Age effect on fibrinogen and albumin synthesis in humans

AIZHONG FU AND K. SREEKUMARAN NAIR
Division of Endocrinology, Mayo Clinic and Foundation, Rochester, Minnesota 55905

Fu, Aizhong, and K. Sreekumaran Nair. Age effect on fibrinogen and albumin synthesis in humans. Am. J. Physiol. 275 (Endocrinol. Metab. 38): E1023–E1030, 1998.—A strong association has been reported between atherosclerotic diseases and fibrinogen levels, and a decreased whole body protein synthesis has also been reported with aging. We investigated the effect of age on fractional synthesis rates (FSR) of fibrinogen and albumin in 12 human subjects of young (20–30 yr), middle (45–60 yr), and old (65–79 yr) age by use of L-[1-13C]leucine and L-[15N]phenylalanine as tracers. An age-related decline in FSR of fibrinogen (P < 0.01) was observed with use of both tracers, with the maximal decrease (average 37% with α-[13C]ketoisocaproate as the precursor) occurring by middle age and with no further changes thereafter. In contrast, plasma concentrations of fibrinogen increased with age (P < 0.002). There was no age-related change in synthesis rate and concentrations of albumin. An age-related decline in fibrinogen FSR, but not FSR of albumin, indicates a differential effect of age on synthesis rate of these two liver proteins. This study also demonstrated that the increased circulating levels of fibrinogen represent a slower rate of disposal of fibrinogen rather than an increased production rate.

HUMAN AGING is associated with a generalized decline in synthesis rates of muscle proteins and a decline in overall physical performance (4, 36). We recently reported an age-related decline in whole body protein synthesis in human subjects (4), whereas there are contradictory reports on this matter in the literature (19, 33, 35, 43). Because muscle contributes only <30% to whole body protein synthesis (32) (an average measure of synthesis rates of different proteins in the body), it is possible that the measurements of whole body protein synthesis may not be sensitive enough to detect small changes occurring in many individual proteins. It is now increasingly clear that decreased synthesis rates of several muscle proteins occur with aging in humans (36, 43–45). Very little information is available on the effect of aging on synthesis of liver proteins. Animal studies showing a decline in liver weight and a generalized atrophy of liver with aging suggest a compromised capacity for protein synthesis in liver (41). Animal studies also have shown a generalized decline in protein synthesis in liver, as reviewed by Ward and Richardson (42), although conflicting reports on this matter exist in literature (21, 30). The effect of age on synthesis of specific liver proteins, such as albumin and fibrinogen, in healthy humans remains to be clearly defined.

Both albumin and fibrinogen are essential liver proteins with specific functions. Changes in circulating levels of both of these proteins have potential implications in health and diseases. A decrease in plasma level of albumin has been used as a marker of mortality in both clinical and population studies (12, 29, 37). Increased plasma fibrinogen concentrations in obesity, diabetes mellitus, and cardiovascular diseases have prompted us to link fibrinogen as an independent risk factor for increased cardiovascular morbidity and mortality in these conditions (5, 7, 23, 27, 28). Although an age-related increase in blood fibrinogen has been reported in major epidemiological studies (8, 10, 16, 22, 24, 25), little is known about the biochemical mechanism of this increase in fibrinogen levels with age.

Measurements of concentrations of the circulating fibrinogen have provided important information on the potential association between increase of the plasma level of this protein and aging. Plasma concentrations of fibrinogen are determined by both synthesis and disposal of this protein. It is not known whether the reported increase in plasma concentration of fibrinogen is related to increased synthesis rate or reduced disposal rate. Whereas fibrinogen levels are reported to increase with age, no age-related changes are reported for circulating levels of albumin, which is also synthesized in the liver.

The purpose of the current study was to measure the fractional synthesis rates (FSR) of albumin and fibrinogen to determine the effect of age on synthesis rates of these two liver proteins.

SUBJECTS AND METHODOLOGY

Materials. L-[1-13C]leucine, α-[13C]ketoisocaproate ([13C]KIC), and L-[15N]phenylalanine (99% atom percent excess [APE]) were purchased from Cambridge Isotope Laboratories (Andover, MA). Mouse monoclonal antibody against human plasma albumin (Sigma Chemical, St. Louis, MO) was produced at the Immunochemical Core Facility of the Mayo Clinic (Rochester, MN). Monoclonal antibody to human plasma fibrinogen was produced from hybridoma cells purchased from the American Type Culture Collection (ATCC, Rockville, MD) at the Immunochemical Core Facility of the Mayo Clinic. Antibody was produced according to the accompanying protocols. The antibody against human fibrinogen binds to the α-chain of human plasma fibrin and fibrinogen, but not fibrinogen degradation products.

Subjects. Thirty-six healthy volunteers were the subjects in this study. Their ages spanned from 20 to 79 yr. They were a part of a larger study protocol that assessed the mechanism of sarcopenia in aging (4, 36). Only healthy subjects with normal results from a physical examination and a blood biochemical analysis were included in this study. Postmenopausal women on estrogen replacement, subjects taking β-adrenergic blockers or steroids, and subjects exercising more than twice per week for 20 min or more were also excluded from this study. Detailed information of these subjects are summarized in Table 1. The study protocol was...
approved by the Institutional Human Investigation Committee of the University of Vermont and the Mayo Clinic and Mayo Foundation, and written informed consent was obtained from each volunteer.

Study protocol. Five days before the study, all subjects were given at the Clinical Research Center a diet that was estimated to meet their daily energy and nutrient needs as assessed on the basis of their body weight and height. This diet consisted of a calorie ratio of carbohydrate, fat, and protein of 50:35:15. None of the subjects lost any weight during the 5-day period. All subjects received a standard meal at 10:00 PM on the day before the study to avoid prolonged fasting during the 5-day period. The entire process was performed at room temperature.

Table 1. Characteristics of study subjects

<table>
<thead>
<tr>
<th></th>
<th>Young</th>
<th>Middle Age</th>
<th>Old Age</th>
<th>Effect (P Value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Females Males</td>
<td>Females Males</td>
<td>Females Males</td>
<td>Females Males</td>
</tr>
<tr>
<td>Age, yr</td>
<td>25±1.7 26.3±1.8</td>
<td>56±1.2 50.7±1.5</td>
<td>70.3±2.2 73.5±2.0</td>
<td>0.345 0.003</td>
</tr>
<tr>
<td>Body wt, kg</td>
<td>57.6±5.9 70.1±4.8</td>
<td>84.2±6.6</td>
<td>72.2±5.4 78.6±5.5</td>
<td>0.563 0.001</td>
</tr>
<tr>
<td>Height, cm</td>
<td>162±2.6 170±2.5</td>
<td>176±3.0</td>
<td>163.4±1.7 176.1±2.1</td>
<td>0.158 0.246</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>21.9±0.9 23.9±1.4</td>
<td>27.0±1.7 25.2±1.3</td>
<td>83.7±4.6 89.5±3.2</td>
<td>0.724 0.889</td>
</tr>
<tr>
<td>Glucose, mg/100 ml</td>
<td>83.7±3.0 87.4±3.4</td>
<td>82.8±2.2</td>
<td>7.8±1.7 6.5±1.3</td>
<td>0.704 0.288</td>
</tr>
<tr>
<td>Fasting insulin, µU/ml</td>
<td>11.8±7.2 4.1±0.7</td>
<td>93.9±19</td>
<td>88.0±5.5 99.7±6.1</td>
<td>0.255 0.889</td>
</tr>
<tr>
<td>Fasting glucagon, pg/ml</td>
<td>98.3±1.9 85.4±7.9</td>
<td>81.2±24</td>
<td>81.2±24 89.7±26.8</td>
<td>0.255 0.889</td>
</tr>
</tbody>
</table>

Values are means ± SE. BMI, body mass index.

The separated albumin and fibrinogen were precipitated with trichloroacetic acid. The precipitates were recovered by centrifugation and then hydrolyzed in 1 ml 6 N HCl at 110°C for 18–20 h. The hydrolysates were further purified by passing through a column containing 1 ml Bio-Rad AG 50W-X8, 100–200 mesh, H⁺ form, and were dried in a SpeedVac apparatus (Savant Instruments). The dried amino acid hydrolysates were derivatized to be analyzed by mass-spectrometric (MS) measurement of [13C]leucine and [15N]phenylalanine enrichments and were stored at −80°C in 0.1 ml of 0.1 N HCl if not immediately derivatized.

The purity of the proteins separated by this technique was assessed by analytic electrophoresis followed by silver stain (Bio-Rad Laboratories). An electrophoresis gel of both proteins showed a single band for albumin and three bands for fibrinogen after denaturation (18). The process was highly reproducible with a coefficient of variation of <5% of five repeatedly measured aliquots of one blood sample.

Measurements of isotopic enrichment in precursor and proteins. Hydrolysates of albumin and fibrinogen were derivatized by first reacting with 4 M methanol-HCl and then derivatized to their heptafluorobutyric acid ester (17) for measurement of [13C]leucine enrichment by gas chromatography-isotope ratio MS (GC-IRMS, Finigan-MAT, Bremen, Germany), as previously described (3). Plasma precursor [13C]KIC was derivatized as their trifluoroacetyl isopropyl ester and measured by the positive ion chemical ionization method; plasma [13C]leucine was extracted from plasma and derivatized to its quinoxalinol-trimethylsilyl ester and measured by positive ion chemical ionization (17). Hydrolysates of albumin and fibrinogen were derivatized with 95% N-methyl-N-(t-butyldimethylsilyl)trifluoroacetamide, or MTBSTFA, plus t-butyldimethylchlorosilane and acetonitrile into their t-butyldimethylsilyl ether; then isoene enrichments of [15N]phenylalanine in both proteins were measured by IR-MS (39).

Calculation of FSR of albumin and fibrinogen. FSR of albumin and fibrinogen were calculated by dividing the regression slope of isotope enrichment from 5 to 10 h of isotope infusion by the precursor pool enrichment. We used plasma plateau enrichments of [13C]leucine, [13C]KIC, and plasma [15N]phenylalanine as surrogate measures of the precursor pool enrichment in liver protein synthesis

\[
\text{FSR (%/day)} = \frac{\text{slope of enrichment in protein from 5–10 h (AEP/h)}}{\text{precursor pool enrichment at plateau (MPE)}} \times 100 \times 24
\]

Measurements of glucose, insulin, and glucagon. Plasma glucose was measured enzymatically with an auto-analyzer.
was performed using SAS window version 9.0 on a PC. Statistical analysis procedures were used for assessing effect of age on gender differences. General linear model and regression analysis were used for detecting group and gender differences. A two-tailed t-test was used for detecting group and gender differences. General linear model and regression analysis procedures were used for assessing effect of age on albumin and fibrinogen synthesis. Statistical analysis of data was performed using SAS window version 9.0 on a PC.

RESULTS

Characteristics of study subjects. The general characteristics of the 36 subjects are summarized in Table 1. The age span of the three groups was 20–79 yr. Except for the young male subjects, who were significantly heavier than young female subjects (P < 0.01), body weight was not different between male and female subjects in the middle and old age groups. Male subjects were significantly taller than female subjects in both young and old groups (P < 0.05). Body mass index (BMI) (weight in kg/height in m²) was similar in all age groups and between the two genders. No significant differences were found in fasting blood glucose, plasma insulin, and glucagon levels among the three age groups and between the two genders.

Isotope enrichments of precursors at plateau and in proteins. The precursor isotopes of plasma [13C]leucine, [15N]phenylalanine, and [15N]phenylalanine are presented in Fig. 1. All three precursors reached an isotopic plateau between 5 and 10 h of infusion. The plateau was defined here as the time when the slope of the isotopic enrichment, plotted against time, did not significantly differ from zero. The plateau values between 5 and 10 h for the three precursors were used for calculation of FSR of albumin and fibrinogen. Figures 2 and 3 depict the increment of tracer enrichments in fibrinogen and albumin between 5 and 10 h of isotope infusion. A linear increment in isotope enrichment was observed for both proteins, which enabled calculation of FSR of both proteins by use of the regression slope divided by its precursor enrichment.

FSR values and blood levels. The FSR of fibrinogen (Fig. 4 and Table 2) ranged between 13 and 23%/day on the basis of calculation with plasma [13C]leucine and [15N]phenylalanine as precursors, and between 17 and 29%/day with plasma [13C]KIC as the precursor. A significant effect of age on the FSR of fibrinogen was demonstrated (P < 0.001) irrespective of the precursors used for the calculations. The young subjects had significantly higher values of FSR (for example, 28.5 ± 4.3%/day, with plasma [13C]KIC as precursor) of fibrinogen than middle-aged (18.0 ± 1.0%/day) and old subjects (17.9 ± 1.1%/day) (P < 0.05). There was no change from middle to old age. There was no gender effect on FSR of fibrinogen (20.6 ± 2.3%/day for females and 19.2 ± 1.1%/day for males).

Plasma concentration of fibrinogen increased progressively with age (P < 0.0003; Fig. 5 and Table 2). It was higher in old and middle-aged subjects than in young subjects (P < 0.01). Although there was a tendency to increase from middle to old age, no statistically significant change occurred. There was no gender effect on plasma fibrinogen concentration.

![Fig. 1. Isotopic enrichments of the 3 plasma tracers. Molar percent excess (MPE) of all 3 precursors reached a plateau between 5 and 10 h of continuous infusion. Isotopic enrichment of plasma [13C]ketoisocaproate(13C)KIC was lower than that of [13C]leucine. Average enrichment values within 5–10 h were used for calculating fractional synthesis rates (FSR) of both proteins. Values are means ± SE for young male (Y), young female (YF), middle-aged male (M), middle-aged female (MF), older male (O), and older female (OF) subjects.](http://ajpendo.physiology.org/doi/10.1152/ajpendo.00032.2017)
FSR values of albumin are summarized in Table 3. The values are similar when they are calculated with plasma $[^{13}\text{C}]$leucine or $[^{15}\text{N}]$phenylalanine as precursors, whereas higher values were observed when they were calculated with plasma $[^{13}\text{C}]$KIC. No significant differences were found for FSR or plasma concentration of albumin among young, middle-aged, and old subjects. Male subjects had higher FSR of albumin ($P < 0.05$) on the basis of calculations with $[^{13}\text{C}]$leucine and $[^{15}\text{N}]$phenylalanine as precursors, but no differences were observed when $[^{13}\text{C}]$KIC was used as precursor label.

Table 4 presents the ASR of fibrinogen per kilogram body weight; it was not significantly different among the three age groups. A putative measure of WBPS (nonoxidative leucine flux) based on leucine kinetics was significant from middle age onward, with the young having a WBPS of $137.6 \pm 4.2\text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ vs. $95.9 \pm 8.6\text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ in the middle-aged and $103.7 \pm 4.1\text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ in the older subjects ($P < 0.01$).

DISCUSSION

The novel observation of the current study is that there is an age-related decline in FSR of fibrinogen, an essential component of blood clotting and a cardiovascular risk factor in humans. In contrast, no age effect on FSR and plasma concentration of albumin was observed. An increased fibrinogen concentration, along with an age-related decline in the FSR of this protein, provides new insight into the mechanism of increased circulating fibrinogen concentration in humans.

The biochemical mechanism of increased fibrinogen concentration cannot be attributed to an increased fibrinogen synthesis, because the FSR values of fibrinogen are lower in middle-aged and older people. The blood concentrations of fibrinogen, like many substrates, are ultimately determined by the balance between synthesis and disposal of this protein. The current study demonstrated that the FSR values of fibrinogen in the older people are lower than those of young people and therefore that the increased fibrinogen levels in the middle-aged and older people could occur only if the fractional disappearance rate of fibrinogen were substantially lower than the synthesis rate. The FSR is a measure of the fraction of the total fibrinogen in the body synthesized every day and is independent of any body composition parameters. We also estimated the absolute amount of fibrinogen synthesized per day per kilogram of body weight, which is also not different among the three age groups, again indicating that the increased plasma fibrinogen levels cannot be explained by the ASR of fibrinogen in older people. Unlike the measurements of FSR, the estima-
tion of ASR of fibrinogen is influenced by the changes in body composition. Because the composition of body weight and fat-free mass differs in the young and the old, the estimation of plasma volume and normalization of ASR for body weight may cause some potential errors in this measurement. It is likely that plasma volume is overestimated in the older people, because they are likely to have increased extracellular water, and a dual-energy X-ray absorptiometry-based fat-free mass measurement does not distinguish between water and lean tissue. When fibrinogen synthesis is expressed per kilogram body weight, it may introduce an error because the body weight includes fat, which is higher in the older people. Irrespective of these problems, it is clear that the difference between fibrinogen synthesis rate and disposal rate is greater in the older people than in the young. Increased fibrinogen concentration occurs only if its disposal rate is lower than its synthesis rate; therefore, fibrinogen is likely to have a longer half-life in the older people. The implications of this “older” circulating fibrinogen in the pathogenesis of atherosclerosis and thromboembolic episodes remain to be investigated.

Aging is associated with a decline in synthesis rate of muscle proteins, such as myosin heavy chain (4) and mitochondrial protein (36). There is evidence that there is a generalized decline of whole body protein turnover (4, 43) and mixed muscle proteins (43–45). Together, these data suggest a generalized decline in the remodeling process of tissues with aging. Because the continuous processes of breakdown and synthesis of new tissue proteins are essential for the remodeling process in the body, this generalized decline in protein synthesis with aging may be an underlying mechanism of human aging. Previous works (42) also showed by use of the classic radioactive isotopic approach that aging resulted in a generalized decline in liver protein synthesis in animals. The limitation of this approach is that the results are an average of synthesis rate of mixed proteins in liver.

Although the effect of age on fibrinogen synthesis has not been reported in humans, Andrew et al. (2) observed that the young healthy newborn lamb has significantly higher turnover rate of fibrinogen than the adult lamb. The current study further extended this observation in animals to humans and demonstrated that fibrinogen turnover rate is slower among adults as they get older. In addition, it is also demon-

![Fig. 4. FSR of fibrinogen by age groups as calculated with different precursor enrichment at plateau. There is a significant age effect on fibrinogen synthesis, with a decreasing trend with advanced age. This decrease in synthesis rate was observed from young to both middle age (\(^*P < 0.001\)) and old age (\(^*P < 0.001\)). There was no significant difference between middle and old age groups.](image)

![Fig. 5. Plasma fibrinogen concentrations increased significantly with age (\(^*P < 0.003\)). Middle and old age groups had higher concentrations than the young group. There was no difference between middle and old age groups.](image)
Table 3. Effects of age and gender on FSR and concentrations of albumin

<table>
<thead>
<tr>
<th>Age Effect (P Value)</th>
<th>Young (Values are means ± SE)</th>
<th>Middle (Values are means ± SE)</th>
<th>Old (Values are means ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLM</td>
<td>0.685</td>
<td>0.933</td>
<td></td>
</tr>
<tr>
<td>Reg</td>
<td>0.244</td>
<td>0.136</td>
<td></td>
</tr>
</tbody>
</table>

Table 4. ASR of fibrinogen and albumin by age groups

<table>
<thead>
<tr>
<th>ASR of fibrinogen by age groups (Values are means ± SE expressed in mg·day⁻¹·kg body wt⁻¹)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>[¹⁵N]Phe</td>
<td>0.9307</td>
</tr>
<tr>
<td>[¹³C]KIC</td>
<td>0.8095</td>
</tr>
<tr>
<td>[¹³C]Leu</td>
<td>0.7809</td>
</tr>
<tr>
<td>Albumin (Values are means ± SE expressed in mg·day⁻¹·kg body wt⁻¹)</td>
<td></td>
</tr>
<tr>
<td>[¹⁵N]Phe</td>
<td>0.6490</td>
</tr>
<tr>
<td>[¹³C]KIC</td>
<td>0.1641</td>
</tr>
<tr>
<td>[¹³C]Leu</td>
<td>0.0740</td>
</tr>
</tbody>
</table>

Values are means ± SE expressed in mg·day⁻¹·kg body wt⁻¹. ASR, absolute synthesis rates. *Significant difference between young and older at P < 0.05.
that arterial plasma KIC enrichment better represents the hepatic leucine enrichment than that of arterial plasma leucine. It is therefore likely that the calculations of FSR values of proteins of hepatic origin based on plasma KIC enrichment as the precursor pool are more correct than those based on plasma leucine and phenylalanine enrichment. The conclusion that the FSR values of fibrinogen decrease with age is supported equally by the calculations based on three different precursor labels. Although VLDL apolipoprotein B-100 (apoB-100) has been proposed as an appropriate precursor pool label for assessing liver protein synthesis (9, 34), our preliminary data indicate that tracer enrichment in VLDL apoB-100 is much lower than aminoacyl-tRNA and tissue fluid (1). Thus calculations of liver protein synthesis with VLDL apoB-100 as precursor may overestimate liver protein synthesis rate.

In summary, the present study demonstrated that age has a differential effect on the FSR of albumin and fibrinogen in healthy humans, whereas the ASR of fibrinogen is not significantly different among age groups. The finding that FSR of fibrinogen are decreased while its concentration increased with age in healthy humans indicates a decreased disposal rate of fibrinogen in older people. The implication of this slower disposal rate than synthesis rate of fibrinogen with aging in healthy humans remains to be investigated.

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