Protein requirements and muscle mass/strength changes during intensive training in novice bodybuilders

PETER W. R. LEMON, MARK A. TARNOPOLSKY, J. DUNCAN MACDOUGALL, AND STEPHANIE A. ATKINSON
Schools of Biomedical Sciences and Physical Education, Recreation, and Dance, Kent State University, Kent, Ohio 44242; and Departments of Pediatrics, Physical Education, and Medicine, McMaster University, Hamilton, Ontario L8N 3Z5, Canada

LEMON, PETER W. R., MARK A. TARNOPOLSKY, J. DUNCAN MACDOUGALL, AND STEPHANIE A. ATKINSON. *Protein requirements and muscle mass/strength changes during intensive training in novice bodybuilders.* J. Appl. Physiol. 73(2): 767–775, 1992.—This randomized double-blind cross-over study assessed protein (PRO) requirements during the early stages of intensive bodybuilding training and determined whether supplemental PRO intake (PRO_{IN}) enhanced muscle mass/strength gains. Twelve men [22.4 ± 2.4 (SD) yr] received an isoenergetic PRO (total PRO_{IN} 2.62 $g \cdot kg^{-1} \cdot day^{-1}$) or carbohydrate (CHO; total PRO_{IN} 1.35 $g \cdot kg^{-1} \cdot day^{-1}$) supplement for 1 mo each during intensive (1.5 h/day, 6 days/wk) weight training. On the basis of 3-day nitrogen balance (NBAL) measurements after 3.5 wk on each treatment (8.9 ± 4.2 and −3.4 ± 1.9 g N/day, respectively), the PRO_{IN} necessary for zero NBAL (requirement) was 1.4–1.5 $g \cdot kg^{-1} \cdot day^{-1}$. The recommended intake (requirement + 2 SD) was 1.6–1.7 $g \cdot kg^{-1} \cdot day^{-1}$. However, strength (voluntary and electrically evoked) and muscle mass [density, creatinine excretion, muscle area (computer axial tomography scan), and biceps N content] gains were not different between diet treatments. These data indicate that, during the early stages of intensive bodybuilding training, PRO needs are ~100% greater than current recommendations but that PRO_{IN} increases from 1.35 to 2.62 $g \cdot kg^{-1} \cdot day^{-1}$ do not enhance muscle mass/strength gains, at least during the 1st mo of training. Whether differential gains would occur with longer training remains to be determined.

recommended protein intakes; weight-lifting exercise; protein supplements; nutritional supplements; strength exercise

THE PROTEIN (PRO) requirements for strength athletes have been a subject of debate for many years (8, 18, 27). Current recommendations for men >19 yr of age are 0.86 (25) and 0.85 $g \cdot kg^{-1} \cdot day^{-1}$ (13). There is no additional allowance for those involved in regular physical activity. However, evidence is growing to support the concept that both endurance (5, 14, 22) and strength (9, 11, 31, 34) exercise increase PRO requirements.

Suggested PRO intakes (PRO_{IN}) for strength athletes range widely, from levels just above (31) to approximately four times (9) current recommendations. This wide discrepancy may relate to differences in training intensity, energy and/or carbohydrate content of the diet, adaptation to a given training load (early training vs. habitual) (18), or possibly the confounding effect of anabolic steroids (16).

Strength/bodybuilder athletes habitually consume PRO_{IN} as high as 2–4 $g \cdot kg^{-1} \cdot day^{-1}$ (9, 29, 31), despite equivocal evidence to suggest that this quantity of PRO would have a positive effect on lean body/muscle mass accretion (11, 27, 31, 33). Although excessive PRO_{IN} could potentially have negative health consequences (4, 38), this has not been documented in otherwise healthy strength athletes. However, high PRO_{IN} may decrease the percent energy intake ($\%E_{IN}$) from carbohydrates (CHO) and increase the $\%E_{IN}$ from fats, both of which are associated with increased health risk (25). In addition, PRO is an expensive macronutrient (cost of animal protein is usually in excess of isoenergetic amounts of fat or CHO foodstuffs). Thus it is important to determine the PRO requirement for strength athletes to avoid a state of nutrient excess and, conversely, to avoid a nutrient deficiency state, with the associated potential negative health consequences, including impaired immune function (1), decreased oxygen transport capacity [sports anemia (36)], and/or suboptimal muscle growth.

The purpose of this study was to assess the PRO requirements for strength athletes performing intensive resistance exercise in the early stages of training and to determine whether a very high PRO_{IN} (2.62 $g \cdot kg^{-1} \cdot day^{-1}$) would result in greater muscle mass/strength gains than a lower PRO_{IN} (1.35 $g \cdot kg^{-1} \cdot day^{-1}$).

METHODS

Subjects. Informed consent was obtained from 14 healthy young male subjects in accordance with the guidelines of the Kent State University Human Subjects Review Board and the McMaster University Research Project Advisory Committee for Clinical Studies. The subjects were active physical education students who had not participated in a regular weight-training program for 12 mo before the study. None reported use of anabolic steroids. Two of the 14 subjects who began were unable to complete the study (one because of an automobile accident and one as a result of relocation to another city). The characteristics of the remaining 12 subjects are given in Table 1.

Experimental protocol. Each of the subjects completed two 1-mo dietary treatment periods (double-blind counterbalanced cross-over design) separated by a 7-day ad libitum diet washout period. For the entire 2-mo period,

TABLE 1. *Subject characteristics*

Age, yr	22.4±2.4
Weight, kg	81.9±11.3
Height, cm	180.8±6.1
Body fat,* %	10.1±6.1

Values are means ± SD for 12 subjects. * Determined from hydrostatic weighing and equation of Brozek et al. (6).

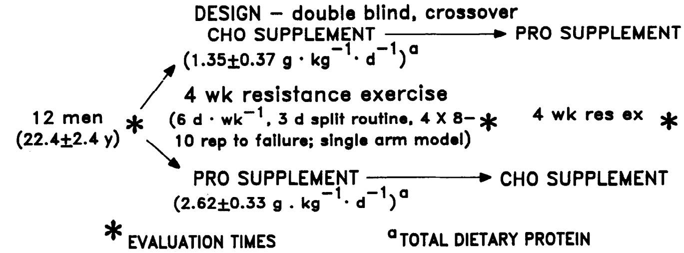

FIG. 1. Experimental design. CHO, carbohydrate; PRO, protein. Values are means ± SD.

each subject consumed, in addition to his habitual diet, a daily isoenergetic supplement (1.5 g/kg) of either CHO (maltodextrin) or PRO (calcium caseinate and free amino acids). To facilitate ingestion of the quantity of supplement required, each subject consumed 30 g of free amino acids (Table 2) or maltodextrin in gelatin capsules and the remaining supplement in a flavored drink.

The composition and energy content of each subject's habitual diet were assessed (Table 3) before the study and during the 4th wk of each treatment by use of 3-day computerized diet record assessments (Analyze, McMaster University, Hamilton, Ontario). On the basis of these dietary assessments, a 3-day diet, similar in both composition and energy content (Table 3), was prepared and distributed in prepackaged form to be consumed during the nitrogen balance (NBAL) phase (see below).

The NBAL phase was completed after 3.5 wk on each treatment to allow sufficient time for adaptation to the different PRO_{IN}. During this phase, all subjects were instructed to consume only the foods and liquids provided. Compliance (>97%) was maximized by providing each day's food in separate bags and by having the subjects check off, from a list, all foods immediately after consumption.

The subjects participated in an intensive (6 days/wk) bodybuilding program that was supervised by professional bodybuilders. The training was a 3-day split routine (*day 1*: chest/back; *day 2*: legs; *day 3*: shoulders/arms) with 1 day of rest per week. Each training session included a warm-up set followed by four sets of ≤10 repetitions per set (5–8 exercises) with use of the heaviest weight possible [70–85% of the individual's one repetition maximum (1 RM)]. Subjects trained with partners and were encouraged to increase weight as their strength progressed. Before the study, the subjects received instruction in the proper technique of each lift and completed 1 wk of training with increasing resistance in an attempt to minimize soreness once the actual training began. The training program involved all major muscle groups; in addition, the effect of the supplement-training interaction on muscle was assessed directly by use of a single-arm training model (each subject trained the elbow flexors of one arm while on the CHO supplement and those of the other arm while on the PRO supplement).

TABLE 2. *Composition of amino acid capsules*

Amino Acid	Content, mg/5 capsules
Leucine	610
Aspartate	340
Glycine	230
Glutamine	140
Histidine	75
Threonine	90
Alanine	80
Arginine	140
Tyrosine	390
Valine	490
Methionine	70
Cystine	270
Isoleucine	10
Tryptophan	80
Lysine	420

Values were determined in duplicate by high-pressure liquid chromatography.

Measurements of the effects of diet and/or training on indexes of muscular development were assessed before and after a 1-mo training period on each supplement and included lean body mass (hydrostatic weighing); midarm and midthigh circumferences; computerized axial tomography (CAT) scans of the midarm and midthigh; NBAL; 1 RM contraction strength for bench press and leg squat exercises; neuromuscular properties of the forearm flexors, including peak twitch tension (PTT), maximal isometric contraction force (MVC), posttetanic PTT (30 s after MVC), and percent motor unit activation (%MUA); and total nitrogen content from a biceps brachii muscle biopsy (Fig. 1).

Lean body mass. Lean body mass (LBM) was determined by hydrostatic weighing. Residual lung volume was assessed using a helium-dilution method (W. E. Collins, Braintree, MA). Percent body fat was estimated by the equation of Brozek et al. (6). Subjects were weighed using an electronic scale accurate to ±10 g (Mott Scales, Brantford, Ontario). By this method the coefficient of variation (CV) for determining LBM was 1.2% for 31 subjects tested >10 days apart with two or three determinations for each subject.

CAT scans and circumferences. Detailed morphometric anthropometry was completed for the midthigh and midarm by use of CAT scanning (fourth-generation high-resolution scanner, Ohio Nuclear, 20/20). A single scan was taken midway between the acromion process and the lateral epicondyle for the arm to be trained and another midway between the superior aspect of the greater trochanter and the lateral knee jointline for the thigh measurement. Negative photographic slides were made from the CAT scan films, from which muscle, limb, and fat cross-sectional areas were determined using planimetric computerized digital analysis (Sigma Scan, Jandel Scientific, Sausalito, CA). In addition, muscle density (in Hounsfield units) was assessed at three randomly selected places in both the arm and thigh musculature. All CAT scans were analyzed by the same individual, who was unaware of either dietary treatment (CHO vs. PRO) or training (pre vs. post) conditions. Limb circumference measurements were made using the same landmarks and a steel tape measure.

1 RM. One RM was taken as the maximal weight (free weights) lifted (bench press or leg squat) by a concentric contraction after a brief warm-up.

Neuromuscular function. This was determined for the forearm flexors (primarily biceps brachii) by use of a custom-made dynamometer. The arm was firmly strapped to the measuring device with Velcro straps at a fixed elbow joint angle of 110° and the shoulder at 90° of flexion. The initial arm (for 6 subjects the dominant arm, for 6 the nondominant) to be trained was tested before and after the first 1-mo training period, and the contralateral arm was tested before and after the second training period. The measurements included PTT, MVC, and %MUA [the latter of the biceps brachii by use of the interpolated twitch technique (2)].

Muscle biopsy. A percutaneous needle biopsy sample (~100 mg) was obtained from the lower-lateral quadrant of the biceps brachii under local anesthesia (12). Four biopsies were taken (2 from each muscle: 1 before and 1 after each 1-mo training period). The sample was immediately quenched in liquid nitrogen for subsequent analysis of total nitrogen (TN) content by use of flash combustion-gas chromatography-thermal conductivity (Perkin-Elmer 2400 CHN elemental analyzer) with acetanilide as a standard.

NBAL. From the individual computerized analysis of the 3-day food records (Analyze, McMaster University) collected just before each of the NBAL periods (after 3 wk on the dietary intervention), an individual diet was prepared for each subject to match his mean E_{IN} and be representative of the $\%E_{IN}$ derived from fat, CHO, and PRO. Subjects continued to consume either their PRO or CHO supplements, and these were included in the determination of their E_{IN}. The subjects were issued their diet: 50% solid foods and 50% defined formula liquid (Ensure, Ross Laboratories, Columbus, OH) in a prepackaged form with each item weighed to ±0.05 g (E400D, OHAUS, Florham Park, NJ). The solid food component consisted of one of five distinct diets composed of various amounts of the following foods: eggs, milk, spaghetti, spaghetti sauce, whole wheat bread, butter, jam, bologna, apples, orange juice, peanut butter, crackers, chocolate chip cookies, and granola bars. Twenty percent of each dietary component from each of the five diets was homogenized for 10 min, lyophilized, ground, and analyzed for TN by the micro-Kjeldahl method and for gross energy content by adiabatic bomb calorimetry. During each 3-day NBAL period, urine was collected in 4-liter containers containing 5 ml glacial acetic acid, and a 72-h fecal collection was made between carmine markers. Carmine markers (500 mg/subject) were provided in gelatin capsules. Total urine volumes were measured, and aliquots of urine were kept at −70°C until subsequent determination of TN, urea nitrogen (UN), and creatinine. Fecal samples were weighed and homogenized with an equal weight of deionized water, and an aliquot was lyophilized and analyzed for TN content. Sweat N loss was measured in three subjects on the CHO treatment and in four subjects on the PRO treatment after a typical training session by use of the washdown method, as previously described (20). Resting sweat N losses were estimated from the results of a recent study on bodybuilding athletes with similar PRO_{IN} (31). An aliquot of the sweat washdown water was frozen at −70°C until analysis for urea N content. The individual sweat values were used for those tested, and the mean values were used for the other subjects. Miscellaneous N losses (semen, toothbrush, toilet paper, plate, hair, N_2 gas) were estimated at 140 mg N/day for each subject in both groups (7). Apparent NBAL was calculated as the difference between N_{IN} (diet) and N excretion (urine + feces + sweat + miscellaneous).

Biochemical analyses. Total N content of the diets, urines, and feces was determined using the micro-Kjeldahl technique. The intra-assay CV for diets, urines, and feces was 4.4, 5.8, and 3.8%, whereas the interassay CV was 9.2, 1.1, and 5.0%, respectively. The mean ratio of the measured-to-calculated N content was 0.91 ± 0.04 for the five standard solid food diets (assuming mixed proteins-16% N by weight). The gross energy content of each diet was determined by adiabatic bomb calorimetry (Parr Instruments, Moline, IL). The intra-assay CV of the bomb calorimeter was 3.1%. To convert from metabolizable energy (diet calculations) to gross energy, the percent metabolizable energy contribution of CHO, fat, and PRO was multiplied by 1.00, 1.03, and 1.43, respectively (23). The ratio of measured-to-calculated gross energy content of each of the five standard solid food diets was 0.94 ± 0.05. All N and energy data given during the NBAL period are corrected for the measured values.

Urine urea N was determined using the urease-phenol method (kit 640, Sigma Chemical, St. Louis, MO). The intra- and interassay CVs were 4.6 and 9.0%, respectively. Creatinine was determined using a colorimetric picric acid method (kit 555, Sigma Chemical). The intra- and interassay CVs were 3.0 and 9.9%, respectively.

Statistical analyses. The effect of diet (CHO vs. PRO) on NBAL and creatinine excretion was determined using a paired *t* test. Regression analysis of NBAL vs. PRO_{IN} was performed to determine the PRO_{IN} necessary for zero NBAL. The effect of the training (pre vs. post variable) and diet treatment (CHO vs. PRO variable) on various indexes of muscle function (neuromuscular function, 1 RM strength tests) and anthropometry (body/muscle density, CAT scans, limb circumferences) was determined using a repeated-measures analysis of variance (SAS Institute). Values are means ± SD.

RESULTS

E_{IN} values were not significantly different between diet periods (Table 3). The PRO_{IN} and percent contribution of PRO to total energy were greater ($P < 0.001$) for the adaptation- and NBAL-PRO periods than for the habitual intake, adaptation-, and NBAL-CHO periods. PRO_{IN} was significantly lower during the NBAL-CHO period than during the adaptation-CHO ($P < 0.05$) and the habitual intake ($P < 0.01$) periods. The habitual intake was not significantly different from that during the adaptation-CHO period. The $\%E_{IN}$ from carbohydrate during

TABLE 3. *Diet summary*

Period	Energy Intake					Protein Intake, $g \cdot kg^{-1} \cdot day^{-1}$
	$kJ \cdot kg^{-1} \cdot day^{-1}$	% PRO	% CHO	% Fat	% EtOH	
Habitual	165±11	14[a]	52[d]	32	2	1.44±0.24[f]
AD-PRO	172±12	26[b]	43[e]	31	0	2.62±0.33[g]
AD-CHO	174±12	13[a]	55[d]	30	2	1.35±0.37[f]
NBAL-PRO	165±13	28[b]	41[e]	31	0	2.67±0.34[g]
NBAL-CHO	161±39	10[c]	60[d]	30	0	0.99±0.29[e]

Values are means ± SD. PRO, protein; CHO, carbohydrate; EtOH, alcohol. Habitual, ad libitum consumption in week before study; AD-PRO and AD-CHO, 1-mo adaptation period on each diet; NBAL-PRO and NBAL-CHO, 3-day nitrogen balance period on each diet. Unlike letters significantly ($P < 0.05$) different from other periods.

the NBAL- and adaptation-PRO periods were both significantly ($P < 0.05$) lower than that of the other periods. Fat and alcohol intake were nonsignificantly different across all periods (Table 3).

The NBAL was significantly more positive for the PRO than for the CHO period (+8.9 ± 4.2 vs. −3.4 ± 1.9 g/day, $P < 0.001$), and all subjects were in negative NBAL while on the CHO treatment (Table 4). In addition, N intake, urinary N losses, sweat + miscellaneous N losses, and total N losses were greater for the PRO than for the CHO treatments ($P < 0.001$; Table 4). Urinary urea losses accounted for 92 and 95% of total urinary N losses on the CHO and PRO treatments, respectively. Adequate adaptation to the N intake during each of the NBAL periods is apparent by the lack of day-to-day change in urinary UN excretion (CV during the CHO and PRO treatments was 6.2 and 4.1%, respectively; Fig. 2).

The PRO_{IN} to achieve NBAL was extrapolated from linear regression analysis of PRO_{IN} ($g \cdot kg^{-1} \cdot day^{-1}$) vs. NBAL (g/day) for each diet treatment (interpolated from both treatments). For the CHO supplement ($y = 0.13x + 1.43$; $r = 0.82$) and for assessment of both treatments ($y = 0.11x + 1.53$; $r = 0.86$) there were significant ($P < 0.01$) correlations; however, there appeared to be no relationship ($r = 0.11$) for the PRO supplement (Fig. 3). The PRO_{IN} to achieve zero NBAL (requirement) was 1.43 $g \cdot kg^{-1} \cdot day^{-1}$ for the CHO treatment and 1.53 $g \cdot kg^{-1} \cdot day^{-1}$ when both groups were combined. The recommended intake (requirement + 2 SD) was computed as 1.63 and 1.73 g $PRO \cdot kg^{-1} \cdot day^{-1}$, respectively.

There were no effects of dietary treatment (PRO vs. CHO) or training (pre vs. post) on body weight (PRO 81.95, CHO 81.95 kg), percent body fat (PRO 10.1, CHO 10.2%), body density (PRO 1.0772, CHO 1.0770 g/ml), LBM (PRO 73.7, CHO 73.6 kg), urinary creatinine excretion (PRO 10.9, CHO 11.5 mmol/day), or biceps muscle N concentration (PRO 14.86, CHO 14.70 g/100 g dry wt^{-1}). Values represent the mean of the pre − post measures (Fig. 4).

There were no effects of diet or training on the neuromuscular properties of the forearm flexors: PTT (PRO 8.05, CHO 8.25 N · m), posttetanic twitch torque (PRO 11.9, CHO 12.2 N · m), and %MUA (PRO 88.5, CHO 90.4%). Values represent the mean of pre − post measures (Fig. 5). There were no effects of diet treatment on indexes of absolute strength; however, training had a significant ($P < 0.05$) effect on maximal voluntary forearm flexor contraction strength [PRO 74.4–77.9 (+4.7%), CHO 72.4–77.4 (+6.9%) N · m], 1 RM bench press strength [PRO 84.6–89.6 (+5.9%), CHO 83.7–91.5 (+9.3%) kg], and 1 RM leg squat strength [PRO 123.7–146.4 (+18.4%), CHO 133.3–136.5 (+2.4%) kg; Fig. 5].

There were no effects of diet treatment on midarm circumference, midarm CAT scan muscle flexor cross-sectional area (Fig. 6), or muscle density (Table 5); however, there was a significant ($P < 0.05$) effect of training on these variables: midarm circumference [PRO 31.5–32.3 (+2.5%), CHO 31.8–32.1 (+0.9%) cm], midarm flexor cross-sectional area [PRO 27.1–29.2, (+7.7%), CHO 26.4–29.0 (+9.8%) cm^2; Fig. 6], and muscle density [arm: PRO 67.3–70.7 (+5.0%), CHO 68.1–72.3 (+6.2%) Houndsfield units; thigh: PRO 60.7–64.3 (+5.9%), CHO 61.5–64.6 (+5.0%) Houndsfield units; Table 5].

There were no effects of diet treatment or training on arm subcutaneous fat cross-sectional area (PRO 16.05, CHO 15.65 cm^2), midthigh circumference (PRO 52.95, CHO 53.15 cm), thigh muscle cross-sectional area (PRO 354.3, CHO 357.7 cm^2), and thigh subcutaneous fat cross-sectional area (PRO 85.65, CHO 85.65 cm^2). Values represent the mean of pre − post measures; Fig. 6).

DISCUSSION

Despite the general belief among bodybuilders and other strength-trained athletes that high PRO_{IN} (2–4 $g \cdot kg^{-1} \cdot day^{-1}$) is necessary during training, few investigations have used objective laboratory as well as performance measures to determine whether this degree of supplemental PRO is necessary or beneficial (18). The purpose of this investigation was to assess the PRO requirements for strength athletes performing intensive resistance exercise in the early stages of training and to determine whether a very high PRO_{IN} (2.62 $g \cdot kg^{-1} \cdot day^{-1}$) would result in greater muscle mass/strength gains than a lower PRO_{IN} (1.35 $g \cdot kg^{-1} \cdot day^{-1}$). Novice bodybuilders were selected in an attempt to maximize observable gains over an experimentally managable duration. Although the question of PRO supple-

TABLE 4. *Nitrogen balance summary*

Treatment Group	N Intake	N Excretion				N Balance
		Urine	Feces	Sweat*	Total	
CHO	12.8±3.1	12.5±1.2	2.0±0.9	1.6±0.2	16.2±1.7	−3.4±1.9
PRO	34.8±4.6†	21.1±5.6†	2.2±1.0	2.5±0.3†	25.8±6.0†	+8.9±4.2†

Values are means ± SD in grams per day. N balance = N intake − total N excretion. CHO, carbohydrate supplement; PRO, protein supplement.
* Includes measured exercise loss + rest estimate (31) and miscellaneous losses (7). † Significantly greater ($P < 0.001$) than CHO.

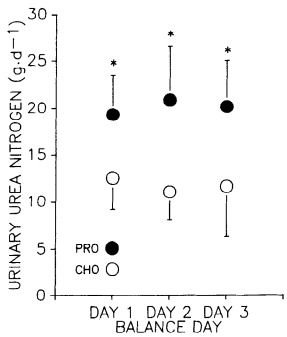

FIG. 2. Effect of dietary treatment on daily urinary urea N excretion (means ± SD). PRO, protein supplement; CHO, carbohydrate supplement. * Significantly greater ($P < 0.01$) than CHO treatment.

mentation is equally important for experienced bodybuilders, we believed that any effect of supplementation on these individuals might be less obvious, because most of the potential gain would already have occurred. CHO was selected to compare with PRO not only because it is the major fuel for intense exercise (32) but also because excess E_{IN} can lead to increased LBM (35).

Despite the relatively short duration of the bodybuilding program in the present study, it is apparent from increased voluntary strength, arm circumferences, and both muscle density and cross-sectional area that a training effect occurred. Such gains were anticipated and essential to determine whether one supplement was more effective than the other.

Although the subjects received a PRO_{IN} exceeding current recommendations during the CHO treatment (157% for 3.5 wk and 115% for the 3-day NBAL period), all were in negative NBAL (−3.4 ± 1.9 g N/day). Because the E_{IN} was more than adequate (Table 3), these data indicate that this PRO_{IN} (0.99 ± 0.29 g/kg) was insufficient for these novice bodybuilders. Thus, over time, this PRO_{IN} would be expected to reduce gains in muscle mass/strength. When established linear regression methodology (13) is used, the PRO_{IN} necessary to produce NBAL in these subjects (Fig. 3) would be ≥1.43 $g \cdot kg^{-1} \cdot day^{-1}$ (166% of current recommendations). To minimize the chances of deficiency in ~95% of the population, PRO_{IN} recommendations are typically based on the mean PRO_{IN} for zero NBAL + 2 SD. For the CHO treatment and for both treatments combined, the recommended PRO_{IN} for the bodybuilders in this study would be 1.63 and 1.73 $g \cdot kg^{-1} \cdot day^{-1}$, respectively. This recommendation not only exceeds the current recommendation for sedentary individuals (by ~100%), but it also exceeds (by ~40%) that measured for elite bodybuilders under similar conditions (31).

Given the limitations of a short-term NBAL study, this recommendation needs to be considered carefully. The number of subjects studied ($n = 12$) is small relative to the total numbers on which current recommendations are based; however, the CV around the zero NBAL was only 13.3%, which compares favorably with the CV of 11.4 and 17% found in determination of the PRO requirements for endurance athletes (22, 31) and with the 12.5% used in setting current recommendations for sedentary individuals (25). When we consider this information and the fact that bodybuilders represent a population totally different from that on which the established recommendations are based, the values reported in this study (1.63–1.73 $g \cdot kg^{-1} \cdot day^{-1}$) appear reasonable.

The increased requirement for the novice bodybuilder may be indicative of a greater stress in the early stages of training to which the athlete subsequently adapts, as has been found for endurance exercise (15, 36). For bodybuilding exercise, there is probably greater myofibrillar PRO turnover and a greater increase in PRO synthesis in the early stages of training, which results in net PRO synthesis and hypertrophy, whereas the experienced bodybuilder is likely at a plateau with relatively little net muscle accretion. This is apparent in the modest gains observed for strength (+4%) and muscle fiber diameter (+5.9%) in elite lifters over a 2-yr period (17) compared with the strength (+7.9%) and midarm flexor area (+8.8%) gains in our novice lifters after only 1 mo of training.

The observed nonsignificant correlation between PRO_{IN} and NBAL with the PRO treatment is interesting and suggests that, at some point above ~1.5–1.8 $g \cdot kg^{-1} \cdot day^{-1}$, this relationship becomes curvilinear. Therefore it appears that the PRO_{IN} of this group (and that of many strength athletes) represents a nutritional overload. This is consistent with animal data demonstrating that when rats are fed PRO exceeding their requirement, muscle PRO synthesis and gain plateau (28), and the extra PRO is metabolized through an upregulated urea cycle (38). This probably means that a considerable portion of the amino acids consumed above ~1.5–1.8 g $PRO \cdot kg^{-1} \cdot day^{-1}$ would be of questionable benefit to the bodybuilder, because they would be oxidized rather than stored as PRO.

Although the PRO supplement produced slightly greater gains in some measures (body density, midthigh muscle area, and leg strength), these differences were small and not statistically greater than those observed with the CHO supplement. These data indicate that during the 1st mo of intensive bodybuilding training, if dietary PRO_{IN} = 1.35 $g \cdot kg^{-1} \cdot day^{-1}$ (157% of current recommendations), isoenergy CHO or PRO supplementation leads to similar gains in muscle mass/strength. This is surprising, given the NBAL results, because over a

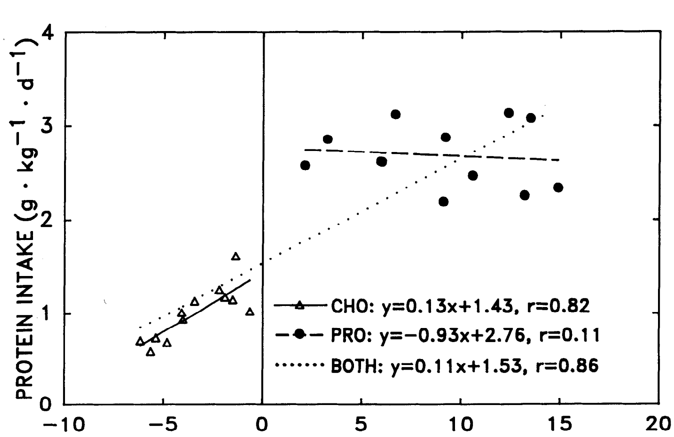

FIG. 3. Predicted protein intake for zero nitrogen balance. Extrapolation was based on regression line calculated for each treatment separately (interpolation when both treatments were combined).

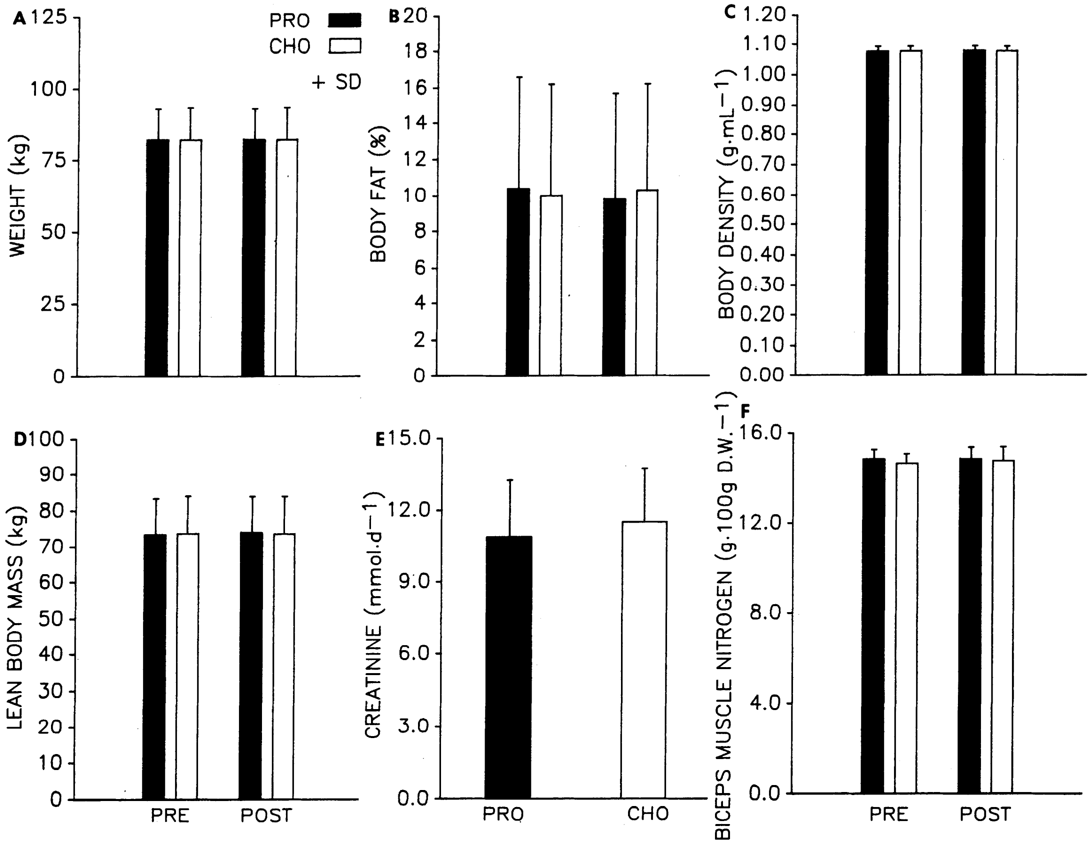

FIG. 4. Selected anthropometric measurements and biochemical indexes of muscle mass: body weight (*A*), percent body fat calculated from body density (Ref. 6; *B*), body density (from hydrostatic weighing; *C*), lean body mass (calculated from *A* and *B*; *D*), urinary creatinine excretion (mean of each 3-day nitrogen balance period; *E*), biceps brachii muscle total nitrogen content (*F*). PRE, pretraining; POST, posttraining.

1-mo period the observed positive NBAL (+8.9 g N/day) should have resulted in a net muscle accretion of ~7.8 kg (assuming wet muscle tissue is 20% protein and protein is 16% N by weight). This increase would have been detectable using the body density method employed (hydrostatic weighing) and definitely did not occur. Although the explanation for this discrepancy is unclear, at least two possibilities exist. First, despite the negative NBAL in the CHO treatment, the anabolic stimulus of the exercise program may have made adequate N available for skeletal muscle from endogenous N stores, e.g., gastrointestinal tract, liver, and kidneys (24). If so, short-term muscle gains during both treatments could be similar. However, such N mobilization (~95 g) could not continue indefinitely, and eventually one would expect to observe reduced gains in muscle mass/strength with the CHO treatment. To confirm this possibility, exercise studies with longer training programs and measures of labile protein mobilization are needed. Second, markedly positive NBAL results without significant increases in tissue mass have been reported previously (26, 31). These could be due, at least partially, to limitations of the NBAL technique, including *1*) overestimation of intake and underestimation of losses, *2*) slow physiological adaptation to altered PRO_{IN}, *3*) confounding effects of E_{IN}, *4*) true N accretion below the limits of detection, and *5*) loss of N as molecular N_2. We believe significant methodological errors in the present study were unlikely, given the lack of difference in creatinine excretion over each of the 3-day NBAL periods, the use of carmine markers to ensure completeness of stool collections, and the measurement of sweat losses. Compliance was maximized by requiring that subjects consume solid palatable foods and use diet checklists. Furthermore, the lack of day-to-day variation in urinary urea excretion provides an objective measure that subjects maintained a consistent PRO_{IN} over the NBAL period. However, a greater contribution from any unmeasured route of N loss during the PRO treatment could explain at least part of the observed very high NBAL. It is also unlikely that the subjects were still adapting to the diets after 3.5 wk of the diet and exercise program. Moreover, there was no evidence of a trend in day-to-day urinary urea excretion over the NBAL period (if subjects were not adapted, urea excretion would be increasing for PRO and decreasing for CHO treatments). Nor could the positive NBAL on the PRO supplement be explained by greater E_{IN}, for these were nearly identical during both NBAL periods (165 ± 13 vs. 161 ± 39 $kJ \cdot kg^{-1} \cdot day^{-1}$). This intake exceeds by 32 $kJ \cdot kg^{-1} \cdot day^{-1}$ current recommendations for a sedentary subject of the age studied (13). This surplus is two to three times the energy needed for the type of training program utilized (30). As well, the contribution of CHO to E_{IN} was greater for the CHO (60%) than for the PRO treatment (41%), which would tend to have a PRO-sparing effect for the CHO and not the PRO supplement. Such differences in CHO content may have important practical implications for the individual who trains on a regular basis,

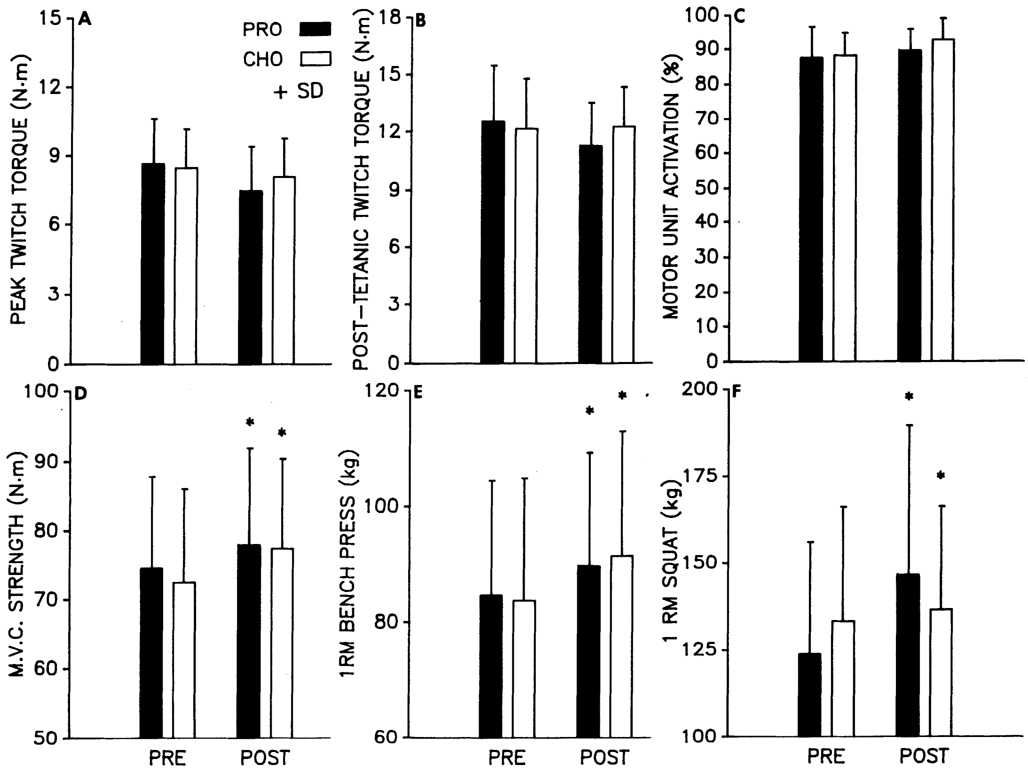

FIG. 5. Strength measurements: peak evoked twitch torque (*A*), peak evoked posttetanic twitch torque (*B*), percent motor unit activation (*C*), maximal voluntary contraction (MVC) strength of arm flexors (*D*), one repetition maximum (1 RM) bench press strength (*E*), 1 RM leg squat strength (*F*). * Significant ($P < 0.05$) training effect.

because it has been demonstrated that male bodybuilders have reduced muscular endurance when performing weight lifting on a hypoenergetic moderate PRO-low CHO (50% E_{IN}) diet compared with a low PRO-high CHO (75% E_{IN}) diet with the same energy content (34). As mentioned above, the hydrostatic weighing method would have detected a 7.8-kg increase in LBM had it occurred. Molecular N_2 losses were estimated (7), and it is unlikely that these could explain the magnitude of the observed differences. In summary, there is no clear explanation for the high NBAL observed on the PRO diet, but it does not appear to represent accretion of skeletal muscle mass. Given that NBAL experiments measure only the net balance between whole body synthesis and breakdown over a period of days, that NBAL can be established at a number of different PRO_{IN} (accommodation to low intakes), and the technical and interpretative concerns expressed above, it may be that amino acid turnover studies provide a more appropriate method for study of the dynamics of protein metabolism (37). For bodybuilders, the optimal PRO_{IN} would likely be the level at which protein synthesis plateaus. Future studies are needed to resolve the observed discrepancy between the NBAL and muscle mass/strength results in this study.

The finding of no effect of PRO supplementation on indexes of muscle mass/strength is in disagreement with the results of several recent studies (10, 11, 21, 33); however, important methodological considerations limit direct comparison of these with the present study.

Consolazio et al. (10) reported significantly greater LBM (densitometry) and cumulative NBAL in male subjects provided 2.8 g PRO $\cdot$ kg^{-1} $\cdot$ day^{-1} (n = 4) than in a matched group given 1.4 g PRO $\cdot$ kg^{-1} $\cdot$ day^{-1} (n = 4) over a 40-day mixed-exercise (walking, running, calisthenics, isometrics, and cycle ergometry) program. This study was of greater duration than the present study and was likely confounded by the varied forms of exercise (resistance and endurance) used, because endurance exercise increases amino acid oxidation (19) whereas resistance exercise does not (30). Furthermore, both resistance and endurance exercise increase muscle PRO synthesis after exercise (3). The additive effect of the two forms of exercise may therefore elevate the PRO requirements of those who perform both during a training program. In addition, this study did not use a repeated-measures design, and the small sample size (n = 4/group) limits its statistical power.

Another similar study reported greater LBM (^{40}K counting) and NBAL in young men performing isometric exercise (75 min/day, 3 times/wk for ~5 wk) who received 1.0 g $\cdot$ kg^{-1} $\cdot$ day^{-1} egg and milk PRO (n = 4) than in seven different subjects who received 0.5 g $\cdot$ kg^{-1} $\cdot$ day^{-1} egg and milk PRO (33). This study also had small subject numbers, did not use a repeated-measures design, and utilized isometric exercises that make it difficult to confirm and/or equate total work loads between the groups. It is also not surprising that there were greater gains in the higher PRO group, for their total PRO_{IN} was 100% greater than that of the low PRO group, whose PRO_{IN} was deficient on the basis not only of the results of the present study but also of current recommendations for sedentary individuals (13, 25).

Marable et al. (21) also reported greater N retention (dietary N − urine N) in young men given a PRO_{IN} of

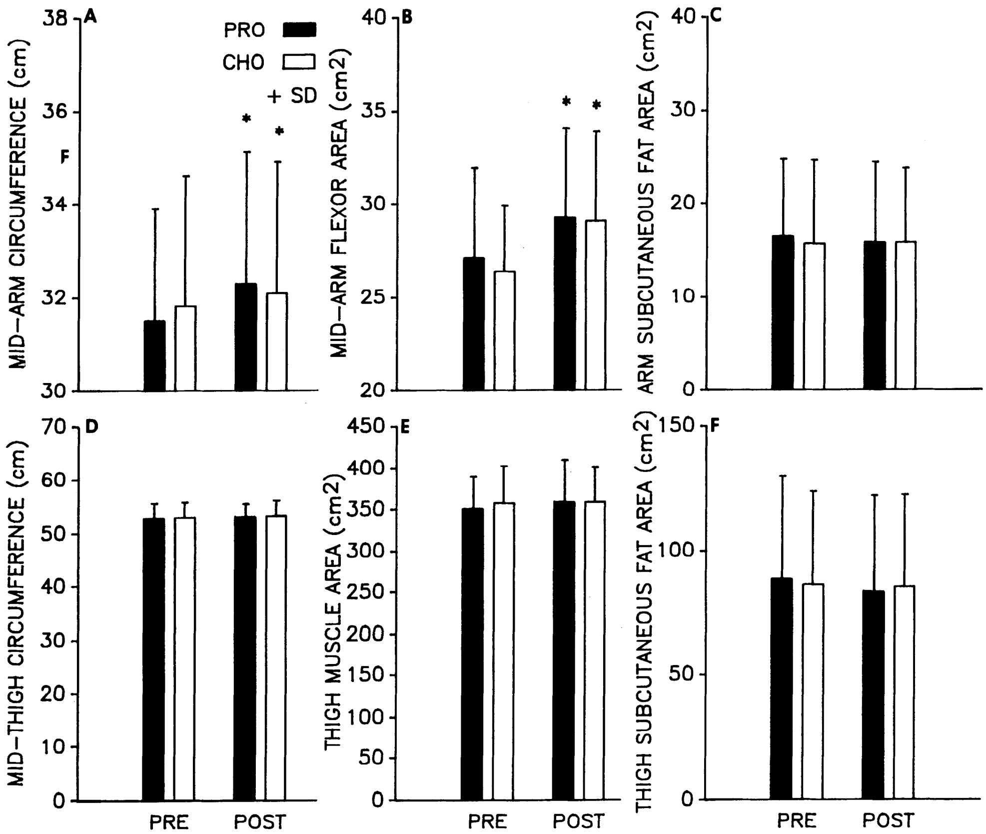

FIG. 6. Limb-specific anthropometry: midarm circumference (tape measure; *A*), computerized axial tomography (CAT) scan-determined flexor muscle cross-sectional area (*B*), CAT scan-determined arm subcutaneous fat cross-sectional area (*C*), midthigh circumference (tape measure; *D*), CAT scan-determined thigh muscle cross-sectional area (*E*), CAT scan-determined thigh subcutaneous fat cross-sectional area (*F*). * Significant ($P < 0.05$) training effect.

279% ($n = 4$) compared with 93% ($n = 2$) of current recommendations consequent to a 4-wk strength training program. In addition to the small sample size and study design concerns (of the previously discussed studies), the study of Marable et al. was limited because the N retention would have been overestimated in the higher PRO group (sweat and fecal measurements were not made) and because the "low" PRO group consumed a PRO_{IN} that was only ~50% of the calculated requirement in the present study.

Finally, Dragan et al. (11) examined the effects of increasing the PRO_{IN} of elite weight lifters from habitual intakes of 2.2 to 3.5 $g \cdot kg^{-1} \cdot day^{-1}$ during several months of training and found significant increases in both muscle strength (+5%) and LBM (+6%, estimated from skinfold measures). However, the study may have been confounded because the subjects were peaking for different competitions and because no information was given with respect to anabolic steroid use.

TABLE 5. *Muscle density*

	Arm		Thigh	
Treatment	Pre	Post	Pre	Post
PRO	67.3±5.8	70.7±5.9*	60.7±16.1	64.3±15.3*
CHO	68.1±5.2	72.3±5.4*	61.5±15.9	64.6±15.0*

Values are means ± SD expressed in Houndsfield units (HU), linear attenuation coefficients of muscle relative to water (0 HU) and air (−1,000 HU). Pre, pretraining; Post, posttraining. * Significantly greater ($P < 0.05$) than Pre.

Although at the PRO_{IN} examined in the present study (1.35 ± 0.37 vs. 2.62 ± 0.33 $g \cdot kg^{-1} \cdot day^{-1}$) muscle mass/strength gains were similar, it is likely that CHO supplementation would be less effective if the PRO_{IN} were closer to the currently recommendated PRO_{IN} (0.86 $g \cdot kg^{-1} \cdot day^{-1}$), because when PRO_{IN} is adequate, CHO overfeeding can increase PRO synthesis and decrease PRO degradation, even without training (35). It is logical to assume that this effect would be greater with bodybuilding training, because this type of exercise also stimulates net PRO synthesis (3). Therefore if the PRO_{IN} in the present study was adequate to provide the necessary amino acids, the excess energy from the CHO supplement could have stimulated muscle development. In an analogous manner, it could be that the PRO supplement provided a similar stimulus. That is, the excess energy from the isoenergy PRO supplement superimposed on an already adequate PRO_{IN} might have enhanced net PRO synthesis to a similar extent. If so, either supplement would be superior to none for the bodybuilding athlete.

In summary, the intensive bodybuilding program studied clearly increased dietary protein needs, at least during the inital stages of training. The PRO_{IN} for zero NBAL (requirement) was 1.43–1.53 $g \cdot kg^{-1} \cdot day^{-1}$, and the recommended PRO_{IN} (requirement + 2 SD) was 1.63–1.73 $g \cdot kg^{-1} \cdot day^{-1}$. Although this recommendation exceeds the subjects' habitual intake by only 13–20%, it is

~40% greater than that measured for elite bodybuilders and is ~100% higher than the current dietary recommendation for sedentary individuals. However, despite this increased protein requirement, increasing PRO_{IN} from 1.35 to 2.62 $g \cdot kg^{-1} \cdot day^{-1}$ did not result in measurable muscle mass/strength gains, at least over the 1st mo of intensive bodybuilding exercise. Whether a similar result would occur over longer training periods remains to be determined.

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Address for reprint requests: P. W. R. Lemon, Applied Physiology Research Laboratory, Kent State University, Kent, OH 44242.

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