test. Strong verbal encouragement was given throughout.

Blood analysis. Blood was centrifuged for 10 min immediately after collection at 4°C at 1800*g* (Centra-CL3R; Thermo IEC, Waltham, MA), transferred into polypropylene tubes, and frozen at −80°C until analysis. Glucose, nonesterified fatty acids (NEFA), glycerol, and urea were analyzed by COBAS MIRA semiautomatic analyzer (La Roche, Basel, Switzerland). Free testosterone (IBL Hamburg, Hamburg, Germany), cortisol (IBL Hamburg), growth

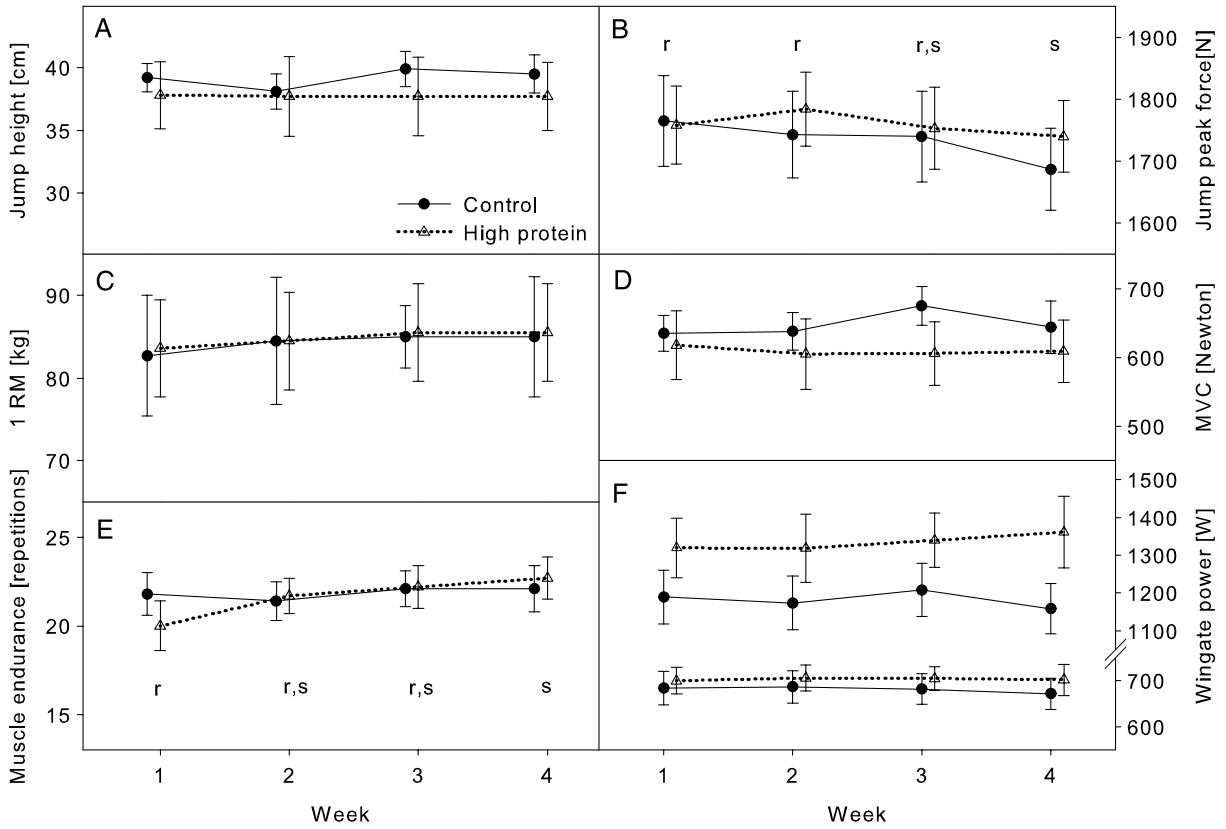

FIGURE 3—Performance data for the control (n = 10) and the high-protein (n = 10) groups during the study. Neither was there a statistically significant difference between the groups nor was there any significant group × time effect. Squat jump height (A), jump peak force (B), 1RM chest press (C), maximal voluntary contraction (MVC) knee extension (D), muscle endurance test chest press (E), and mean power (*lower pair of lines*) and peak power (*upper pair of lines*) of the 30-s Wingate test (F). Values are mean ± SE. r and s, Different letters indicate significant difference (P < 0.05) between time points (effect of time).

TABLE 3. Fasting blood values for the control (n = 10) and the high-protein groups (n = 10).

						Statistics		
		Week 1	Week 2	Week 3	Week 4	*P* (Group)	*P* (Time)	*P* (Group × Time)
Glucose (mmol·L^{-1})	Control	5.26 ± 0.09	5.33 ± 0.08	5.14 ± 0.09	5.05 ± 0.09	0.762	<**0.001**	0.836
	High protein	5.29 ± 0.11	5.31 ± 0.11	5.06 ± 0.13	4.99 ± 0.08			
NEFA (μmol·L^{-1})	Control	311 ± 31	323 ± 23	496 ± 30	430 ± 26	0.529	<**0.001**	**0.005**
	High protein	304 ± 35	402 ± 54	379 ± 49	374 ± 33			
Glycerol (μmol·L^{-1})	Control	88 ± 14	76 ± 9	108 ± 4	110 ± 14	0.714	0.054	0.443
	High protein	74 ± 11	90 ± 15	96 ± 8	97 ± 19			
Urea (mmol·L^{-1})	Control	6.1 ± 0.4	5.9 ± 0.3	5.3 ± 0.4	5.4 ± 0.5	**0.020**	**0.017**	<**0.001**
	High protein	6.6 ± 0.5	5.9 ± 0.4	8.0 ± 0.4	7.9 ± 0.5			
Cortisol (ng·mL^{-1})	Control	137 ± 15	118 ± 11	112 ± 8	123 ± 9	0.971	0.092	0.557
	High protein	130 ± 21	121 ± 16	123 ± 12	119 ± 10			
Free testosterone (μIU·mL^{-1})	Control	58 ± 6	59 ± 8	43 ± 5	36 ± 5	0.757	<**0.001**	0.259
	High protein	55 ± 7	60 ± 9	51 ± 7	41 ± 6			
Free IGF-1 (ng·mL^{-1})	Control	0.82 ± 0.12	0.87 ± 0.10	0.78 ± 0.09	0.75 ± 0.08	0.426	**0.018**	0.186
	High protein	0.72 ± 0.07	0.71 ± 0.07	0.73 ± 0.08	0.67 ± 0.06			
Growth hormone (μIU·mL^{-1})	Control	2.65 ± 1.81	0.45 ± 0.37	2.43 ± 1.49	0.57 ± 0.19	0.446	0.266	0.084
	High protein	2.11 ± 1.94	1.17 ± 0.95	2.27 ± 1.34	8.28 ± 5.66			

Values are mean ± SE. *P* values <0.05 are in bold.

hormone (IBL Hamburg), and free IGF-1 (DSL, Webster, TX) were analyzed by enzyme-linked immunosorbent assay according to the manufacturer's instructions. Blood parameters were analyzed in duplicate, and the average coefficients of variation were 1.8%, 1.2%, 2.3%, 0.7%, 2.4%, 2.8%, 4.6%, and 3.3% for glucose, urea, glycerol, NEFA, free IGF-1, free testosterone, growth hormone, and cortisol, respectively. The mean of the duplicates was used for statistical analysis.

Calculations and statistics. Changes in body composition were calculated by subtracting the average of the week 1 and week 2 measurements (before weight loss) from the week 4 measurement (after weight loss). Using the week 2 measurement only as pre–weight loss value instead of the average of weeks 1 and 2 would not change the outcome and interpretation of the data. Values that were tracked or planned on a daily basis were averaged per subject and week, and weekly averages were used for statistics.

Statistical analysis was performed with SAS for Windows (version 8.2; SAS Institute, Inc., Cary, NC) using ANOVA for repeated measures with Tukey adjustment. $P < 0.05$ was considered significant, although explicit P values are usually presented. Values are presented as mean ± SE.

RESULTS

The loss of total body mass, lean body mass, and fat mass is presented in Figure 2. Total body mass was 78.3 ± 4.3, 78.3 ± 4.3, 76.5 ± 4.1, and 75.3 ± 4.0 kg after weeks 1, 2, 3, and 4, respectively, in the control group and 79.8 ± 2.9, 79.5 ± 2.9, 78.5 ± 2.8, and 78.0 ± 2.9 kg in the high-protein group. Body mass was not different between groups ($P = 0.718$), but there was a significant effect of time ($P < 0.001$) and a group × time interaction ($P = 0.011$). Total body mass did not change from week 1 to week 2 in the control ($P > 0.999$) and high-protein groups ($P = 0.886$). Both groups lost the same amount of fat mass, but the control group lost significantly more lean ($P = 0.006$) and total ($P = 0.036$) body mass than the high-protein group. The relative lean body mass loss in the arms, legs, trunk, and head was 2.2% ± 0.7%, 2.5% ± 0.8%, 2.7% ± 0.6%, and 2.0% ± 1.0%, respectively, for the control group and 1.1% ± 0.8%, 0.2% ± 0.7%, 0.4% ± 0.7%, and 0.8% ± 0.6%,

TABLE 4. Tracking of energy expenditure, satiety, fatigue, and muscle soreness ratings for the control (n = 10) and the high-protein groups (n = 10).

						Statistics		
		Week 1	Week 2	Week 3	Week 4	*P* (Group)	*P* (Time)	*P* (Group × Time)
Energy expenditure[a] (%)	Control	100	101 ± 1	101 ± 1	102 ± 2	0.272	0.827	0.476
	High protein	100	101 ± 2	99 ± 2	100 ± 2			
Satiety whole day (mm)	Control	60 ± 5	57 ± 3	38 ± 2	36 ± 4	0.456	<**0.001**	0.379
	High protein	62 ± 5	65 ± 7	39 ± 5	44 ± 6			
Satiety evening (mm)	Control	62 ± 4	58 ± 3	42 ± 3	38 ± 3	**0.048**	<**0.001**	0.706
	High protein	72 ± 6	72 ± 6	54 ± 7	54 ± 7			
Fatigue	Control	4.9 ± 0.5	4.8 ± 0.5	5.1 ± 0.3	5.6 ± 0.3	0.721	<**0.001**	**0.009**
	High protein	4.3 ± 0.7	4.4 ± 0.6	6.2 ± 0.5	6.4 ± 0.5			
Muscle soreness	Control	4.0 ± 0.6	3.6 ± 0.5	3.6 ± 0.5	4.0 ± 0.4	0.619	0.243	0.478
	High protein	4.1 ± 0.7	3.5 ± 0.5	4.5 ± 0.8	4.6 ± 0.7			
POMS-24[b]	Control	−4.7 ± 1.8	−3.9 ± 2.4	1.6 ± 2.3	−1.7 ± 2.4	0.113	0.082	0.683
	High protein	2.1 ± 4.1	−0.9 ± 2.1	3.6 ± 1.9	2.6 ± 2.2			

Values are mean ± SE.
[a] Energy expenditure is expressed relative to week 1, which is set as baseline (100%).
[b] Total mood disturbance score of the POMS questionnaire.

respectively, in the high-protein group. The relative lean loss in the segments was statistically different between the groups (P = 0.009) but did not differ between the segments.

There were no statistically significant differences between the two groups for any of the performance tests (Fig. 3). There was a statistically significant decrease of 2.8% in peak jump force (P = 0.011) and a 7.2% increase (P = 0.044) in muscle endurance between weeks 1 and 4 with no significant difference between the groups. The maximal voluntary contraction, 1RM, jump peak force, and the Wingate power output can be corrected for body mass. However, there was no effect of group, time, or group × time for the corrected performance values except for the 1RM. Relative to body mass, 1RM increased significantly (P < 0.001) during weeks 3 and 4 compared with weeks 1 and 2, but there was no statistical difference between the control and high-protein groups (data not shown).

Glucose, free testosterone, and free IGF-1 decreased, whereas NEFA and urea increased significantly (P < 0.05) with energy restriction (effect of time). Urea was significantly increased (P = 0.017) in the high-protein group compared with the control group. Urea (P < 0.001) and NEFA (P = 0.005) showed a significant group × time interaction (Table 3).

The results of the energy expenditure tracking, satiety ratings, and the muscle soreness and fatigue ratings are presented in Table 4. There was no effect of group, time, or group × time for the number of training sessions per week, training duration per week, and the average exercise energy expenditure as estimated with the HR monitor (data not shown). There was no effect of the energy restriction *per se* nor was there any difference between the groups with respect to total mood disturbance in the POMS-24 questionnaire (Table 4). There was also no difference between the groups in the A-part of the DALDA. In contrast, results of the B-part of the DALDA questionnaire were influenced by the energy restriction and showed a significant (P < 0.05) increase of the "worse than normal" ratings during the weight loss weeks, including a significant (P = 0.023) group × time effect, that is, the high-protein group showed a larger rise of the "worse than normal" ratings in the weight loss weeks than the control group (Fig. 4).

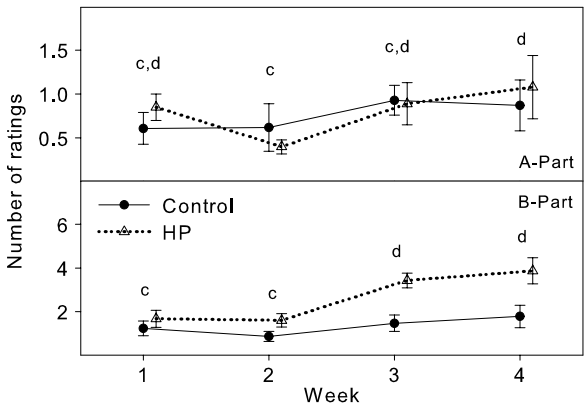

FIGURE 4—"Worse than normal" ratings in the A-part and B-part of the DALDA questionnaire for the control (*solid lines*, n = 10) and the high-protein groups (*dashed line*, n = 10). In the B-part, there was a significant group × time interaction (P = 0.024). c and d, Time points with different letters are significantly different from each other.

DISCUSSION

This study was designed to examine the effect of increased dietary protein composition on lean body mass maintenance during hypoenergetic weight loss in athletes. We found that although fat loss was similar for both the high-protein and the control diet groups, increased protein intake resulted in less lean and total body mass loss. Diet composition did not seem to impact any measured performance parameter, and there were minimal differences, for example, greater urea in the high-protein group, between groups for fasting blood metabolites.

Body composition. There was a significantly reduced loss of lean body mass with ingestion of the high-protein diet (~2.3 $g \cdot kg^{-1} \cdot d^{-1}$) compared with the control diet (~1.0 $g \cdot kg^{-1} \cdot d^{-1}$). These results may be considered similar to those previously reported in overweight and obese subjects (18,19,21,24,28), albeit with some notable differences. We found no difference in fat loss between dietary groups but a significantly elevated loss of lean body mass in the control group, resulting in an elevated loss of total body mass. On the other hand, in obese subjects, the results were reversed. High protein intake resulted in greater total body mass loss due to a larger loss of body fat (18,19). Also in obese subjects, there was a consistently greater loss of fat mass than lean mass, independent of the dietary protein level (18,19,21,24,28). In our healthy, trained, lean subjects, we found a loss of lean body mass, which was substantially larger than the loss of fat mass, when the control diet was consumed. However, with higher protein intake, lean body mass loss was ~20% of that of the control group. The loss of lean body mass did not seem to be particular to any body section but rather seemed to be fairly consistent throughout the whole body, perhaps suggesting that skeletal muscle and splanchnic protein may be affected to a comparable extent.

Another important aspect to consider is the macronutrient composition of the diets other than protein. Whereas previous studies in overweight subjects increased protein intake at the expense of carbohydrate intake (18,19,21,24,28), we chose to balance energy by changing fat intake. Increasing protein intake at the expense of carbohydrates is likely to negatively impact exercise performance and training intensity (9,23). With respect to strength, muscle endurance, and high-intensity performance, carbohydrate intake may be critical if too low (9). Thus, maintenance of carbohydrate intake would be important for this population when energy intake is limited and when maintenance of training levels is a critical aspect. Because dietary carbohydrate intake can

reduce fat oxidation (32), maintenance of carbohydrate intake in the high-protein group may explain the lack of difference in fat loss between groups as was reported from studies with obese subjects where the high protein was balanced with carbohydrates (18,19,21,24,28). However, differences between lean and obese subjects with respect to the metabolic effect of high-protein meals on fuel metabolism and fat oxidation have been reported (16,21). Therefore, it is not intuitively obvious which, if any, of the variables that differ between studies (e.g., subject population, macronutrient composition, and physical activity of the subjects) explain the differential responses of fat and lean mass.

Another aspect to consider is the timing of the protein intake in relation to the training stimulus because this may influence the impact on protein synthesis (38). We did not explicitly advise the subjects to consume a particular amount of protein at a particular time before, during, or after training. However, we distributed the daily protein load as much as possible among the different meals and snacks so that a reasonably steady protein supply during the day was ensured.

Previously, Walberg et al. (40) demonstrated that increased protein intake resulted in greater N balance during weight loss. We did not measure N balance in our study, but clearly, greater N balance in the high-protein group would be important for the lack of loss of lean mass that we observed. However, it is not clear that N balance can be quantitatively associated with lean body mass. Although Walberg et al. (40) found that N balance increased by as much as $\sim$7 g N·d^{-1} on the high-protein diet, there was no increase in fat-free mass. There seems to be an overall discrepancy between positive N balances and body protein accretion in N balance studies (29). Therefore, conclusions regarding protein accretion or loss with varying diets may be qualitatively assessed with N balance, but quantitative conclusions may be best based on body composition measures.

In fact, if the relatively hidden information about body composition (measured by underwater weighing) are extracted from the study of Walberg et al. (40), the results match surprisingly well with our data. These data reveal a similar absolute fat loss of 2.1 versus 2.0 kg in the low-protein group (0.8 g·kg^{-1}·d^{-1}) and the high-protein group (1.6 g·kg^{-1}·d^{-1}), respectively. The mean lean body mass loss, however, was 2.7 versus 1.4 kg. This seems to be in accordance with our data in two important aspects. The lean body mass loss seemed to be influenced by the protein content of the diet, and the lean body mass loss clearly exceeded the fat loss at the lower protein level.

The influence of the dietary energy level and the amount of energy reduction may be further parameters to consider (5,38). Although we have attributed the differences between groups to the obvious difference in protein intake, the vagaries of assessing energy intake make it possible that differences in energy intake may have influenced our results. If energy balance was greater for the high-protein group, then higher body weight and lean body mass in that group may be because of energy as much as protein. However, this explanation seems unlikely. We made extensive attempts to control and assess diet and energy expenditure. Our satiety ratings during week 2, that is, when we established energy intakes, were stable, and energy was carefully adjusted during that week to ensure appropriate levels. Body weight did not change during week 2. Although this time frame may be too short to detect changes in body weight because of smaller, yet significant, energy deficits, at the very least, it excludes large deficits that would account for the changes noted in subsequent weeks. In fact, if anything, the average change in body mass during week 2 was slightly negative, albeit non-statistically significant, for the high-protein group. Finally, fat loss was similar for the two groups. If energy balance was different, it is likely that fat loss would have been greater for the control group. Thus, it seems that differences in energy balance are less likely to account for the differences between groups than differences in protein intake.

Energy intake may have impacted body composition in another manner. It is possible that the drop in protein intake with the simultaneous drop in energy intake might have been two factors, both of which contributed to lean body mass loss in the control group. Quevedo et al. (30) demonstrated that N balance was negative for $\sim$10 d after a reduction in protein intake. However, in practice, when energy intake is restricted, protein intake almost certainly will be reduced unless high-protein foods are specifically selected. However, Friedlander et al. (11) noted comparable lean body mass losses to our control group, which also exceeded fat losses, even when the absolute protein supply was held constant before and during weight loss at 1.2 g·kg^{-1}·d^{-1} in young, lean subjects.

Mourier et al. (26) found no effect of two different nitrogen-enriched diets (high protein and high BCAA intake) on lean body mass loss during a hypoenergetic weight loss in athletes compared with a control group. The reasons for discrepancies between studies are not obvious but may be because of methodological differences influencing the anabolic response of muscle.

Elevated protein intake was accomplished by different food types in these studies. Mourier et al. (26) used soy protein as a major protein source in the high-protein group. Soy protein has been shown to increase protein breakdown in animal and human experiments (3). Therefore, the high-protein diet might not have been as effective as it could have been with other protein sources. We relied more on supplying animal protein sources (dairy, tuna, chicken, and other meat proteins) to increase the protein intake in our study. Recent data indicate that the response of muscle anabolism to resistance exercise is greater when animal protein sources are consumed (43). Thus, maintenance of lean body mass may have been better in our high-protein group owing to the type of protein ingested.

The second nitrogen-enriched group of Mourier et al. (26) received most of the daily nitrogen intake as BCAA (0.9 g BCAA·kg^{-1} body mass, consisting of 76% leucine, 19% isoleucine, and 5% valine). Whereas leucine stimulates protein synthesis (1), increased leucine levels have long been known to decrease levels of other amino acids, especially the other BCAA because of increased activity of branched-chain α-keto acid dehydrogenase (13). Thus, the high levels of BCAA from the supplementation without additional supply of exogenous amino acids may have limited the availability of the other (essential) amino acids in that study (26). Amino acid availability is a key factor for the stimulation of muscle protein synthesis; thus, muscle anabolism may have been limited in that high-BCAA group (26) relative to our high-protein group.

The discussion in the above paragraph is predicated primarily on the notion that the higher protein intake somehow changed muscle protein metabolism resulting in less atrophy during hypocaloric weight loss. Because DXA does not provide an assessment of muscle protein, we cannot rule out the possibility that the differences in lean body mass were due to other factors influencing DXA methodology, for example, differences in body water. However, there does not seem to be an obvious reason for differences in body water with the two diets. Thus, differences in lean body mass are likely accounted for, at least in part, by differences in muscle protein. Walberg et al. (40) reported a comparable result with a completely different body composition method (underwater weighing) with different assumptions.

Whereas no measurement of protein metabolism was made in this study, it is intuitively satisfying to accept that provision of excess protein in combination with the resistance exercise may have resulted in greater magnitude of positive muscle protein balance (2,38), either through longer or more frequent periods of positive balance. The influence of exercise and amino acid provision on muscle protein balance is primarily due to the changes in muscle protein synthesis (2,29,38,43). However, muscle protein breakdown may also have played a role. During muscle atrophy, when amino acid availability is low, myofibrillar proteins seem to be selectively targeted for degradation (45). Thus, differences in lean body mass may have been due to increased muscle protein synthesis in response to the exercise and increased amino acid availability and/or selective degradation of the structural proteins with low amino acid availability.

Blood parameters. Some blood values responded to the energy restriction, but there was no statistical difference between the diets for most parameters. One exception was the NEFA, which showed a group × time effect (i.e., larger increase in NEFA during weight loss in the control compared with the high-protein group). This result was not entirely expected because high-fat diets do not increase resting levels of NEFA in inactive males (36). However, this interaction may be due to the different dietary fat intake during weeks 3 and 4 combined with training.

The other exception was the blood urea, which showed significant group, time, and group × time effects. This result may be explained by the substantial divergent dietary protein intake in weeks 3 and 4. The protein intake in the high-protein group was not only relatively, but even absolutely, higher during the two weight loss weeks compared with the maintenance week despite the energy restriction. Because body composition data indicate that there was no protein accretion, but still a slight loss of lean body mass, some of the protein must have been metabolized, causing increased urea values (10). The time course of the urea values, therefore, may be no real surprise (10). None of the measured anabolic hormones showed a group or group × time effect. Therefore, the blood values cannot help elucidate the difference in lean body mass loss between the two groups. However, this lack of explanatory power should be put into perspective. In this study, we measured only fasting values in the morning. It has been shown that anabolic hormones such as insulin (39), testosterone (35), or IGF-1 (44) respond to the dietary protein level. Thus, given the limitations of our study design, we cannot exclude the possibility that postprandial or whole-day hormonal profiles may be more informative.

Moreover, we have no information regarding responses at the muscle level. Although some anabolic signals such as IGF-1 seem to be reasonably represented in the plasma (27,44), there might be further autocrine or paracrine signaling processes on the muscular level. In fact, the major impact of IGF-1 is related more to the muscle than to the blood levels (27).

Further signaling processes may be going on in the muscle cell, for example, as a consequence of larger amino acid availability in the high-protein diet. It has been shown that leucine, in particular, is a potent stimulator of anabolic signal cascades in the cell (1,20). The increased leucine could have been a possible signal to increase protein synthesis and therewith counterbalance to some point the catabolic signaling of the hypoenergetic diet in the high-protein group (20). In fact, leucine signaling has been suggested to play a major role in preventing lean body mass loss with increased protein intake (17–20). Thus, it is possible that leucine signaling played an important role in our results. However, the role of leucine *per se* in the preservation of lean body mass during weight loss has not yet been systematically investigated. These possibilities need further study; measurement of not only fasting blood values but also whole-day hormonal profiles, including postprandial and postexercise situations, as well as muscle biopsies, could contribute more information about paracrine and other signaling on the muscular level.

Performance. For the most part, the results of the performance tests responded neither to the energy restriction *per se* nor to the composition of the diet. These results are similar to some, but not all, previous studies on performance during weight loss (9). Unfortunately, the disparate subject populations, duration of weight loss, and performance measures make it difficult to draw firm conclusions. However,

taken together, it seems that short periods of hypoenergetic weight loss do not result in dramatic decreases in performance.

There was no difference in measured performance parameters between the control group and the high-protein group. The jump peak force was the only performance parameter that dropped down significantly over time. This decrease may, at least partially, be explained by the lower body weight toward the end of the study, where less force was needed for the same jump height. As peak force in a squat jump increases with increasing external loading and decreases with decreasing external loading (7), a decreasing peak force in the squat jump with the decreased body weight does not necessarily indicate decreasing performance but rather represents an inevitable biomechanical effect. This supposition is supported by the other performance tests that do not decrease significantly. In contrast, the muscle endurance improved significantly. Possible adaptation to the testing procedure cannot be ruled out and may be the more likely explanation for this observation. Indeed, in week 1, muscle endurance was slightly less than in subsequent weeks. From weeks 2 to 4, there was no statistical change in muscular endurance. Taken together, these data support the notion that there may have been a learning effect for this test.

However, the lack of statistical significance does not mean that there is no effect at all. Because there was a significant difference in lean body mass loss between the two groups, it is feasible to speculate that performance may be impacted but not detected by our chosen methods of measurement. Our subjects were trained, but not elite and, except for weight lifting, participated in somewhat varied exercise activities. Continuing these investigations in a more homogeneous group of elite athletes, which could be tested in a sports-specific manner, is likely to provide more definitive results. Potential adaptive mechanisms to the testing sessions would likely be smaller, and the test–retest reliability might be even better than in our less homogenous subjects. Furthermore, accommodation to the testing may be reduced by adoption of more preceding familiarization trials than what we had our subjects perform. In addition, the influence of lean body mass loss on performance may be even more significant if periods of restricted energy intake are repeated, and lean body mass loss potentially accumulates, for example, during a competitive season in sports with weight classes and long-term energy restriction (33). However, this issue would be particularly difficult to test in a research setting.

In summary, the performance tests do not seem to be affected by the energy restriction or by the diet composition in this study. Nevertheless, a small but possibly relevant, influence of the diet composition or reduced energy intake on performance cannot be ruled out.

Other results. The tracking of the energy expenditure by accelerometer and HR monitor indicated that subjects consistently did not change their physical activity level, despite the 40% dietary energy restriction for 2 wk. This was important information because most previous weight loss studies have not monitored this parameter. It should be no surprise that the satiety ratings dropped with the dietary energy reduction; however, it might be considered surprising that satiety was not maintained in the protein group. Whereas protein has been reported to increase satiety in the free-living situation as well as in situations with clamped energy (21,41), we could not detect any difference between the groups in our study population. Leidy et al. (21) reported a significant impact of protein on the postprandial satiety ratings but not on the 24-h satiety. Therefore, it is possible that we missed postprandial effects of protein intake on satiety in our study.

Results of the psychologic assessments suggest that higher protein intake during hypoenergetic weight loss may negatively impact mood. There was a significant group $\times$ time effect for the fatigue rating during training sessions as well as in the B-part of the DALDA questionnaire (i.e., the high-protein group reported a more pronounced increase in the fatigue and the "worse than normal" ratings during weight loss compared with the control group). There is no obvious explanation for these results. The only difference between groups was the fat and protein intake. To our knowledge, there is no association of increased fatigue with reduced fat or increased protein intake while energy and carbohydrate intake is the same—at least to our knowledge, such an association has never been reported. Therefore, these potentially negative effects of the high-protein diet with respect to well-being and fatigue should be noted. Subjective feelings may be more relevant for performance than lean body mass maintenance, at least in the short term. However, it is possible that the decreased feelings of well-being and fatigue might have counteracted any influence of improved lean body mass maintenance on performance in the high-protein group.

Practical implications. Our results indicate that $\sim$2.3 $g \cdot kg^{-1} \cdot d^{-1}$ mass or $\sim$35% energy protein was significantly superior to $\sim$1.0 $g \cdot kg^{-1} \cdot d^{-1}$ or $\sim$15% energy protein for the maintenance of lean body mass. These levels of intake could be considered somewhat extreme. However, 1.0 $g \cdot kg^{-1} \cdot d^{-1}$ is likely sufficient to maintain mass when in energy balance (37) and 2.3 $g \cdot kg^{-1} \cdot d^{-1}$ is approximately three times the US recommended daily allowance. Comparison with previous studies on overweight and obese individuals suggests that this effect might be more pronounced in lean trained athletes compared with obese subjects. However, it is not possible to determine the protein intake needed to produce the maximal positive effect on lean body mass maintenance from our results. More studies with different protein levels and comparing different population groups would be needed to bring more clarification.

The practical implication of these results is that the protein content of a hypoenergetic diet may play a crucial role. Athletes aiming for body weight reduction while maintaining lean body mass may be advised to keep protein intake high.

On the other hand, athletes aiming for maximizing body weight reduction regardless of composition may wish to avoid an elevation of protein intake during hypoenergetic weight loss. However, maintenance of lean body mass might be the most desirable strategy for many athletes, particularly in the long term and/or if periods of restricted energy are repeated. On the other hand, it should be noted that we detected slightly but significantly reduced feelings of well-being and higher fatigue in the high-protein group compared with the control group. Whereas these negative results might be of less significance compared with maintenance of lean body mass with respect to the long-term influence on body composition and performance, reduced well-being might indeed be relevant for performance in the short term. In the relatively short 2-wk duration of our study, these feelings did not seem to impact the ability of the subjects to maintain their training volume and intensity and thus had no measurable impact on performance. Nonetheless, the impact of these diets on well-being should not be ignored when planning hypoenergetic weight loss. It should be mentioned that a high-protein diet may sound particularly attractive to athletes compared with a "standard" diet. We blinded the diets in an attempt to avoid the possibility that subjects in the high-protein diet would be more motivated for the study.

CONCLUSIONS

In conclusion, we found a significantly reduced loss of lean body mass with increased protein ($\sim$2.3 $g \cdot kg^{-1} \cdot d^{-1}$) compared with a normal protein diet ($\sim$1.0 $g \cdot kg^{-1} \cdot d^{-1}$) during short-term weight loss in healthy lean athletes. On the other hand, we detected slightly but significantly reduced feelings of well-being in the high-protein group. Performance was affected neither by the energy restriction nor by the diet composition in this study. Nevertheless, a small but possibly relevant influence of the diet composition or reduced energy intake on performance cannot be ruled out. Further studies are needed to gain more information about the dose response of the protein intake on lean body mass loss, and future studies with a more homogenous group of elite athletes may give more detailed information about the influence of dietary protein and lean body mass loss on performance.

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