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Nutrient Ingestion, Protein Intake, and Sex, but Not Age, Affect the Albumin Synthesis Rate in Humans^{1,2,3}

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Abstract

The aim of this study was to assess the effects of nutrient ingestion, dietary protein intake, age, and sex on the fractional synthesis rate (FSR) of albumin. Thirty-six healthy free-living individuals (8 females and 10 males aged 21–43 y and 9 females and 9 males aged 63–79 y) completed three 18-d periods of controlled feeding with protein intakes of 125% (P125, 1.00 g protein · kg⁻¹ · d⁻¹), 94% (P94, 0.75 g protein · kg⁻¹ · d⁻¹), and 63% (P63, 0.50 g protein · kg⁻¹ · d⁻¹) of the recommended dietary allowance. On d 12 of each trial, postabsorptive (PA) serum albumin concentration was determined and PA and postprandial (PP) albumin FSR were estimated from the rate of L-[1-¹³C] leucine incorporation into plasma albumin during an 8-h infusion. There were no age-related differences in PA and PP albumin FSR. Albumin FSR was higher PP than PA ($P < 0.0001$), and the increase in albumin FSR from PA to PP was smaller as dietary protein intake decreased from P125 to P94 and P63 ($P < 0.05$). Independent of protein intake, males had a higher albumin FSR ($P < 0.05$) and a greater increase in albumin FSR with feeding ($P < 0.05$). There was no age or dietary protein effect on serum albumin concentrations, but males had higher albumin concentrations than females ($P < 0.0001$). These results show that older persons are responsive to nutrient ingestion and dietary protein-related changes in albumin FSR. The greater albumin synthesis rate in males might contribute to a higher albumin concentration set point.

Introduction

Albumin is the most abundant protein in human serum. Numerous health and lifestyle factors influence serum albumin, and low albumin concentration is associated with low protein intake (1), malnutrition (2), liver, and kidney diseases (3), smoking (4), decreased muscle mass (5), strength (6), and functional performance (7,8), and low physical activity and exercise (4). More broadly, serum albumin is inversely related to morbidity and mortality (4,9). Advancing age also contributes to lower serum albumin concentration (4,9–11). However, the reports of an age-related decline in albumin concentration are conflicting (5,12), and these inconsistencies could be due to the confounding effects of one or more

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health and lifestyle factors, inflammation, sepsis, and other medical conditions that can change albumin concentration by altering any of the following: the rate of synthesis, the secretion from liver cells, distribution in body fluids, the rate of degradation, and exogenous loss (13,14).

Hepatic albumin synthesis is suppressed during an extended fasting period and is stimulated with nutrient ingestion. Increased fractional synthesis rate (FSR)⁶ of albumin has been observed following the consumption of a meal in young (15), middle aged [~50–62 y, (16,17)], and older persons (18). Caso et al. (18) reported that younger and older adults have comparable postabsorptive (PA) state and postprandial (PP) state (ingestion of meals containing a mixture of carbohydrate, fat, and protein or only protein) albumin FSR. The lack of difference in PP FSR when an equivalent amount of protein was consumed in combination with other macronutrients or alone, underscores that amino acid availability is an important determiner of the FSR of albumin (18). Findings from limited research suggest that a person's age might influence whether the rate of hepatic albumin synthesis is responsive to changes in dietary protein intake. One study showed no difference in the FSR of albumin between younger and older men fed a diet containing adequate protein ($1.5 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$) (10). However, when a diet containing protein below the recommended dietary allowance (RDA) was consumed, the albumin FSR decreased in younger men (10,19,20) but remained the same in older men (10). Gersovitz et al. (10) suggested that older persons may have a blunted protein synthesis response because albumin FSR remained constant when inadequate protein was consumed. The stable isotope procedure used (oral administration of ¹⁵N-glycine every 3 h over a 60-h period) did not provide an opportunity to estimate PA and PP FSR separately. The apparent age-related differences in albumin synthesis sensitivity to changes in dietary protein intake have not been further assessed in healthy free-living older men or women.

Sex may be an influential factor in age-related and dietary protein-related effects on albumin FSR, but relatively little research has examined the sex-related difference in albumin FSR. One study reported that males had a greater albumin FSR than females, independent of age (12). To our knowledge there are no studies comparing the sex-related differences in the metabolic response of albumin to nutrient intake.

The primary purpose of this study was to assess the influences of dietary protein intake and age on albumin FSR in both the PA and PP states. The secondary purpose of this study was to determine whether sex has an effect on the above variables. Based on Gersovitz et al. (10), we hypothesized that albumin FSR would decrease with decreasing dietary protein intake in younger, but not older, males and females.

Methods

Subjects and preliminary testing

Fifty-eight subjects were recruited to participate in this study. Prior to starting the study, each subject completed an evaluation that included a resting-state electrocardiogram, routine clinical blood and urine chemistries, and a written medical history. All subjects had clinically normal heart, liver, and kidney functions and did not have diabetes mellitus. Each subject received written and oral descriptions of the protocol, signed a written informed consent agreement, and received a monetary stipend for participating. The study protocol and consent form were approved by the Institutional Review Boards at Purdue University, West Lafayette, IN and the University of Arkansas for Medical Sciences, Little Rock, AR.

⁶Abbreviations used: BUN, blood urea nitrogen; FSR, fractional synthesis rate; KIC, α -keto-isocaproic acid; P63, 63% of the RDA; P94, 94% of the RDA; P125, 125% of the RDA; PA, postabsorptive; PP, postprandial; RDA, recommended dietary allowance.

Forty-eight subjects successfully completed the study, and 10 subjects voluntarily discontinued participation due to scheduling conflicts and personal circumstances unrelated to the study. Data from 36 participants were used for the analyses: 8 females and 10 males aged 21–43 y (younger group) and 9 post menopausal females and 9 males aged 63–79 y (older group). The reasons for excluding data of 12 subjects from the 48 who completed the study were: incomplete blood sampling or inadvertently lost samples (3 subjects), inadvertently providing the subjects with the wrong beverage for the PP hourly feedings during one of the infusion testing days (3 subjects), and nonphysiological changes in albumin FSR (negative change) from the PA to the PP states during one or more of the subject's infusion procedures (6 subjects).

Experimental design and dietary control

Each subject was provided a controlled diet for three 18-d periods in a randomized, crossover design consisting of 3 levels of dietary protein intake: 125% (P125, 1.00 g protein · kg body wt⁻¹ · d⁻¹), 94% [P94, 0.75 g protein · kg⁻¹ · d⁻¹ (21)], and 63% [P63, 0.50 g protein · kg⁻¹ · d⁻¹ (22)] of the RDA. Each subject's meals were customized to provide sufficient energy for body weight maintenance and a nonprotein energy content of 65% carbohydrate and 35% fat. The subject's total energy requirement was estimated to equal their resting energy expenditure [calculated using a sex-specific Harris Benedict equation (23)] times 1.75 (energy expenditure of physical activity factor). The 1.75 activity factor was used instead of the more commonly used 1.50–1.60 factors to compensate for potential overestimation of actual metabolizable energy intakes when using the Atwater energy equivalents of 16.7, 16.7, and 37.7 kJ/g of protein, carbohydrate, and fat, respectively, to calculate the energy contents of the diets provided to the subjects (24). Energy, macronutrient, and micronutrient components of the diet were calculated using Nutritionist Pro computer software, version 1.5 (First Databank). Dietary proteins were provided during each trial (P125, P94, and P63) from the following sources: for the men, dairy (27, 29, and 12% of protein, respectively), egg (4, 3, and 2%, respectively), grains and legumes (58, 55, and 65%, respectively), and fruits and vegetables (11, 13, and 21%, respectively); for the women, dairy (29, 25, and 13%, respectively), a whey protein supplement (22, 11, and <1%, respectively), grains and legumes (29, 38, and 47%, respectively) and fruits and vegetables (20, 26, and 39%, respectively). Muscle-tissue-containing foods were excluded from the meals due to their high protein content. Subject instructions and setting for meal consumption and protocol for alcohol, water, and multivitamin and multi-mineral ingestion were described previously (25,26). Fasting-state nude body weight was determined on each weekday throughout the study using a digital platform scale (model ES200L; Ohaus).

The current study was a portion of a larger 18-d nitrogen balance study. All measurements for the results reported here were made on d 5–12 of each trial, as described below. A washout period occurred between each trial lasting a minimum of 1 wk during which time participants consumed their self-selected habitual diet. All younger women began the controlled diet for each trial 5–7 d following the onset of their menstrual cycle.

Urine collections and analyses

Twenty-four-hour urine collections were obtained on d 7–10 of each trial. The urine samples were kept refrigerated at 4°C during the day of collection and aliquots frozen at –20°C for later testing. Total urinary nitrogen concentrations were measured using a nitrogen analyzer (model FP-528, Leco).

Infusion-day testing procedures and collections

On d 12 of each trial, after an overnight fast of at least 10 h, each participant completed the testing protocol shown in Supplemental Figure 1. One catheter was placed in an anticubital

vein and used to administer the isotopes during the infusion procedure. A second catheter was placed retrograde in a hand vein and used for blood collections. The catheter in the hand was maintained between blood collections with a saline drip and the hand was continuously kept warm with a heating pad to facilitate the collection of “arterialized” venous blood. Following the collection of baseline blood samples, each subject received 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 (7.6 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$), and intravenous L-[1- ^{13}C] leucine infusion. Each subject remained in a PA state during the first 3 h of the infusion (0–180 min) and was in a PP state during the remaining 5 h (180–480 min). The PP state was induced through consumption of 5 hourly beverages (min 180, 240, 300, 360, and 420), each containing one-twelfth of the individual’s daily protein and energy intakes. Beverages consisted of Ensure Plus, Polycose (Abbott Laboratories) and a nondairy liquid (Coffee Rich, Morningstar Foods).

Aliquots of the baseline (fasting) blood samples were analyzed for leukocyte content and the concentrations of serum urea nitrogen (BUN), albumin, and aspartate aminotransferase using standard clinical procedures at Pathology and Laboratory Medicine Service, Central Arkansas Veterans Healthcare System, Little Rock, AR or at Laboratory Corporation of America (LabCorp).

Blood samples were collected at the time points indicated in Supplemental Figure 1 and placed into heparin-coated tubes, the tubes were centrifuged at $3000 \times g$ for 10 min at 4°C , aliquots of plasma were prepared and placed into cryovials and stored at -80°C until thawed and used for analysis. Plasma α -keto-isocaproic acid (KIC) was extracted and chemically derivatized, as described (27). Plasma ^{13}C -KIC enrichment was quantified using GC-MS (28).

Albumin isolation and quantification

Albumin was isolated from the plasma samples collected at min 0 (baseline), 180, and 480 using the protocol described previously (29,30) with a few modifications. Briefly, 0.25 mL of plasma was deproteinized with 0.25 mL of 20% trichloroacetic acid. After removing the supernatant, the precipitate was re-suspended and disrupted in 1 mL of 10% trichloroacetic acid to remove free labeled leucine. The pellet was dissolved in absolute ethanol and the supernatant was retained and dried in a CentriVap (Labconco). The sample was hydrolyzed with 2 mL 6 mol/L HCl for 24–36 h at 110°C . The hydrolysate was filtered through a 0.2 μm syringe filter onto a column containing 1 mL AG-50W-X8 (100–200 mesh) cation exchange resin and washed 3 times with 2 mL of 1 mmol/L HCl. The amino acids were eluted with 3 mL 12 mol/L ammonium hydroxide. The sample was dried in a Savant Speed Vac drier (Crawley). The N-acetyl o-propyl ester was formed as previously described (31). ^{13}C -leucine enrichment in plasma albumin was quantified using GC-combustion-isotope ratio MS using the protocols described previously (29,32).

Calculation of albumin fractional synthesis rate

Albumin FSR was calculated for both the PA and the PP states using the following equation (29):

$$FSR = \frac{(\Delta[1-^{13}\text{C}]\text{leucine atom \% excess} \cdot 100) \cdot 24 \text{ h}}{[1-^{13}\text{C}]\text{KIC atom \% excess} \cdot \text{elapsed time (h)}}$$

The $\Delta[1-^{13}\text{C}]$ leucine atom % excess represents the change in albumin-bound 1- ^{13}C -leucine from the start to end of the elapsed time, 0–180 min for PA and 180–480 for PP. The mean ^{13}C -KIC enrichment from the 4 samples collected between 120–180 and 420–480 min

was used to represent the hepatic precursor pool enrichment for albumin synthesis during the PA and PP states, respectively (33). Δ FSR is the difference between the PA and the PP states; determined by subtracting PA FSR from the PP FSR.

Statistical analyses

Values are expressed as means \pm SD, unless otherwise indicated. For the diet, urine, and blood parameters, a 3-factor, repeated measures ANOVA was used to assess the main effects of dietary protein intake (P125, P94, and P63; within-subject effect), age (younger and older; between-subject effect), sex (males and females; between-subject effect), and their interactions on the dependent variables. Age was assessed as both a continuous and a categorical variable. A 4-factor repeated measures ANOVA was used to assess the main effects and interactions of nutrient ingestion (PA and PP; within-subject effect), dietary protein, age, and sex on albumin FSR. Post hoc analyses were conducted using the Tukey adjustment and least square difference. A linear regression analysis was used to assess all relations. SAS software, version 9.1 (SAS Institute) was used for the statistical analysis of all parameters. Significance was determined at $P < 0.05$.

Results

Subject characteristics and dietary intervention

For both sexes, there was not a significant difference in height and weight between younger and older subjects. The BMI of younger females was lower than older females ($P < 0.05$; Table 1) and that between younger and older males did not differ. Blood leukocyte content and serum aspartate aminotransferase concentration, markers of infection and liver function, respectively, were within the range of clinical normalcy for all groups at all trials (Table 1). They were higher in the males than females ($P < 0.05$) and did not differ among trials or between younger and older subjects.

As planned, protein intake (on a $\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ basis) did not differ between younger and older subjects or between males and females and decreased from P125 to P94 to P63 (Table 2). Dietary intakes of energy, carbohydrate, fat, and fiber were higher in younger than in older subjects, independent of sex and trial ($P < 0.01$). Dietary intakes of energy (on a MJ/d basis), carbohydrate, and fiber were lower in females than in males, independent of age and trial ($P < 0.0001$, Table 2).

Among all trials combined, the older adults had a higher BUN concentration than younger adults ($P < 0.0001$) and females had a lower BUN concentration than males ($P = 0.003$). There was a trial-specific change in BUN concentration and urinary total nitrogen excretion; as dietary protein intake decreased, BUN and urinary total nitrogen excretion also decreased ($P < 0.0001$; Table 2). Changes in BUN and total nitrogen excretion demonstrate a change in dietary protein intake.

Albumin fractional synthesis rate and serum concentration

Preinfusion baseline plasma ^{13}C -albumin enrichments did not differ among trials (P125, 1.085 ± 0.008 ; P94, 1.086 ± 0.006 ; P63, $1.083 \pm 0.007\%$; $P > 0.05$). They differed among the numeric order in which the 3 trials were performed (1.079 ± 0.004 , 1st infusion; 1.085 ± 0.004 , 2nd infusion; $1.090 \pm 0.007\%$, 3rd infusion; $P < 0.05$); however, the albumin FSR calculation corrects for these expected differences using baseline ^{13}C -albumin abundance.

Steady state (plateau plasma ^{13}C -KIC enrichment) was achieved in the PA state from min 60–180 for both ages and sexes during each of the protein trials with exception of the younger men during trial P125 (Supplemental Figure 2). The steady-state enrichment was

achieved in all groups from min 120 to 180 of the PA state for all protein trials (P125, 6.615 ± 0.893 ; P94, 6.834 ± 1.002 ; P63, 6.764 ± 0.957 ; $P > 0.05$). For the PP state, the steady state was achieved for all groups across the 3 trials except the younger and older females during trial P125 from min 300 to 480. All groups during each protein trial achieved steady state from min 420 to 480 of the PP state (P125, 5.473 ± 0.612 ; P94, 6.176 ± 0.852 ; P63, 6.650 ± 0.877 ; $P > 0.05$).

Nutrient ingestion and dietary protein

For all subjects and trials combined, the FSR of albumin was greater overall in the PP state ($14.7 \pm 5.6\%/d$) than in the PA state ($11.8 \pm 4.2\%/d$; $P < 0.0001$). The FSR of albumin was comparable among the 3 trials (P125, P94, and P63) in the PA (11.7 ± 4.9 , 11.5 ± 3.7 , $12.2 \pm 4.1\%/d$, respectively) and PP states (15.7 ± 6.2 , 14.2 ± 4.6 , $14.3 \pm 5.8\%/d$, respectively) when all subjects were included. As protein intake decreased from P125 to P94 and P63 there were smaller changes in FSR (Δ FSR) from the PA to PP state (P125 $\Delta = 4.0 \pm 3.4$, P94 $\Delta = 2.7 \pm 2.0$, and P63 $\Delta = 2.1 \pm 2.6\%/d$, $P < 0.05$), independent of age and sex. The Δ FSR did not differ between trials P94 and P63. The amount of protein consumed in the hourly meals was correlated with FSR and Δ FSR; as the total amount of protein consumed (g/h) decreased during the PP state, the PP albumin FSR and Δ FSR decreased ($r^2 = 0.12$, $P = 0.0004$ and $r^2 = 0.19$, $P < 0.0001$, respectively). Fasting-state albumin concentration did not change with dietary protein intake (Table 1).

Age

Age did not affect albumin FSR (Supplemental Table 1) or fasting serum albumin concentration (Table 1), independent of nutrient ingestion, sex, and dietary protein intake. Comparable results were obtained when age was considered as a continuous and categorical variable.

Sex

The overall mean albumin FSR was higher in males ($14.8 \pm 4.9\%/d$) than in females ($11.5 \pm 4.8\%/d$; $P < 0.05$; Supplemental Table 1), independent of age, dietary protein intake, and nutrient ingestion. The change in albumin FSR from the PA to the PP state was greater for males ($\Delta = 3.7 \pm 3.1\%/d$) than in females ($2.1 \pm 2.1\%/d$; $P < 0.05$), independent of age and dietary protein intake. Males (44 ± 3 g/L) had a higher serum albumin concentration than females (33 ± 4 g/L; $P < 0.0001$; Table 1).

Discussion

Contrary to the findings of Gersovitz et al. (10) that albumin FSR was not responsive to changes in dietary protein intake in older persons, which was the foundation of our hypothesis, the results from the current study indicate that the albumin FSR of healthy free-living younger and older adults respond comparably to changes in chronic dietary protein intake in both the PA and the PP states. The study by Gersovitz et al. (10) used younger (19–25 y) and older (64–78 y) men who consumed an adequate protein diet (1.5 g protein \cdot kg $^{-1}$ \cdot d $^{-1}$) for 7 d followed by an inadequate protein diet (0.4 g protein \cdot kg $^{-1}$ \cdot d $^{-1}$) for 14 d. The FSR was determined during the adequate and inadequate protein diets using 15 N-glycine (dosed orally every 3 h during a 60-h period), which estimates composite albumin FSR, and not PA and PP FSR separately. The researchers found, similar to the present findings, no age-related difference in the FSR of albumin when the younger and older men consumed a diet containing adequate protein. However, contrary to the current study, they found an age effect of albumin synthesis when the men consumed inadequate protein; the FSR decreased following the inadequate protein diet in the younger men, but did not change in the older men. A potential reason for differential results from the current study is that whereas the

older men studied by Gersovitz et al. (10) were deemed healthy, the clinical comments indicate that the older men had acute stress and/or inflammation, which might alter the synthesis rate (13).

The results of the current study show that the FSR of albumin does not change with advancing age in free-living healthy individuals: there was no difference in albumin FSR between the younger and older subjects (age used as a categorical variable) or with chronological aging (age used as a continuous variable) during the PA and PP states. The PA findings agree with those of Fu and Nair (12) who reported no difference in the fasting albumin FSR among healthy young, middle-aged, or older (total age range of 20–79 y) individuals who consumed a controlled diet (50:35:15, carbohydrate, fat, and protein, respectively) for 5 d prior to a 10-h infusion of [1-¹³C] leucine and ¹⁵N-phenylalanine (12); the PP findings are novel.

The differences in daily dietary protein intake among the trials in the current study did not affect the PA state FSR of albumin. These results contradict the findings of Jackson et al. (19) who showed that a decrease in dietary protein from 175 to 75% of the RDA for 7 d decreased the fasted-state FSR of albumin, derived with VLDL apolipoprotein B-100, from 13.7 ± 1.02 to $8.22 \pm 0.61\%/d$ ($P < 0.001$) in younger adults. The subjects in the current study consumed the dietary protein intake for 12 d, compared with 7 d of inadequate dietary protein intake in the Jackson et al. study (19), which provided them with more time to adapt and reestablish metabolic steady state, reflected by a plateau of urinary nitrogen excretion (34–36).

The stimulation of albumin synthesis with nutrient ingestion did not change with advanced age. This response was recently reported in an acute study by Caso et al. (18) in younger (25 ± 1 y) and older (68 ± 2 y) adults who consumed, in randomized crossover design, 3 different liquid beverages: 1) water; 2) a mixture of carbohydrate, fat, and protein (55, 30, and 15% of total energy, respectively); and 3) protein only (isonitrogenous but not isoenergetic to the mixed macronutrient beverage). The younger and older adults had a comparable PA state FSR of albumin, determined with the water trial, and a comparable increase of albumin FSR following the consumption of both the mixed macronutrient and protein only beverages with no difference between the beverages (18).

The albumin FSR results reported in the current study are quantitatively comparable with findings from other studies. Loudon et al. (16) reported a similar albumin FSR during the PA ($12.9 \pm 0.6\%/d$) and PP states ($15.2 \pm 0.6\%/d$) using [1-¹³C] leucine in healthy adults aged 52 ± 3 y. Barber et al. (17) also reported a similar differential response of albumin FSR between the PA (5.5–14.8%/d) and the PP states (11.0–15.7%/d) in healthy adults (aged 50–62 y). In the current study the smaller differential response of albumin FSR from PA to PP state with decreasing dietary protein intake from above the RDA (P125) to protein intake below the RDA (P94 and P63) was a likely response because of the smaller amino acid substrate available for protein synthesis. Other researchers have not tested this protein-related response to meal consumption, but have reported an increase in albumin protein synthesis during the PP state compared with the PA state (15–17).

The effect of sex on albumin FSR was not a primary outcome of this study; however, there were sex-related differences. Consistent with Fu and Nair (12), males had greater albumin FSR than females. Results from the present study show that males also had a greater feeding-induced change in albumin FSR from the PA to the PP state, independent of age and dietary protein intake; a finding not previously seen. The higher albumin concentration and FSR for males observed in this study might suggest that males have a higher “set point” for albumin concentration than females and therefore have a higher rate of synthesis per day.

This study showed that albumin concentration is not affected by age, and the results both confirm (12) and conflict (10,11) with other studies. Numerous physiological factors influence serum concentration of albumin (e.g., anemia, cancer, increasing age, hormone levels, physical activity limitations, decreased muscle mass and strength, and cigarette smoking decrease albumin concentrations). The age-related decrease in serum albumin concentrations found in other studies (10) could be confounded by the advancement of stress, inflammation, and/or other health- or lifestyle-related factors. The lack of difference in albumin concentration among the 3 protein intakes was expected because albumin has a relatively long half-life of 17–19 d, and therefore, changes in the concentration of albumin may not be measurable after 2 wk of inadequate dietary protein. Indeed, albumin concentration was maintained within the range of clinical normalcy in older women who consumed 56% of the RDA for protein for 10 wk (22,37). Protein intakes that span the range of adequacy did not compromise albumin status in healthy, community-dwelling older persons (19,38), but was clinically low in long-stay institutionalized geriatric patients (1) and during periods of extreme malnutrition (2). Therefore, the change in FSR of albumin might be a better indicator of the responses of albumin to dietary protein, particularly during short-term changes in intake. Lastly, sex affected albumin concentration; a difference that occurs until adults reach an “old” age (75–76 y), at which time there may not be a difference in albumin concentration between the sexes as suggested by Hostmark et al. (11).

Study limitations

One study limitation is the time points used for the albumin FSR calculation. The FSR of albumin should be calculated during periods of time that are sufficiently long for the changes in albumin-bound L-¹³C-leucine to be quantified and when the precursor pool is in steady state, reflected by constant plasma ¹³C-KIC enrichment. When the precursor pool is not in steady state, the PA and PP FSR of albumin could be underestimated and overestimated, respectively. To attempt to achieve these conditions within the framework of an 8-h infusion protocol that included both PA and PP metabolic states, the decision was made to quantify the incorporation of L-¹³C-leucine into albumin from 0 to 180 min (PA) and from 180 to 480 (PP) (the maximum amounts of time in the PA and PP states, respectively) and to use the mean ¹³C-KIC enrichments at 120 to 180 min (PA) and 420 to 480 min (PP) as the steady-state precursor pool values. Results from the ¹³C-KIC enrichment data show that the precursor pool was in steady state from 60 to 180 min (PA) and from 300 to 480 (PP) for the majority of the groups during each of the trials, and it was in steady state from 120 to 180 min (PA) and from 420 to 480 min (PP) for all subjects during all trials. These results indicate that the desired steady-state conditions existed throughout most of the PA and a large part of the PP time periods and that the ¹³C-KIC enrichments used to calculate the FSR of albumin reflected steady states. The limitation rests with the lack of plasma samples (they were not collected) to document if steady-state conditions existed during the first 60 min of the PA period and the first 120 min of the PP period. Forslund et al. (39) showed that steady-state enrichment of the KIC precursor pool could be achieved within 1 h after commencing hourly meals that contained the same dietary protein intake as trial P125 (1.0 g · kg⁻¹ · d⁻¹); therefore the subjects in the current study were most likely close to the steady-state plateau within 1 h after the onset of the hourly meals. Although the changes that were observed in albumin FSR from the PA to the PP states were probably an overestimation, the error between the estimates reported in the current study and “true” estimates for PA and PP state FSR was most likely <10% across all trials, and the error would have decreased with decreasing protein intake. This small error would not be sufficient enough to change our conclusions. Also, we are confident that our results represent reasonably accurate albumin FSR estimates because they are comparable to results from other studies (16,17) in healthy subjects.

In summary, these findings support that chronological age does not affect the estimated FSR of albumin in healthy free-living individuals. The protein-dependent differential albumin FSR response to nutrient ingestion (PA vs. PP states) suggests that decreasing the availability of amino acid substrate by consuming meals with inadequate protein reduces the rate of albumin synthesis. The small, nonsignificant difference ($P > 0.05$) between trials P94 and P63 for Δ FSR could be indicative of an exponential fed-state protein synthesis response to dietary protein intakes ranging from below the RDA (P94 and P63) to slightly above the RDA (P125). Additional research of the effects of dietary protein ingestion over a range of quantities on the FSR of albumin is warranted. These results could indicate a potential decreased tolerability for metabolically stressful situations such as disease or inflammation, especially for older adults who routinely consume below the RDA for dietary protein. Males have greater albumin FSR than females, which might contribute to the higher “set point” for albumin concentration demonstrated in this study.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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TABLE 1

Descriptive subject characteristics of younger and older males and females during the postabsorptive state that consumed 125, 94, and 63% of the RDA for dietary protein¹

	Trial ³	Group ²			
		YM	OM	YF	OF
<i>n</i>		10	9	8	9
Age, y		28 ± 6 ^a	73 ± 6 ^b	31 ± 8 ^a	74 ± 4 ^b
Height, cm		179.9 ± 5.5 ^j	173.6 ± 4.4 ^j	169.0 ± 5.6 ^k	162.0 ± 5.0 ^k
Weight, kg		82.3 ± 14.2 ^j	77.6 ± 10.8 ^j	64.4 ± 8.5 ^k	72.1 ± 13.5 ^k
BMI, kg/m ²		25.4 ± 4.2 ^{ab}	25.6 ± 2.5 ^{ab}	22.5 ± 2.5 ^a	27.4 ± 4.4 ^b
Serum albumin, g/L	P125	46 ± 2 ^j	43 ± 2 ^j	32 ± 5 ^k	33 ± 3 ^k
	P94	45 ± 3 ^j	41 ± 4 ^j	33 ± 4 ^k	34 ± 3 ^k
	P63	46 ± 2 ^j	42 ± 2 ^j	32 ± 7 ^k	34 ± 2 ^k
Leukoocytes, U/L	P125	6.2 ± 1.2 ^j	5.8 ± 1.3 ^j	5.2 ± 1.3 ^k	5.0 ± 1.0 ^k
	P94	6.4 ± 1.1 ^j	5.7 ± 1.5 ^j	5.2 ± 1.2 ^k	5.1 ± 1.0 ^k
	P63	6.1 ± 1.4 ^j	5.7 ± 1.3 ^j	4.7 ± 1.4 ^k	5.1 ± 1.1 ^k
AST, U/L	P125	22.5 ± 5.0 ^j	24.8 ± 14.2 ^j	20.1 ± 6.0 ^k	17.4 ± 2.1 ^k
	P94	21.7 ± 5.7 ^j	23.6 ± 10.6 ^j	19.8 ± 5.1 ^k	17.4 ± 5.2 ^k
	P63	21.6 ± 5.7 ^j	27.8 ± 8.3 ^j	20.4 ± 4.4 ^k	18.3 ± 2.3 ^k

¹ Values are mean ± SD. Means for a variable with superscripts without a common letter differ by age (a and b) and by sex (j and k), $P < 0.05$. Post-hoc analysis using Tukey was performed to determine difference between protein trials. Trials within a variable with superscripts without a common letter differ, $P < 0.05$.

² YM, younger males; OM, older males; YF, younger females; OF, older females; and AST, aspartate aminotransferase.

³ Protein trial: P125 = 125%; P94 = 94%; and P63 = 63% of the RDA for dietary protein.

TABLE 2

Controlled energy and macronutrient intakes and nitrogen assessments of younger and older males and females with 125, 94, and 63% of the RDA for dietary protein¹

<i>n</i>	Trial ³	Group ²			
		YM	OM	YF	OF
		10	9	8	9
Energy intake, MJ/d	P125	13.6 ± 2.7 ^{uj}	11.7 ± 1.9 ^{bj}	10.3 ± 0.7 ^{ak}	9.2 ± 0.9 ^{bk}
	P94	13.5 ± 2.5 ^{uj}	11.3 ± 1.6 ^{bj}	10.3 ± 0.7 ^{ak}	9.2 ± 0.9 ^{bk}
	P63	14.0 ± 2.7 ^{uj}	11.1 ± 1.8 ^{bj}	10.3 ± 0.7 ^{ak}	9.2 ± 0.9 ^{bk}
Energy intake, kJ · kg ⁻¹ · d ⁻¹	P125	165.3 ± 22.7 ^a	151.0 ± 28.9 ^b	160.1 ± 13.4 ^a	130.8 ± 14.8 ^b
	P94	166.1 ± 24.3 ^a	145.9 ± 14.7 ^b	161.0 ± 14.6 ^a	130.3 ± 14.5 ^b
	P63	171.4 ± 24.1 ^a	145.0 ± 22.2 ^b	161.9 ± 15.1 ^a	130.4 ± 14.2 ^b
Protein intake, g · kg ⁻¹ · d ⁻¹	P125 ^x	1.01 ± 0.01	1.01 ± 0.02	0.97 ± 0.02	1.01 ± 0.04
	P94 ^y	0.76 ± 0.02	0.76 ± 0.02	0.73 ± 0.01	0.76 ± 0.03
	P63 ^z	0.51 ± 0.01	0.51 ± 0.01	0.51 ± 0.5	0.51 ± 0.02
Fat intake, g · kg ⁻¹ · d ⁻¹	P125 ^x	1.37 ± 0.21 ^a	1.24 ± 0.24 ^b	1.35 ± 0.12 ^a	1.05 ± 0.13 ^b
	P94 ^x	1.42 ± 0.22 ^a	1.24 ± 0.14 ^b	1.39 ± 0.13 ^a	1.09 ± 0.14 ^b
	P63 ^y	1.15 ± 0.24 ^a	1.28 ± 0.20 ^b	1.44 ± 0.14 ^a	1.09 ± 0.13 ^b
Carbohydrate intake, g · kg ⁻¹ · d ⁻¹	P125 ^x	5.81 ± 0.89 ^{uj}	5.25 ± 1.20 ^{bj}	5.61 ± 0.54 ^{ak}	4.25 ± 0.52 ^{bk}
	P94 ^x	6.00 ± 0.97 ^{uj}	5.18 ± 0.57 ^{bj}	5.81 ± 0.58 ^{ak}	4.40 ± 0.50 ^{bk}
	P63 ^y	6.32 ± 0.90 ^{uj}	5.39 ± 0.88 ^{bj}	6.02 ± 0.61 ^{ak}	4.65 ± 0.51 ^{bk}
Fiber intake, g/d	P125 ^x	34 ± 5 ^{uj}	34 ± 4 ^{bj}	24 ± 2 ^{ak}	18 ± 2 ^{bk}
	P94 ^y	35 ± 5 ^{uj}	28 ± 2 ^{bj}	22 ± 1 ^{ak}	18 ± 2 ^{bk}
	P63 ^z	28 ± 5 ^{uj}	25 ± 4 ^{bj}	20 ± 2 ^{ak}	19 ± 2 ^{bk}
Urinary total nitrogen, g/d	P125 ^x	8.4 ± 2.4	8.9 ± 2.3	7.4 ± 1.8	8.4 ± 1.8
	P94 ^y	6.7 ± 2.0	7.3 ± 1.8	6.0 ± 0.7	6.6 ± 1.7
	P63 ^z	5.0 ± 0.8	5.7 ± 0.8	4.9 ± 0.6	5.0 ± 0.9
BUN, mmol/L	P125 ^x	4.1 ± 1.0 ^{uj}	5.9 ± 1.1 ^{bj}	3.1 ± 0.8 ^{ak}	5.4 ± 1.4 ^{bk}
	P94 ^y	3.5 ± 0.9 ^{uj}	4.8 ± 1.3 ^{bj}	2.4 ± 0.5 ^{ak}	4.2 ± 0.7 ^{bk}
	P63 ^z	2.9 ± 1.2 ^{uj}	4.0 ± 1.5 ^{bj}	2.0 ± 0.5 ^{ak}	3.1 ± 0.8 ^{bk}

¹ Values are means \pm SD. Means for a variable with superscripts without a common letter differ by age (a and b) and by sex (j and k), $P < 0.05$. Post-hoc analysis using Tukey was done to determine difference between protein trials. Trials within a variable with superscripts without a common letter (x, y, and z) differ, $P < 0.05$.

² Group: YM, younger males; OM, older males; YF, younger females; and OF, older females.

³ Protein trial: P125 = 125%; P94 = 94%; and P63 = 63% of the RDA for dietary protein.