Age effect on fibrinogen and albumin synthesis in humans

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.

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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.

The separated albumin and fibrinogen were precipitated with trichloroacetic acid. The precipitants 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 cation exchange column (containing 1.0 ml Bio-Rad AG 50W-X8, 50–200 mesh, H+ form) and were dried in a SpeedVac apparatus (Savant Instruments). The dried amino acid hydrolysates were ready to be derivatized for 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 pool enrichment at plateau (MPE) was measured by the positive ion chemical ionization method; plasma [13C]leucine was extracted from plasma and derivatized to its trifluoroacetyl isopropyl ester and measured by the positive ion chemical ionization method; plasma [15N]phenylalanine was extracted from plasma and derivatized to its quinoxalin-1- trimethylsilyl ether and measured by positive ion chemical ionization (17). Hydrolysates of albumin and fibrinogen were derivatized with N-methyl-N-(t-butyldimethylsilyl)trifluoroacetamide, or MTBSTFA, plus t-butyldimethylchlorosilane and acetonitrile into their t-butyldimethylsilyl ether; then isotope enrichments of [15N]phenylalanine in both proteins were measured by IRMS-MS (39).

Calculation of FSR of albumin and fibrinogen. FSR of albumin and fibrinogen were calculated by dividing the regression slope of isotopic enrichment from 5 to 10 h by the precursor pool enrichment in liver protein synthesis.

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

Measurements of glucose, insulin, and glucagon. Plasma glucose was measured enzymatically with an auto-analyzer.
Measurements of plasma albumin and fibrinogen concentrations. Plasma albumin was measured by a modification of the bromcresol green binding assay (COBAS/MIRA System, Roche, NJ), and fibrinogen was measured using a commercially available immunochemical kit (cat. no. KAI-035, Kamiya Biomedical Company, Seattle, WA).

Measurement of absolute synthesis rate of fibrinogen and albumin. The absolute synthesis rate (ASR) was calculated on the basis of FSR of both proteins multiplied by plasma concentrations, further multiplied by plasma volume, and then expressed as milligrams per kilogram body weight. Plasma volume was based on 45 ml plasma volume per kilogram fat-free mass.

Measurement of whole body protein synthesis. Whole body protein synthesis (WBPS) was calculated on the basis of plasma leucine kinetics as presented in Ref. 4. Plasma \([^{13}\text{C}]\text{KIC}\) and \([^{15}\text{N}]\) phenylalanine is presented in Fig. 1. All three precursors reached an isotopic plateau between 5 and 10 h of infusion. The plateau is 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 \([^{13}\text{C}]\text{leucine and}^{[^{13}\text{C}]\text{KIC}}\) and \([^{15}\text{N}]\) phenylalanine as precursors, and between 17 and 29%/day with plasma \([^{13}\text{C}]\text{KIC}\) 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 \(\pm\) 4.3%/day, with plasma \([^{13}\text{C}]\text{KIC}\) as precursor) of fibrinogen than middle-aged \((18.0 \pm 1.0\%)\) and old subjects \((17.9 \pm 1.1\%)\) \((P<0.05)\). There was no change from middle to old age. There was no gender effect on FSR of fibrinogen \((20.6 \pm 2.3\%)\) for females and \((19.2 \pm 1.1\%)\) 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.

**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 m\(^{2}\)) 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 \([^{13}\text{C}]\) leucine, \([^{13}\text{C}]\) KIC, and \([^{15}\text{N}]\) phenylalanine are presented in Fig. 1. All three precursors reached an isotopic plateau between 5 and 10 h of infusion. 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.

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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.
FSR values of albumin are summarized in Table 3. The values are similar when they are calculated with plasma $^{13}$Cleucine or $^{15}$Nphenylalanine as precursors, whereas higher values were observed when they were calculated with plasma $^{13}$CKIC. 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 $^{13}$Cleucine and $^{15}$Nphenylalanine as precursors, but no differences were observed when $^{13}$CKIC 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 $137.6 \pm 4.2$ mg·kg$^{-1}$·h$^{-1}$ vs. $95.9 \pm 8.6$ mg·kg$^{-1}$·h$^{-1}$ in the middle-aged and $103.7 \pm 4.1$ mg·kg$^{-1}$·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, making the increased blood fibrinogen level a potential risk factor for developing cardiovascular disease, atherosclerosis, and thromboembolic episodes (7, 27). 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 (\(\ast P < 0.001\)) and old age (\(\ast\ P < 0.001\)). There was no significant difference between middle and old age groups.](image)

![Fig. 5. Plasma fibrinogen concentrations increased significantly with age (\(\ast P < 0.003\)). Middle and old age groups had higher concentrations than the young group. There was no difference between middle and old age groups.](image)

| Table 2. Effects of age and gender on FSR and concentration of fibrinogen |
|--------------------------|---------------------|---------------------|---------------------|---------------------|
|                         | **FSR of fibrinogen by precursors** | **Fibrinogen Concentration, mg/100 ml** | **Age Effect (P Value)** |
|                         | **Young** | **Middle** | **Old** | **GLM** | **Reg** |
| Plasma \([{}^{15}\text{N}]\text{Phe}\) | 22.946 ± 3.289 | 17.283 ± 1.344 | 15.321 ± 0.904 | 0.0419 | 0.0087 |
| Plasma \([{}^{13}\text{C}]\text{Leu}\) | 19.162 ± 2.428 | 14.084 ± 0.819 | 13.687 ± 0.834 | 0.0011 | 0.0005 |
| Plasma \([{}^{13}\text{C}]\text{KIC}\) | 28.536 ± 4.280 | 18.002 ± 0.992 | 17.877 ± 1.077 | 0.0088 | 0.0030 |
| Fibrinogen Concentration, mg/100 ml | 263.3 ± 33.1 | 368.3 ± 26.5 | 414.0 ± 24.8 | 0.0022 | 0.0003 |

**Gender Effect (P Value)**

<table>
<thead>
<tr>
<th></th>
<th><strong>Female</strong></th>
<th><strong>Male</strong></th>
<th><strong>Gender Effect (P Value)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma ([{}^{15}\text{N}]\text{Phe})</td>
<td>20.460 ± 2.388</td>
<td>16.574 ± 0.937</td>
<td>0.139</td>
</tr>
<tr>
<td>Plasma ([{}^{13}\text{C}]\text{Leu})</td>
<td>15.735 ± 1.686</td>
<td>15.553 ± 1.010</td>
<td>0.338</td>
</tr>
<tr>
<td>Plasma ([{}^{13}\text{C}]\text{KIC})</td>
<td>20.572 ± 2.257</td>
<td>19.186 ± 1.109</td>
<td>0.180</td>
</tr>
<tr>
<td>Fibrinogen concentration, mg/100 ml</td>
<td>336.0 ± 28.8</td>
<td>361.1 ± 25.7</td>
<td>0.520</td>
</tr>
</tbody>
</table>

Values are means ± SE. Fractional synthesis rates (FSR) are expressed as percent/day. Age effect for fibrinogen was calculated on the basis of general linear model analysis (GLM) and regression analysis (Reg) procedures. \([{}^{13}\text{C}]\text{KIC}, [{}^{13}\text{C}]\text{ketoisocaproate.}\)
Table 3. Effects of age and gender on FSR and concentrations of albumin

<table>
<thead>
<tr>
<th>FSR of albumin by precursors</th>
<th>Young</th>
<th>Middle</th>
<th>Old</th>
<th>GLM</th>
<th>Reg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma $[{}^{15}\text{N}]\text{Phe}$</td>
<td>5.785 ± 0.379</td>
<td>5.350 ± 0.373</td>
<td>5.745 ± 0.413</td>
<td>0.685</td>
<td>0.933</td>
</tr>
<tr>
<td>Plasma $[{}^{13}\text{C}]\text{Leu}$</td>
<td>5.920 ± 0.286</td>
<td>5.038 ± 0.369</td>
<td>5.246 ± 0.270</td>
<td>0.244</td>
<td>0.136</td>
</tr>
<tr>
<td>Plasma $[{}^{13}\text{C}]\text{KIC}$</td>
<td>7.368 ± 0.352</td>
<td>6.451 ± 0.463</td>
<td>6.833 ± 0.304</td>
<td>0.128</td>
<td>0.065</td>
</tr>
<tr>
<td>Albumin concentration, g/l</td>
<td>3.958 ± 0.125</td>
<td>4.092 ± 0.106</td>
<td>3.817 ± 0.075</td>
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</tbody>
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<table>
<thead>
<tr>
<th>FSR of albumin by precursors</th>
<th>Female</th>
<th>Male</th>
<th>Gender Effect (P Value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma $[{}^{15}\text{N}]\text{Phe}$</td>
<td>5.075 ± 0.264</td>
<td>6.178 ± 0.307</td>
<td>0.010</td>
</tr>
<tr>
<td>Plasma $[{}^{13}\text{C}]\text{Leu}$</td>
<td>5.023 ± 0.185</td>
<td>5.780 ± 0.302</td>
<td>0.040</td>
</tr>
<tr>
<td>Plasma $[{}^{13}\text{C}]\text{KIC}$</td>
<td>6.624 ± 0.299</td>
<td>7.143 ± 0.324</td>
<td>0.248</td>
</tr>
<tr>
<td>Albumin concentration, g/l</td>
<td>4.0 ± 0.0897</td>
<td>3.9 ± 0.08</td>
<td>0.476</td>
</tr>
</tbody>
</table>

Values are means ± SE.

strated that, like muscle proteins, age affects the FSR of some individual liver proteins, like fibrinogen.

It is, however, intriguing that the synthesis rate of another liver protein, albumin, is not affected by aging, thereby demonstrating a differential effect of age on the FSR values of different individual proteins in the same organ. Similar differential effects of age on synthesis rate of muscle proteins have previously been observed (4). Whereas the synthesis rate of myosin heavy chain and mitochondrial protein decreased with age, that of sarcoplasmic protein was unaffected (4). The mechanism of this differential effect of age on various proteins remains to be clearly understood.

An important question is whether any hormonal changes that occur with age could cause such an effect on fibrinogen synthesis. Insulin secretion and insulin sensitivity may be attenuated in aging (11), and it has been observed that deprivation of insulin increases fibrinogen synthesis (13, 14). Glucagon has also been reported to affect the fibrinogen synthesis rate (40). It is unlikely that insulin, glucagon, and glucose hemostasis had a role in the decreased fibrinogen synthesis or increased blood levels with aging in this study population, because no difference in blood concentrations of glucose, insulin, and glucagon was observed in this study. Measured BMI is also not related to changes in either blood concentration or FSR of fibrinogen in the current study.

Contrary to a previous hypothesis that aging results in a decreased albumin synthesis because of a reduced nutritional demand (15), no age effect on albumin synthesis was observed in the current study. Of note, albumin synthesis rates expressed as either FSR or ASR were higher in men than in women. The observation in the present study suggests that albumin synthesis is unrelated to aging, which is further supported by the finding that the plasma concentration of albumin is not different among the three age groups. An early study (20) observed both a lower albumin synthesis rate measured with urinary $[{}^{15}\text{N}]\text{glycine}$ as precursor enrichment and a decreased serum concentration in elderly subjects. Decreased serum albumin with age has also been reported in a study by Baumgartner et al. (6) in which serum albumin levels are correlated to skeletal muscle in elderly men and women. The difference between the present study and the previous ones may be due to the selection of subjects and the techniques employed. We studied healthy older and young subjects and studied them while they were on a normal diet that was expected to provide energy and protein based on their body weight. The results of the present study represent findings from the healthy and well-nourished human subjects. When elderly subjects have chronic diseases or nutritional deficiencies with a decreased albumin serum level, the albumin synthesis rate may be decreased.

It is reassuring that the measurements based on two different amino acid tracers gave similar results. The FSR values of both fibrinogen and albumin were lower when plasma leucine and phenylalanine labels were used as the precursor pool labels in the calculations than when plasma $[{}^{13}\text{C}]\text{KIC}$ was used as the precursor pool. Previously, whole body studies demonstrated that plasma KIC enrichment or specific activity represents intracellular leucine enrichment or specific activity better than plasma leucine enrichment or specific activity (38). In skeletal muscle there is a substantial heterogeneity of various precursor pools of leucine (26). Splanchnic studies have demonstrated that arterial plasma leucine and phenylalanine enrichment values are higher than those of hepatic venous leucine and phenylalanine (31). Hepatic venous leucine enrichment is very close to that of arterial plasma KIC, suggesting
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 are 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 apoipoprotein 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 amino acyl-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 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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