Response of albumin synthesis to oral nutrients in young and elderly subjects\textsuperscript{1–3}

Giuseppe Caso, Joshua Feiner, Izolda Mileva, Leslie J Bryan, Patricia Kelly, Karen Autio, Marie C Gelato, and Margaret A McNurlan

ABSTRACT

Background: The synthesis of albumin after oral ingestion of nutrients provides a means of storing amino acids, which can be made available during periods of fasting.

Objective: This study was undertaken to see whether the response of albumin synthesis to the oral intake of nutrients is compromised in elderly subjects.

Design: Albumin synthesis was determined from the incorporation of 43 mg L-\textsuperscript{[2H\textsubscript{5}]}phenylalanine/kg body wt. Eight elderly subjects (aged ≥60 y) and 8 young subjects (aged 21–35 y) were studied on 3 separate occasions: after the intake of water, a liquid meal (with 15\% of energy from protein, 30\% of energy from fat, and 55\% of energy from carbohydrate), or an isonitrogenous but not isocaloric meal containing only protein.

Results: Mean (±SEM) albumin synthesis, expressed as an absolute rate (ie, the amount of albumin synthesized per day), was significantly lower in elderly subjects (108 ± 7 mg · kg body wt\textsuperscript{−1} · d\textsuperscript{−1}) than in young subjects (141 ± 7 mg · kg body wt\textsuperscript{−1} · d\textsuperscript{−1}). In response to the complete meal, albumin synthesis was significantly increased in both the elderly (144 ± 7 mg · kg body wt\textsuperscript{−1} · d\textsuperscript{−1}) and the young (187 ± 11 mg · kg body wt\textsuperscript{−1} · d\textsuperscript{−1}) subjects. The protein component of the meal was sufficient to stimulate albumin synthesis in both the elderly (147 ± 14 mg · kg body wt\textsuperscript{−1} · d\textsuperscript{−1}) and the young (182 ± 6 mg · kg body wt\textsuperscript{−1} · d\textsuperscript{−1}) subjects.

Conclusions: Elderly subjects have lower rates of albumin synthesis than do young subjects during fasting, but they stimulate albumin synthesis proportionately in response to the oral ingestion of protein. The intakes of additional fat and carbohydrate do not stimulate albumin synthesis further. Am J Clin Nutr 2007;85:446–51.

KEY WORDS Aging, albumin synthesis, dietary protein, feeding, L-\textsuperscript{[2H\textsubscript{5}]}phenylalanine, nutrients

INTRODUCTION

The synthesis and degradation of albumin may be important in the regulation of the amino acid supply of a whole organism. Albumin synthesis by the liver is acutely stimulated by feeding (1–3), and it has been suggested that increased postprandial albumin synthesis may serve as temporary storage for dietary essential amino acids, thus preventing their oxidative loss (1, 3). Albumin catabolism during the postabsorptive period may then release essential amino acids, making them available to muscle and other tissues to maintain protein synthesis. The increase in the albumin synthesis rate after a meal therefore may have the important physiologic function of preventing oxidation of essential amino acids from the diet and may have an important role in the long-term maintenance of body protein and muscle mass.

In young subjects, albumin synthesis is stimulated after ingestion of a meal containing protein, carbohydrate, and fat (1–3). Many studies, conducted both in animals and humans, have clearly shown that the protein content of ingested nutrients has an important role in the regulation of albumin synthesis, with a decrease in albumin synthesis in response to a reduction in protein intake (4–11). Indeed, amino acids can have a direct effect on hepatocytes in vitro, up-regulating the synthesis of albumin (9, 12–14). The ingestion of carbohydrates and fat can also, in the absence of protein, stimulate postprandial albumin synthesis (3), with further enhancement of albumin synthesis in response to the addition of amino acids (3, 15).

In older subjects, the response of albumin synthesis to nutrient intake, particularly protein, is less clearly delineated. Moreover, because of the age-related loss of body protein (16–21), conservation of dietary amino acids is of even greater importance in older subjects than in younger subjects. A positive relation between plasma albumin concentration and muscle mass in elderly subjects has been shown (22, 23), and there is also an indication that albumin synthesis in elderly subjects may be less responsive to changes in the protein content of the diet than in young subjects (5).

Given the progressive decline in muscle mass and a possible defective response of albumin synthesis to feeding with aging, the present study was undertaken to characterize the nutritional factors regulating the acute response of albumin synthesis to oral nutrients and to assess whether the response of albumin synthesis decreases with age. An assessment of the ability of the protein component of the meal alone or in combination with carbohydrate and fat in eliciting postprandial stimulation of albumin synthesis in both young and elderly subjects was also undertaken.\textsuperscript{1–3}

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TABLE 1
Subject characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Young subjects</th>
<th>Elderly subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (F/M)</td>
<td>2/6</td>
<td>2/6</td>
</tr>
<tr>
<td>Age (y)</td>
<td>25 ± 1</td>
<td>68 ± 2</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.73 ± 0.03</td>
<td>1.73 ± 0.03</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>78.8 ± 4.5</td>
<td>78.3 ± 4.2</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.1 ± 0.9</td>
<td>26.1 ± 0.7</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.88 ± 0.07</td>
<td>4.08 ± 0.10</td>
</tr>
<tr>
<td>Prealbumin (mg/dL)</td>
<td>28.8 ± 1.4</td>
<td>25.4 ± 1.6</td>
</tr>
<tr>
<td>RBP (mg/dL)</td>
<td>4.5 ± 0.3</td>
<td>4.9 ± 0.4</td>
</tr>
<tr>
<td>Triacylglycerols</td>
<td>98 ± 11</td>
<td>103 ± 22</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>145 ± 9</td>
<td>154 ± 11</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>43 ± 4</td>
<td>50 ± 3</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>81 ± 10</td>
<td>84 ± 13</td>
</tr>
</tbody>
</table>

1 n = 8. RBP, retinol-binding protein.
2 ± SEM (all such values).
3 Significantly different from the young subjects, P < 0.001 (unpaired t test).

SUBJECTS AND METHODS

Subjects

Eight young (aged 21–35 y) and 8 elderly (aged >60 y) subjects without disease participated in the present study (Table 1). The subjects were screened and excluded for clinical evidence of cardiac, vascular, hepatic, renal or endocrine disease, unintended weight loss, coagulopathy, or other serious medical conditions. All volunteers gave written informed consent before being enrolled in the study, and the study protocols were approved by the Committee on Research Involving Human Subjects at Stony Brook University.

Experimental design

Enrolled subjects (n = 16) were admitted to the General Clinical Research Center at Stony Brook University for 3 separate visits ≈ 1 wk apart. For 3 d before each visit, the subjects were asked to fill in a dietary diary, to drink no alcohol, and to refrain from heavy or prolonged physical exercise. On each visit, albumin synthesis rates were measured after an overnight fast (water meal); after the ingestion of a liquid meal containing protein, carbohydrate, and fat (complete meal); after consumption of only the protein portion of the complete meal (protein meal); after the ingestion of a liquid meal containing protein, carbohydrate, and fat (complete meal); or after consumption of only the protein portion of the complete meal (protein meal). The response to the 3 meals was assessed in a random order.

For each test, the subjects were admitted at 1700 h the day before the study day and assessed clinically for acute illness. Only water, but no food or other drinks, was allowed after 2200. At 0700 the next morning, 2 intravenous cannulas were inserted into contralateral forearm veins. A pretest blood sample was taken, then 1 of the 3 meals in form of a liquid diet was provided. The complete meal consisted of whey protein (Bi-Pro; Davisco, Le Sueur, MN), carbohydrates in the form of maltodextrin (Moducal; Mead Johnson, Evansville, IN), and fat in the form of canola oil. The energy content of the complete meal represented one-third of the estimated daily energy expenditure for each subject. Energy expenditure was estimated by multiplying the basal metabolic rate [calculated from 1985 Food and Agricultural Organization equations by using the subject’s sex, age, weight, and height (24)] by a factor of 1.5. The energy content of the meal was derived 15% from protein, 30% from fat and 55% from carbohydrates. For the protein meal, only the protein component of the complete meal was provided. The complete meal and the protein meal were therefore isonitrogenous, but not isocaloric. For the fasting test, water was given in a volume similar to the other 2 meals.

Exactly 30 min after the ingestion of meals, albumin synthesis was measured with the flooding method with the stable isotope labeled amino acid, L-[2H5]phenylalanine, as previously described (2, 4, 25, 26). After taking a baseline blood sample for the measurement of plasma insulin and amino acid concentrations, a sterile and pyrogen-free solution containing L-[2H5]phenylalanine (Cambridge Isotopes, Andover, MA) and unlabeled phenylalanine (Ajinomoto, Tokyo, Japan) was infused over 10 min (total phenylalanine given: 43 mg/kg BW). Sequential blood samples were taken over 90 min for determination of the enrichment of L-[2H5]phenylalanine in the albumin and in the plasma-free amino acid pool (2, 4, 25, 26). The enrichment of the injected isotope solution was 10, 20, and 40 mol%/ on study day 1, 2, and 3, respectively.

Analytic methods

Enrichment of phenylalanine in albumin and in plasma

Albumin was isolated from plasma samples by differential solubility in ethanol then extensively washed in cold perchloric acid (30 g/L) and hydrolyzed for 24 h in 6 mol HCl/L for 24 h (25). Phenylalanine enrichment in the albumin hydrolyzate was assessed by mass spectrometry with an MD800 gas chromatography-mass spectrometer (Fisons Instruments, Beverly, MA), as previously described (26, 27). The enrichment of phenylalanine in plasma was assessed with EZ:faast amino acid analysis kit (Phenomenex, Torrance, CA) by monitoring the ions at mass-to-charge ratio (m/z) 206 and 211 on a gas chromatograph mass spectrometer (MD800; Fisons Instruments).

Other analytic procedures

Plasma albumin concentration was measured with an automated bromocresol green method (28). Plasma amino acid concentrations were measured by HPLC with fluorescence detection of o-phthalaldehyde derivatives (Waters 2690, Milford, MA), as previously described (29, 30). Plasma insulin concentration was measured by radioimmunoassay (Diagnostics Products Corporation, Los Angeles, CA).

Calculation of albumin synthesis rate

Fractional albumin synthesis rates (FSRs), ie, the percentage of the intravascular albumin mass synthesized per day (%/d), was calculated from the enrichment of albumin-bound phenylalanine and the area under the curve of the plasma free phenylalanine enrichment (precursor pool) versus time, as previously described in detail (2, 4, 25, 26). The absolute albumin synthesis rates (ASR), ie, the total amount synthesized per day (mg · kg⁻¹ · d⁻¹), was measured by multiplying the FSR by the intravascular albumin mass and normalized for body weight. Intravascular albumin mass was assessed from the plasma albumin concentration and plasma volume, which was predicted from sex, age, and weight by using a nomogram (31).

Statistics

Data are expressed as means ± SEMs. The differences in variables between the 3 diets in the young and elderly groups
RESULTS

The characteristics of the subjects in the present study are presented in Table 1. Elderly (aged >60 y) and young (aged 21–35 y) subjects did not differ significantly in sex, height, weight, body mass index, albumin, prealbumin, retinol-binding protein, triacylglycerols, cholesterol, and LDL cholesterol. Five of the 8 elderly subjects were on a 3-hydroxy-3-methylglutaryl-coenzyme A inhibitor for lipid lowering. No evidence of malnutrition was observed in the subjects; this was ascertained from prealbumin or retinol-binding protein concentrations.

The basal rate of albumin synthesis, ie, after the water meal, tended to be lower in the elderly subjects (6.17 ± 0.4%/d) than in the young subjects (7.71 ± 0.4%/d), although this difference was not statistically significant (repeated-measures ANOVA for age effect, P = 0.16) when albumin synthesis was expressed as a fractional rate (FSR ie, the proportion of the intravascular albumin mass renewed each day). However, when albumin synthesis was expressed as an absolute rate (ASR, ie, the amount of albumin synthesized per day), then the rate in the elderly subjects (108 ± 7 mg·kg⁻¹·d⁻¹) was significantly lower (repeated measures ANOVA for age effect, P = 0.003, with no age by meal interaction, P = 0.75) than in the young subjects (141 ± 7 mg·kg⁻¹·d⁻¹), due primarily to a lower plasma volume in elderly subjects (3104 ± 130 mL) than in young subjects (3544 ± 150 mL, P < 0.05).

When young subjects consumed the complete meal, the rate of albumin synthesis was significantly increased (P = 0.001) to 10.09 ± 0.49%/d, or 32 ± 8% (Figure 1A). Albumin synthesis, expressed in absolute terms (ASR), increased from 141 ± 7 to 187 ± 11 mg albumin·kg⁻¹·d⁻¹ (P = 0.002) or 31 ± 9% (Figure 2A). Similarly, when elderly subjects consumed the complete meal, albumin FSR was also significantly increased to 9.22 ± 0.42%/d (P = 0.005), an increase of 43 ± 9.5% (Figure 1B). Albumin ASR was also significantly increased to 144 ± 7 mg·kg⁻¹·d⁻¹ (P = 0.02, Figure 2B). In both young and elderly subjects, consuming only the protein portion of the meal in the young subjects (9.91 ± 0.3 in the young subjects and 9.50 ± 0.65%/d in the elderly subjects, P < 0.005) and ASR (182 ± 6 in the young subjects and 147 ± 14 mg·kg⁻¹·d⁻¹ in the elderly subjects, P < 0.05) to levels that were not different to those achieved with the complete meal (Figures 1 and 2).

In the young subjects, plasma insulin concentrations increased from 4.5 ± 1.4 μU/mL during fasting to 24.7 ± 3.8 μU/mL after the protein meal, with a further increase to 62.7 ± 11.5 μU/mL after the complete meal (P < 0.05). In the elderly subjects, circulating concentrations of insulin showed a similar pattern, with an increase from 2.4 ± 0.8 during fasting, to 26.2 μU/mL ± 6.2 μU/mL after the protein meal (P < 0.05), and a further significant increase to 45.0 ± 6.8 μU/mL after the complete meal.

The changes in plasma concentrations of amino acids in response to the ingestion of nutrients are shown in Table 2. The amino acids have been grouped into nonessential (alanine, aspartate, asparagine, cysteine, glutamate, glutamine, glycine, serine, and tyrosine), essential (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine), and branched-chain amino acids (leucine, isoleucine, and valine). In the young subjects, there was no significant change in the concentration of nonessential amino acids with either the protein or complete meal ingestion in both the young and elderly subjects, P < 0.05 (ANOVA with Tukey’s test). The age-by-meal interaction and the main effect of age were not significant.

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intake. In the earlier study, albumin synthesis was assessed from incorporation of $^{15}$N-glycine (as $^{15}$N-guanidino arginine), with the enrichment of urinary urea used to estimate the enrichment of intrahepatic $^{15}$N-arginine. Although this early study showed the utility of stable isotopes for the assessment of protein kinetics, the albumin FSR were considerably lower (3–4%/d) than that observed in the present study and in other studies (2–4, 15, 26, 32–34).

In a comparison of various studies reporting the rates of albumin synthesis in young subjects and its response to nutrients, the consistency among studies with different methodologies is quite remarkable. Although the studies have all assessed albumin synthesis from the incorporation of a labeled amino acid into albumin, the tracer amino acid has been given either as a continuous infusion (3, 15, 32–35) or as a flooding amount (2, 4, 25, 26). In the studies with continuous infusion of labeled leucine, the labeling of plasma ketoisocaproate (derived from muscle) is often taken to represent the precursor labeling within the liver; in studies with the flooding technique, the labeling of the plasma amino acid is taken to represent precursor labeling. In studies where the tracer amino acid is given as a continuous infusion, the requirement for steady-state conditions imposes a regimen on the oral intake of either intragastric infusion or consumption of frequent, small meals. Because the flooding technique does not require steady-state conditions, protocols can include single larger meals. Comparisons of albumin synthesis in older and younger subjects made with the continuous infusion technique (32, 34, 35) suggested comparable FSRs between the subjects with somewhat lower ASR observed in elderly subjects, though not all studies showed a statistically significant difference between the elderly and young subjects.

Although albumin FSR did increase in the elderly subjects in response to nutrient intake, the increase was proportional to the fasting FSR, which was lower in the elderly subjects than in the younger subjects (Figure 2). Consequently, the absolute amount of albumin synthesized by the elderly subjects both during fasting and in response to a complete meal remained below the amount synthesized by the younger subjects. With a smaller intravascular albumin mass and a lower albumin FSR, elderly subjects have a smaller albumin reserve and thus potentially greater risk of the catabolism of body protein during times, such

### Table 2

Amino acid concentrations in plasma of the subjects after consumption of a water, protein, or complete meal

<table>
<thead>
<tr>
<th></th>
<th>NEAA</th>
<th>EAA</th>
<th>BCAA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Young subjects</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water meal</td>
<td>1590 ± 116</td>
<td>1057 ± 51</td>
<td>503 ± 36</td>
</tr>
<tr>
<td>Protein meal</td>
<td>1919 ± 273</td>
<td>1764 ± 254</td>
<td>893 ± 136</td>
</tr>
<tr>
<td>Complete meal</td>
<td>1545 ± 184</td>
<td>1254 ± 92</td>
<td>609 ± 71</td>
</tr>
<tr>
<td><strong>Elderly subjects</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water meal</td>
<td>1333 ± 69</td>
<td>1059 ± 92</td>
<td>488 ± 67</td>
</tr>
<tr>
<td>Protein meal</td>
<td>1480 ± 110</td>
<td>1548 ± 210</td>
<td>772 ± 130</td>
</tr>
<tr>
<td>Complete meal</td>
<td>1415 ± 58</td>
<td>1157 ± 90</td>
<td>578 ± 55</td>
</tr>
</tbody>
</table>

*All values are $\bar{x}$ ± SEM; $n = 8$. NEAA, nonessential amino acids; EAA, essential amino acids; BCAA, branched-chain amino acids. Means with different superscript letters are significantly different, $P \leq 0.05$ (ANOVA with Tukey’s test).
as the catabolic state accompanying trauma, when amino acids stored as albumin may be needed. The increase in albumin FSR and ASR of 30—40% with consumption of oral nutrients in both the young and elderly subjects observed in the current study was similar to the effect we (2) and others (3) have observed previously in young subjects. In the present study, consumption of only the protein component of the meal was sufficient to stimulate albumin FSR and ASR, suggesting that additional energy from fat and carbohydrate was not required for maximal stimulation of albumin synthesis. The study by Volpi et al (3) showed a stimulation of albumin synthesis with the consumption of a liquid diet containing carbohydrates and fat, but no amino acids, with further stimulation when amino acids were added. Similarly, Cayol et al (15) showed that albumin synthesis was higher in subjects fed small meals containing protein than in those fed an isoenergetic formula without the protein component.

With the consumption of only the protein component of the meal, not only were plasma amino acid concentrations increased (Table 2), but the concentration of insulin in plasma also increased. Both plasma insulin and amino acid concentrations play an important role in the regulation of albumin synthesis. Insulin has been shown to stimulate albumin synthesis both in vitro and in vivo (33, 36, 37). Although in vivo amino acids may enhance albumin synthesis indirectly by stimulating insulin release, studies performed in isolated perfused liver indicate a direct effect of amino acids on albumin synthesis (12, 14). In the study by Volpi et al (3), the stimulation of albumin synthesis in response to insulin was additive to the effect of amino acids. In our study, the elevation of plasma insulin concentration with the intake of protein was sufficient, along with the increase in plasma concentrations of amino acids, to provide for maximal stimulation of albumin synthesis.

In conclusion, the present study showed that the ingestion of a meal is followed by an increase in the hepatic synthesis of albumin, and that ingestion of the protein component of meal alone is as effective in stimulating albumin synthesis as is ingestion of a complete meal that also included carbohydrates and fat. The proportional increase of albumin synthesis to food intake as well as to dietary protein alone was comparable in young and elderly subjects, indicating that the response of albumin synthesis to nutrient intake is maintained in the elderly. However, because the basal amounts of albumin produced per day (fasting ASR) are lower in older subjects, the total amounts of albumin synthesized in response to food intake were smaller in the elderly than in the young subjects. This suggests the possibility of a smaller albumin reserve available to elderly subjects.

We thank Jeanne Kidd for her help in recruiting the study participants, Dawn Sasvary and Yuqun Hong for their expert technical assistance, and the nursing staff at the Stony Brook University General Clinical Research Center (GCRC).

GC and MAM were responsible for the study design, preparation of experimental diets, data collection, sample and data analysis and writing of the manuscript. JF participated in the data collection, sample analyses and writing of the manuscript. IM was responsible for the sample analyses by mass spectrometry, LJB and KA were involved in the data collection and sample analyses. PK participated in the medical monitoring of the participants and data collection. MCG oversaw the clinical aspects of the study including health screening, determination of eligibility of participants, and medical monitoring. The authors had no conflicts of interest.

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