Feeding Acutely Stimulates Fibrinogen Synthesis in Healthy Young and Elderly Adults\textsuperscript{1,2}

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Abstract

Fibrinogen is a positive acute-phase protein and its hepatic synthesis is enhanced following inflammation and injury. However, it is not clear whether fibrinogen synthesis is also responsive to oral nutrients and whether the response to a meal may be affected by age. Our aim in this study was to investigate the acute effect of oral feeding on fibrinogen synthesis in both young and elderly men and women. Fibrinogen synthesis was determined in 3 separate occasions from the incorporation of \( \text{L}^{[2\text{H}]5}\text{phenylalanine} \) (43 mg/kg body weight) in 8 young (21–35 y) and 8 elderly (>60 y) participants following the ingestion of water (control), a complete liquid meal (15% protein, 30% fat, and 55% carbohydrate), or only the protein component of the meal. The ingestion of the complete meal enhanced fibrinogen fractional synthesis rates (FSR) by 17 ± 6% in the young and by 38 ± 10% in the elderly participants compared with the water meal (\( P < 0.02 \)). A comparable stimulation of FSR occurred with only the protein component of the meal in both young (29 ± 7%) and elderly participants (41 ± 9%) compared with the water meal (\( P < 0.005 \)). Similar results were obtained when fibrinogen synthesis was expressed as absolute synthesis rates (i.e. mg kg\(^{-1}\) d\(^{-1}\)). The results demonstrate that fibrinogen synthesis is acutely stimulated after ingestion of a meal and that this effect can be reproduced by the protein component of the meal alone, both in young and elderly adults. J. Nutr. 139: 2032–2036, 2009.

Introduction

Fibrinogen is a coagulation protein involved both in thrombogenesis and atherogenesis processes and it has been recognized as a major independent risk factor for cardiovascular disease. Elevated plasma fibrinogen concentrations are strongly associated with increased risk of coronary heart disease, stroke, and mortality from vascular events (1–4). The plasma fibrinogen concentration increases with aging, obesity, physical inactivity, and smoking (5–10). However, the factors regulating in vivo fibrinogen metabolism have not been fully elucidated.

Fibrinogen is a positive-acute phase protein and its hepatic synthesis greatly increases with inflammatory stimuli. A stimulation of fibrinogen synthesis has been reported in animal models of inflammation (11,12) and in patients following injury and trauma (13–15). The stimulation of fibrinogen synthesis during an acute-phase reaction is mediated by cytokines and interleukin 6 (IL-6)\textsuperscript{6} in particular has a prominent role in the enhancement of fibrinogen synthesis by directly upregulating fibrinogen gene expression (12,16–18).

Although regulation of fibrinogen synthesis by inflammation is well documented, it is not clear whether fibrinogen synthesis is also responsive to nutrient intake. Previous studies indicate that fibrinogen synthesis rates are unaltered (19) or only tend to be increased (20) with feeding in healthy humans. However, a significant stimulation with feeding has been reported in cancer patients (20).

In aging, elevated circulating levels of fibrinogen relative to younger individuals have been reported (5–10), but no age-related changes in the basal rate of fibrinogen synthesis have been documented (21). The impact of aging on the response of fibrinogen synthesis to nutrient intake is not known.

Therefore, our aim in this study was to examine the acute effect of feeding on fibrinogen synthesis in both younger and older subjects. To explore the role of specific nutrients, particularly of dietary protein in eliciting the effects, fibrinogen synthesis was assessed following the ingestion of a complete meal alone, both in young and elderly adults. J. Nutr. 139: 2032–2036, 2009.

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\textsuperscript{6} Abbreviations used: ASR, absolute synthesis rate; FSR, fractional synthesis rate; IL-6, interleukin 6; RBP, retinol binding protein.
meal (i.e. containing protein, carbohydrate, and fat) or of the protein component alone.

Data on albumin synthesis from the same study, including some participants’ characteristics, have been previously published (22).

Methods

Participants and experimental design

Healthy, nonsmoking young (age 21–35 y, n = 8) and elderly (age > 60 y, n = 8) volunteers took part in the study. Elderly participants were recruited and then young participants were matched for gender and BMI. After giving written informed consent, participants were admitted to the General Clinical Research Center at Stony Brook University Medical Center for a screening visit consisting of a complete medical history, physical examination, and routine blood testing for general chemistry, hematologic, hepatic, and renal function. Participants with clinical evidence of cardiac, vascular, hepatic, renal, or endocrine disease were excluded from the study.

Enrolled subjects were studied 3 times ~1 wk apart. On each study visit, fibrinogen synthesis rates were assessed following the ingestion of either water (i.e. postabsorptive state, water meal), or a liquid meal containing protein, carbohydrates, and fat (complete meal) or only protein (protein meal). Participants were asked to not make changes in habitual dietary intake or drink alcohol and to avoid any heavy or prolonged physical exercise for 3 d before each test.

On each study visit, participants were admitted to the General Clinical Research Center at 1700 h, examined for any acute illness, and provided a standard dinner. No food or drinks, except for water, were allowed after 2200. The following morning at 0700 after taking a pretest blood sample, volunteers consumed 1 of the 3 experimental meals, which were given in random order.

The complete meal contained whey protein (Bi-Pro, Davisco), carbohydrates in the form of maltodextrin (Moducal, Mead Johnson), and fat in the form of canola oil. The meal supplied one-third of participants’ daily energy expenditure, estimated by multiplying by a factor of 1.5 the basal metabolic rate predicted from 1985 FAO equations (23), and provided 15% of energy as protein, 30% as fat, and 55% as carbohydrates.

The protein meal contained only the protein component of the complete meal and it was therefore isonitrogenous, but not isocaloric. The water meal consisted of water given in a volume similar to the other 2 meals.

Thirty minutes after the ingestion of meals, fibrinogen synthesis was measured using the flooding technique (13,22,24,25). A sterile and pyrogen-free solution containing t-[2H5]phenylalanine (Cambridge Isotopes) and unlabeled phenylalanine (Ajinomoto) (45 mg/kg body weight) was infused at a constant rate over 10 min. Blood samples were then taken over 90 min for determination of the enrichment of t-[2H5]phenylalanine in plasma fibrinogen and in the plasma phenylalanine pool. The enrichment of the injected isotope solution was 10, 20, and 40 mol%, respectively, on study d 1, 2, and 3. All protocol were approved by the Committee on Research Involving Human Subjects at Stony Brook University.

Analytical methods

Enrichment of fibrinogen. Fibrinogen was isolated from plasma by repeated precipitation with ammonium sulfate and solubilization in sodium citrate, as previously described (13,24,25). Fibrinogen was then washed several times with cold perchloric acid (30 g/L) and hydrolyzed with 6 mol/L hydrochloric acid for 24 h at 110°C. t-[2H5]Phenylalanine enrichment was determined by monitoring ions with mass-charge ratios 106 (m+2) and 109 (m+5) of the n-heptafluorobutyryl derivative of 3-phenylethylamine on a MD800 GC-MS (Fisons Instruments) (13,22,26,27).

Enrichment of plasma phenylalanine. Plasma free phenylalanine was purified and derivatized using an EZ:faast amino acid analysis kit (Phenomenex). Enrichment was assessed by monitoring the ions at mass:charge ratios 206 and 211 on a GC-MS (MD800, Fisons Instruments) (22).

Other analytical procedures. The plasma fibrinogen concentration was measured using an ACL Advance/Futura coagulometer (Instrumentation Laboratory) (28). The plasma albumin concentration was measured with an automated bromocresol green method (29). Serum prealbumin and retinol binding protein (RBP) concentrations were determined by nephelometry using a BN II nephelometer (Dade Behring). Serum lipid panel was assessed at the Stony Brook University Medical Center Clinical Lab using a Roche Modular Automated test system. Plasma IL-6 concentration was determined by ELISA using a commercially available kit (R&D Systems). Plasma insulin and glucagon concentrations were measured by RIA (Diagnostics Products and ALPCO Diagnostics).

Calculations

The fractional rates of fibrinogen synthesis (FSR), i.e. the percentage of the intravascular fibrinogen mass synthesized per day, were calculated from the enrichment of t-[2H5]phenylalanine in fibrinogen and the area under the curve of the plasma free phenylalanine enrichment (precursor pool) compared with time, as previously described in detail (13,24,25,30).

Fibrinogen synthesis was also calculated as absolute synthesis rates (ASR), i.e. the total amount synthesized per day, by multiplying the FSR by the intravascular fibrinogen mass and normalizing for body weight (mg·kg⁻¹·d⁻¹). The fibrinogen intravascular mass was estimated from the plasma fibrinogen concentration and plasma volume, which was predicted from sex, age, and weight by using a nomogram (31).

Statistics

All data are expressed as means ± SE. The data analysis was undertaken using SPSS software (version 15.0; SPSS). The differences in characteristics between young and elderly groups were analyzed with an independent 2-sample t test. Repeated-measures ANOVA was undertaken to ascertain age, meal, and meal × age interactions. The within-subject differences for the 3 dietary conditions were analyzed with post hoc comparisons with Bonferroni adjustment. Differences were considered significant if P < 0.05.

Results

The participants in the young and elderly groups did not differ for gender, height, weight, and BMI (Table 1). The plasma concentrations of albumin, prealbumin, RBP, triacylglycerols, and total, LDL, and HDL cholesterol were also comparable in the 2 groups (Table 1). Five elderly participants were taking lipid-lowering medications (3-hydroxy-3-methylglutaryl-coenzyme A inhibitors), which were not discontinued or modified over the duration of the study. Plasma fibrinogen concentrations in young and elderly participants did not differ (Table 1). Plasma IL-6 concentrations were higher in the elderly than in the young group (P < 0.005; Table 1).

Fibrinogen synthesis in the postabsorptive state after the water meal was lower in the elderly than in the young when expressed as the FSR (P < 0.05; Table 1). However, when basal fibrinogen synthesis was expressed as the ASR, younger and older participants did not differ (Table 1).

Synthesis of fibrinogen was stimulated by feeding both in young and elderly participants (Fig. 1). FSR was affected by meal consumed (P < 0.001) and age (P = 0.04), but there was no meal × age interaction (P = 0.4). After consumption of the complete meal, the fibrinogen FSR in the young participants was 17 ± 6% greater than after the water meal (P < 0.02) and in elderly participants, 38 ± 10% greater (P < 0.01) (Fig. 1A). ASR was affected by the meal consumed (P < 0.001) but not by age or the meal × age interaction. In the young and elderly groups combined, fibrinogen synthesis, expressed as the ASR,
Fibrinogen ASR,

mg

Fibrinogen FSR,

LDL cholesterol,

mmol/L

Total cholesterol,

mmol/L

mmol/L

Triglycerides,

IL-6,

ng/L

RBP,

mg/L

Fibrinogen,

g/L

BMI,

kg/m²

Albumin,

g/L

was 34 ± 8% greater after the complete meal than after the water meal \(P = 0.002\).

The stimulation of fibrinogen synthesis after consuming only the protein component of the meal was comparable to that following the complete meal (Fig. 1). Fibrinogen FSR was 29 ± 7% greater in the young after the protein meal than after the water meal \(P < 0.005\) and was 41 ± 9% greater in the elderly participants \(P < 0.002\). Fibrinogen ASR was 30 ± 6% greater after consumption of the protein meal than after the water meal \(P < 0.001\) and did not differ from the stimulation after consumption of the complete meal.

Plasma insulin concentrations were greater following the ingestion of the protein meal than after the water meal in both the young \((32.8 ± 8.8 \text{ to } 171.3 ± 26.2 \text{ pmol/L})\) and elderly \((19.0 ± 4.5 \text{ to } 182.2 ± 43.3 \text{ pmol/L})\) participants \(P < 0.01\). After the complete meal, insulin levels were even higher in both young \((435.7 ± 80.2 \text{ pmol/L})\) and elderly \((312.3 ± 46.9 \text{ pmol/L})\) participants \(P < 0.001\). In contrast, the response of plasma glucagon was higher after the protein meal and blunted after the complete meal. Plasma glucagon concentrations were greater following the ingestion of the protein meal than after the water meal in both the young \((219.4 ± 12 \text{ to } 259.5 ± 17.3 \text{ ng/L})\) and elderly \((191.1 ± 7.2 \text{ to } 332.9 ± 45.8 \text{ ng/L})\) participants \(P < 0.05\). However, plasma glucagon concentrations following the complete meal were comparable to basal concentrations in the young \((198.8 ± 9.2 \text{ ng/L})\) or 15% higher in the elderly \((219.4 ± 14.0 \text{ ng/L})\) than following the water meal \(P < 0.05\).

**Discussion**

The results of this study demonstrate that hepatic synthesis of fibrinogen is acutely stimulated by feeding. Following the ingestion of a mixed meal, the amount of fibrinogen synthesized (i.e., ASR) increased by 34 ± 8% compared with postabsorptive values \(n = 16; P < 0.005\). A similar stimulation of fibrinogen synthesis \(30 ± 6\%; \ n = 16; \ P < 0.001\) was observed after consumption of the protein component of the meal alone, showing that dietary fat and carbohydrates may not be required for eliciting the meal effect. The results also indicate that proportional stimulation of fibrinogen synthesis by feeding does not differ in young and elderly subjects, suggesting that the response of fibrinogen synthesis to nutrients is maintained with aging.

Fibrinogen is a positive acute phase protein and its hepatic synthesis is greatly enhanced following inflammation and trauma \((11,13–15)\), a response mainly mediated by cytokines \((12,16–18)\). However, previous studies exploring whether fibrinogen synthesis is also sensitive to feeding have been equivocal. In contrast with the results of the present study, De Feo et al. \((19)\) did not observe any increase in fibrinogen synthesis in healthy volunteers during a 6-h intraduodenal infusion of glucose and amino acids compared with the fasting state. Barber et al. \((20)\) detected a stimulation of fibrinogen synthesis in cancer patients fed small hourly meals. However, in healthy volunteers, the increase in fibrinogen synthesis during feeding was smaller and did not reach significance \((14\%; \ P = 0.12)\), leading the authors to the conclusion that fibrinogen synthesis is sensitive to nutrient intake in cancer patients but not in healthy participants \((20)\). Some of the apparent discrepancies between the present study and those of De Feo et al. \((19)\) and Barber et al. \((20)\) may be in part explained by differences in feeding regimens and the quantitative and qualitative composition of the experimental diets.

In this study, the response of fibrinogen synthesis to feeding was assessed after consumption of a large single meal, equivalent
to one-third of the participants’ daily requirements, which more closely mimics a normal daily feeding pattern. Assessment of a single, large meal was possible, because fibrinogen synthesis was assessed with the flooding technique, which does not require attainment of isotopic steady-state conditions and can allow the investigation of acute changes in protein synthesis (32). In the other studies, for methodological reasons (19) or because of experimental choice (20), nutrients were provided by continuous enteral infusion or with small frequent meals, which reproduce a constant feeding state. Feeding a single large meal is likely to result in a more acute and pronounced changes in the concentration of the absorbed nutrients and postprandial hormonal levels in the portal circulation, which can both contribute to the stimulation of fibrinogen synthesis. The enhancement of fibrinogen synthesis by feeding may therefore be part of the physiological response of liver protein synthesis to the availability of nutrients and the degree of response may be related to the size of the meal and the amount of nutrients absorbed.

The acute stimulatory effect of feeding on fibrinogen synthesis is comparable to that previously reported in the same experiment for the major liver export protein, albumin (22). However, although the synthesis of both plasma proteins was acutely enhanced by the meal in this study, other studies suggest there may be a difference in the sensitivity of the response of fibrinogen and albumin to the intake of nutrients or to diet composition. In studies with smaller meals than the present study, albumin synthesis was more responsive than fibrinogen (19,20). Chronic dietary changes such as changes in dietary protein quality for 10 d resulted in significant changes in albumin (30) but not fibrinogen synthesis rates in healthy volunteers (33). Similarly, in piglets, feeding a protein-deficient diet for 4 wk resulted in a slower rate of synthesis of albumin, but fibrinogen synthesis was not affected (11). However, when feeding the protein-deficient diet was prolonged for 8 wk, both albumin and fibrinogen synthesis were affected (34).

The greater sensitivity of albumin synthesis to changes in dietary intake may reflect the role in preventing oxidation of essential amino acids from the diet. In contrast, fibrinogen is primarily involved in the acute-phase response and coagulation.

The response of fibrinogen synthesis to the acute intake of nutrients is more likely to reflect an overall anabolic response of the liver rather than a specific stimulation of fibrinogen synthesis. The changes in fibrinogen synthesis that occur in response to inflammation and injury are on the order of 2- to 3-fold (13), reflecting the primary physiological role of this protein. The difference in sensitivity of response of albumin and fibrinogen synthesis to nutrients and dietary composition may, therefore, reflect the difference in physiological function.

The stimulation of fibrinogen synthesis after a complete meal could be reproduced with the consumption of the protein component of the meal alone (Fig. 1), suggesting the importance of dietary amino acids in the regulation of postprandial liver protein synthesis. Plasma concentrations of essential and branched-chain amino acids acid were elevated following the complete meal and to a greater extent following the protein meal (22). The upregulation of fibrinogen synthesis after the meal may therefore be the direct result of the increased amino acid supply, possibly also involving alteration in hormone levels in the postprandial period. Although insulin increased after both the complete meal and the protein meal, it is unlikely that elevated insulin was responsible for the stimulation of fibrinogen synthesis. In healthy volunteers, physiological hyperinsulinemia depresses fibrinogen synthesis (35) and insulin deficiency stimulates fibrinogen synthesis in diabetic patients (36). Perhaps more important to the stimulation of fibrinogen synthesis, glucagon concentration was elevated to a greater extent following the protein meal compared with the complete meal. Acute elevation of plasma glucagon concentrations has been shown to significantly enhance fibrinogen synthesis (37).

The present study and that of Fu and Nair (21) suggest that fibrinogen synthesis in the postabsorptive state is not altered by age. Although postabsorptive fibrinogen FSR was lower in older than in young participants (Fig. 1A), the total amount of fibrinogen synthesized per day (ASR) was comparable in both young and elderly participants. Not only did age not affect postabsorptive fibrinogen synthesis rates, but the increase in fibrinogen synthesis after a meal was also comparable in young and elderly participants, indicating that the postprandial anabolic response to nutrients is maintained with aging.

Plasma fibrinogen concentrations, regulated by rates of synthesis and disposal, generally tend to increase with aging (5–10). The findings of the present study along with that of Fu and Nair (21) suggest that the age-related increase in fibrinogen plasma concentration is not due to higher basal synthesis rates but may be rather due to slower rates of fibrinogen disposal.

Given the importance of inflammation on the synthesis of fibrinogen (11,13–15), the present study examined IL-6 concentrations in younger and older participants as a marker of inflammation. Although they were higher in the older participants (Table 1), the magnitude of elevation was not sufficient to affect the rates of fibrinogen synthesis in the postabsorptive and postprandial states, which were comparable in younger and older participants. Because postabsorptive and postprandial states were comparable in older and younger participants despite the significantly higher levels of IL-6 in the elderly, it seems reasonable to conclude that the low levels of inflammation in these elderly subjects did not significantly affect the rates of fibrinogen synthesis.

Five of 8 participants in the present study were taking statins. Because statin use has been shown to reduce inflammation (i.e. lower C-reactive protein), it is possible that the fibrinogen concentrations in these elderly participants would be higher in individuals not taking statins. This possibility seems unlikely given the meta-analysis of over 5000 volunteers from Balk et al. (38) indicating that statin use was not associated with a significant effect on plasma fibrinogen concentrations.

In conclusion, this study demonstrated that fibrinogen synthesis is acutely enhanced after the consumption of a meal and that the protein component of the meal alone was sufficient to elicit stimulation comparable to a complete meal, suggesting an important role of dietary amino acids in regulating postprandial protein synthesis in the liver. The increase in fibrinogen synthesis may be part of an overall response of hepatic protein synthesis to the increased nutrient supply, particularly amino acids, possibly modulated by changes in glucagon in response to the intake of amino acids. The study also showed that the amount of fibrinogen synthesized per day (i.e. ASR) is comparable in young and elderly and that aging does not affect the acute response of fibrinogen synthesis to nutrients.

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Literature Cited


