Protein to Maximize Whole-Body Anabolism in Resistance-trained Females after Exercise

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ABSTRACT

MALOWANY, J. M., D. W. D. WEST, E. WILLIAMSON, K. A. VOLTERMAN, S. ABOU SAWAN, M. MAZZULLA, and D. R. MOORE. Protein to Maximize Whole-Body Anabolism in Resistance-trained Females after Exercise. *Med. Sci. Sports Exerc.*, Vol. 51, No. 4, pp. 798–804, 2019. **Introduction**: Current athlete-specific protein recommendations are based almost exclusively on research in males. **Purpose**: Using the minimally invasive indicator amino acid oxidation technique, we determined the daily protein intake that maximizes whole-body protein synthesis (PS) and net protein balance (NB) after exercise in strength-trained females. **Methods**: Eight resistance-trained females (23 ± 3.5 yr, 67.0 ± 7.7 kg, 163.3 ± 3.7 cm, 24.4% ± 6.9% body fat; mean ± SD) completed a 2-d controlled diet during the luteal phase before performing an acute bout of whole-body resistance exercise. During recovery, participants consumed eight hourly meals providing a randomized test protein intake (0.2–2.9 $g \cdot kg^{-1} \cdot d^{-1}$) as crystalline amino acids modeled after egg protein, with constant phenylalanine (30.5 $mg \cdot kg^{-1} \cdot d^{-1}$) and excess tyrosine (40.0 $mg \cdot kg^{-1} \cdot d^{-1}$) intakes. Steady-state whole-body phenylalanine rate of appearance (Ra), oxidation (Ox; the reciprocal of PS), and NB (PS − Ra) were determined from oral [^{13}C] phenylalanine ingestion. Total protein oxidation was estimated from the urinary urea–creatinine ratio (U/Cr). **Results**: A mixed model biphase linear regression revealed a break point (i.e., estimated average requirement) of 1.49 ± 0.44 $g \cdot kg^{-1} \cdot d^{-1}$ (mean ± 95% confidence interval) in Ox ($r^2 = 0.64$) and 1.53 ± 0.32 $g \cdot kg^{-1} \cdot d^{-1}$ in NB ($r^2 = 0.65$), indicating a saturation in whole-body anabolism. U/Cr increased linearly with protein intake ($r^2 = 0.56$, $P < 0.01$). **Conclusions**: Findings from this investigation indicate that the safe protein intake (upper 95% confidence interval) to maximize anabolism and minimize protein oxidation for strength-trained females during the early ~8-h postexercise recovery period is at the upper end of the recommendations of the American College of Sports Medicine for athletes (i.e., 1.2–2.0 $g \cdot kg^{-1} \cdot d^{-1}$). **Key Words:** PROTEIN RECOMMENDATIONS, WOMEN, RECOVERY, PROTEIN SYNTHESIS, RESISTANCE EXERCISE, MUSCLE HYPERTROPHY

APPLIED SCIENCES

Dietary protein ingestion enhances whole-body and skeletal muscle protein synthesis (PS) during recovery from resistance exercise, which ultimately supports the net accretion of lean mass with training (1). Thus, dietary protein recommendations are of particular interest to athletes aiming to support postexercise recovery and training-induced anabolism, which is primarily driven by changes in PS that ultimately results in a positive net protein balance (NB) (i.e., the algebraic difference between PS and protein breakdown) (2). The importance of dietary protein for athletes is reflected in current sports consensus statements that recommend 1.2–2.0 $g \cdot kg^{-1} \cdot d^{-1}$ (3), which is greater than the current recommended dietary allowance (RDA) of 0.8 $g \cdot kg^{-1} \cdot d^{-1}$ for the general population (4). Recommended protein intakes for strength-trained athletes range from ~1.3 $g \cdot kg^{-1} \cdot d^{-1}$ of protein based on nitrogen studies (5) to ~2.2 $g \cdot kg^{-1} \cdot d^{-1}$ based on the indicator amino acid oxidation (IAAO) technique (6). A recent meta-analysis suggested that lean mass growth, which is an important training adaptation for many strength-trained athletes, is maximized at a protein intake of ~1.6 $g \cdot kg^{-1} \cdot d^{-1}$ (1). This suggested intake is at odds with the general propensity of strength athletes to consume higher than average habitual protein intakes (e.g., ~2.1 $g \cdot kg^{-1} \cdot d^{-1}$) (5). Importantly, dietary amino acids consumed in excess of their capacity to be incorporated into new protein are ultimately deaminated before their oxidative catabolism, a situation that results in the production of the nitrogen end-product urea (7,8). Previous prospective research on protein intake recommended for strength-trained athletes has exclusively been performed in males (6,9,10), which exemplifies the sex disparity in sports science research (11). Given the inconsistent recommendations for strength-trained athletes and the complete lack of comparable data in females, additional research is needed to determine the protein intake that maximizes anabolism to support postexercise repair and recovery.

Bandegan et al. (6) recently assessed the dietary protein requirement of young male body builders with the IAAO technique, a method considered advantageous over traditional

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nitrogen balance methodology in that it identifies the protein intake that maximizes whole-body PS (6). These researchers observed an estimated average requirement (EAR) of 1.7 $g \cdot kg^{-1} \cdot d^{-1}$ with a safe intake (i.e., upper 95% confidence interval [CI]) of 2.2 $g \cdot kg^{-1} \cdot d^{-1}$ (6), which were greater than previous estimates by IAAO in nonexercising adults (i.e., 0.93 and 1.2 $g \cdot kg^{-1} \cdot d^{-1}$, respectively) (12). However, kinetic assessments in that study (6) were assessed at least 48 h after their last training bout, which is outside the window during which postexercise muscle protein remodeling is elevated (13). Given that resistance exercise increases the intramuscular reuse of amino acids (13) and the sensitivity to dietary amino acids (9), it is unclear whether estimates of protein needed on nontraining days are adequate for athletes (especially females) who generally train most days of the week (either whole-body or split routines) in an attempt to maximize training adaptations (14). Therefore, the aim of the present study was to use the IAAO method to determine the protein intake that maximizes whole-body PS and NB in resistance-trained females on a training day. We hypothesized that the protein intake to maximize whole-body PS in strength-trained females would be lower than that previously determined in resistance-trained males on a nontraining day (1.7 $g \cdot kg^{-1} \cdot d^{-1}$) (6) but within broad, nonspecific recommendations of the American College of Sports Medicine for athletes (i.e., 1.2–2.0 $g \cdot kg^{-1} \cdot d^{-1}$) (4,15).

METHODS

Participants

Eight healthy, young adult self-reported females with regular menstrual cycles and who perform whole-body resistance exercise regularly were recruited to participate in the study (Table 1). Participants were required to have trained each major muscle group (i.e., chest, back, and legs) at least twice per week consistently for at least 1 yr before recruitment. Training could not include more than 30 min of continuous endurance training per exercise session to ensure the primary training focus was on resistance exercise. Strength training status was confirmed by achieving a minimum one-repetition maximum (1RM) bench press and leg press of 0.7 and 2.3 × bodyweight, respectively (adapted from Morton et al. [16]). Exclusion criteria included the following: (i) being sedentary or abstaining from resistance training for greater than 1 month in the last 6 months before the study, (ii) non–weight stable within the past month (on the basis of a questionnaire that participants have not been engaging in intentional weight loss or weight gain programs), (iii) tobacco use, (iv) on any medications known to alter protein metabolism (e.g., corticosteroids), (v) previous and current use of anabolic steroids, and (vi) using supplements with creatine or beta-alanine, or any additional amino acid or macronutrient/micronutrient supplements in the last 30 d. Finally, participants were required to have no current use, or discontinuation of use within the last 3 months, of hormonal contraceptives, as well as a predictable menstrual cycle during the previous year as determined by the calendar method (17) 3 months before study enrolment and during the study participation.

TABLE 1. Characteristics of participants.

Participant Characteristics (n = 8)	Mean ± SD
Age (yr)	23 ± 3.5
Height (cm)	163.3 ± 3.7
Body mass (kg)	67.0 ± 7.7
FFM (kg)	50.4 ± 3.8
Body fat (%)	24.4 ± 6.9
Bench press 1RM (kg)	59.1 ± 7.9
Leg press 1RM (kg)	211.8 ± 53.5
Habitual dietary protein intake ($g \cdot kg^{-1} \cdot d^{-1}$)	1.91 ± 0.53

Ethics Statement

The study was approved by the University of Toronto Health Sciences Research Ethics Board (REB no. 33462) and conformed to the standards set by the latest revision of the Declaration of Helsinki. All participants were informed of the purpose of the study, the experimental protocols, and the potential associated risks involved and informed consent was obtained. The study has been registered under ClinicalTrials.gov (ID no. NCT03397043).

Study Protocol

Baseline testing and familiarization. Eligible participants were fitted with an accelerometer (ActiGraph, Pensacola, FL) for 3 d to assess habitual caloric energy expenditure, coinciding with a 3-d diet log to assess habitual energy and protein intake. On a separate day, participants completed a body composition assessment (fat mass [FM] and fat-free mass [FFM]) by air displacement plethysmography (BOD-POD; COSMED USA Inc., Chicago, IL) after avoiding food, water, and exercise for ≥4 h. After body composition testing, participants were familiarized with the whole-body resistance exercise protocol and subsequently underwent 3RM testing to determine their 1RM (18). Upon completion of the testing, all participants were provided with a brief explanation of the trial day testing protocol before returning for their metabolic trials.

Experimental design. Each participant completed six to seven metabolic trials (n = 50 for total trials completed) during the luteal phase, which was defined as the second half of the menstrual cycle (17). Menstrual cycle was standardized as the requirement for some amino acids (e.g., lysine) may vary slightly between phases (19). Given that a 3-d isotope "washout" period is sufficient for the IAAO method (20), up to two metabolic trials could be completed within the same luteal phase. After scheduling around participants' training/competition requirements and/or menstrual cycle, the average duration to complete the study was 86 ± 19 d, which is similar to a previous study estimating lysine requirements across the menstrual cycle in nonexercising healthy adult females (21). On each metabolic trial day, participants were randomly assigned a protein

intake ranging from deficient to excess (0.2–2.90 $g \cdot kg^{-1} \cdot d^{-1}$) (Table 2) similar to IAAO test intakes previously used in sedentary men (12).

Adaptation period. Two days before each metabolic trial, all participants were required to complete a prescribed but self-monitored whole-body resistance exercise session, which was modeled off a previous study in males (22). Briefly, participants performed a brief warm-up before performing a whole-body resistance exercise protocol involving the following: (i) barbell bench press and latissimus dorsi ("lat") pull down superset, (ii) standing overhead barbell press and seated cable row superset, (iii) leg press, and (iv) leg extension. Each exercise was performed at 75% 1RM for 4 sets of 8–10 repetitions with ~90-s rest intervals between sets. On the second day (i.e., 1 d before the metabolic trial), participants were instructed to refrain from physical activity to mimic a "rest" day. This was done to replicate the general training pattern that resistance athletes tend to follow (e.g., training day, rest day, training day, etc.) (15). In addition, participants were required to consume a 2-d adaptation diet containing 1.2 $g \cdot kg^{-1} \cdot d^{-1}$ of protein during the 2-d controlled training period before each metabolic trial. This adaptation diet intake was selected to minimize metabolic variability during trial day (23) while providing an intake that was previously determined to be sufficient for nonexercising men by IAAO (12), within the recommended range for athletic populations (4), and which was consistent with our previous study in active, variable intensity female athletes (24). Dietary carbohydrate intake was set at 4 $g \cdot kg^{-1} \cdot d^{-1}$, which is consistent with current intakes for resistance-trained athletes (25) and similar to their habitual intake. The remaining energy was supplied from fat. Adaptation diets contained sufficient energy to satisfy individual resting energy expenditure (REE), as determined by the energy expenditure estimates from the BOD-POD analysis (COSMED USA Inc.), with a moderate activity correction factor of 1.5. Diet record logs were completed by each participant for each adaptation period to ensure they consumed the provided meals. On the third day, participants reported to the Goldring Centre for High Performance Sport to complete their metabolic trial.

Metabolic trial days. Metabolic trial day methods were consistent with previous IAAO work in nonexercising populations (12) except for the addition of an exercise stimulus (24,26). On the day of the metabolic trial, after an overnight fast, participants consumed a protein-free liquid carbohydrate beverage (1 g carbohydrate$\cdot kg^{-1} \cdot d^{-1}$ as a 1:1 ratio of maltodextrin [Polycal®; Nutricia, Amsterdam, Netherlands] and sports drink powder [Gatorade® Endurance Formula; PepsiCo, Purchase, NY]) approximately 1 h before the exercise bout (as described above) to provide exogenous carbohydrate energy to fuel the exercise bout (25). Immediately after the completion of the exercise bout, participants consumed their first of eight isocaloric hourly meals containing a randomly assigned protein intake (Table 2).

TABLE 2. Test protein intakes consumed by individual participants.

Participant No.	Test Protein Intakes ($g \cdot kg^{-1} \cdot d^{-1}$)
1	0.20, 0.66, 1.08, 1.64, 2.25, 2.60
2	0.55, 0.97, 1.25, 1.97, 2.60, 2.67
3	0.34, 0.80, 1.21, 1.74, 2.11, 2.36
4	0.52, 0.69, 1.32, 1.81, 2.04, 2.46
5	0.24, 0.90, 1.67, 2.08, 2.18, 2.53
6	0.27, 0.83, 1.39, 1.53, 1.90, 2.32
7	0.48, 0.76, 1.04, 1.46, 1.94, 2.63, 2.90
8	0.22, 0.41, 0.62, 1.11, 1.50, 2.08, 2.39

Each meal provided one twelfth of the participant's total daily energy requirement and included protein-free cookies (27) and test drinks, the latter of which included protein free powder (PFD-1; Mead Johnson, Evansville, IN), flavoring crystals (Tang; Kraft, Don Mills, Canada), grape seed oil, maltodextrin (Polycal®), and a crystalline amino acid mixture (Ajinomoto North America, Inc., Raleigh, NC). The composition of the amino acid mixture was modeled on the basis of egg protein except phenylalanine and tyrosine, which were both held constant at intakes of 30.5 and 40.0 $mg \cdot kg^{-1} \cdot d^{-1}$, respectively. Tyrosine was provided in excess to ensure that the hydroxylation of the carboxyl carbon of phenylalanine was at a minimum and was instead directed toward PS or oxidation (28).

During the fifth hour of feeding, a priming dose of $NaH^{13}CO_3$ (0.176 $mg \cdot kg^{-1}$) and L-[1-^{13}C]phenylalanine (1.86 $mg \cdot kg^{-1}$) were ingested with all subsequent test drinks during the metabolic trial, including 1.20 $mg \cdot kg^{-1}$ of L-[1-^{13}C]phenylalanine as part of the total phenylalanine intake to model steady-state phenylalanine metabolism (see below). The trial day energy intake was estimated as follows: (REE × 1.5) + [(0.05 × BM × 45) × 1.1], whereby REE represents the REE (kcal) determined from the energy expenditure results of the BOD-POD (COSMED USA Inc.), 1.5 represents a moderate activity factor, 0.05 represents the average energy expenditure ($kcal \cdot kg^{-1} \cdot min^{-1}$) of the whole-body resistance exercise protocol for females (29), BM is body mass (kg), 45 is the duration of the whole-body resistance exercise protocol (min), and 1.1 accounts for a 10% "buffer" to ensure participants were in a positive energy balance in light of potential individual differences in energy expenditure.

Sample Collection and Analysis

Breath and urine samples. Sample collection was identical with previous studies from our laboratory (24,26). Briefly, three baseline breath samples (Easy-Sampler, QuinTron Instrument Company, Milwaukee, WI) were collected in a sterile vacutainer, and two baseline urine samples were collected at 15- and 30-min increments, respectively, starting 15 min after the fourth meal and before the ingestion of the L-[1-^{13}C]phenylalanine tracer in the fifth meal. Beginning 30 min after the seventh meal, five breath and three urine samples were collected at isotopic plateau according to the same frequency as baseline samples. Breath and urine samples were collected to determine $^{13}CO_2$ and L-[1-^{13}C]phenylalanine enrichment, respectively, at baseline and plateau. Steady-state CO_2 production ($\dot{V}CO_2$), as previously established after exercise (24), was

measured over two 10-min periods approximately 30 min after the fifth or sixth test drink, by indirect calorimetry (IX-TA-220; iWorx Systems Inc., Dover, NH). With L-[1-^{13}C]phenylalanine tracer addition in the fifth to eighth drinks, our estimate of whole-body PS reflects 4–8 h postexercise.

Breath samples were stored at room temperature before analysis of $^{13}CO_2$ enrichment by continuous-flow isotope ratio mass spectrometry (ID-Microbreath; Compact Science Systems, Newcastle, UK). Urine samples were stored at −80°C before analysis of [^{13}C]phenylalanine enrichment by liquid chromatography tandem mass spectrometry (1290 HPLC, Agilent Technologies, Santa Clara, CA; Q-Trap MS, Sciex, Framingham, MA) by the Analytical Facility for Bioactive Molecules, The Hospital for Sick Children, Toronto, Canada. Phenylalanine flux (Ra, $\mu mol \cdot kg^{-1} \cdot h^{-1}$), $^{13}CO_2$ excretion ($F^{13}CO_2$), and phenylalanine oxidation (Ox; $\mu mol \cdot kg^{-1} \cdot h^{-1}$) were calculated as previously detailed (12,26,30). Using standard equations (30), whole-body protein breakdown was assumed to reflect Ra, and PS was calculated as the difference between Ra and Ox. Whole-body phenylalanine NB was calculated as the difference between PS and Ra.

Urinary urea concentrations were measured using a colorimetric assay according to manufacturer's instructions (QuantiChrom Urea Assay Kit; BioAssay Systems, Hayward, CA). Creatinine concentrations were measured using a colorimetric assay according to manufacturer's instructions (QuantiChrom Creatinine Assay Kit, BioAssay Systems). Coefficients of variation were <5% for urea and creatinine assays. The ratio of urinary urea to creatinine (U/Cr) was completed to examine the excretion of nitrogen via urea with increasing protein intakes, which is reflective of amino acid excretion.

Statistical analysis. The use of the IAAO method has been applied extensively to estimate dietary indispensable amino acid and protein requirements with six to eight participants consuming five to seven test protein intakes (i.e., a total of ~40 metabolic trials) (12,21,31). The present study included $n = 8$ participants with each participant consuming six to seven unique protein intakes (i.e., a total of 50 metabolic trials), consuming unique protein intakes, which is a more robust approach to estimate requirements than providing identical intakes across participants. With $n = 50$ intakes, our 95% CI was ~29% of our EAR (see below), which is comparable with previous studies that used $n = 56$ trials at seven identical/defined intakes (i.e., ~30%) (12) or $n = 56$ random intakes (i.e., ~19%) (31).

Changes in Ra, $F^{13}CO_2$, Ox, and NB were determined as a function of protein intake using a mixed linear model with the participants as a random variable using Proc Mixed program (SAS university version; SAS Institute Japan, Toyo, Japan). Consistent with previous literature (12,26,31), the upper 95% CI was used to approximate the RDA. In addition, changes in U/Cr were determined as a function of protein intake using a linear regression model with the participants as a random variable using GraphPad Prism (V6; GraphPad Software, La Jolla, CA).

To determine whether the break point determined herein (EAR_1) differed from that of previously published nonexercising men (EAR_2) (12), we first converted the 95% CI to SE (SE_1 and SE_2, respectively) by dividing the difference between the upper and the lower 95% CI of each EAR by 3.92. The extent of overlapping CI was determined by the following: $(EAR_1 - EAR_2) \pm 1.96 [\sqrt{(SE_1^2 + SE_2^2)}]$, whereby the null hypothesis was rejected if the interval did not contain zero.

RESULTS

Phenylalanine flux. The average phenylalanine flux was 56.7 ± 1.4 $\mu mol \cdot kg_{BM}^{-1} \cdot h^{-1}$ or 74.4 ± 1.9 $\mu mol \cdot kg_{FFM}^{-1} \cdot h^{-1}$. The slope [−4.7 ($\mu mol \cdot kg_{BM}^{-1} \cdot h^{-1}$)·(g of protein·$kg_{BM}^{-1} \cdot d^{-1}$)$^{-1}$] of the Ra against protein intake was significantly different from zero ($P = 0.003$) (Fig. 1).

$F^{13}CO_2$ and phenylalanine oxidation. The $F^{13}CO_2$ displayed a biphasic linear regression with a break point of 1.60 ± 0.40 $g \cdot kg_{BM}^{-1} \cdot d^{-1}$ ($r^2 = 0.55$) and 2.39 ± 0.66 $g \cdot kg_{FMM}^{-1} \cdot d^{-1}$ ($r^2 = 0.5$).

The Ox break point followed a biphasic linear regression ($r^2 = 0.64$) with an EAR of 1.49 ± 0.44 $g \cdot kg^{-1} \cdot d^{-1}$ (Fig. 2). The Ox break point determined in the present study was greater ($P < 0.05$) than that previously determined in nonexercising men (i.e., 0.93 ± 0.31 $g \cdot kg^{-1} \cdot d^{-1}$) (12). The FFM-normalized Ox ($r^2 = 0.62$) break point was 2.13 ± 0.65 $g \cdot kg_{FFM}^{-1} \cdot d^{-1}$.

Whole-body NB. The NB break point followed a biphasic linear regression ($r^2 = 0.65$) with a break point of 1.53 $g \cdot kg_{BM}^{-1} \cdot d^{-1}$ and an upper 95% CI of 1.85 $g \cdot kg_{BM}^{-1} \cdot d^{-1}$ (Fig. 3). The NB break point followed a biphasic linear regression ($r^2 = 0.63$) with a break point of 2.03 $g \cdot kg_{FFM}^{-1} \cdot d^{-1}$ and an upper 95% CI of 2.47 $g \cdot kg_{FFM}^{-1} \cdot d^{-1}$ (data not shown).

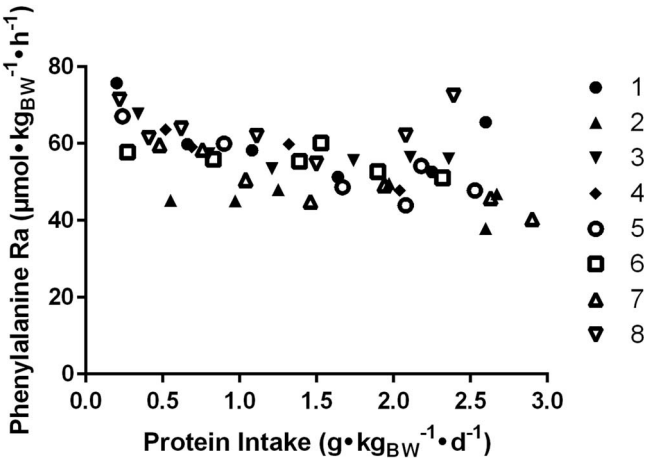

FIGURE 1—Relationship between protein intake and urinary phenylalanine rate of appearance (Ra). Eight participants completed six to seven metabolic trials each ($n = 50$ trials or individual protein intakes). Each participant is represented by a unique symbol, and each data point represents the Ra for one metabolic trial. Average phenylalanine flux was 56.7 ± 1.4 $\mu mol \cdot kg^{-1} \cdot h^{-1}$. The slope of the line is significantly different from zero ($P < 0.01$).

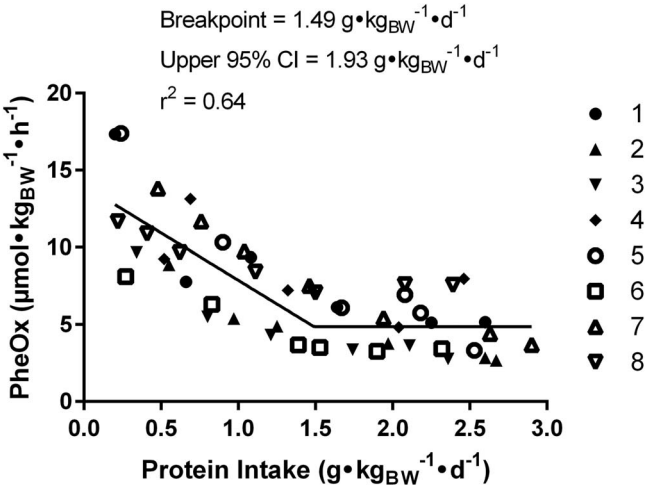

FIGURE 2—Relationship between protein intake and phenylalanine oxidation (PheOx). Eight participants completed six to seven metabolic trials each (n = 50 trials or individual protein intakes). Each participant is represented by a unique symbol, and each data point represents the PheOx for one metabolic trial. The PheOx break point followed a biphasic linear regression (r^2 = 0.64) and reveals an average dietary protein requirement of 1.49 g·kg^{-1}·d^{-1} and a recommended daily allowance (i.e., upper 95% CI) of 1.93 g·kg^{-1}·d^{-1}.

Urinary urea–creatinine ratio. The urea–creatinine ratio showed a positive linear regression with protein intake (r^2 = 0.56, P < 0.001) (Fig. 4).

DISCUSSION

The purpose of the present study was to use the IAAO method to determine the protein intake that maximizes whole-body PS and NB in strength-trained females on a training day. We have demonstrated that on a day in which an acute bout of whole-body resistance exercise is performed, the EAR and the safe protein intake (i.e., upper 95% CI) are 1.49

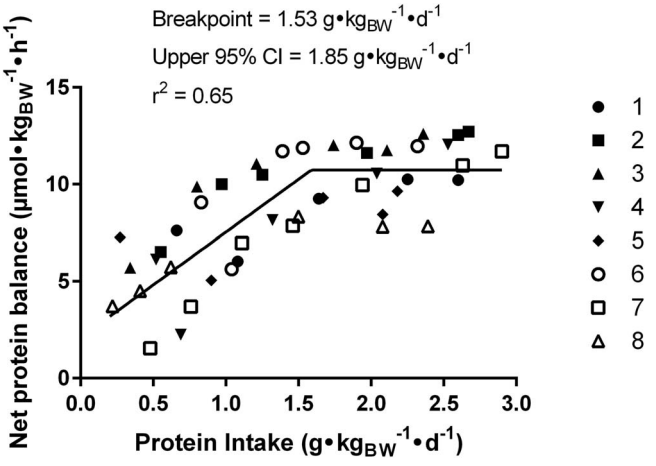

FIGURE 3—Relationship between protein intake and whole-body NB. Eight participants completed six to seven metabolic trials each (n = 50 trials or individual protein intakes). Each participant is represented by a unique symbol, and each data point represents the NB for one metabolic trial. The NB break point followed a biphasic linear regression (r^2 = 0.65) with a break point of 1.53 g·kg^{-1}·d^{-1} and an upper 95% CI of 1.85 g·kg^{-1}·d^{-1}.

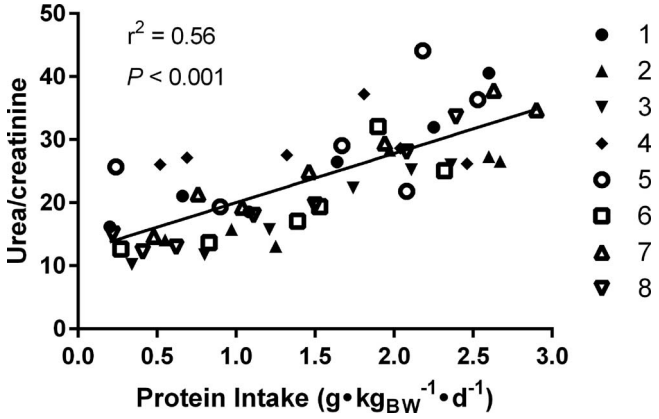

FIGURE 4—Relationship between protein intake and urinary urea–creatinine ratio. Eight participants completed six to seven metabolic trials each (n = 50 trials or individual protein intakes). Each participant is represented by a unique symbol, and each data point represents the NB for one metabolic trial. The urea–creatinine ratio showed a positive linear regression with protein intake (r^2 = 0.56, P < 0.001).

and 1.93 g·kg^{-1}·d^{-1}, respectively, in strength-trained females according to the biphase linear break point analysis of phenylalanine oxidation. Traditional IAAO methodology suggests that the break point in $F^{13}CO_2$ reflects the protein intake that maximizes whole-body PS (32); however, this conventional approach presumes that phenylalanine flux is uninfluenced by the protein intake, which would translate into a stable estimate of the immediate precursor enrichment for PS. In the present study, there was a slight decrease in Ra with increasing protein intakes such that it declined across the ~1- to 2-g·kg^{-1}·d^{-1} range defined by the 95% CI by ~8%. This decreased whole-body flux may be related in part to a slight anticatabolic effect of high-protein intakes (33). Nevertheless, although average phenylalanine flux was similar in the present study compared with strength-trained (6) and nonexercising males (12), the protein-dependent decrease may introduce error in the EAR when determined by $F^{13}CO_2$, which does not account for differences in precursor pool enrichment. For example, the goodness of fit for the biphase linear model was lower (i.e., r^2 = 0.56 vs 0.64, respectively), whereas the percent variation in the 95% CI relative to the EAR was higher (i.e., ~48 vs ~29%, respectively) when determining the recommended protein intake by $F^{13}CO_2$ compared with phenylalanine oxidation. Given phenylalanine oxidation takes into consideration the estimate of the intracellular precursor for PS (34), we believe that the protein intake determined by phenylalanine oxidation is a more accurate estimate of the intake that maximizes whole-body PS in the present study.

Consistent with the general recommendation that higher protein intakes are recommended for athletes compared with their sedentary counterparts (3), the EAR in the present study was ~60% greater than similar IAAO-determined estimates in nonactive males (12). By contrast, intakes noted in our study are slightly lower than that previously reported to maximize whole-body PS in strength-trained males on a nontraining day (EAR = 1.49 vs 1.70 g·kg^{-1}·d^{-1},

respectively [6]). One interpretation of these findings is that protein intake to maximize whole-body PS may be lower on training days; this approach would be consistent with the anabolic nature of resistance exercise and its ability to increase the sensitivity to dietary amino acids (13,35). When the EAR was normalized to metabolically active FFM, estimates for protein intake to support maximal rates of PS were similar for females and males (EAR = 2.2 vs 2.1 $g \cdot kg_{FFM}^{-1} \cdot d^{-1}$, respectively [6]), suggesting that the metabolic requirement of dietary protein to maximize whole-body PS is elevated in resistance-trained populations irrespective of sex. This would generally be consistent with the similar postexercise muscle protein synthetic response between sexes (36) and that resistance exercise has little effect on whole-body amino acid oxidation (37), the latter of which represents the primary sexual dimorphism in amino acid metabolism (38).

Maximizing muscle PS is of primary importance to a trained population aiming to enhance the recovery from and adaptation to exercise. The repeated (i.e., every ~3 h) ingestion of moderate doses (i.e., ~0.25 $g \cdot kg^{-1}$) of high-quality protein has been shown to support greater postexercise rates of muscle PS in trained men than an equivalent amount of protein consumed in smaller, more frequent doses (i.e., ~0.13 $g \cdot kg^{-1}$ over 1.5 h) (39). Compared with consuming approximately five equally spaced, moderate protein meals (i.e., ~1.25 $g \cdot kg^{-1} \cdot d^{-1}$ total protein), the EAR in the present study (i.e., ~1.49 $g \cdot kg^{-1} \cdot d^{-1}$) that maximized whole-body PS in our population may reflect, in part, that a higher protein intake will optimize muscle PS. However, strength-trained athletes, including those in the present study, generally consume high habitually protein intakes (i.e., ≥1.9 $g \cdot kg^{-1} \cdot d^{-1}$) (6). Excess dietary protein consumption results in a greater capacity to oxidize amino acids (10), which may take at least 5 d to adapt to lower intakes (40). Therefore, the possibility exists that increased protein recommendation for strength athletes compared with untrained individuals as determined by the IAAO may be related in part to an upregulated amino acid oxidative capacity secondary to the relatively higher habitual protein intakes of this population.

Training-induced hypertrophy and lean mass growth are undermined by a persistent positive NB, which ultimately represents the algebraic difference between PS and protein breakdown. NB in the present study increased to and plateaued at a protein intake of 1.53 $g \cdot kg^{-1} \cdot d^{-1}$ (95% CI = 1.85 $g \cdot kg^{-1} \cdot d^{-1}$), which is similar to the protein intake that maximizes PS. Moreover, there was a linear increase in the urinary urea–creatinine ratio, which would indicate that a greater proportion of amino acids are being deaminated with their carbon skeletons ultimately directed toward gluconeogenesis and/or oxidative catabolism (7). The break point in NB concomitant with a linear increase in urea production in the present study is consistent with a plateau in whole-body anabolism in strength-trained females. Our data suggest that whole-body anabolism is primarily regulated by changes in PS and that, contrary to previous suggestions (33), a maximal anabolic response to dietary protein intake possibly exists. Interestingly, our acute results align with the results of a recent meta-analysis that suggests training-induced lean mass accretion plateaus at ~1.6 $g \cdot kg^{-1} \cdot d^{-1}$ (1). Therefore, collectively our data and that of others (1,6,10) do not support the consumption of very high (e.g., ≥2.2 $g \cdot kg^{-1} \cdot d^{-1}$) habitual protein intakes as a requirement to support training-induced anabolism. Findings in the present study suggest or indicate that protein intakes greater than 1.53 $g \cdot kg^{-1} \cdot d^{-1}$ (95% CI = 1.85 $g \cdot kg^{-1} \cdot d^{-1}$) will be diverted to amino acid oxidative disposal and provide no further benefit to the anabolic potential in this strength population.

In summary, our study is the first to prospectively estimate the protein intake that maximizes whole-body PS in a strength-trained female population using the IAAO technique. Given that females and women are a regrettably underrepresented population in sport and exercise science research (11), our study addresses this research sex and gender bias and provides valuable population-specific nutritional information suggesting that that an average protein intake of ~1.5 $g \cdot kg^{-1} \cdot d^{-1}$ and a safe intake (i.e., upper 95% CI) of ~1.9 $g \cdot kg^{-1} \cdot d^{-1}$ would maximize whole-body PS and NB strength-trained females on a day of training. This intake exceeds the current RDA (0.8 $g \cdot kg^{-1} \cdot d^{-1}$), is slightly lower than previous IAAO-determined estimates in strength-trained males on a training day (1.7 ± 0.5 $g \cdot kg^{-1} \cdot d^{-1}$) (6), and is at the upper end of the American College of Sports Medicine Position Stand on protein requirements for active populations based primarily on research in males (i.e., 1.2–2.0 $g \cdot kg^{-1} \cdot d^{-1}$) (4,15).

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