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Dietary modulation of apolipoprotein serum amyloid A (apoSAA) metabolism and prevention of amyloidosis in aging C57BL/6J and SJL/J mice

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The specific effect of acute inflammation on apoSAA and lipoprotein metabolism was determined in two strains of mice that are prone to develop spontaneous age-associated systemic amyloidosis. C57BL/6J and SJL/J mice were maintained on purified diets, relatively rich in fat and differing only with respect to protein constituents, i.e., casein (20%) versus soy protein (20%). After 18 months, aging mice of either strain and on either diet were injected subcutaneously with $AgNO_3$ thus inducing a powerful acute phase response as evidenced by increases in plasma LDL and apoSAA-rich HDL. None of the mice in each of the dietary groups developed amyloidosis. Cholesterol levels were elevated during the acute phase response in both mouse strains, although the hypercholesterolemia was less pronounced in C57BL/6J mice and in SJL/J mice fed soy protein compared with casein. Control plasma triglyceride levels were lower in C57BL/6J mice in both dietary groups compared to the SJL/J strain. By contrast, C57BL/6J mice fed either protein increased the triglyceride concentration, whereas in SJL/J mice, triglyceride levels were not altered by casein but were decreased significantly by soy protein diets. Our findings show for the first time that dietary protein intake modulates the acute phase response in rodents. Furthermore, the data support the concept that alterations in lipid metabolism during the acute phase response may represent a protective mechanism whereby HDL-cholesterol and phospholipids are directed to sites of inflammation for connective tissue repair. (J. Nutr. Biochem. 8:328–333, 1997) © *Elsevier Science Inc. 1997*

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Introduction

In man and many other vertebrate species exposure to inflammatory or immune stimuli results in an acute phase response (APR) that encompasses dramatic changes in many areas of metabolism. The changes in plasma protein profiles and concentrations that result primarily from alterations in hepatic synthesis have been studied widely. In man and rodents (with the exception of the rat) levels of plasma apoSAA can increase more than 20 to 40 fold within 8 hr of an appropriate stimulus.[1] Although there have been numerous studies of apoSAA metabolism with regard to its role in experimental models of amyloidosis, a critical question remains: What is the normal physiological function of apoSAA? Recent studies from our laboratory have revealed profound changes in cholesterol metabolism and disruption of plasma apolipoprotein profiles during the APR in young male hamsters.[2] The association of apoSAA with HDL has given rise to several interesting hypotheses, chief of which are: (1) that apoSAA plays an important pathogenic role in atherogenesis and (2) that it may play a pivotal role in transporting cholesterol and other lipids to sites of tissue

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Table 1 Composition of diets rich in casein or soy protein

Ingredients (gm/100 gm)	Casein-rich diet	Soy protein-rich diet
Casein	22.0	0
Soy protein	0	22.0
Sucrose	14.3	14.3
Cellulose	15.0	15.0
Cornstarch	22.0	22.0
Fat[a]	20.0	20.0
Mineral mix[b]	5.0	5.0
Vitamin mix[c]	1.2	1.2
Choline chloride	0.3	0.3

[a]Fat blend contains 10.4% of butter; 6.4% of canola oil; and 3.2% of corn oil; and provides about 40% of total energy.
[b]Ausman-Hayes Mineral Mix (BioServ, Frenchtown, NJ USA) contains the following gm/kg of mix: magnesium oxide, 32; calcium carbonate, 290; potassium phosphate dibasic, 312; calcium phosphate dibasic, 72.6; magnesium sulfate, 98.7; sodium chloride, 162.4; ferric citrate, 27; potassium iodine, 0.8; manganese sulfate, 1.22; zinc chloride, 0.92; cupric sulfate, 0.29; chromium acetate, 0.044; and sodium selenite, 0.0004.
[c]Hayes-Cathcart vitamin mix contains the following gm/kg of mix: dL-α-tocopheryl acetate (500 IU/g) 15; inisotol, 5; niacin, 3; calcium pantothentate, 1.6; retinyl palmitate (500,000 IU/gm), 1.5; cholecalciferol (400,000 IU/gm), 0.1; menadione, 0.2; biotin, 0.02; folic acid, 0.2; riboflavin, 0.7; thiamin, 0.6; pyridoxine HCL, 0.7; cyanocobolamine, 0.001; and dextrin, 972.

damage after acute inflammation or injury from various causes. Because the difference of dietary protein on lipoprotein metabolism is firmly established, the present study was designed to test the hypothesis that plant-derived soy protein might ameliorate the specific effect of acute inflammation on apoSAA and lipoprotein responses in C57BL/6J and SJL/J mice. Accordingly, mice were maintained for 18 months on identical diets in which the protein source was either casein or soy protein. C57BL/6J mice were chosen because they are prone to develop spontaneous AA-type amyloidosis with aging[3] and because they are more susceptible to atherosclerosis than other strains of mice when placed on a high-cholesterol diet.[4–6] SJL/J mice were selected because in all previous studies mice of this strain invariably developed systemic amyloidosis by 1 year of age.[7]

Methods and materials

Animals, diets and basic design

C57BL/6J and SJL/J mice were purchased from Charles River Laboratories under the Animal Procurement Program of NCI. Mice were maintained under 12-hr light/dark cycle in the Animal Research Facility at the ENRM VA Hospital, Bedford, MA. The composition of the purified diets (*Table 1*) was identical except that either casein or soy protein was the protein source. Starting at 8 weeks of age two groups of C57BL/6J mice and SJL/J mice (n = 18 per group) were fed either a soy protein-rich diet or a casein-rich diet for 18 months. At the end of the experiment, each group was divided in two subgroups according to the strain and the diet (C57BL/6J versus SJL/J and soy protein versus casein) and the acute phase response (APR) was induced by sc. injection of 0.5 mL 2% $AgNO_3$ in half of the animals. After an overnight fast, mice were killed by CO_2 inhalation and bled by cardiac puncture.

Density gradient centrifugation

Lipoproteins were isolated from fresh plasma by discontinuous gradient as described previously.[8] Pooled plasma (3.0 mL) was adjusted to a density of 1.21 g/mL by addition of solid KBr. Sudan black was added to each plasma sample. The total volume was adjusted to 4.0 mL by addition of d = 1.21 KBr. This was overlaid with 3.0 ml KBr (density 1.063), 3.0 mL KBr (density 1.019), and 2.3 mL KBr (density 1.006) solutions. Plasma was then centrifuged for 26 hr at 35,000 rpm in a SW41 swinging-bucket rotor at 12°C. After centrifugation the lipoprotein fractions outlined by Sudan black staining were removed by aspiration.

Plasma lipid analyses

Aliquots of individual plasma samples were analyzed for total cholesterol and triglyceride using commercially available methods (Sigma Chemical Co., St. Louis, MO USA). Total cholesterol was also measured in each ultracentrifugation fraction. The protein concentration was measured using the Markwell modification of the Lowry protein determination.[9] The volume of each fraction was measured and used to determine total amounts of each constituent and to calculate the inclusive densities of each fraction. Fractions were dialyzed vs. 0.15 M NaCl; 0.05 M Tris buffer, pH 8.8, using 3500 mw cutoff dialysis membranes (Spectrapor, Spectrum Medical Industries, Inc., Los Angeles, CA USA). Each fraction was analyzed for content of apoSAA protein by an ELISA method as described previously.[10] Specificity of the assay was assured by using polyclonal antiserum raised against synthetic peptides and directed towards the C-terminus of $apoSAA_1$ and $apoSAA_2$ isoforms.

Agarose gel electrophoresis

Plasma samples and density gradient fractions corresponding to LDL and HDL fractions were analyzed by agarose gel electrophoresis using commercially available methods (Titan Gel Multi-Slot Lipo-17, procedure No. 3095, Helena Laboratories, Beaumont, TX USA). Electrophoretograms were stained with fat Red 7B to visualize lipoproteins according to the manufacturer's directions.

Statistical analyses

Mean differences between groups were analyzed by two-way analysis of variance (ANOVA). Where appropriate based on the ANOVA, significance of the differences was determined using Tukey's protected t-test at the 0.05 level.

Results

Mean body weight

C57BL/6J mice fed the casein-based diet weighed less (33.7 ± 3.3 gm) than did those fed the soy protein-based diet (41.9 ± 7.7 gm) ($P < 0.01$). The diet had no effect on growth of SJL/J mice (23.9 ± 2.5 gm for casein-fed mice versus 25.2 ± 2.8 gm for soy protein-fed mice).

Plasma cholesterol

C57BL/6J mice tended to have higher plasma cholesterol levels than SJL/J mice but this difference was significant ($P < 0.01$) only in soy-fed animals (*Table 2*). Induction of the APR resulted in significant increases in cholesterol levels in both strains and with both dietary regimens. Responses in C57Bl/6 and SJL/J were not significantly

Table 2 Effect of casein and soy diet on acute phase- versus control total plasma cholesterol levels (mmol/L)

Dietary protein	C57BL/6J		SJL/J	
	Acute phase	Control	Acute phase	Control
Casein	3.0 ± 0.1[a]	1.7 ± 0.2	2.6 ± 0.1[a]	1.0 ± 0.1
Soy	2.3 ± 0.1[ab]	1.8 ± 0.1[c]	1.8 ± 0.1[ab]	1.3 ± 0.1

Values are means ± SE; $n = 9$ per group.
[a]Control animals of the same strain and dietary regimen ($P < 0.01$).
[b]Casein-fed mice of the same strain ($P < 0.01$).
[c]Soy-fed control mice of the same strain ($P < 0.01$).

different from one another but hypercholesterolemia was considerably less ($P < 0.01$) in both strains maintained on the soy protein versus casein diet (*Table 2*).

Plasma triglyceride levels

In control animals, plasma triglyceride levels were not different between casein-fed and soy-fed mice of the same strain. However, strain differences were evident in both the baseline levels and in the APR. In contrast to cholesterol levels in which the C57BL/6J control levels were higher than those in SJL/J mice, control triglyceride levels were significantly lower in C57BL/6J mice in both diet groups compared to the SJL/J strain (*Table 3*). The acute phase in both casein- and soy-fed C57BL/6J mice was accompanied by significantly increased total plasma triglyceride levels ($P < 0.01$). By contrast, in the SJL/J strain, triglyceride levels were not altered in casein-fed animals but were significantly decreased in the soy-fed group ($P < 0.05$).

Plasma apoSAA levels

Acute phase animals showed the expected marked increase in plasma apoSAA levels in response to the inflammatory stimulus (*Table 4*). No dietary effect on apoSAA levels were noted in control mice, but among the C57BL/6J mice the response to casein was significantly greater than that to soy protein.

Lipoprotein fractions

Plasma lipoprotein fractions from C57BL/6J mice were separated by agarose gel electrophoresis. The APR in both dietary groups revealed a band migrating in the LDL region that was not apparent in control plasma. In addition,

Table 3 Effect of casein or soy protein based diets on acute phase versus control total plasma triglyceride levels (mmol/L)

Dietary protein	C57BL/6		SJL/J	
	Acute phase	Control	Acute phase	Control
Casein	0.86 ± 0.04[a]	0.63 ± 0.04	1.11 ± 0.03	1.16 ± 0.06
Soy	0.98 ± 0.06[b]	0.80 ± 0.05[c]	0.96 ± 0.08[b]	1.16 ± 0.06

Values are means ± SE, $n = 9$ per group.
[a]Control animals of the same strain and dietary regimen ($P < 0.01$).
[b]Different from casein-fed animals ($P < 0.05$).
[c]Soy-fed mice of the SJL/J strain ($P < 0.01$).

Table 4 Effect of casein or soy protein based diets on acute phase versus control total plasma apoSAA levels (mg/L)

Dietary protein	C57BL/6		SJL/J	
	Acute phase	Control	Acute phase	Control
Casein	471 ± 11.6[ab]	13 ± 3.5	462 ± 8.5[a]	28 ± 10.1
Soy	436 ± 13.0[a]	11 ± 2.0	438 ± 9.7[a]	26 ± 8.5

Values are means ± SE; $n = 9$ per group.
[a]Control mice of the same strain and dietary group ($P < 0.01$).
[b]Soy-fed mice of the same strain ($P < 0.01$).

migration of the HDL band was retarded in the APR compared to the controls (*Figure 1*).

To define further the lipoprotein profile observed on agarose gel, plasma from both strains and both diets was separated by density gradient ultracentrifugation and distribution of cholesterol among the different lipoprotein classes was measured (*Table 5*). One difference between acute phase and control plasma was that HDL from APR-mice transported a smaller percentage of cholesterol (60% versus 70% in C57BL/6J; 53% versus 64% in SJL/J mice) with the balance carried in the more buoyant fractions, especially LDL. On the other hand, no marked differences were noted between dietary groups with regard to distribution of cholesterol among lipoprotein fractions.

Discussion

Although the pathogenesis of murine AA-type amyloidosis is not fully understood, amyloid fibril formation in vivo is probably the end-result of impaired proteolysis of the serum precursor protein, apoSAA, by tissue macrophages.[11] We originally postulated that repeated exposure to casein, either by multiple sc. injections or even after prolonged oral ingestion might overwhelm a mononuclear phagocytic system already depleted by aging.[12] This could cause incomplete degradation of circulating amyloid precursors apoSAA and eventually lead to β-pleated sheet fibril deposition in

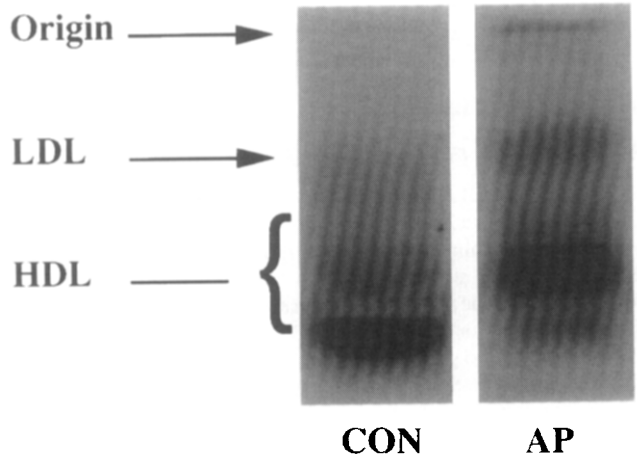

Figure 1 Agarose gel electrophoresis of plasma lipoproteins demonstrates the increased intensity of the LDL fraction and retarded migration of the HDL fraction in acute phase (AP)- versus control (CON)-mice.

Table 5 a Cholesterol concentration (mmol/L) in plasma lipoproteins of C57BL/6 mice fed casein or soy protein diet

	VLDL		LDL		HDL	
	Acute phase	Control	Acute phase	Control	Acute phase	Control
Casein	0.10 ± 0.01[a]	0.01 ± 0.01	0.73 ± 0.1[a]	0.22 ± 0.07	1.89 ± 0.04[a]	1.34 ± 0.07
Soy	0.11 ± 0.01[a]	0.02 ± 0.01	0.50 ± 0.05	0.25 ± 0.05	1.49 ± 0.08[ab]	1.42 ± 0.02

b Cholesterol concentration (mmol/L) in plasma lipoprotein classes of SJL/J mice fed casein or soy protein diet

	VLDL		LDL		HDL	
	Acute phase	Control	Acute phase	Control	Acute phase	Control
Casein	0.23 ± 0.02[a]	0.08 ± 0.01	0.66 ± 0.06[a]	0.21 ± 0.01	1.33 ± 0.04[a]	0.87 ± 0.02
Soy	0.13 ± 0.01[ab]	0.08 ± 0.01	0.47 ± 0.04[a]	0.18 ± 0.01	0.88 ± 0.02[ab]	0.90 ± 0.01

Values are mean ± SE; $n = 9$ per group.
[a]Control animals of the same strain and dietary regimen ($P < 0.05$).
[b]Casein-fed mice of the same strain ($P < 0.05$).

the extracellular space. Biochemical studies in SJL/J mice as well as the senescence accelerated mouse (SAM) strain have shown that amyloid deposits in these mice are derived from mutant forms of apoA-II rather than apoSAA, the known precursor of secondary amyloid deposits.[7,13] Interestingly, SAM mice appear to have a normal life span and fail to develop amyloidosis when fed a low-calorie diet.[14] Casein was restricted to less than 15% w/w so that either low-calorie or low-protein intakes may have been beneficial.

Because earlier studies from our laboratory have shown that C57BL/6J mice on a regular chow diet spontaneously develop amyloidosis at 9 months of age,[3] it was surprising to find that older mice of the same inbred strain remained amyloid-negative in the present study. Even more puzzling was the discovery that none of the SJL/J mice used in this study developed age-associated systemic amyloidosis that has been observed in several laboratories, including our own.[7] Based on these findings, one must assume that the environmental circumstances of the present study, presumably semisynthetic diets fortified by relatively high fat concentrations, favored normal apoSAA and apoA-II homeostasis thus preventing the onset of systemic amyloidosis. In this regard it should be noted that the rapid induction of secondary AA-type amyloidosis in young CBA/J mice was significantly retarded when diets enriched with either n-3 or n-6 polyunsaturated fatty acids were substituted for a diet containing 20% coconut oil.[15] In light of the latest observations regarding C57BL/6J and SJL/J mice, many previous publications using lab chow diets in experimentally induced amyloidosis should be revisited. Indeed, Newberne and McConnel pointed out some time ago that lab chow, in addition to containing a number of carcinogens, also contains a variety of fungal and microbial toxins that could conceivably impact on the APR.[16] Although it seems logical to assume that dietary protein per se may have been instrumental in maintaining apoSAA and apoSAA-II homeostasis, it is conceivable that high concentrations of fat used in the present study contributed at least in part to the findings reported here. Hence, in the absence of a 10% fat diet control, the question remains unresolved as to whether differences between the chow-fed and semisynthetic diets were based solely on the casein or soy protein content.

These studies are the first to demonstrate subtle but significant alterations in plasma apoSAA and lipid metabolism induced by changes in dietary protein during the acute phase response. Whereas basal cholesterol levels tended to be lower during soy protein intake in control mice, systemic stress induced by the APR highlighted striking differences between soy-fed and casein-fed animals. Although both dietary groups in each strain showed marked increases in plasma cholesterol levels, the hypercholesterolemic response was significantly less in mice of either strain fed the soy protein-based diet compared with casein-fed animals. In this respect 18-month-old mice closely resemble young hamsters that manifest a sharp increase in plasma cholesterol levels after subcutaneous injections of $AgNO_3$.[2] Similarly, strain differences in mice were apparent with respect to triglyceride responses.

Feeding casein, compared to soy protein, results in hyperlipidemia in many animal species.[17] Rabbits are particularly susceptible to diets containing casein, developing hypercholesterolemia and atherosclerosis even without supplementary cholesterol in the diet.[18–22] In monkeys[23,24] and rats[25,26] dietary casein results in increased cholesterol levels compared with dietary soy protein, but in these species the effect typically depends on the presence of cholesterol in the diet. In many animal species and in human subjects during acute inflammatory episodes, pronounced hypertriglyceridemia has been reported and attributed to depressed lipoprotein lipase activity.[27] Hypertriglyceridemia has most often been associated with sepsis induced by gram negative bacterial cell wall components, especially bacterial lipopolysaccharide (LPS).[28–30] Although large doses of LPS may produce hypertriglyceridemia in mice, previous studies in our laboratory indicated that young CBA/J mice given $AgNO_3$ as an inflammatory stimulus, demonstrate a net decrease in plasma triglyceride levels within 24 hr.[31]

C57BL/6J mice gained significantly more weight on the soy protein-based diet than on the casein-based diet, whereas the weights SJL/J mice fed the two diets did not

differ. This suggests that the difference between casein and soy protein groups may reflect the lack of sulfur-containing amino acids that have been recommended as a supplement to casein for the past 20 years. Although the mice were fed the same diets ad libitum and food disappearance patterns seemed similar, we did not monitor food consumption per se. This difference is especially intriguing in view of minimal dietary differences between basal (control) concentrations for either cholesterol or triglyceride. If the differences in body weight were real, it suggests that alternative pathways in lipid metabolism, including adipose storage, were not reflected in increased serum lipid levels. At the present time we have no explanation for the strain differences in weight gain.

The dramatic increase in plasma apoSAA levels in response to inflammatory stimuli or tissue trauma has been well documented. ApoSAA is carried almost exclusively on HDL particles, from which it displaces apoA-I.[32] We and others have shown that acute phase HDL containing apoSAA binds more avidly to tissue macrophages[2,31,33] where it is then degraded in vitro by neutral serine proteases secreted into the extracellular fluid.[34] Although increased plasma apoSAA levels were evident in all four acute phase groups, there were 10% higher apoSAA concentrations in casein-fed versus soy-fed C57BL/6J mice. Reduced clearance of cholesterol-rich HDL in casein-fed mice would also be consistent with higher levels of both cholesterol and apoSAA seen in this group. The appearance of increased amounts of cholesterol in the LDL region of the lipoprotein profiles derived from density gradient ultracentrifugation suggests that LDL receptors may be down-regulated during the APR as suggested by previous hamster data.[2] The accumulation of cholesterol in LDL could result in decreased shuttling of cholesterol from HDL and seems a reduction in HDL clearance.

To date, the physiological functions of the primary acute phase proteins, apoSAA and the major pentraxins (C-reactive protein in man, amyloid P component in the mouse and female protein in the hamster), are largely unknown. Similarly, the role of observed changes in lipid metabolism during the APR are speculative. Although it seems logical to infer that changes associated with the APR are inherently protective, an opposing body of evidence suggests that increased expression of apoSAA may be pro-inflammatory, acting as a powerful chemoattractant for blood monocytes, polymorphonuclear leukocytes and T cells,[35,36] or even facilitating aortic fatty streak formation in mice.[6,37,38] The findings reported here, particularly the striking alterations in cholesterol and triglyceride metabolism in 18-month-old C57BL/6J mice, do not refute the pro-inflammatory aspect of atherogenesis, but tend to support our overall hypothesis that selective cellular affinity for apoSAA containing HDL permits more efficient delivery of cholesterol and phospholipids needed for tissue repair and should not be viewed as proinflammatory per se. Finally, our study demonstrates that even in normal lipoprotein metabolism, dietary history can influence specific patterns of cholesterol transport and disposition when the host is faced with a stressful situation such as the APR that challenges homeostasis. Thus, acute or even chronic episodes of inflammation and tissue necrosis, including myocardial infarction, could result in different outcomes depending on the dietary habit of the individual patient.

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