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Level of dietary protein impacts whole body protein turnover in trained males at rest

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Abstract

The current investigation examined the effect of variations in protein intake on Whole body protein turnover (WBPTO) at rest in endurance-trained males. Whole body protein turnover is influenced by both diet and exercise. Whether endurance athletes require more protein than the non-exerciser remains equivocal. Five male runners (21.3 ± 0.3 years, 179 ± 2 cm, 70.6 ± 0.1 kg, 8.7% ± 0.4% body fat, 70.6 ± 0.1 $\dot{V}O_2$max) participated in a randomized, crossover design diet intervention where they consumed either a low-protein (LP; 0.8 g/kg), moderate-protein (MP; 1.8 g/kg), or high-protein (HP; 3.6 g/kg) diet for 3 weeks. Whole body protein turnover (Ra, leucine rate of appearance; NOLD, nonoxidative leucine disposal; and Ox, leucine oxidation), nitrogen balance, and substrate oxidation were assessed at rest following each dietary intervention period. The HP diet increased leucine Ra (indicator of protein breakdown; 136.7 ± 9.3, 129.1 ± 7.4, and 107.8 ± 3.1 μmol/[kg · h] for HP, MP, and LP diets, respectively) and leucine Ox (31.0 ± 3.6, 26.2 ± 4.3, and 18.3 ± 0.6 μmol/[kg · h] for HP, MP, and LP diets, respectively) compared with LP diet ($P < .05$). No differences were noted in nonoxidative leucine disposal (an indicator of protein synthesis) across diets. Nitrogen balance was greater for HP diet than for MP and LP diets (10.2 ± 0.7, 1.8 ± 0.6, and −0.3 ± 0.5 for HP, MP, and LP diets, respectively). Protein oxidation increased with increasing protein intake (54% ± 6%, 25% ± 1%, and 14% ± 2% for HP, MP, and LP diets, respectively). Findings from this study show that variations in protein intake can modulate WBPTO and that protein intake approximating the current recommended dietary allowance was not sufficient to achieve nitrogen balance in the endurance-trained males in this investigation. Our results suggest that a protein intake of 1.2 g/kg or 10% of total energy intake is needed to achieve a positive nitrogen balance. This is not a concern for most endurance athletes who routinely consume protein at or above this level.

1. Introduction

Whole body protein utilization is affected by energy intake [1], level of protein intake [2,3], and endurance exercise [4,5]. The important relationship between energy and protein has been recognized for many years and is documented in both early nitrogen balance studies and more recent studies in which nitrogen retention and protein balance improved in periods of energy balance vs energy deficit [1,6,7]. Adequate energy reduces the reliance on amino acids for fuels and allows the body to use protein for non–energy-yielding functions.

Level of dietary protein consumption influences whole body protein utilization. With increasing dietary protein there is an increase in nitrogen retention [8-11], an increase in the oxidation of leucine [2], and changes in rates of protein turnover [9,10]. Although the implications of noted changes in rates of protein turnover in response to protein intake are not fully understood, it is clear that increases in dietary protein influence rates of protein turnover [2,10].

Whole body protein turnover (WBPTO) is also affected by a bout of endurance exercise. Several studies have found whole body protein breakdown to increase during exercise [12-14], with rates postexercise either decreasing [15] or being no different than those noted at rest [16]. Whole body protein synthesis has been found to decrease [14,16], or not change during exercise [13,17], but increase in the period

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after exercise [16,18]. Leucine oxidation increases during exercise in an intensity-dependent manner and may contribute 2% to 6% of total energy expended during an exercise session [2,3,13,19,20].

Given that endurance exercise impacts whole body protein metabolism, investigators have questioned whether the current recommended level of protein is adequate for those persons who consistently engage in high-intensity endurance exercise. Although some studies have found optimal protein intakes to be 1.0 to 1.8 g/kg of body weight for endurance-exercising individuals [8,9,11,13,21], there are studies that oppose this view and hypothesize that with the maintenance of energy balance, protein intake of 0.8 g/kg of body weight is sufficient for this population [1,6].

The recommended range for protein intake, according to the new dietary reference intakes (DRIs), is 10% to 35% of total energy [22]. For a 70-kg man, consuming 10460 kJ/d, this equates to a protein intake ranging from 0.89 to 3.1 g/kg per day. There are currently no studies that have examined protein intakes across this recommended range in endurance athletes. We hypothesized that an increase in protein intake would lead to an increase in whole body protein turnover (WBPTO). Therefore, the purpose of this investigation was to examine the impact of increasing protein intake from 0.8 to 1.8 to 3.6 g/kg per day on leucine kinetics, nitrogen balance, and substrate oxidation in weight-stable endurance athletes.

2. Methods

2.1. Subjects

After project approval by the institutional review board at the University of Connecticut, 5 male endurance athletes aged 22 to 29 years were recruited from the university community and local health/running clubs to participate in the study. Each participant provided a complete medical history, training schedule, and a record of dietary intake. Subjects were required to be running a minimum of 35 miles/wk for inclusion in the study. Individuals reporting metabolic or cardiovascular abnormalities, gastrointestinal disorders (ie, lactose intolerance), use of nutritional/sports supplements or anabolic steroids, or who considered themselves vegetarians were excluded from the study. Informed, written consent was obtained from all subjects.

This study was a crossover design with volunteers serving as their own controls. After initial baseline testing, subjects were randomly assigned to a diet containing 0.8 g (low protein [LP]), 1.8 g (moderate protein [MP]), or 3.6 g (high protein [HP]) of protein per kilogram of body weight (g/kg per day) for 3 weeks. After 3 weeks on the diet, WBPTO was assessed at rest using a primed continuous infusion of [1-^{13}C]leucine. After an approximate 2-week "wash-out" period, athletes crossed over to another diet intervention for 3 weeks and all measurements were repeated. Baseline testing included assessment of aerobic capacity (maximal oxygen uptake [$\dot{V}O_2$peak]), anthropometry (height and weight), body composition (hydrostatic weighing), resting energy expenditure (REE) (indirect calorimetry), 3-day diet records, and training records. Descriptions of these measures are given below.

2.2. Anthropometry

Body mass and height was measured using a balance beam scale equipped with a measuring rod (Health-o-Meter, Bridgeview, IL). Body weight was assessed at baseline and twice weekly at the same time of day under the same conditions throughout the study to ensure body weight maintenance. Percentage of body fat was estimated through hydrostatic weighing, and values were calculated from body density according to equations by Brozek et al [23].

2.3. Maximal oxygen uptake

Maximal graded exercise cardiopulmonary testing was conducted before the start of the study. $\dot{V}O_2$peak was determined via breath by breath analysis of expired gases during testing using an open circuit respiratory apparatus (MedGraphics CPX/D, Medical Graphics, St Paul, MN) on a treadmill (MedTrack ST55, Quinton, Bothell, WA) containing a ventilation flow meter, oxygen analyzer, and carbon dioxide analyzer.

2.4. Baseline dietary records

Three-day dietary records were collected from all study participants to assess their baseline nutrient intake for energy, carbohydrates, protein, and fat. All dietary records were entered into a computer and analyzed using Nutritionist Pro Software (N^2 Computing, Salem, OR).

2.5. Training journal

All athletes were instructed to keep training journals that detailed their daily and weekly totals for running mileage. These were collected weekly throughout each of the diet interventions.

2.6. Dietary intervention

Protein intakes were set at a "low," "moderate," or "high" protein intake level (0.8, 1.8, or 3.6 g/kg per day for LP, MP, and HP diets, respectively). Diet interventions were designed such that the percentage of total energy contributed by the macronutrients approximated 60% carbohydrate, 30% fat, and 10% protein for LP diet; 55% carbohydrate, 30% fat, and 15% protein for MP diet; and 40% carbohydrate, 30% fat, and 30% protein for HP diet. The diets were eu energetic with an isoenergetic exchange between protein and carbohydrate. The predominant protein source at each meal was beef. In addition, participants on the HP diet received 2 commercially available protein bars (Met-Rx Protein Plus, Irvine, CA) per day that provided approximately 1255 kJ and 32 g of protein (15 g carbohydrate,

Table 1
Baseline subject characteristics

Age (y)	21.3 ± 0.3
Height (cm)	179.1 ± 1.6
Weight (kg)	70.6 ± 0.1
Body fat (%)	8.7 ± 0.4
$\dot{V}O_2$max (mL/[kg · min])	70.6 ± 0.1
Running distance (miles/wk)	52 ± 2

Values are mean ± SEM (n = 5).

8 g fat) to increase their protein intake to the prescribed level. Menus incorporated food item exchange lists to meet the specified diet prescription for each individual and to ensure body weight maintenance.

Participants were fed at the metabolic kitchen in the Department of Nutritional Sciences and at a designated dining room through the Department of Catering at the University of Connecticut. Research assistants were present at all meals to weigh and serve the appropriate foods for each participant. Participants were not food restricted, per se, with any food eaten in excess or less than that prescribed at each meal recorded for that participant.

2.7. Apparent nitrogen balance

After 3 weeks of each dietary intervention, participants collected a pooled 24-hour urine sample for determination of nitrogen balance. Total nitrogen content of the urine was determined using a micro-Kjeldahl apparatus (Tecator Kjeltec System, Hoganus, Sweden). Nitrogen intake was determined from the nutrient analysis of the subjects' food intakes for the respective 24-hour period. Apparent nitrogen balance was calculated as the difference of nitrogen intake minus urinary nitrogen excretion plus estimated integumental losses [8].

2.8. Resting energy expenditure and substrate oxidation

Resting energy expenditure was estimated by open-circuit indirect calorimetry using a metabolic cart (MedGraphics CPX/D, Medical Graphics) before initiating dietary interventions. Subjects were driven to the metabolic laboratory immediately after waking in the morning after an overnight fast and rested quietly for 10 to 15 minutes before REE was measured. Resting energy expenditure was assessed for 20 minutes with the subject lying in a quiet, temperature-regulated room. Substrate oxidation was calculated using software supplied by the metabolic cart manufacturer after input of 24-hour urinary nitrogen data.

2.9. Infusate preparations

Stock solutions of all stable isotopes were created and certified to be sterile and pyrogen-free by the Department of Laboratory Medicine, University of Connecticut Health Center, Farmington. The stable isotopes tested were all commercially available products (Cambridge Isotope Laboratories, Cambridge, MA).

2.10. Study protocol

Subjects trained and competed throughout the study, but refrained from exercise the day before the infusion protocols to minimize the influence of the last exercise session on WBPTO. On study days, subjects were transported to the Metabolic Assessment Laboratory in the Department of Nutritional Sciences at ~7:00 AM after an overnight fast (≥10 hours). After REE determination, a 20-gauge Teflon catheter (7.78 cm, Jelco, Critikon, Tampa, FL) was inserted into an antecubital vein for isotope infusion. Another catheter (3.97 cm) was placed in the contralateral hand, and the hand heated with a heating pad, for the sampling of "arterialized" blood.

After providing baseline blood and breath samples, a primed continuous infusion of L-[1-^{13}C]leucine (4 μmol/kg; 4.8 μmol/kg per hour) (Cambridge Isotope, Andover, MA) was initiated (Razel Syringe Pump, Razel Scientific Instruments, Stamford, CT) and continued for 180 minutes. Beginning at 120 minutes, blood and breath measurements were collected at 15-minute intervals for 1 hour. Plasma samples were stored at −80°C for subsequent analyses. For $^{13}CO_2$ breath enrichments, subjects breathed into a Douglas bag for 2 minutes at specified time intervals. Breath samples were then transferred to 20-mL Venoject containers for isotope ratio analysis. Plasma ^{13}C-KIC and $^{13}CO_2$ enrichments were determined by gas chromatography and infrared mass spectroscopy, respectively, by a commercial laboratory (Metabolic Solutions, Nashua, NH).

Blood ^{13}C-KIC and breath $^{13}CO_2$ data for the 5 time points (120, 135, 150, 165, and 180 minutes) were evaluated to confirm steady-state conditions. Steady-state conditions were assumed when the coefficient of variation of the atom percent excess values at isotopic plateau was less than 10%. Data from the 5 time points were averaged for each subject and group means determined. Leucine Ra (Leucine rate of appearance; indicator of protein breakdown), Ox (leucine oxidation), and NOLD (nonoxidative leucine disposal; indicator of protein synthesis) were then calculated using the reciprocal pool model.

2.11. Insulin analysis

Plasma insulin was assessed throughout the infusion protocols. All blood samples were centrifuged (~5000 rpm) and transferred to appropriate cryotubes for storage at −80°C until processed. Insulin concentrations were determined in

Table 2
Actual dietary intakes for LP, MP, and HP diets

	kcal	Carbohydrate (g)	Fat (g)	Protein (g)
LP	14636 ± 138	584 ± 4†,‡	107 ± 3	64 ± 1†,‡
MP	14489 ± 364	524 ± 13*,‡	98 ± 3*	125 ± 3*,‡
HP	14004 ± 79	391 ± 10*,†	97 ± 2*	220 ± 3*,†

Values are mean ± SEM.
* $P < .05$, significantly different from LP.
† $P < .05$, significantly different from MP.
‡ $P < .05$, significantly different from HP.

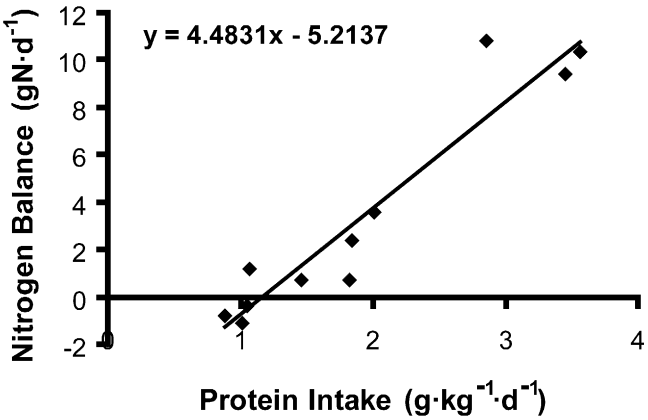

Fig. 1. Nitrogen balance is plotted against protein intakes for LP, MP, and HP diets in trained males. Extrapolated data indicate a protein intake of 1.2 g/kg per day is required to achieve zero nitrogen balance in these subjects.

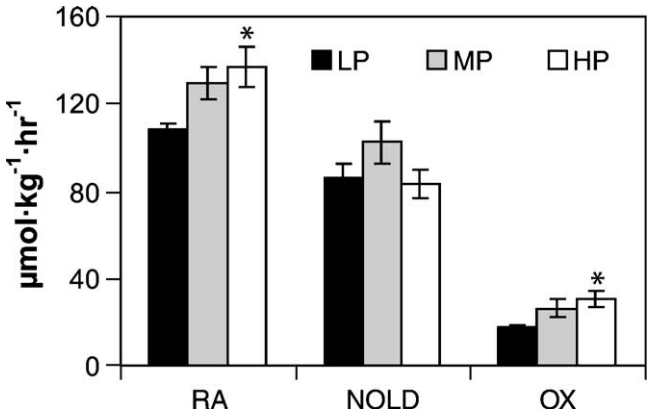

Fig. 3. Whole body protein turnover in trained males at rest after 3 weeks on the LP, MP, and HP diets. Values are means ± SEM. *$P < .05$, different from LP.

duplicate from lithium heparin–processed samples using solid-phase, double-antibody radioimmunoassay (DSL-1600, Diagnostic Systems Laboratories, Webster, TX).

2.12. Nutrient analyses

All daily menus were analyzed for energy and macronutrient composition using Nutritionist Pro Software (N^2 Computing). Once all menus were inputted, each participant's dietary analysis for determination of actual energy and nutrient intakes throughout the study was corrected for deletions, substitutions, and/or additions to the respective meal.

2.13. Statistical analysis

Primary outcome measures were WBPTO (Ra, Ox, and NOLD), substrate oxidation, and nitrogen balance after 3 weeks on each of the dietary interventions. Group means for Ra, Ox, and NOLD were compared using repeated-measures analysis of variance with group differences determined using Tukey post hoc analysis with an α level of .05. Simple linear regression analysis was used to characterize the relationships between protein intake and nitrogen balance as well as REE and protein turnover. Data were analyzed using SPSS 11.0 Statistical Software (SPSS, Chicago, IL). All data are presented as mean ± SEM.

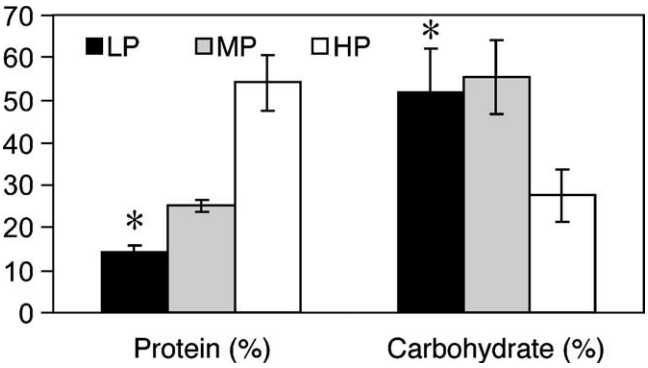

Fig. 2. Protein and carbohydrate oxidation in trained males at rest after 3 weeks on LP, MP, and HP diets. Values are means ± SEM. *$P < .05$, different from MP and HP diets.

3. Results

3.1. Subject characteristics

A homogenous group of 5 fit young men participated in this study (Table 1). Baseline dietary intakes revealed that the runners consumed ~11845 kJ, 52% carbohydrate (5.4 g/kg), 17% protein (1.7 g/kg), and 31% fat.

3.2. Diet intervention

The macronutrient breakdown was 48% CHO, 26% fat, and 26% protein for HP diet; 60% CHO, 26% fat, and 14% protein for MP diet; and 66% CHO, 27% fat, and 7% protein for LP diet. Mean protein intake in grams per kilogram of body weight was 0.87, 1.78, and 3.12, and carbohydrate intake was 8.3, 7.4, and 5.4 for LP, MP, and HP diets, respectively (Table 2).

3.3. Apparent nitrogen balance

Apparent nitrogen balance for HP diet (10.2 ± 0.7) was significantly higher ($P < .01$) than for both MP (1.8 ± 0.6) and LP (−0.3 ± 0.5) diets. A predicted protein intake to achieve zero nitrogen balance was calculated using a regression equation for group data based on data points for each subject (Fig. 1). The extrapolated protein intake for zero nitrogen balance was determined to be 1.2 g/kg per day.

3.4. Substrate oxidation

Resting energy expenditure after 3 weeks on each of the dietary treatments did not differ and was 7878 ± 385, 7644 ± 155, and 7406 ± 71 kJ/d for HP, MP, and LP diets, respectively (Fig. 2). As protein intake increased, protein oxidation increased, with each level of protein being significantly different from each other (54% ± 6% vs 25% ± 1% vs 14% ± 2% for HP, MP, and LP diets,

respectively; $P < .01$). On the HP diet, carbohydrate oxidation was significantly less than that noted for the MP and HP diets (28% ± 6% vs 55% ± 9% vs 52% ± 10% for HP, MP, and LP diets, respectively; $P \leq .01$).

3.5. Leucine kinetics

Leucine Ra for LP diet (107.8 ± 3.1 μmol/kg per hour) was significantly lower ($P < .05$) than for HP diet (136.7 ± 9.3 μmol/kg per hour) (Fig. 3). No differences in Ra between MP diet (129.1 ± 7.4 μmol/kg per hour) and LP or HP diet were found. Leucine oxidation was significantly lower ($P < .05$) for LP diet (18.3 ± 0.6 μmol/kg per hour) than for HP diet (31.0 ± 3.6 μmol/kg per hour). No differences in Ox between MP diet (26.2 ± 4.3 μmol/kg per hour) and LP or HP diet were found. There were no differences noted in NOLD across all 3 interventions (85.6 ± 6.7, 102.3 ± 9.4, and 83.2 ± 6.5 μmol/kg per hour for LP, MP, and HP diets, respectively). There was a significant positive correlation between increases in Ra and REE ($r = 0.7$, $P < .05$).

3.6. Plasma insulin

Plasma insulin concentrations decreased with increasing dietary protein with LP diet significantly higher than MP and HP diets (7.1 ± 1.0, 3.3 ± 0.4, and 2.3 ± 0.1 μIU/mL for LP, MP, and HP diets, respectively; $P < .01$).

4. Discussion

This study examined the effect of increasing dietary protein from 0.8 to 1.6 to 3.6 g/kg of body weight on resting whole body leucine kinetics, substrate oxidation, and nitrogen balance in male endurance athletes. This investigation is unique in that both contemporary (stable isotope methodology) and traditional (nitrogen balance) techniques were used in combination with controlled feeding protocols to assess protein turnover and nitrogen retention in the same, weight-stable, individuals, habitually consuming 3 levels of dietary protein that span the current DRI for protein [22].

The results of the present investigation indicate that variations in habitual protein intake influence measurements of WBPTO at rest in endurance-trained males. The highest level of dietary protein resulted in elevated rates of leucine Ra (protein breakdown) and leucine Ox compared with the lowest protein intake. Protein oxidation and nitrogen balance were also greater at the highest protein intake.

The premise for our investigation regarding how variations in protein intake influence protein turnover was based upon previous investigations that found leucine oxidation to increase during endurance exercise [2,3,13,14,16,24]. Evans and colleagues [25] demonstrated that a single endurance exercise bout (2 hours at 55% $\dot{V}O_2$peak) is associated with oxidation of as much as 86% of the daily requirement for leucine. Although this may not impact protein requirements for the recreational athlete, individuals who train daily at high intensities may have increased requirements because of long-term losses of this essential amino acid. Indeed, several previous studies of endurance-exercising individuals found that subjects were in negative nitrogen balance when consuming the recommended dietary allowance (RDA) for protein [22]. Positive nitrogen balance was only achieved as protein intakes were increased and ranged from 0.94 to 1.6 g/kg per day [8,9,11,13]. Therefore, the current study examined how the habitual consumption of varied levels of protein influences WBPTO parameters and contributes to the ongoing discussion as to whether recommendations for increased dietary protein are warranted for endurance athletes.

The significantly greater nitrogen balance noted with HP diet was not accompanied by an increase in whole body protein synthesis. Instead, the highest protein intake resulted in the greatest rates of whole body protein breakdown (leucine Ra) and leucine oxidation. These observations may suggest that at the lowest protein intake there is a greater efficiency in protein utilization or a down-regulation in turnover due to inadequate dietary amino acids. In addition, although there were no significant differences in REE across the 3 diets, the mean was ~460 kJ greater for subjects consuming the HP diet compared with the LP diet. This observed increase in REE was positively correlated with increases in leucine Ra. A relationship between protein turnover and resting metabolic rate has been observed previously in our laboratory [26] and others [27]. Our findings support the notion of a conservation of energy when exogenous protein stores are limited. It should also be noted that plasma insulin was greatest on the LP diet, and this may have contributed to the observed attenuation in protein breakdown.

Nitrogen balance increased with each level of protein, with positive values occurring only when subjects were consuming the MP and HP diets. In the current study, despite adequate energy intake, the LP diet was not sufficient for subjects to achieve a positive nitrogen balance. Although nitrogen balance was significantly more positive for HP diet compared with both MP and LP diets, these findings must be interpreted with caution given that the correction factor used for integumental losses may have underestimated increased fecal and integumentary nitrogen losses that often accompany HP diets [28].

In a study similar to the current investigation, Meredith et al [9] evaluated the effect of 3 levels of protein (0.6, 0.9, and 1.2 g/kg per day) on nitrogen balance in 12 weight-stable, endurance-trained men. A protein intake of 0.6 g/kg per day resulted in a negative nitrogen balance. With increasing protein intake to 0.9 g/kg per day, 7 of the 12 subjects remained in negative nitrogen balance. However, at an intake of 1.2 g/kg per day, subjects were in positive nitrogen balance. From these data, the authors determined a minimum protein requirement of 0.94 g/kg per day, a value slightly greater than the current RDA. In both the current

study and that of Meredith et al [9], dietary protein intakes less than 0.9 g/kg per day were not sufficient to achieve a positive nitrogen balance in all subjects. It is important to note that both of these studies were performed in endurance-trained males. In contrast, a study by Todd et al [1] and a recent study from our laboratory [26] found 0.8 to 0.9 g/kg per day of protein to be sufficient for achieving positive nitrogen balance in untrained individuals participating in moderate exercise, so long as subjects were in energy balance (as indicated by maintenance of body weight). Discrepancies between these studies highlight the importance of considering individuals who differ in training state and in the volume and intensity of training as distinct populations, especially when making protein recommendations for persons who endurance train. Because amino acid oxidation increases with increasing exercise intensity and duration [3,5,29], it could be assumed that this would result in differences in WBPTO when looking at differences in long-term training.

Our findings suggest that there are no advantages to consuming a level of protein higher than 1.8 g/kg per day. In fact, extrapolation of the nitrogen balance data predicted that the protein intake needed to achieve zero nitrogen balance in our study population was 1.2 g/kg per day. When consuming ~14644 kJ (the amount required for weight maintenance in these males), this equates to approximately 10% of energy intake or the low end of the current DRI for protein [22].

In addition, from a practical perspective, protein intakes above 1.8 g/kg per day present the concern for maintaining sufficient dietary carbohydrates for replenishment of muscle glycogen as well as issues regarding proper hydration (see Ref [30]) in competitive endurance athletes. In terms of performance, these issues remain a priority. Although it is generally recommended that endurance athletes consume 6 to 10 g of carbohydrate per kilogram of body weight, on the HP diet, despite an intake of ~14644 kJ, subjects were consuming only 5.6 g carbohydrate per kilogram of body weight [31]. However, on both the LP and MP diets, subjects did meet carbohydrate recommendations, with intakes of 8.3 and 7.5 g/kg, respectively.

In summary, this study placed special emphasis on the control of energy and protein intakes to characterize changes in protein utilization in response to varied levels of dietary protein in highly trained runners. Our findings suggest a down-regulation of protein turnover with protein intakes approximating the current RDA. The impact that routine consumption of this amount of dietary protein may have on the synthesis and repair of body tissues in endurance-trained individuals is not known. Practically speaking, this is not a typical concern for these types of athletes when consuming adequate energy. In fact, baseline protein intakes of our subjects reflected those noted by other researchers in similar populations (ie, 1.8 g/kg per day). Regardless, it is important to note that 0.87 g/kg per day appeared to be insufficient for athletes in energy balance for maintenance of nitrogen balance in this study. Therefore, in situations where endurance athletes restrict energy intake or fail to consume sufficient energy to achieve energy balance, special emphasis should be placed on consumption of higher levels of protein. Finally, our findings provide further support of current recommendations for protein intakes that approximate 1.5 g/kg for endurance athletes [32], as we observed that consumption of a diet containing greater than 1.8 g/kg per day provided no advantage to the endurance athlete with regard to whole body protein utilization.

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