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

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 food-stuff 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, ...]
protein breakdown] in response to three levels of dietary protein intake [low protein (LP) = 0.86 g·kg⁻¹·day⁻¹; moderate protein (MP) = 1.40 g·kg⁻¹·day⁻¹; high protein (HP) = 2.4 g·kg⁻¹·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 “strengh 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.

### TABLE 1. Physical characteristics of the subjects

<table>
<thead>
<tr>
<th></th>
<th>SA (n = 7)</th>
<th>S (n = 6)</th>
<th>Pt</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, yr</strong></td>
<td>21.6±1.5</td>
<td>24.5±3.6</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Weight, kg</strong></td>
<td>85.4±7.3</td>
<td>84.9±11.4</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Height, cm</strong></td>
<td>179.7±7.9</td>
<td>178.9±8.1</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Body fat, %</strong></td>
<td>9.8±1.2</td>
<td>20.8±2.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Lean mass, kg</strong></td>
<td>77.2±6.5</td>
<td>66.9±7.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><strong>Physical activity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration, h/wk</td>
<td>9.7±2.8</td>
<td>0.2±0.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Length, m</td>
<td>6.6±3.7</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Strength, N·m</td>
<td>32.9±1.75</td>
<td>68.2±15.0</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

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.

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, CO₂ production, O₂ 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 O₂ analyzer (Rapox, Bilthoven, The Netherlands), and a CO₂ analyzer (Hewlett-Packard, Palo Alto, CA). The system was calibrated with a known O₂-CO₂ gas mixture before each measurement. The intrainject (39 × 4 measures every 30 min) coefficient of variation (CV) of this system was 7.1% and the interject (13 subjects × 3 trials separated by ~20 days) CV was 7.9% for the determination of CO₂ production. Two gas samples were injected into 20-ml evacuated tubes (Vacutainer, Rutherford, NJ) for subsequent determination of background breath ¹³C₀₂/¹²C₀₂ 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-[¹³C]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% ¹³C isotopes (MSD Isotopes, Pointe Claire, Quebec), L-[¹³C]leucine (1 mg/kg) and [¹³C]NaHCO₃ (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-[¹³C]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-[¹³C]KIC enrichment. All gas samples were stored at room temperature, and the CO₂ was cryogenically trapped within 7 days for subsequent determination of breath ¹³CO₂/¹²CO₂ 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, Brain-tree, 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 5A 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, N₂ 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 (N₀) (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 (K₂SO₄-Se catalyst, Kjeltex 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-[13C]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 (Q) was calculated using the reciprocal pool model (from α-[13C]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 13CO2/12CO2 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 13CO2 enrichment significantly (P < 0.01) (LP, increase of 0.0018 APE during first 120 min to 0.0027 APE by end of 210 min; HP, increase of 0.0018 APE during first 120 min to 0.0027 APE by the end of 210 min; 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 in background breath 13CO2/12CO2 enrichment.

Values are means ± SD. HAB, habitual intake; LP, low protein; MP, medium protein; HP, high protein. %Energy intake of total protein, carbohydrate, fat, and alcohol, respectively, in parentheses. *Within-subject comparisons; values within each subject group with different superscript letters are significantly (P < 0.05) different from each other. 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 EIN, but, as expected, the EIN 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 %EIN from protein, carbohydrate, and fat. The %EIN from protein increased significantly (P < 0.05) and the %EIN 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.
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·kg$^{-1}$·day$^{-1}$) vs. NBAL (mg·N·kg$^{-1}$·day$^{-1}$) for both groups (SA: NBAL = −71.6 + 50.9 (protein intake), SD = 9.99, $r^2$ = 0.56; 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·kg$^{-1}$·day$^{-1}$ for S and 0.69 g·kg$^{-1}$·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·kg$^{-1}$·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. 3A). 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. 3B). There was a significant correlation between leucine oxidation and urinary urea excretion (r = -0.965, P < 0.001).

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

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·kg$^{-1}$·h$^{-1}$.

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

<table>
<thead>
<tr>
<th></th>
<th>SA (n - 7)</th>
<th>S (n - 6)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lean body mass, kg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LP</td>
<td>77.3±1.7</td>
<td>66.6±7.9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>MP</td>
<td>77.7±6.6</td>
<td>67.3±28.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>HP</td>
<td>76.6±6.7</td>
<td>67.9±8.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Creatinine excretion, mmol/day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LP</td>
<td>14.0±3.6</td>
<td>10.7±2.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>MP</td>
<td>14.6±3.8</td>
<td>11.3±3.4</td>
<td>&lt;0.01</td>
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<tr>
<td>HP</td>
<td>13.4±2.4</td>
<td>11.2±2.2</td>
<td>&lt;0.01</td>
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<tr>
<td>Isokinetic arm-flexor strength, N·m</td>
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<tr>
<td>0°/s</td>
<td>92.7±6.1</td>
<td>65.5±15.4</td>
<td>&lt;0.01</td>
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<td>30°/s</td>
<td>90.0±3.7</td>
<td>75.1±14.2</td>
<td>&lt;0.01</td>
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<td>180°/s</td>
<td>74.1±7.4</td>
<td>55.3±10.6</td>
<td>&lt;0.01</td>
</tr>
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<td>CPK activity, U/l</td>
<td>183±124</td>
<td>60±50</td>
<td>&lt;0.01</td>
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<tr>
<td>LP</td>
<td>183±99</td>
<td>57±41</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>MP</td>
<td>181±132</td>
<td>66±51</td>
<td>&lt;0.01</td>
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</table>

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

### Table 4. Nitrogen balance summary

<table>
<thead>
<tr>
<th>N Intake, g/day</th>
<th>Urine</th>
<th>Feces</th>
<th>Sweat + miscellaneous losses</th>
<th>Total losses</th>
<th>Balance</th>
</tr>
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<tbody>
<tr>
<td>SALP</td>
<td>12.9±1.0*</td>
<td>11.0±2.4*</td>
<td>2.0±0.5*</td>
<td>1.64</td>
<td>14.6±2.6*</td>
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<td>SAMP</td>
<td>19.3±2.4*</td>
<td>15.3±2.4*</td>
<td>2.1±0.5*</td>
<td>1.57</td>
<td>15.5±2.6*</td>
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<tr>
<td>SAHP</td>
<td>31.9±2.8*</td>
<td>23.3±3.1*</td>
<td>2.6±0.8*</td>
<td>1.96</td>
<td>26.3±3.2*</td>
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<tr>
<td>SLP</td>
<td>12.2±1.6*</td>
<td>9.8±1.3*</td>
<td>1.5±0.1*</td>
<td>0.49</td>
<td>11.8±1.3*</td>
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<tr>
<td>SMP</td>
<td>19.6±2.9*</td>
<td>15.3±2.5*</td>
<td>1.7±0.2*</td>
<td>0.87</td>
<td>17.2±2.6*</td>
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<tr>
<td>SHP</td>
<td>32.2±4.4*</td>
<td>25.0±4.2*</td>
<td>2.3±1.4*</td>
<td>1.42</td>
<td>28.8±4.2*</td>
</tr>
</tbody>
</table>

Values are means ± SD. N, nitrogen. SAMP, SLP, 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. 9, 26, and 28. 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. *Urine 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 μmol kg⁻¹ h⁻¹. The values for 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 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 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) (2a). 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 plateau 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 which the Canadian RNI (0.86 g kg⁻¹ day⁻¹) 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-
et al. 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. 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 ($P < 0.001$) greater for both groups on HP diet vs. MP and LP diets. There were no between-group differences.

**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·kg⁻¹·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 O₂ 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 anabolic concept that the protein requirement for SA was elevated primarily because of habitual activity and not merely an increased LBM.

The calculated protein intake for zero NBAL was 1.41 g·kg⁻¹·day⁻¹ for SA and 0.69 g·kg⁻¹·day⁻¹ for S; with a safety margin of ±1 SD, the estimated requirements were 1.76 and 0.89 g protein·kg⁻¹·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 ±2 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·kg LBM⁻¹·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.
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 26% 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., wind-sprints/throwing football) would have some aerobic component; this may have slightly increased the protein requirement 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.

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Address for reprint requests: S. A. Atkinson, Faculty of Health Sciences, McMaster University, 1200 Main Street West, Km. 3V42, Hamilton, Ontario L8N 3S5, Canada.

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