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Age and sex affect protein metabolism at protein intakes that span the range of adequacy: comparison of leucine kinetics and nitrogen balance data[☆]

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Abstract

Research suggests that changes in leucine oxidation (leu_{ox}) with feeding may reflect adult protein requirements. We evaluated this possibility by assessing the effects of age, sex, and different protein intakes on whole-body leucine kinetics and nitrogen balance. Thirty-four young ($n = 18$, 22–46 years) and old ($n = 16$, 63–81 years) men and women completed three 18-day trials with protein intakes of 0.50, 0.75 and 1.00 g protein·kg body weight⁻¹·d⁻¹. Fasting and fed-state leucine kinetics were quantified on day 12 of each trial using a primed, constant infusion of L-[1-13C]leucine. Protein requirement was estimated using classical nitrogen balance measurements and calculations. Leucine kinetics parameters were influenced by age and sex across all protein intakes. With feeding, leu_{ox} increased more in old vs. young adults. Independent of age, fasting and fed-state leu_{ox} were lower, and net leucine balance (fasting+fed-state) was higher in women vs. men. Among all subjects and protein intakes, nitrogen balance was correlated with fed-state leu_{ox} ($r=0.39$), fed-state leucine balance ($r=0.60$), net leucine balance ($r=0.49$) and the change in leu_{ox} from the fasting to fed state ($r=0.49$) ($P<.05$ for all results). At the highest protein intake, the change in leu_{ox} with feeding was inversely correlated with protein requirement ($r=-0.39$). These findings indicate that leucine kinetics, especially leu_{ox} , reflect nitrogen balance-based estimates of the need for dietary protein and generally support the view that protein requirement is comparable between young and old adults.

Keywords

Dietary protein; Protein adequacy; Protein metabolism

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1. Introduction

The dietary protein needs of old men and women are not known with confidence [1], and discussions continue about adequate and “optimal” protein intakes for this important segment of the population [1-4]. Numerous physiological and metabolic changes occur with advancing age, including alterations in the rate and efficiency of protein metabolism and sarcopenia, which may influence dietary protein requirements [5-7]. However, establishing age-specific dietary protein requirements has been challenging; some studies support higher [8-11], unchanged [1,12-14] or lower [2,15] protein requirements for old vs. young adults. The 2002/2005 Institute of Medicine report on human dietary energy and macronutrient needs [16] emphasized the importance and need for more research to determine the dietary protein needs of old adults. We recently published nitrogen balance data suggesting that the protein needs of old adults are not different from those of young adults and not different from the recommended dietary allowance (RDA) of 0.8 g protein·kg body weight (BW)⁻¹·d⁻¹[3].

While the nitrogen balance technique is the standard approach for establishing the adult RDA for protein [1,16], it has well-defined limitations [17,18], so the Institute of Medicine report emphasized the need to evaluate and validate new methods for determining protein requirements [16]. Recent investigations have attempted to determine protein needs [15,19,20] by measuring whole-body protein metabolism using stable isotope-labeled amino acids [15,21,22]. However, the transition from using nitrogen balance to amino acid kinetics to estimate dietary protein needs has not been directly compared in the same subjects. To this end, we measured whole-body leucine kinetics in conjunction with the nitrogen balance-based assessment of protein requirements [3].

The primary purpose for this research project was to assess the effects of age and sex on fasting and fed-state protein metabolism using a primed constant infusion of L-[1-¹³C]leucine at three protein intakes that span the range of adequacy. A second purpose was to compare data from the stable isotope-labeled amino acid assessment with nitrogen balance data collected in the same subjects.

1.1. Subjects

Fifty-seven individuals were recruited to participate in this study via community postings and advertisements in local newspapers in Little Rock, AR, and West Lafayette, IN. The data from 40 subjects were included in the leucine kinetics analysis (11 young men, 9 old men, 11 young women and 9 old women) (Table 1). Data from 34 subjects with complete nitrogen balance and leucine kinetics data were used to compare the leucine kinetics findings to the nitrogen balance data (10 young men, 7 old men, 8 young women, and 9 old women). All subjects completed a prestudy evaluation that included a written medical history, clinical blood and urine chemistries and a resting-state electrocardiogram. Each subject had a clinically normal serum albumin concentration, no diabetes mellitus and clinically normal heart, liver, kidney and thyroid functions. Oral and written explanations of the study purpose and procedures were provided, and each subject signed an informed consent document. All subjects received monetary reimbursement for their participation, and the study protocol, advertisements and informed consent documents were approved by the institutional review boards of the University of Arkansas for Medical Sciences, Little Rock, AR, and Purdue University, West Lafayette, IN.

2. Methods and materials

All subjects completed three 18-day trials of strict dietary control in random order, with a minimum of 1 week of unrestricted habitual food intake between each trial. All young

women began each trial 5 to 7 days after the onset of their menstrual cycle. The procedures and testing were the same for each trial, except for the macronutrient distribution of the diets provided. The three trials were identified by the amount of protein that each subject was provided to consume: lower protein (LPro; 0.50 g protein·kg BW⁻¹·d⁻¹, 63% of the RDA), medium protein (MPro; 0.75 g protein·kg BW⁻¹·d⁻¹, 94% of the RDA) and higher protein (HPro; 1.00 g protein·kg BW⁻¹·d⁻¹, 125% of the RDA).

2.1. Diet

All subject's meals were provided by using a 3-day rotation of menus, and each daily menu was customized to provide sufficient total energy to meet the individual subject's energy requirement as previously described [3]. Subjects were provided a very-low-protein diet (<0.2 g protein·kg BW⁻¹·d⁻¹) on the first day of each trial to accelerate the metabolic adjustments to the subsequent protein intakes [23]. The menus used from days 2 to 18 during each of the three trials included highly digestible, egg (LPro, 1.9% of total protein; MPro, 3.0%; HPro, 4.2%) and dairy (LPro, 12.0%; MPro, 29.3%; HPro, 26.6%) protein sources but were void of meats because the high-protein content of skeletal muscle makes it difficult to incorporate into lower-protein menus. The nonprotein energy content of each menu within a trial was maintained at 65% carbohydrate and 35% fat during all three trials. The energy and macronutrient contents of the menus were calculated by using Nutritionist Pro computer software (version 1.5; First Databank Inc., San Bruno, CA, USA).

2.2. Body composition

Fasting-state nude BW (total weight minus robe weight) was measured each weekday during the three trials with a digital platform scale (model 15S or ES200L; Ohaus Corporation, Pine Brook, NJ, USA). Standing height without shoes was measured with a wall-mounted stadiometer. Body mass index (BMI) was calculated as BW divided by height squared (kg/m²). Whole-body volume and mass were measured by using a plethysmography system (Bod Pod; Life Measurement Instruments, Concord, CA, USA) on days 7 and 14 of each trial, and fat-free mass, fat mass and percentage body fat were calculated from body density by using Siri's [24] two-compartment model equation.

2.3. Food, urine and stool collections

During each trial, food, urine and stool samples were collected and processed, and aliquots were stored at -20°C as previously described [3]. The accuracy of the stool collections was enhanced by requiring each subject to orally consume a stool dye marker composed of encapsulated food coloring (either FD and CBlue No. 1 Alum Lake 11%–13% or Carmine Red; Warner-Jenkinson Co, St. Louis, MO, USA) at the beginning and end of the 3-day collection periods. Samples were continuously collected until the end-of-collection marker was visually identified by a research technician. Eight 24-h urine collections were made during days 7–10 and 14–17 of each trial. Urine total nitrogen excretion from the days 14–17 collections were used for the nitrogen balance calculations as the subjects had longer time to establish a steady state [3].

2.3. Nitrogen analysis

Food, stool and urine aliquots were analyzed for total nitrogen concentration with a Leco model FP-528 analyzer (St. Joseph, MI, USA) as previously described [3]. The instrument was calibrated daily with the use of an EDTA standard, and a soy flour standard was used to confirm accuracy.

2.4. Nitrogen balance and calculations of protein requirement

Each subject's apparent nitrogen balance ($\text{mg nitrogen} \cdot \text{kg BW}^{-1} \cdot \text{d}^{-1}$) during the LPro, MPro and HPro trials was calculated as $\text{IN} - (\text{UN} + \text{FN} + \text{MN})$, where IN is daily dietary nitrogen intake, UN is daily urinary nitrogen excretion, FN is daily fecal nitrogen excretion and MN is daily miscellaneous nitrogen excretions, assumed to be $5 \text{ mg N} \cdot \text{kg BW}^{-1} \cdot \text{d}^{-1}$ as previously described [3]. Each subject's nitrogen balance data from all three trials were linearly regressed with dietary protein intake, and inverse prediction [25] was used to estimate the protein intake that corresponded with nitrogen equilibrium. This value was used to represent the subject's protein requirement. The nitrogen balance data are published [3] and are described here only as necessary for comparison with the leucine kinetics data.

2.5. Leucine kinetics: infusion procedures, sample collections and calculations

On day 12 of each trial, a primed [$7.6 \mu\text{mol} \cdot \text{kg}^{-1}$ and $\text{NaH}^{13}\text{CO}_3$ ($2.35 \mu\text{mol} \cdot \text{kg}^{-1}$)] 8-h constant infusion $7.6 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ of L-[1- ^{13}C]leucine was used to determine fasting and fed-state whole-body leucine kinetics as previously described [26,27] (Fig. 1). The subjects remained in the fasting state during the first 3 hours and were in a fed state during the last 5 h of the 8-h infusion. The fed state was established by having each subject consume hourly (hours 5, 6, 7 and 8 of the infusion procedure) a formula beverage that contained one twelfth of his or her daily protein and energy intakes set for that trial. Blood and expired breath samples were obtained at 15-min intervals during hours 2–3 (fasting) and 7–8 (fed) of the infusion and processed as described [28,29]. The rate of carbon dioxide production was measured using a ventilated hood indirect calorimeter prior to and during the third and eighth hours of the infusions.

One week prior to an infusion procedure, each subject consumed the formula beverage hourly for 5 h, and breath samples were collected on the same schedule as during the infusion procedure. Exhaled $^{13}\text{CO}_2$ enrichment was quantified and subtracted from the corresponding breath samples collected on the infusion day to account for background changes in $^{13}\text{CO}_2$ associated with consuming the formula beverages. Other procedures performed on the infusion testing days (catheter placement, indirect calorimetry, etc.) were not done on this day.

The ^{13}C -enrichment of plasma α -ketoisocaproic acid was quantified using gas chromatography mass spectrometry (model HP 5989; Hewlett-Packard, Palo Alto, CA, USA), and the $^{13}\text{CO}_2$ enrichment in exhaled breath samples was quantified using isotope ratio mass spectrometry (VG Isogas, model SIRA 2; VG Instruments, Middlewich, UK). The isotope enrichment of each sample was calculated as atom percentage excess [29].

All kinetics parameters are expressed as $\mu\text{mol leucine} \cdot \text{kg BW}^{-1} \cdot \text{h}^{-1}$. Leucine turnover was calculated using a two-pool steady-state approach [30,31] as previously described [26]. In a steady state, the components of leucine turnover are represented by the following equation: $\text{turnover} = \text{oxidation} + \text{synthesis} = \text{dietary intake} + \text{infusate intake} + \text{breakdown}$. Leucine incorporation into protein (synthesis or nonoxidative leu disposal) was calculated as turnover minus oxidation. Leucine release from protein (breakdown) was calculated as turnover minus dietary leucine intake minus infusate leucine content. In the fasting state, dietary leucine intake was zero, and breakdown equaled turnover minus infusate leucine content. Fasting and fed leucine balances were calculated from intake (diet+infusate) minus oxidation. Net leucine balance was calculated as fasting leucine balance plus fed-state leucine balance. Net leucine balance was used to derive a term for nitrogen balance by converting leucine balance values to a nitrogen equivalent assuming that body protein contains 8% leucine and 16% nitrogen [32].

2.6. Clinical blood variables

Fasting blood samples collected on day 12 of each trial were analyzed for leukocyte content, and aliquots of serum were used to determine the concentrations of urea nitrogen (blood urea nitrogen), albumin and aspartate aminotransferase. These analyses were performed using standard clinical procedures at Pathology and Laboratory Medicine Service, Central Arkansas Veterans Healthcare System (Little Rock, AR, USA) or at the Laboratory Corporation of America (LabCorp, Burlington, NC, USA).

2.7. Statistical analysis

Subjects included in the leucine kinetics analysis had complete data for all three dietary trials ($n = 40$). Subjects included in the leucine kinetics and nitrogen balance comparisons had complete data sets for both analyses ($n=34$). Each value is expressed as mean $\pm$ S.E. To document the effect of feeding on leucine kinetics, a four-way repeated-measures analysis of variance (ANOVA) with a mixed-model approach was conducted to assess the effects of age (between-subject), sex (between-subject), metabolic state (fasting and fed state; within-subject) and dietary protein intake (LPro, MPro and HPro; within-subject) and their interactions on the dependent variables. If no interactions were observed, the main effects are reported. Post hoc analyses were conducted using Bonferroni correction. A two-way ANOVA with factors of age and sex was used to compare subject characteristics. To control for a potentially confounding subject effect in correlation, partial correlations were computed between nitrogen balance and leucine kinetics data to assess relationships between these outcomes. Statistical significance was considered as $P<.05$. Statistical Analysis Software version 9.2 was used for all statistical analysis (SAS Institute, Inc., Cary, NC, USA).

3. Results

3.1. Subject characteristics, dietary intakes and clinical blood profiles

BW did not differ between the young and old adults, whereas percentage body fat and BMI were lower and fat-free mass was higher in the young adults (Table 1). BW and fat-free mass were higher and body fat percentage was lower in men vs. women (Table 1) and in young men vs. young women. Old women had the highest percentage of body fat and lowest amount of fat-free mass vs. all other groups. By design, protein intake (g protein·kg BW⁻¹·d⁻¹) was not different among groups. Trial-specific differences in protein intake were confirmed by blood urea nitrogen and urinary total nitrogen excretion, which increased significantly with increasing protein intake [3,33]. Blood urea nitrogen was higher in men compared to women and was lower in young vs. old adults. The dietary intakes of energy, carbohydrate and fat were higher for young vs. the old adults, higher for men vs. women and not significantly different among the three trials. Serum albumin concentration, blood leukocyte content and serum aspartate aminotransferase concentration (markers of protein status, infection and liver function, respectively) were within the ranges of clinical normalcy for each group at all trials. Clinical blood profile data are not shown but were reported previously [33].

3.2. Effect of metabolic state and protein intake

For all subjects, the transition from the fasting to the fed states increased leucine turnover [2.1 $\pm$ 1.3, 11.3 $\pm$ 1.2 and 21.0 $\pm$ 1.6 μ mol leucine·kg BW⁻¹·h⁻¹; LPro (fed–fasted), MPro (fed–fasted) and HPro (fed–fasted)], oxidation (5.0 $\pm$ 0.6, 8.8 $\pm$ 0.6 and 13.7 $\pm$ 0.8 μ mol leucine·kg BW⁻¹·h⁻¹), balance (23.0 $\pm$ 0.7, 31.3 $\pm$ 0.7 and 38.6 $\pm$ 0.9 μ mol leucine·kg BW⁻¹·h⁻¹) and reduced protein breakdown (–25.9 $\pm$ 1.4, –28.8 $\pm$ 1.2 and –31.3 $\pm$ 1.5 μ mol leucine·kg BW⁻¹·h⁻¹), at each of the three protein intakes ($P=.002$), with synthesis increasing at MPro

and HPro (2.6 ± 1.0 and 7.3 ± 1.2 $\mu\text{mol leucine} \cdot \text{kg BW}^{-1} \cdot \text{h}^{-1}$) (Supplemental Table 1). Protein intake significantly altered leucine kinetics within the fasted and fed states, independent of age and sex (main effect of protein). In the fasted state, turnover (112.6 ± 2.0 $\mu\text{mol leucine} \cdot \text{kg BW}^{-1} \cdot \text{h}^{-1}$ vs. 109.5 ± 2.3 and 109.6 ± 2.1 $\mu\text{mol leucine} \cdot \text{kg BW}^{-1} \cdot \text{h}^{-1}$), oxidation (17.4 ± 0.7 $\mu\text{mol leucine} \cdot \text{kg BW}^{-1} \cdot \text{h}^{-1}$ vs. 16.0 ± 0.6 and 15.2 ± 0.7 $\mu\text{mol leucine} \cdot \text{kg BW}^{-1} \cdot \text{h}^{-1}$), breakdown (105.3 ± 2.0 $\mu\text{mol leucine} \cdot \text{kg BW}^{-1} \cdot \text{h}^{-1}$ vs. 102.2 ± 2.3 and 102.3 ± 2.1 $\mu\text{mol leucine} \cdot \text{kg BW}^{-1} \cdot \text{h}^{-1}$) and balance (38.6 ± 0.90 $\mu\text{mol leucine} \cdot \text{kg BW}^{-1} \cdot \text{h}^{-1}$ vs. 31.3 ± 0.7 and 23.1 ± 0.7 $\mu\text{mol leucine} \cdot \text{kg BW}^{-1} \cdot \text{h}^{-1}$) were greater at HPro compared to MPro and LPro ($P < .05$). Fed-state turnover (111.7 ± 2.1 $\mu\text{mol leucine} \cdot \text{kg BW}^{-1} \cdot \text{h}^{-1}$ vs. 120.8 ± 2.4 and 133.6 ± 2.3 $\mu\text{mol leucine} \cdot \text{kg BW}^{-1} \cdot \text{h}^{-1}$; LPro, MPro and HPro), oxidation (20.2 ± 0.7 $\mu\text{mol leucine} \cdot \text{kg BW}^{-1} \cdot \text{h}^{-1}$ vs. 24.8 ± 0.7 and 31.1 ± 1.0 $\mu\text{mol leucine} \cdot \text{kg BW}^{-1} \cdot \text{h}^{-1}$), synthesis (91.6 ± 1.8 $\mu\text{mol leucine} \cdot \text{kg BW}^{-1} \cdot \text{h}^{-1}$ vs. 96.1 ± 2.2 and 102.5 ± 2.0 $\mu\text{mol leucine} \cdot \text{kg BW}^{-1} \cdot \text{h}^{-1}$) and balance (15.2 ± 0.8 $\mu\text{mol leucine} \cdot \text{kg BW}^{-1} \cdot \text{h}^{-1}$ vs. 22.6 ± 0.8 and 28.5 ± 1.1 $\mu\text{mol leucine} \cdot \text{kg BW}^{-1} \cdot \text{h}^{-1}$) increased with each successive protein intake (LProMProHPro), as did net balance ($P < .05$).

3.3. Effect of age

The young adults had higher rates of turnover, synthesis and breakdown, compared to the old adults, in the fasting and fed states ($P = .002$) (Supplemental Table 1). There was no effect of age on leucine oxidation or balance in the fed state. The change in oxidation from the fasting to the fed states was greater in old vs. young adults (Fig. 2); however, the change in leucine balance was greater in young vs. old adults ($P < .05$).

3.4. Effect of sex

Men exhibited higher rates of oxidation leading to a lower net protein balance compared with women ($P < .05$). In the fasting state, men had higher rates of turnover, oxidation and breakdown and a more negative balance compared with women ($P = .04$), independent of age and protein intake (Supplemental Table 1). In the fed state, oxidation was higher and balance was lower in men vs. women ($P = .002$). Net leucine balance was lower in men vs. women ($P < .05$) (Fig. 3).

3.5. Relationship between leucine kinetics and classical nitrogen balance

Correlations were observed between the change in nitrogen balance from the lowest to highest protein intake (HPro–LPro) and fasting leucine turnover ($R^2 = 0.12$, $P < .05$), oxidation ($R^2 = 0.12$, $P < .05$) and breakdown ($R^2 = 0.34$, $P < .01$), with a positive correlation with leucine balance ($R^2 = 0.12$, $P < .05$). Using data from all subjects and protein intakes, nitrogen balance was positively correlated with fed-state leucine balance ($R^2 = 0.36$, $P < .05$), net leucine balance (net leucine balance converted to isotope-derived nitrogen balance) ($R^2 = 0.40$, $P < .05$; Fig. 4) and the change in leucine oxidation from the fasted to fed states ($R^2 = 0.45$, $P < .05$; Fig. 5). The partial correlations between net leucine balance and nitrogen balance for old women, young women, old men and young men are 0.76 ($P = .0002$), 0.76 ($P < .0001$), 0.30 ($P = .22$) and 0.66 ($P = .03$), respectively. The partial correlations between nitrogen balance and change in leucine oxidation for old women, young women, old men and young men are 0.82 ($P < .0001$), 0.59, ($P = .005$), 0.61 ($P = .007$) and 0.73 ($P = .01$), respectively. For the HPro trial, the change in leucine oxidation from the fasted to the fed state was negatively correlated with the subject's protein requirement estimated from nitrogen balance ($R^2 = 0.16$, $P < .05$; Fig. 6).

4. Discussion

The current study is the first to directly compare leucine kinetics and nitrogen balance data in young and old men and women at dietary protein intakes that span the range of adequacy.

Our results confirm the notion that age and sex significantly impact protein metabolism as assessed by stable isotope-labeled amino acid kinetics. The primary finding was that assessments of protein metabolism based on stable isotope-labeled amino acid (leucine) kinetics were in good agreement with nitrogen balance-based estimates of protein requirement. These findings provide important information regarding the compatibility of labeled amino acid and nitrogen balance data in young and old men and women at dietary protein intakes that span the range of adequacy. In addition, our findings help to better describe the protein needs of young and old adults.

As expected, acute feeding caused marked changes in protein oxidation, turnover and synthesis [30,31,34,35], resulting in a transition toward a positive net leucine balance when all groups and treatments were combined [15,31]. Within each metabolic state, the level of dietary protein intake significantly influenced protein metabolism in accordance with existing literature. In the fasted state, turnover, oxidation, breakdown and balance were increased at HPro vs. MPro and LPro, supporting previous reports that changes in fasting state kinetics are altered in response to changes in habitual protein intake, specifically at HPro intakes [36,37].

Aging is associated with numerous metabolic and physiological changes that may alter dietary protein requirements with increasing age. These alterations may include changes in the proportion of lean vs. fat mass as well as alterations in interorgan amino acid utilization [35,38]. The impact of age on protein metabolism continues to be debated, with some investigators observing deficiencies in basal and postfeeding protein metabolism [7,39,40], while others reported no differences between young and old adults [5,41,42]. The current data show an age-related difference in whole-body protein metabolism, with old adults exhibiting reduced rates of protein turnover, synthesis and breakdown in the fasting and fed states compared with young adults per kg BW. Although age-related differences in protein metabolism are well documented, [39,40,43], based on the nitrogen balance [3,12] and kinetics data [2,44] in the literature and in the current study, there are no compelling data that the dietary protein needs of old people are different than those of young people when expressed per kg BW.

The use of postprandial leucine oxidation as an index of dietary protein adequacy is based on observations that the rate of oxidation remains low and relatively constant at protein intakes at or below the protein requirement and increases at protein intakes above the protein requirement [19,20,36,37]. For individuals with an a lower protein requirement, leucine oxidation would disproportionately increase for a given increase in protein intake (and nitrogen balance) from below to above the person's need. When all protein intakes were considered, the change in leucine oxidation from the fasted to the fed state was greater in old vs. young adults, with a trend observed for greater oxidation in old adults at HPro ($P<.10$; Fig. 2). These findings do not support the notion of a higher protein requirement in old adults but are consistent with a similar or reduced protein need in this population. This finding is in accord with previously published nitrogen balance data [3,12] and in agreement with previous reports using a novel metabolic approach [15]. The agreement of leucine kinetics and nitrogen balance data collected in the same subjects further supports the notion of a similar protein requirement for younger and older adults.

In addition to age, differences in skeletal muscle size and lean mass stores between men and women imply that there are inherent sex differences in the regulation of protein metabolism. Published findings in this area are inconsistent; several studies report sex differences in leucine oxidation at rest and during aerobic exercise [45-49], while others have found no significant differences [50-52] in protein metabolism when data were normalized to fat-free mass. The current finding of an increased rate of fed-state oxidation in men vs. women is

novel and expands upon previously reported sex differences in fasting protein kinetics [48,49,53,54]. There was no difference in the change in leucine oxidation from the fasting to the fed states between men and women, suggesting that the greater fed-state rate of leucine oxidation was influenced by the higher fasting oxidation rate in men. Currently, few studies have examined the influence of subject sex on leucine oxidation without exercise. A mechanism mediating the higher fasting and fed rates of leucine oxidation in men is unclear, and the impact of greater leucine oxidation on protein requirement in men is not known.

To date, very few investigations have concomitantly examined nitrogen balance and amino acid kinetics in the same group of subjects. As a result, our understanding of the conformity of these techniques in assessing protein balance and requirement is limited. Our results indicate that the change in leucine oxidation from the fasted to fed states at the highest protein intake was negatively correlated with nitrogen balance-based estimates of protein requirement (Fig. 6). This finding supports the notion that protein oxidation increases at protein intakes that exceed dietary need and provides evidence for a qualitative link between nitrogen and isotope-labeled amino acid based assessments of protein requirement as previously reported [19,37,55]. This notion is also supported by the positive correlation observed between the change in leucine oxidation from the fasted to fed states and nitrogen balance across all protein intakes (Fig. 5) and is in line with previous reports [56,57]. The leucine oxidation data from the current study in combination with the noted relationships between leucine kinetics and nitrogen balance data support the nitrogen balance-derived estimates of protein needs in young and old adults.

Qualitative similarities between data sets were also observed when protein balance was considered, as classically assessed nitrogen balance data were positively correlated with nitrogen balance estimates derived from leucine balance data (Fig. 4), as previously reported [56]. Nitrogen balance calculated from net leucine balance data was more positive than the classic nitrogen balance data, a finding possibly influenced by the length and nature of the feeding trial. Specifically, assessment of nitrogen balance is conducted during consecutive 24-h periods encompassing the fasting and fed states over multiple days. In contrast, nitrogen balance estimates derived from leucine balance data represent estimates of the daily protein balance extrapolated from short-term assessments of fasting and fed-state leucine kinetics. The assessment of fasting and fed-state leucine oxidation over a short period assumes that the measured rate of oxidation represents the average rate of oxidation over the entire 12-h period.

Nitrogen and leucine balance data may also be impacted by the nature of the feeding associated with each trial. Studies investigating both leucine [56-58] and nitrogen balance [56,59] were shown to be directly impacted by the frequency of protein feeding. In the current study, subjects consumed meals three times daily on days in which samples were collected for calculation of nitrogen balance. In contrast, on the day of the stable isotope infusion trial, the subject received hourly meals equal to one twelfth of the total daily energy requirement during the fed state. These differences in feeding frequency may impact nitrogen and leucine balance results and underscore the importance of evaluating quantitative comparisons between nitrogen balance and stable isotope-labeled leucine kinetics-based assessments cautiously.

Regarding the relationships between the change in nitrogen balance from the lowest to highest protein intake and fasting leucine kinetics parameters, it is interesting to note that the R^2 tended to be higher for breakdown ($R^2=0.34$) than for turnover ($R^2=0.12$) and oxidation ($R^2=0.12$). The apparently stronger correlation for breakdown may occur because the rate of isotope infusion, which contributes to the overall variability of the procedure, is accounted for when breakdown is calculated.

5. Summary and conclusions

This study provides a unique assessment of the effects of age and sex on protein metabolism and dietary requirement, comparing nitrogen balance and leucine kinetics data across a range of protein intakes that span the range of adequacy. Our results indicate that leucine kinetics data are closely related to and support the nitrogen balance-derived estimates of protein needs of young and old men and women.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Experimental design: priming doses of $7.6 \mu\text{mol}\cdot\text{kg}^{-1}$ L-[1-¹³C]leucine and $2.35 \mu\text{mol}\cdot\text{kg}^{-1}$ NaH¹³CO₂ were immediately followed by a continuous infusion of $7.6 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ L-[1-¹³C]leucine for 8 h. *Beverages contained one twelfth of the subject's daily protein and energy intakes.

Fig. 2.

Change in leucine oxidation between the fasting and fed states at each protein intake. Old vs. young adults were significantly different ($P < .05$). Error bars represent the standard error of the change in leucine oxidation from the fasting to the fed states. *Main effect of age (different between young and old, $P < .05$). The change in leucine oxidation was greater in old vs. young across all three protein intakes. The x-axis represents the change in leucine oxidation at each protein intake (0.5, 0.75 and 1.00 (g protein · kg BW⁻¹ · d⁻¹)). A three-way repeated-measures ANOVA with a mixed-model approach was used.

Fig. 3.

Net leucine balance at each protein intake. Women vs. men were significantly different ($P < .05$). Error bars represent the standard error of net leucine balance. †Main effect of sex (different between young and old, $P < .05$). Net leucine balance was greater in women vs. men across all three protein intakes. The x -axis represents the net leucine balance at each protein intake (0.5, 0.75 and 1.00 (g protein • kg BW⁻¹ • d⁻¹)). A three-way repeated-measures ANOVA with a mixed-model approach was used.

Fig. 4. Relationship between classical nitrogen balance and isotopically derived nitrogen balance data combining all protein intakes ($R^2=0.40$, $P<.05$). Partial correlation was used.

Fig. 5.

Relationship between classical nitrogen balance and the change in leucine oxidation from the fasting to the fed states combining all protein intakes. The change in leucine oxidation from the fasted to fed states increased as nitrogen balance increased ($R^2=0.45$, $P<.05$). Partial correlation was used.

Fig. 6. Relationship between estimated protein requirements and the change in leucine oxidation from the fasting to the fed states during the HPro treatment. The change in leucine oxidation from the fasted to the fed states decreased at the highest protein intake with increasing protein requirement (estimated from classical nitrogen balance data, ($R^2=0.16$, $P<.05$). Partial correlation was used.

Table 1

Subject characteristics

	Young men	Old men	Young women	Old women
<i>n</i>	11	9	11	9
Age, years	29.5±2.0*	72.6±2.2	28.9±2.0	74.4±1.4
Weight, kg	80.4±4.6	77.6±4.0	65.7±3.0 [†]	73.3±3.8
BMI, kg/m ²	25.0±1.3*	25.7±0.9	22.2±0.9	27.4±1.1
FFM, kg	61.2±2.8*	55.2±1.9	45.0±0.9 [†]	38.9±1.5 ^a
Body fat, %	22.5±2.1*	28.1±2.3	28.5±2.2 [†]	45.3±1.6 ^a

Values are means±SE; two-way-ANOVA (age and gender). FFM, fat-free mass.

* Old and young differ ($P<.05$).

[†] Men and women differ ($P<.05$).

^a Old women different from all groups ($P<.05$).