Evaluation of protein requirements for trained strength athletes

M. A. TARNOPOLSKY, S. A. ATKINSON, J. D. MACDOUGALL, A. CHESLEY, S. PHILLIPS, AND H. P. SCHWARCZ
Departments of Pediatrics, Physical Education, Biochemistry, and Geology, McMaster University, Hamilton, Ontario L8N 3Z5, Canada

TARNOPOLSKY, M. A., S. A. ATKINSON, J. D. MACDOUGALL, A. CHESLEY, S. PHILLIPS, AND H. P. SCHWARCZ. *Evaluation of protein requirements for trained strength athletes.* J. Appl. Physiol. 73(5): 1986–1995, 1992.—Leucine kinetic and nitrogen balance (NBAL) methods were used to determine the dietary protein requirements of strength athletes (SA) compared with sedentary subjects (S). Individual subjects were randomly assigned to one of three protein intakes: low protein (LP) = 0.86 g protein · kg^{-1} · day^{-1}, moderate protein (MP) = 1.40 g protein · kg^{-1} · day^{-1}, or high protein (HP) = 2.40 g protein · kg^{-1} · day^{-1} for 13 days for each dietary treatment. NBAL was measured and whole body protein synthesis (WBPS) and leucine oxidation were determined from L-[1-^{13}C]leucine turnover. NBAL data were used to determine that the protein intake for zero NBAL for S was 0.69 g · kg^{-1} · day^{-1} and for SA was 1.41 g · kg^{-1} · day^{-1}. A suggested recommended intake for S was 0.89 g · kg^{-1} · day^{-1} and for SA was 1.76 g · kg^{-1} · day^{-1}. For SA, the LP diet did not provide adequate protein and resulted in an accommodated state (decreased WBPS vs. MP and HP), and the MP diet resulted in a state of adaptation [increase in WBPS (vs. LP) and no change in leucine oxidation (vs. LP)]. The HP diet did not result in increased WBPS compared with the MP diet, but leucine oxidation did increase significantly, indicating a nutrient overload. For S the LP diet provided adequate protein, and increasing protein intake did not increase WBPS. On the HP diet leucine oxidation increased for S. These results indicated that the MP and HP diets were nutrient overloads for S. There were no effects of varying protein intake on indexes of lean body mass (creatinine excretion, body density) for either group. In summary, protein requirements for athletes performing strength training are greater than for sedentary individuals and are above current Canadian and US recommended daily protein intake requirements for young healthy males.

L-[1-^{13}C]leucine; protein turnover; resistance athletes; weight lifting; bodybuilders

THE CANADIAN recommended nutrient intake (RNI) for dietary protein does not make allowance for an effect of habitual physical activity (2a). However, there is now substantive evidence to indicate that exercise increases protein requirements above the Canadian RNI (6, 15, 27–29). It also appears, however, that there is no advantage to strength athletes of protein consumption at a level exceeding intakes of 1.2–1.62 g protein · kg^{-1} · day^{-1} (27, 28).

Nitrogen balance (NBAL) methodology has traditionally been used to establish the protein requirements of both sedentary (23) and athletic populations (28, 29). However, there have been a number of concerns about the interpretation of NBAL studies (7, 33, 34). Among these are *1*) the ability to establish NBAL at protein intakes that are arguably suboptimal (accommodation) and *2*) the well-documented positive NBAL apparent at very high nitrogen intakes, which does not result in the expected lean body mass accretion. These issues are of particular importance for the athlete involved in the strength-resistance type of exercise. A protein intake that is too low may result in suboptimal strength/lean mass accretion despite maintenance of a positive NBAL (accommodation). Although an excessively high protein intake (nutrient overload) has not been demonstrated to have short- or long-term negative health consequences in otherwise healthy people, protein is an expensive foodstuff to produce and consume.

The NBAL method provides information on the net balance of all protein synthetic and catabolic processes in the body, but not their quantitation. This limitation, in combination with the interpretive concerns outlined above, has led to an interest in the use of stable isotope methods to determine amino acid (33, 34) and protein requirements (15, 35). It has been suggested that, when stable isotope methods are used, the determination of the protein intakes at which amino acid oxidation exponentially increases and/or the intake at which protein synthesis plateaus would be a more "physiological" measure of protein requirements than NBAL methods (35).

Urea excretion has been shown to increase in response to an elevated dietary protein intake in resistance-trained athletes (8, 28), suggesting that amino acid oxidation was increased. It is also known that the activity of branched-chain ketoacid dehydrogenase is increased in response to a high protein intake (1). This would facilitate the oxidation and subsequent excretion of the increased amino group delivered to the liver. These observations suggest that protein consumed in excess of need is oxidized and excreted and is not stored as lean body/muscle mass. Therefore, the point at which amino acid oxidation and/or urea production increases disproportionately to additional protein intake may help to further define protein requirements (35).

The purpose of the present study was to determine the protein requirements for both sedentary and strength-training men by examining NBAL and leucine turnover [oxidation, whole body protein synthesis (WBPS), flux,

TABLE 1. *Physical characteristics of the subjects*

	SA (n = 7)	S (n = 6)	P‡
Age, yr	21.6±1.5	24.5±3.6	NS
Weight, kg	85.4±7.3	84.9±11.4	NS
Height, cm	179.7±7.9	178.9±8.1	NS
Body fat, %	9.8±2.7	20.8±2.3	<0.001
Lean mass, kg	77.2±6.5	66.9±7.5	<0.01
Physical activity*			
Duration, h/wk	9.7±2.8	0.2±0.1	<0.001
Length, mo	6.6±3.7	NS	NS
Strength,† N·m	92.9±7.5	68.2±15.0	<0.001

Values are means ± SD for all diet periods. SA, strength athletes; S, sedentary control subjects. * Consistent weight training immediately before study. † Isometric dominant-arm flexor strength (joint angle = 100°). ‡ Between-group comparisons.

protein breakdown] in response to three levels of dietary protein intake [low protein (LP) = 0.86 $g \cdot kg^{-1} \cdot day^{-1}$; moderate protein (MP) = 1.40 $g \cdot kg^{-1} \cdot day^{-1}$; high protein (HP) = 2.4 $g \cdot kg^{-1} \cdot day^{-1}$].

METHODS

Subjects. Two groups of young males volunteered for the study, and informed written consent was obtained after subjects were advised of the risks and benefits of participation in accordance with the McMaster University Research Advisory Committee. One group (n = 7) habitually (>4 days/wk) performed exercise to increase strength (>70% of total exercise as circuit weight training) for ≥2 mo before the study and were designated as "strength athletes" (SA) because of the nature of their training. Two subjects were football players, two were rugby players who were lifting weights in the "off season," and the other three performed weight lifting exclusively for 3 mo before the study. A second group (n = 6) consisted of sedentary age-matched control subjects (S). All subjects were apparently healthy, as determined by medical history and semiquantitative urinalysis [pH, leukocytes, protein, glucose, ketones, blood, and hemoglobin (Chemstrip 5L, Boehringer Mannheim, Laval, Quebec)]. Group characteristics are given in Table 1.

Experimental protocol. All subjects participated in three different experiments, each of 13 days, with a mean 8-day (range 3- to 30-day) ad libitum washout diet period. During each experimental period the subjects were randomly (counterbalanced) assigned dietary protein at one of three levels: LP, a protein intake considered low for SA (28) and equal to the Canadian RNI for protein (0.86 g protein $\cdot kg^{-1} \cdot day^{-1}$) (20); MP, a moderate protein intake considered adequate for SA (1.4 g protein $\cdot kg^{-1} \cdot day^{-1}$) (28); and HP, a high intake considered to be a nutrient overload for SA (2.4 g protein $\cdot kg^{-1} \cdot day^{-1}$) (27, 28). The diets provided energy to match each individual's habitual intake as determined from weighed food records collected immediately before the study, for which nutrient analysis was performed using the computer program N-Squared Nutritionist III (Silverton, OR).

All subjects completed the three 13-day experimental periods. Each 13-day period began with a 6-day phase, when subjects were instructed to follow provided food exchanges to modify their habitual diet to match the protein and energy intake of the given diet period. They then consumed a 4-day meat-free diet from a checklist of foods that was identical in composition to that given in the final 3-day NBAL period. During the checklist period, subjects were given scales and measuring cups, and ~30% of their energy and protein was provided as a defined formula and supplement (see below). During the 4-day checklist period, it was determined from the food records that they consumed 99.5 ± 2.2% of the required protein intake.

During the final 3-day NBAL period, food was provided in a prepackaged form, with each item weighed to ±0.05 g on a digital scale (E400D, Ohaus, Florham Park, NJ). Each diet consisted of a combination of three major food categories: *1*) defined formula diet (Nutren, Clintec Nutrition, Mississauga, Ontario) supplying 25–50% of the total energy intake (E_{IN}); *2*) supplement of either glucose polymers (Polycose, Ross Laboratories, Columbus, OH; 0–30% of E_{IN}) or whey protein hydrolysate (Ross Laboratories; 0–32% of E_{IN}); and *3*) miscellaneous foods (spaghetti, jam, whole wheat bread, cookies, margarine, apple juice, lettuce, corn flake cereal, 2% milk, peanut butter; 35–65% of E_{IN}). Subjects were instructed to adhere strictly to each diet; they were permitted to consume water ad libitum and could have up to three cups of coffee and/or 750 ml of diet soda per day (except on the morning of the final test day). Subjects recorded consumption of all foods and liquids, and reported protein intake compliance was >98%. Compliance was maximized by having the subjects check off all foods immediately after consumption and report to the testing center at least twice over the NBAL period to deliver urine samples.

Group S maintained their physical inactivity during each 13-day diet treatment and remained sedentary (no activities except those required to perform activities of daily living) during each 4-day adaptation and NBAL period. *Group SA* performed their habitual activity during the adaptation phases (10 days, 70% circuit weights/30% rugby drills in 4 subjects and 100% circuit weights in the other 3 subjects). During *day 1* of the NBAL period, *group SA* performed their habitual circuit weight routine and kept a training log to minimize intertest variability. On *day 2* of the NBAL period, SA subjects performed a standardized whole body circuit-weight routine on a multistation apparatus (Global Gym, Weston, Ontario). The routine has recently been described in detail (28); it consisted of three groups of three exercises performed at 75% of each individual's one repetition maximum (3 sets of 10 repetitions). Each circuit group of three exercises was completed before the next circuit group was begun. The routine was completed in ~1 h, and subjects finished 23.0 ± 2.1 h before the midpoint of the leucine infusion protocol (see below) on NBAL *day 3*.

On *day 3* of the NBAL period, each subject reported to the testing center at 0830 h in the fed state (because of scheduling problems 1 subject in each group began at 1630 h under all 3 diet treatments). A portion of defined formula with or without glucose polymers or protein powder was consumed at 30-min intervals 2 h before the start of the infusion protocol (see below) and continuous

for 4.5 h. The total energy and protein consumed over the period was equivalent to 50% of each individual's daily protein and energy intake during the given diet allocation.

Infusion protocol. Subjects rested in a supine position at an ambient temperature of 23–25°C for 10 min before a 5-min gas sample was obtained in a 150-liter meteorological balloon. Over the 5-min period, CO_2 production, O_2 uptake, and the respiratory exchange ratio (RER) were determined from nine consecutive 20-s on-line measurements by use of an open-circuit computerized acquisition system (Alkin Systems, Hamilton, Ontario) interfaced with a pneumotachometer (Validyne, Northridge, CA), and a O_2 analyzer (Rapox, Bilthoven, The Netherlands), and a CO_2 analyzer (Hewlett-Packard, Palo Alto, CA). The system was calibrated with a known O_2-CO_2 gas mixture before each measurement. The intratest (39 × 4 measures every 30 min) coefficient of variation (CV) of this system was 7.1% and the intertest (13 subjects × 3 trials separated by ~20 days) CV was 7.9% for the determination of CO_2 production. Two gas samples were injected into 20-ml evacuated tubes (Vacutainer, Rutherford, NJ) for subsequent determination of background breath $^{13}CO_2$-to-$^{12}CO_2$ ratios as described below.

A 22-gauge plastic catheter was inserted into a dorsal hand vein, and an arterialized (60 ± 5°C, hot box) sample was obtained for subsequent determination of plasma α-[^{13}C]ketoisocaproic acid (α-KIC) enrichment and creatine phosphokinase (CPK) activity (see below). A 20-gauge plastic catheter was inserted in a retrograde manner into a contralateral proximal forearm vein for isotope infusion. Priming doses of 99% ^{13}C isotopes (MSD Isotopes, Pointe Claire, Quebec), L-[1-^{13}C]leucine (1 mg/kg) and [^{13}C]$NaHCO_3$ (0.295 mg/kg), were mixed immediately before administration and infused over 1 min to prime the respective pools (26, 31). These were immediately followed by a continuous infusion of L-[1-^{13}C]leucine (1 mg · kg^{-1} · h^{-1}) for 3.5 h, delivered by a calibrated syringe pump (Harvard Apparatus, Boston, MA). All isotopes were from the same batch, and leucine was diluted to ~15 mg/ml with sterile normal saline and microfiltered (0.2 μm, Gelman Sciences, Ann Arbor, MI) immediately before infusion.

After 2 h [to establish an isotopic plateau (26, 31)], gas and blood samples were obtained every 30 min for 90 min (t = 0, 30, 60, 90). At isotopic plateau, the CV of the plasma α-KIC enrichment was 6.4 and 3.8% for the breath enrichment over the duration of the experiment. All blood samples were collected in heparinized tubes, centrifuged immediately, and stored at −70°C for subsequent determination of α-[^{13}C]KIC enrichment. All gas samples were stored at room temperature, and the CO_2 was cryogenically trapped within 7 days for subsequent determination of breath $^{13}CO_2/^{12}CO_2$ enrichment by use of a gas-isotope ratio mass spectrometer (VG Isogas, SIRA 10, Cheshire, UK) at a mass-to-valence ratio (m/z) of 44/45, as described previously (26). Our intra-assay CV was 0.89%, and the interassay CV was 1.25% over a range of sample enrichments from 0.002 to 0.025 atom percent excess (APE).

Strength measurements. After the isotope infusion, the isokinetic elbow flexor strength was determined for the dominant arm at 0°/s (joint angle = 100°), 30°/s, and 180°/s on an isokinetic dynamometer (Cybex II, Ronkonkoma, NY).

Lean body mass. Lean body mass (LBM) was determined by hydrostatic weighing, and residual lung volume was estimated by helium dilution (W. E. Collins, Braintree, MA). The percent body fat was estimated from the equation: [495/(density)] − 450 (28). The subjects were weighed in the same shorts and tee shirt (measured weight of 300 g) each time on an electronic scale accurate to ±10 g (Mott Scales, Brantford, Ontario). The CV for determining LBM with this method was 1.3% for a total of 13 subjects tested >13 days apart with three determinations for each subject.

NBAL. During the 3-day NBAL period, daily urine samples were collected in 4-liter containers pretreated with 5 ml of glacial acetic acid. Samples were kept at <4°C by the subjects and delivered to the testing center within 48 h. After volume determination, aliquots were taken and stored at −70°C for subsequent determination of total nitrogen (TN), urea nitrogen (UN), and creatinine. Compliance was determined by creatinine excretion (no significant within-group diet differences), and successful adaptation to each diet was confirmed by daily UN measurements (no significant slope in day-to-day within-group measurements).

Fecal samples, collected between carmine markers, were kept frozen by the subjects and delivered to the testing center within 48 h of completion of the period. Each collection was weighed, diluted with distilled water (~50%), homogenized, lyophilized, ground, and stored for TN analysis.

Exercise and resting sweat nitrogen (N) losses at different protein intakes were estimated for SA from the mean data of several recent studies (27, 28). Resting sweat N losses were estimated for *group S* from recent data (28). Miscellaneous N losses (from semen, tooth brush, toilet paper, plate, hair, blood sampling, N_2 gas) were estimated at 140 mg N/day per subject in both groups (2).

Three diets (~3,000 kcal), representative of each of the three protein intakes, were prepared from the same type of foods that were consumed during the study period. Twenty percent of each dietary component was homogenized for 10 min, lyophilized, ground, and analyzed for TN and combustible energy content.

Apparent NBAL was calculated as the difference between nitrogen intake (N_{IN}) (diet) − N excretion (urine + feces + sweat + miscellaneous losses) (26).

Biochemical analyses. TN content of the diet, urine, and fecal samples was determined by use of the micro-Kjeldahl technique (K_2SO_4-Se catalyst, Kjeltec System, Hoganas, Sweden). The intra-assay CVs for diet, urine, and feces were 4.4, 5.8, and 3.8%, respectively, and the interassay CVs were 9.2, 1.1, and 5.0%, respectively. The ratio of the measured to calculated N was 1.06, 0.98, and 0.99 for the LP, MP, and HP diets, respectively (with the assumption of mixed proteins as 16% N by weight).

The combustible energy content of each diet was determined by adiabatic bomb calorimetry (Parr Instruments, Moline, IL). To convert from metabolizable energy (diet calculations) to gross energy, the percent me-

tabolizable energy contributions of carbohydrate, fat, and protein were multiplied by 1.00, 1.03, and 1.43, respectively (16). The intra-assay CV of the bomb calorimeter was 1.6%. The ratio of measured to calculated gross energy content of the LP, MP, and HP diets was 0.99, 0.95, and 1.08, respectively. All N and energy data given in tables and figures are expressed as the measured values.

Urine UN was determined using the urease-phenol method (kit 640, Sigma Chemical, St. Louis, MO). The intra- and interassay CVs were 4.0 and 7.6%, respectively. Creatinine was determined using a colorimetric picric acid method (kit 555, Sigma Chemical). These intra- and interassay CVs were 3.0 and 9.9%, respectively.

Plasma α-[^{13}C]KIC enrichment in *o*-trimethylsilyl quinoxalinol derivatives was determined by capillary gas chromatography-mass spectroscopy [GC-MS (model 5890, Hewlett-Packard GC, Avondale, PA); VG trio-2 MS (Cheshire, UK)] with electron impact ionization (m/z = 232.1/233.1), as described previously (26). The *o*-trimethylsilyl quinoxalinol derivative was prepared using a modification of standard procedures, as previously described (26). The intra- and interassay CVs were 0.81 and 0.42%, respectively.

Calculations. Whole body leucine kinetics-total leucine flux ($\dot{Q}$) was calculated using the reciprocal pool model (from α-[^{13}C]KIC values) (11) at isotopic plateau, as previously described (11, 14, 26). Leucine oxidation was calculated from established equations (14, 26), with use of a fed state bicarbonate retention factor of 0.83 (28).

The leucine oxidation values were corrected for changes in background breath $^{13}CO_2/^{12}CO_2$ enrichment due to the dietary intervention (24, 30) by studying two subjects under LP and HP diet conditions with no tracer infusion. The acute effect of the dietary intervention was to increase breath $^{13}CO_2$ enrichment significantly ($P < 0.01$) (LP, increase of 0.0018 APE during first 120 min and to 0.0027 APE by the end of 210 min; HP, increase of 0.0017 APE at first 120 min and to 0.0024 APE by end of 210 min) with no between-trial effects. During the LP diet these changes accounted for a maximum of 15.8%, and during the HP diet for 9.0%, of the APE changes during the leucine infusion. A mean of the two values was used for the MP diet trial.

The rate of appearance of endogenous leucine was determined from total leucine flux ($\dot{Q}$) − dietary leucine intake [I(1 − fo)]. The I value was derived from the expected rate of appearance of exogenous α-KIC into the hepatic vein after an oral feeding (fo) with casein and whey protein (85% of dietary leucine; I of 0.85) (3, 9). Therefore, the rate of endogenous leucine appearance was used as an indicator of whole body protein breakdown, and nonoxidative leucine disposal (NOLD) was used to estimate WBPS (14, 25) according to the model in which $\dot{Q}$ = synthesis (S) + oxidation (O) = dietary intake (I) + protein breakdown (B) (14), with an average tissue leucine content of 590 μmol leucine/g protein (14, 31). The validity of NOLD as an indicator of WBPS has been established in rats by use of a protein synthesis inhibitor (25).

Statistical analyses. The physical characteristics of the subjects were compared using an independent *t* test. All other data were analyzed using a two-way between-within split-plot analysis of variance with diet (LP vs. MP vs. HP) as the within-subject variable and group (SA vs. S) as the between-subject variable. When a significant within-group *F* ratio was obtained, the location of pair-wise differences was performed with the Tukey post hoc test. $P \leq 0.05$ was taken to indicate significance. All data are means ± SD.

TABLE 2. *Habitual and treatment dietary characteristics*

	SA (n = 7)	S (n = 6)	P^e
Energy intake, kcal · kg^{-1} · day^{-1}			
HAB	43.0±9.4	30.0±4.6	<0.01
LP	42.1±8.7	31.6±5.4	<0.01
MP	43.7±9.0	31.3±3.9	<0.01
HP	43.6±9.2	34.0±2.7	<0.01
Protein intake, g · kg^{-1} · day^{-1}			
HAB	1.77±0.38[a]	1.21±0.29[a]	<0.05
LP	0.89±0.02[b]	0.90±0.02[b]	NS
MP	1.42±0.08[a]	1.41±0.04[c]	NS
HP	2.32±0.09[c]	2.37±0.03[d]	NS
%Energy intake			
HAB	(16, 49, 32, 3)	(16, 48, 34, 2)	NS
LP	(08, 65, 27, 0)	(11, 66, 23, 0)	NS
MP	(14, 60, 27, 0)	(19, 56, 25, 0)	NS
HP	(22, 48, 30, 0)	(28, 42, 30, 0)	NS

Values are means ± SD. HAB, habitual intake; LP, low protein; MP, medium protein; HP, high protein. %Energy intake of total from protein, carbohydrate, fat, and alcohol, respectively, in parentheses. [a-d] Within-subject comparisons; values within each subject group with different superscript letters are significantly ($P < 0.05$) different from each other. [e] Between-subject comparisons.

RESULTS

Dietary analysis. The data for the habitual intakes and the intakes of subjects during each diet phase are given in Table 2. There were no significant effects of diet treatment on total E_{IN}, but, as expected, the E_{IN} was significantly ($P < 0.01$) greater for SA than for S. SA habitually consumed more protein than did the S ($P < 0.05$). There were no differences between the groups in measured protein intake during the experimental protocol. For the SA, the habitual protein intake was not different from the MP diet but was greater ($P < 0.05$) than for the LP diet and less ($P < 0.05$) than for the HP diet. For S, the habitual protein intake was significantly different ($P < 0.05$) from all of the diet treatments. There were no significant group differences for the $\%E_{IN}$ of protein, carbohydrate, and fat. The $\%E_{IN}$ from protein increased significantly ($P < 0.05$) and the $\%E_{IN}$ from carbohydrate decreased significantly ($P < 0.05$) for both groups from LP to MP to HP diets (Table 2).

LBM, CPK activity, and strength. There were no effects of diet treatment on either LBM or urinary creatinine excretion for either group (Table 3). The CV for creatinine excretion over the diet treatments was 4.3% for SA and 2.9% for S. Both LBM and urinary creatinine excretion values were significantly greater for SA than for S ($P < 0.01$). Isokinetic strength was greater in SA than S on all diet treatments and at all velocities, but there were no diet treatment effects on strength for either group

TABLE 3. *Effects of dietary treatment and activity on lean body mass, urinary creatinine excretion, isokinetic strength, and plasma CPK activity*

	SA (n = 7)	S (n = 6)	P*
Lean body mass, kg			
LP	77.3±7.1	66.6±7.9	<0.01
MP	77.7±6.6	67.3±8.4	<0.01
HP	76.6±6.7	67.9±8.5	<0.01
Creatinine excretion, mmol/day			
LP	14.0±3.6	10.7±2.4	<0.01
MP	14.6±3.8	11.3±3.4	<0.01
HP	13.4±2.4	11.2±2.2	<0.01
Isokinetic arm-flexor strength, N·m			
0°/s			
LP	92.7±6.1	65.5±15.4	<0.01
MP	95.9±6.0	64.0±15.6	<0.01
HP	90.0±9.7	75.1±14.2	<0.05
30°/s			
LP	81.4±10.5	54.8±15.6	<0.01
MP	76.6±11.2	53.0±15.0	<0.05
HP	74.1±7.4	55.5±10.6	<0.01
180°/s			
LP	55.3±4.6	39.3±12.9	<0.05
MP	58.6±7.2	41.5±7.7	<0.05
HP	55.9±7.1	43.3±6.1	<0.05
CPK activity, U/l			
LP	163±124	60±50	<0.01
MP	163±99	57±41	<0.01
HP	186±132	66±51	<0.01

Values are means ± SD. CPK, creatine phosphokinase. Isokinetic strength decreased significantly ($P < 0.05$) for both groups as speed increased. * Between-subject comparisons; there were no significant within-subject (diet effect) differences for any of these variables.

(Table 3). SA had significantly ($P < 0.01$) greater plasma CPK activity than S, but there were no diet treatment effects (Table 3). This finding helped to confirm the consistency of SA training and the fact that S did not do any strenuous activity.

NBAL. The mean NBAL was negative for SA on the LP diet (5 of 7 subjects negative) and was positive for S (Table 4). N_{IN}, urinary N excretion, TN excretion, and NBAL increased significantly ($P < 0.01$) from LP to MP to HP diets for both groups, with no between-group differences (Table 4). For SA there was no effect of N_{IN} (i.e., diet) on fecal N loss, but for S, fecal N losses were greater for the HP diet than for MP and LP diets ($P < 0.05$; Table 4). Adaptation to the N_{IN} during each of the NBAL periods was confirmed by the lack of a significant day-to-day change in urinary UN excretion. In addition, the slopes of the lines joining the days for each diet, in each subject group, were not significantly different from zero. Urinary UN excretion was significantly ($P < 0.01$) greater for HP than for MP and LP diets (Fig. 1). Completeness of the 3-day pooled urine sample between diet periods was confirmed by the low CVs for urinary creatinine excretion of 4.2 and 2.9% for SA and S, respectively, and by the lack of a significant ($P > 0.05$) within-treatment day-to-day effect.

The protein intake to achieve NBAL was interpolated from multiple regression analysis of N_{IN} ($g \cdot kg^{-1} \cdot day^{-1}$) vs. NBAL (mg $N \cdot kg^{-1} \cdot day^{-1}$) for both groups (SA: NBAL = −71.6 + 50.9 (protein intake), SD = 9.99, r^2 = 0.58; S: NBAL = −16.8 + 24.3 (protein intake), SD = 5.47, r^2 = 0.55; Fig. 2). The protein intake to achieve zero NBAL was 1.41 $g \cdot kg^{-1} \cdot day^{-1}$ for SA and 0.69 $g \cdot kg^{-1} \cdot day^{-1}$ for S (104% greater for SA). With a safety margin of ±1 SD, the requirement was calculated to be 1.76 and 0.89 g protein $\cdot kg^{-1} \cdot day^{-1}$ for SA and S, respectively (98% greater for SA).

Leucine turnover. WBPS was significantly greater for SA than for S ($P < 0.05$). There was a significant interaction for WBPS between treatment and subject groups ($P < 0.05$). Post hoc analysis revealed that there was a significant increase ($P < 0.01$) in WBPS for SA from LP to both MP and HP, with no significant difference between MP and HP diets (Fig. 3*A*). There were no differences in leucine oxidation between the groups, and both demonstrated significantly ($P < 0.001$) increased oxidation for the HP diet compared with both MP and LP diets (Fig. 3*B*). There was a significant correlation between leucine oxidation and urinary urea excretion (r = 0.965, $P < 0.001$).

$\dot{Q}$ was significantly ($P < 0.001$) greater for SA than S. $\dot{Q}$ for SA was significantly greater for HP than for MP and LP ($P < 0.01$) and for MP than for LP ($P < 0.05$). $\dot{Q}$ for S was significantly greater for HP than for MP and LP ($P < 0.05$) (Fig. 4*A*). Protein breakdown was significantly ($P < 0.01$) greater for SA than for S (Fig. 4*B*).

The protein turnover data were also analyzed relative to LBM of the subjects. The values for WBPS were SALP = 187.0, SAMP = 235.9, SAHP = 261.3, SLP = 153.8, SMP = 185.2, and SHP = 190.2 $mg \cdot kg^{-1} \cdot h^{-1}$.

TABLE 4. *Nitrogen balance summary*

	N Intake, g/day	N Excretion, g/day				
		Urine	Feces	Sweat + miscellaneous losses	Total losses	Balance
SALP	12.2±1.0[a]	11.0±2.4[a]	2.0±0.5[a]	1.64	14.6±2.6[a]	−2.4±3.1[a]
SAMP	19.9±2.4[b]	15.3±2.4[b]	2.1±0.5[a]	1.87	19.2±2.3[b]	+0.7±1.5[b]
SAHP	31.9±3.2[c]	23.5±3.1[c]	2.6±0.2[a]	1.99	28.1±3.2[c]	+3.8±2.3[c]
SLP	12.2±1.6[a]	9.8±1.3[a]	1.5±0.1[a]	0.49	11.8±1.3[a]	+0.4±1.2[a]
SMP	19.6±2.9[b]	15.3±2.5[b]	1.7±0.2[a]	0.87	17.9±2.6[b]	+1.7±1.2[b]
SHP	32.2±4.3[c]	25.0±4.2[c]	2.3±1.4[b]	1.42	28.8±4.2[c]	+3.4±1.4[c]

Values are means ± SD. N, nitrogen. SALP, SAMP, and SAHP, strength athletes on low protein, moderate protein, or high protein diets, respectively; SLP, SMP, and SHP, sedentary subjects on LP, MP, or HP diets, respectively. Sweat and miscellaneous values were obtained from Refs. 2, 26, and 27. Within-group comparisons; values within groups with different superscript letters are significantly ($P < 0.01$) different from each other.

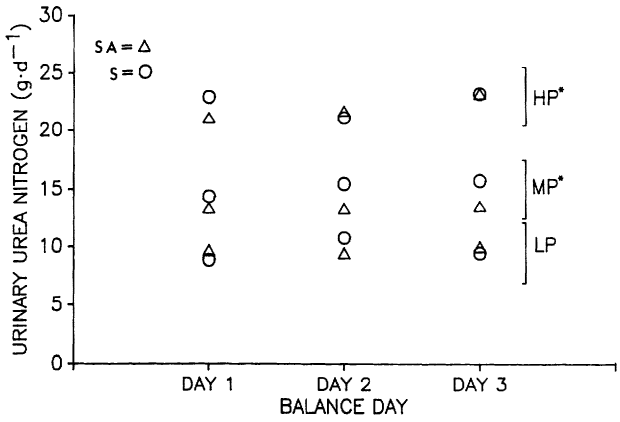

FIG. 1. Effect of dietary intervention and habitual activity (subject group) on daily urinary urea nitrogen excretion during nitrogen balance (NBAL) periods. SA, bodybuilders; S, sedentary control subjects; LP, low protein diet; MP, medium protein diet; HP, high protein diet. * Urinary urea nitrogen excretion increased significantly ($P < 0.01$) from LP to MP to HP diets. There were no significant day-to-day changes in urinary urea excretion, indicating dietary adaptation.

The values for protein breakdown were SALP = 205.1, SAMP = 266.6, SAHP = 252.8, SLP = 180.9, SMP = 178.9, and SHP = 173.1 mg·kg^{-1}·h^{-1}. The values for $\dot{Q}$ were SALP = 159.4, SAMP = 201.6, SAHP = 266.7, SLP = 148.7, SMP = 178.8, and SHP = 224.8 μmol·kg^{-1}·h^{-1}. There were no effects on the results of the statistical analyses, but the between-group differences were not as great. For WBPS, protein breakdown, and $\dot{Q}$, LBM accounted for 24, 28, and 42%, respectively, of the observed between-group differences. These results are important for the interpretation of the data, but the values in the text and figures have been expressed relative to total body weight for interstudy comparison purposes. It should be noted that WBPS and protein breakdown are expressed in milligrams per kilogram per day, whereas leucine oxidation and $\dot{Q}$ are expressed in micromoles per kilogram per day.

DISCUSSION

The current findings demonstrated that young men performing predominantly strength exercise required a habitual dietary protein intake greater than that required by age-matched sedentary control subjects and greater than the protein intake considered safe for 97.5% of the Canadian population (0.86 g·kg^{-1}·day^{-1}) (20). The protein turnover data supported the calculated protein intake determined by NBAL methods. In addition, the protein turnover data provided information to support the argument that an excessive (2.4 g protein·kg^{-1}·day^{-1}) protein intake compared with an adequate (1.4 g protein·kg^{-1}·day^{-1}) does not result in an increased WBPS in strength-trained athletes. Dietary protein consumed in excess of requirements is oxidized as energy and is not deposited as body proteins, a finding that supports previous observations (33, 34).

The lower rate of WBPS for SA on the LP diet compared with MP and HP diets provided evidence that a protein intake of 0.89 g·kg^{-1}·day^{-1} was inadequate for young men performing strength exercise. This level of protein likely resulted in an accommodated state for SA, which might lead to suboptimal protein (muscle) gains in the long term. In addition, five of the seven SA were in negative NBAL on the LP diet, which confirmed that such a protein intake level was suboptimal during strength training for most individuals. The protein intake for the LP diet was very close to the Canadian RNI of 0.86 g protein·kg^{-1}·day^{-1} for men ≥19 yr of age (2a). This implies that the Canadian RNI for protein may not be an optimal or "safe" dietary intake for young men habitually performing strength exercise.

For the SA group, the MP diet (1.41 g protein·kg^{-1}·day^{-1}) was likely close to the optimal physiological requirement (adaptation), whereas the HP diet (2.32 g protein·kg^{-1}·day^{-1}) represented a nutrient overload (34) inasmuch as an increase in WBPS from MP to HP diets did not occur. The fact that the S group did not show a significant increase in WBPS with increasing protein intake led us to conclude that the LP diet (0.90 g protein·kg^{-1}·day^{-1}) was at or above their physiological protein requirement. This is an expected finding for the S subjects because the Canadian RNI for protein is 0.86 g·kg^{-1}·day^{-1} (2a). In humans, the determination of the dietary protein intake at which WBPS plateaus may be a useful indicator of protein requirements (33–35). In fed sedentary males, WBPS increased with graded protein intake, and no plateau was observed (17). However, intakes of 0.1, 0.6, and 1.5 g protein·kg^{-1}·day^{-1} were used in that study, and the 0.6 g·kg^{-1}·day^{-1} level may have been marginal, resulting in an accommodated state with a reduction in WBPS despite a positive NBAL (clearly the 0.1 g·kg^{-1}·day^{-1} diet was deficient in protein) (17). The results of the present and the aforementioned (17) studies support the prediction that a protein intake at about the Canadian RNI (0.86 g·kg^{-1}·day^{-1}) (2a) is appropriate for sedentary men in the age range studied (19–30 yr).

The quantitative measures of neither LBM nor arm muscle strength showed reductions consequent to the 13 days on the LP diet. This may indicate either a preservation of muscle mass at the expense of other body protein compartments or an inability to detect LBM changes over the 13-day period with the methods employed. Both explanations are plausible. First, it is known that rat skel-

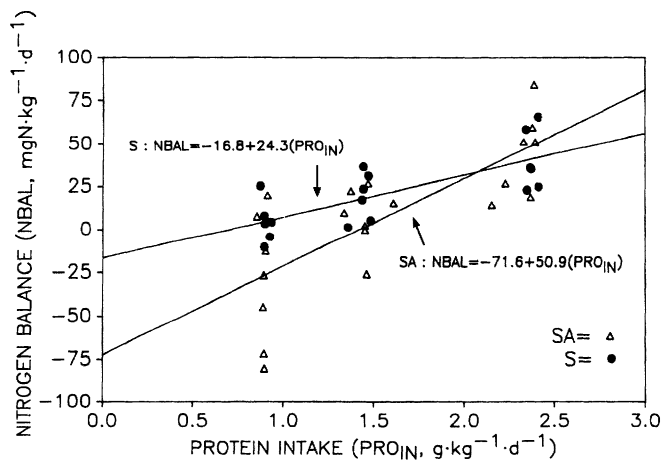

FIG. 2. Plot of protein intake vs. nitrogen balance for both groups. Predicted protein intake for 0 NBAL was interpolated from generated regression equations. See Fig. 1 legend for definitions and text for calculated protein intakes.

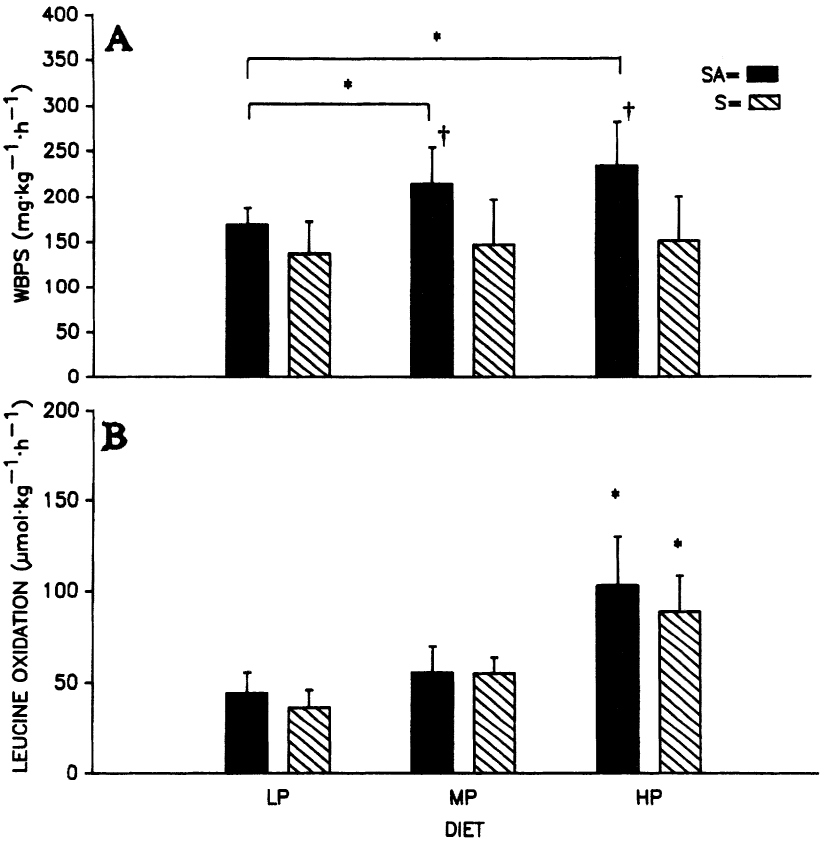

FIG. 3. *A*: whole body protein synthesis (WBPS). * For SA, WBPS increased significantly on diets MP and HP vs. diet LP. † WBPS was significantly ($P < 0.05$) greater for SA than for S. *B*: leucine oxidation. * Leucine oxidation was significantly ($P < 0.01$) greater for both groups on HP diet vs. MP and LP diets. There were no between-group differences.

etal muscle can hypertrophy under the extreme conditions of starvation, diabetes, and hypophysectomy in response to tenotomy of synergistic muscles (4, 5). Thus muscle contraction per se may have a sparing effect on skeletal muscle at the expense of other tissues during periods of protein deprivation. Second, the NBAL data would predict a loss of 1.13 kg LBM for the SA group on the LP (vs. MP) diet, but the measured LBM decreased

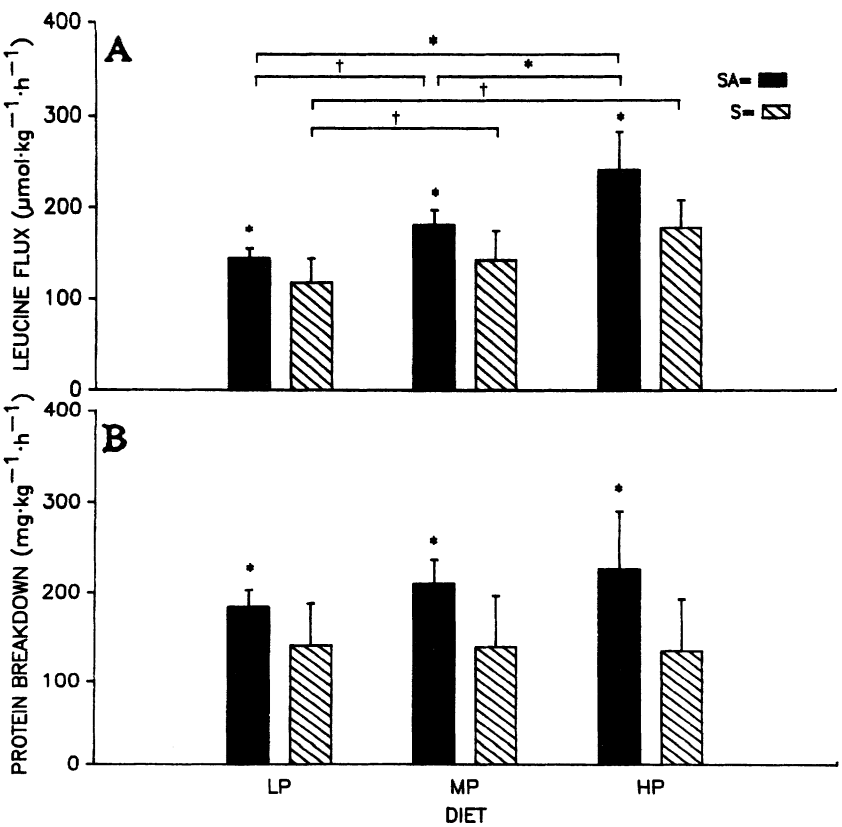

FIG. 4. *A*: total leucine flux. Flux was significantly (* $P < 0.01$) greater for SA than for S. For S, flux was significantly († $P < 0.05$) greater on MP and HP diets than on LP diet. For SA, flux was significantly († $P < 0.05$) greater for MP than LP diet and was significantly (* $P < 0.01$) greater for HP than for either MP or LP diet. *B*: protein breakdown. Rate of endogenous leucine appearance was significantly (* $P < 0.01$) greater for SA than for S.

by 0.4 kg, leaving 0.73 kg unaccounted for. Because the CV for the determination of LBM was 1.3%, with a mean LBM of 77.5 kg, the discrepancy of 0.73 kg could be accounted for by variation in the LBM determination alone.

If we assume that muscle mass was maintained on the LP diet for the SA, the reduction in WBPS per se is of concern. In the long term a negative impact on LBM may become apparent, and reductions in WBPS are characteristic of short-term starvation at rest (10) and during exercise (12). It has been suggested that reductions in WBPS and amino acid flux may have a negative impact on health status because of reductions in lymphocyte amino acid flux (19) and a reduction in the availability of metabolic intermediates for biosynthetic pathways (19, 33).

In support of the fact that the HP diet was a nutrient overload was the finding of a significant increase in leucine oxidation for both groups on the HP diet compared with the LP or MP diets. This demonstrated that protein consumed in excess of needs was oxidized as energy rather than stored as lean tissue. Young et al. (35) stated that the determination of the protein intake at which amino acid oxidation starts to increase significantly (inflection point) may be indicative of an adequate or safe protein intake and thus can be useful as a determinant of dietary protein requirements. From our data, we are not able to determine the point at which amino acid oxidation increased, but it was between 1.4 and 2.4 $g \cdot kg^{-1} \cdot day^{-1}$ for both groups.

A high correlation was found between leucine oxidation and urinary urea excretion ($r = 0.97$), which would be expected inasmuch as leucine oxidation is preceded by intracellular transamination with subsequent hepatic ureagenesis. Wolfe et al. (31) reported that during very light endurance (~30% maximal O_2 consumption) exercise, stable isotope measurements of leucine oxidation and ureagenesis were discordant and suggested that the measurement of either ureagenesis or leucine oxidation was in error. The results of our study support the fact that leucine oxidation and ureagenesis are closely linked processes, and the results of Wolfe et al. (31) may merely indicate that the two processes are not cotemporal.

The finding of a significantly (42%) greater WBPS for SA than for S was expected, because it has been well documented that people who engage in heavy resistance training have an increased muscle mass (13, 28) due to an increased muscle protein synthesis (32). The increased WBPS demonstrated for SA (24 h postexercise) is likely to be a chronic phenomenon, because similar absolute WBPS levels are present both during and for 2 h after circuit weight-training exercise (26). Part of the difference in WBPS between the groups was likely due to the greater LBM of the SA group, for LBM per se accounted for ~24% of the between-group differences (see RESULTS).

In addition to increased WBPS, a significant increase in protein breakdown was observed in the SA compared with the S group. Simultaneous increases in protein synthesis and breakdown have been observed during hypertrophy of skeletal muscle in animal models (5). Part of the increase can also be explained by the greater LBM of SA, for this accounted for 28% of the between-group differences in protein breakdown.

The calculated protein intake for zero NBAL was 1.41 $g \cdot kg^{-1} \cdot day^{-1}$ for SA and 0.69 $g \cdot kg^{-1} \cdot day^{-1}$ for S; with a safety margin of ±1 SD, the estimated requirements were 1.76 and 0.89 g protein $\cdot kg^{-1} \cdot day^{-1}$, respectively. Regression analysis of NBAL data to determine protein requirements has been well established (2a, 3, 15, 23, 28) and was used in the current study so that interstudy comparisons could be made. At zero NBAL, the estimate was 104%, and with ±1 SD the estimate was 98% greater for SA than for S. Inclusion of ±1 SD, in contrast to the usual ±2 SD used by national (2a) and international (3) bodies in setting protein recommendations, is made on the basis of the larger biological variation obtained with small subject numbers and with protein intakes considerably above and below the estimated requirement, as previously described (28). The use of ±2 SD would have yielded a falsely greater protein requirement for these reasons. A further consideration in the interpretation of the estimated protein required is that the protein quality of the HP diet was slightly greater by addition of whey protein powder than for either the MP or LP diet, which would result in a slight underestimation of protein requirements. Given these considerations, the calculated protein requirements must be considered estimates and not definitive levels. Because the SA group had a significantly greater LBM than the S group, it may be informative to express the protein requirements relative to LBM rather than to total mass, which would make the zero intercept protein requirements 1.56 and 0.88 g protein $\cdot$ kg $LBM^{-1} \cdot day^{-1}$ for SA and S, respectively. The difference between the two groups would be 77% greater for SA than for S on the basis of LBM, but 104% greater on the basis of total mass. Although correction for differences in LBM does bring the estimates of protein requirements between the groups closer together, the difference is still substantial and provides further support for the concept that the protein requirement for SA was elevated primarily because of habitual activity and not merely an increased LBM.

The fact that the estimate of 0.89 $g \cdot kg^{-1} \cdot day^{-1}$ for the S group in this study is very close to the Canadian RNI of 0.86 $g \cdot kg^{-1} \cdot day^{-1}$ provides further evidence in support of the validity of the calculated NBAL-derived estimates. It should also be noted that the NBAL study was conducted with excellent subjective (checklist protein compliance >98%) and objective/biochemical (mean CV of creatinine excretion over diet treatments = 3.6%; Table 3) evidence of compliance. The adaptation period of 10 days should be adequate for both an increasing and decreasing protein intake (21), and adaptation was confirmed by the lack of a significant slope in the urinary UN excretion over each of the 3-day NBAL periods by either group (Fig. 1). In addition, the mean energy intake for both S [32.3 kcal $\cdot kg^{-1} \cdot day^{-1}$ (22)] and SA [43.2 kcal $\cdot kg^{-1} \cdot day^{-1}$ (28)] would be adequate for their respective activity patterns. The absence of a significant change in weight, LBM, and estimated body fat for either group provided further support for the adequacy of the dietary E_{IN}.

The estimated protein requirements from the NBAL

data are in agreement with those estimated from the tracer data. For SA, the WBPS and leucine oxidation predicted that the protein requirement would be at or just above 1.41 g protein · kg^{-1} · day^{-1}, and the NBAL was 1.76 (±1 SD) g · kg^{-1} · day^{-1}. The WBPS was 8.7% lower on the MP than on the HP diet (a nonsignificant difference), which may indicate that the true protein requirement is closer to the NBAL-derived value of 1.76 g · kg^{-1} · day^{-1}; however, this can be determined only by studying leucine turnover between these ranges of protein intake. The WBPS data for S predicted that the protein requirement would be at or below 0.90 g · kg^{-1} · day^{-1}, and the NBAL-derived value was 0.89 g · kg^{-1} · day^{-1}. It was hypothesized a priori that the S group would have significantly greater leucine oxidation on the MP than on the LP diet, indicating that the MP diet delivered excessive protein, but this was not found to be significant. However, the oxidation did increase 50% for S and only 25% for SA from the LP to the MP diet.

From the NBAL data, it was estimated that the SA group would have gained ~1.1 kg of LBM, when in fact they lost 1.1 kg, leaving a discrepancy of 2.2 kg. Hence the LBM data did not support the classical interpretation of the positive NBAL data. A failure to consider the leucine turnover data and rely only on the NBAL data may lead one to conclude that an excessive protein intake is of ergogenic benefit to those involved in strength/resistance training. The persistently positive NBAL observed in this study has been demonstrated (21, 28) and commented upon (7, 34) before and appears to result from an inherent error in the method. In a recent study of endurance athletes who consumed protein intake above calculated requirements, NBAL was positive and WBPS plateaued (15). The present study and that of Meredith et al. (15) support the previously stated weaknesses of the NBAL method (7, 34) and further demonstrate the need to combine NBAL methodology with tracer techniques to gain a comprehensive understanding of protein metabolism to determine the protein requirements for humans (15, 17, 33–35).

The protein requirement for the SA group was 79 and 8.6% greater than previously derived protein intake estimates for elite and novice bodybuilders, respectively (27, 28). The protein requirements in these two studies were determined in a manner identical to those in the present study. The 79% greater protein requirements of this study compared with the earlier study of elite bodybuilders (28) probably relate to a phenomenon of adaptation to the stress of activity. The athletes in this study had experience with weights for 3–9 mo, whereas the elite bodybuilders (28) trained for 3 yr. The adaptation of NBAL to the stress of training was shown in a short-term longitudinal study by Gontzea et al. (6). In a previous study (27) we found that novice athletes actively increasing muscle mass with a free-weight body-building program and the subjects in the current study required about the same amount of dietary protein. The novice bodybuilders studied previously (27) could have been increasing muscle mass at a greater rate than the subjects in the current study. Some of the drills habitually performed by the subjects in the current study (i.e., windsprints/throwing football) would have some aerobic component; this may have slightly increased the protein requirements in four of the seven SA subjects (28). However, during the NBAL period only circuit weight training was performed.

The present study clearly demonstrated the need to combine NBAL studies with tracer methods to comprehensively determine protein requirements for humans. The estimated protein requirement for athletes performing circuit weight training (SA) was ~98% greater than for age-matched sedentary control subjects (1.76 vs. 0.89 g · kg^{-1} · day^{-1}, respectively), and these estimates were in agreement with the interpretation of the tracer data. Protein consumed in excess of requirements resulted in a plateau in WBPS and a significant increase in leucine oxidation but did not result in an increased accretion of LBM in bodybuilders.

We acknowledge the gifts of the Nutren formula diet from Clintec Nutrition (Mississauga, Ontario) and the glucose polymers (Polycose) and whey protein concentrate from Ross Laboratories (Montreal, Canada and Columbus, OH).

M. A. Tarnopolsky is supported by a National Institute of Nutrition (Canada) postdoctoral fellowship. S. A. Atkinson holds a Career Scientist Award at McMaster University supported by the Ontario Ministry of Health.

Address for reprint requests: S. A. Atkinson, Faculty of Health Sciences, McMaster University, 1200 Main Street West, Rm. 3V42, Hamilton, Ontario L8N 3Z5, Canada.

Received 27 August 1991; accepted in final form 26 May 1992.

REFERENCES

1. BLOCK, K. P., S. SOEMITRO, B. W. HEYWOOD, AND A. E. HARPER. Activation of liver branched-chain α-keto acid dehydrogenase in rats by excess of dietary amino acids. *J. Nutr.* 115: 1550–1561, 1985.
2. CALLOWAY, D. H., A. C. F. ODELL, AND S. MARGEN. Sweat and miscellaneous nitrogen losses in human balance studies. *J. Nutr.* 101: 775–781, 1971.
2a. DEPARTMENT OF NATIONAL HEALTH AND WELFARE. *Nutrition Recommendations: The Report of the Scientific Review Committee.* Ottawa, Canada: Dept. Natl. Health Welfare, Canadian Government Publishing Centre, 1990.
3. FAO/WHO/UNU. *Energy and Protein Requirements.* Geneva: WHO, 1985. (WHO Tech. Rep. Ser. 724)
4. GOLDBERG, A. L. Influence of insulin and contractile activity on muscle size and protein balance. *Diabetes* 28, *Suppl.* 1: 18–24, 1979.
5. GOLDSPINK, D. F., P. J. GARLICK, AND M. A. MCNURLAN. Changes in protein turnover in the soleus muscle during compensatory growth. *Biochem. Soc. Trans.* 10: 169–170, 1982.
6. GONTZEA, I., P. SUTZESCU, AND S. DUMITRACHE. The influence of adaptation to physical effort on nitrogen balance in man. *Nutr. Rep. Int.* 22: 231–236, 1975.
7. HEGSTED, D. M. Balance studies. *J. Can. Diet. Assoc.* 36: 110–117, 1975.
8. HICKSON, J. F., K. HINKELMANN, AND D. L. BREDLE. Protein intake level and introductory weight training exercise on urinary total nitrogen excretions from untrained men. *Nutr. Res.* 8: 725–731, 1988.
9. HOERR, R. A., D. E. MATTHEWS, D. M. BIER, AND V. R. YOUNG. Leucine kinetics from [$^2H_3$]- and [^{13}C]leucine infused simultaneously by gut and vein. *Am. J. Physiol.* 260 (*Endocrinol. Metab.* 23): E111–E117, 1991.
10. HOFFER, L. J., AND R. A. FORSE. Protein metabolic effects of a prolonged fast and hypocaloric refeeding. *Am. J. Physiol.* 258 (*Endocrinol. Metab.* 21): E832–E840, 1990.
11. HORBER, F. F., C. M. HARBER-FEYDER, S. KRAYER, W. F. SCHWENK, AND M. W. HAYMOND. Plasma reciprocal pool specific activity predicts that of intracellular free leucine for protein synthesis. *Am. J. Physiol.* 257 (*Endocrinol. Metab.* 20): E385–E399, 1989.
12. KNAPIK, J., C. MEREDITH, B. JONES, R. FIELDING, V. YOUNG, AND W. EVANS. Leucine metabolism during fasting and exercise. *J. Appl. Physiol.* 70: 43–47, 1991.

13. MACDOUGALL, J. D., D. G. SALE, J. R. MOROZ, G. C. B. ELDER, AND H. HOWALD. Mitochondrial volume density in human skeletal muscle following heavy resistance training. *Med. Sci. Sports* 11: 164–167, 1979.
14. MATTHEWS, D. E., K. J. MOTIL, D. K. ROHRBAUGH, J. F. BURKE, V. R. YOUNG, AND D. M. BIER. Measurement of leucine metabolism in man from a primed, continuous infusion of L-[1-^{13}C]leucine. *Am. J. Physiol.* 238 (*Endocrinol. Metab.* 1): E473–E479, 1980.
15. MEREDITH, C. N., M. J. ZACKIN, W. R. FRONTERA, AND W. J. EVANS. Dietary protein requirements and body protein metabolism in endurance-trained men. *J. Appl. Physiol.* 66: 2850–2856, 1989.
16. MERRILL, A. L., AND B. K. WATT. Energy value of foods: basis and derivation. *Agriculture Handbook No. 74.* Washington, DC: Dept. of Agriculture, 1973.
17. MOTIL, K. J., D. E. MATTHEWS, D. M. BIER, J. F. BURKE, H. N. MUNRO, AND V. R. YOUNG. Whole body leucine and lysine metabolism: response to dietary protein intake in young men. *Am. J. Physiol.* 240 (*Endocrinol. Metab.* 3): E712–E721, 1981.
18. NAIR, K. S., D. HALLIDAY, AND R. C. GRIGGS. Leucine incorporation into mixed skeletal muscle protein in humans. *Am. J. Physiol.* 254 (*Endocrinol. Metab.* 17): E208–E213, 1988.
19. NEWSHOLME, E. A., B. CRABTREE, AND M. S. M. ARDAWI. Glutamine metabolism in lymphocytes: its biochemical, physiological and clinical importance. *Q. J. Exp. Physiol.* 70: 473–489, 1985.
21. ODDOYE, E. A., AND S. MARGEN. Nitrogen balance studies in humans: long-term effect of high nitrogen intake on nitrogen accretion. *J. Nutr.* 109: 363–377, 1979.
22. PELLETT, P. L. Food energy requirements in humans. *Am. J. Clin. Nutr.* 51: 711–722, 1990.
23. PELLETT, P. L. Protein requirements in humans. *Am. J. Clin. Nutr.* 51: 723–737, 1990.
24. SCHOELLER, D. A., P. D. KLEIN, J. B. WATKINS, T. HEIM, AND W. C. MACLEAN, JR. ^{13}C abundances of nutrients and the effect of variations in ^{13}C isotopic abundances of test meals formulated for $^{13}CO_2$ breath tests. *Am. J. Clin. Nutr.* 33: 2375–2385, 1980.
25. SCHWENK, W. F., E. RUBANYI, AND M. W. HAYMOND. Effect of a protein synthetic inhibitor on in vivo estimates of protein synthesis in dogs. *Am. J. Physiol.* 252 (*Endocrinol. Metab.* 15): E595–E598, 1987.
26. TARNOPOLSKY, M. A., S. A. ATKINSON, J. D. MACDOUGALL, B. B. SENOR, P. W. R. LEMON, AND H. P. SCHWARCZ. Whole body leucine metabolism during and after resistance exercise in fed humans. *Med. Sci. Sports Exercise* 23: 326–333, 1991.
27. TARNOPOLSKY, M. A., P. W. R. LEMON, J. D. MACDOUGALL, AND S. A. ATKINSON. Effect of body building exercise on protein requirements (Abstract). *Can. J. Appl. Sport Sci.* 15: 22S, 1990.
28. 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.
29. TORUN, B., N. S. SCRIMSHAW, AND V. R. YOUNG. Effect of isometric exercises on body potassium and dietary protein requirements of young men. *Am. J. Clin. Nutr.* 30: 1983–1993, 1977.
30. WOLFE, R. R., J. H. F. SHAW, E. R. NADEL, AND M. H. WOLFE. Effect of substrate intake and physiological state on background $^{13}CO_2$ enrichment. *J. Appl. Physiol.* 56: 230–234, 1984.
31. WOLFE, R. R., M. H. WOLFE, E. R. NADEL, AND J. H. F. SHAW. Isotopic determination of amino acid-urea interactions in exercise in humans. *J. Appl. Physiol.* 56: 221–229, 1984.
32. YARASHESKI, K. E., J. A. CAMPBELL, M. J. RENNIE, J. O. HOLLOSZY, AND D. M. BIER. Effect of strength training and growth hormone administration on whole body and skeletal muscle leucine metabolism (Abstract). *Med. Sci. Sports Exercise* 22: S85, 1990.
33. YOUNG, V. R., AND D. M. BIER. A kinetic approach to the determination of human amino acid requirements. *Nutr. Rev.* 45: 289–298, 1987.
34. YOUNG, V. R., D. M. BIER, AND P. L. PELLETT. A theoretical basis for increasing current estimates of amino acid requirements in adult man, with experimental support. *Am. J. Clin. Nutr.* 50: 80–92, 1989.
35. YOUNG, V. R., N. S. SCRIMSHAW, AND D. M. BIER. Whole body protein and amino acid metabolism: relation to protein quality evaluation in human nutrition. *J. Agric. Food Chem.* 29: 440–446, 1981.