# Methionine Deficiency in Rats Fed Soy Protein Induces Hypercholesterolemia and Potentiates Lipoprotein Susceptibility to Peroxidation

Corinne Moundras, Christian Rémésy, Marie-Anne Levrat, and Christian Demigné

**A number of studies have provided evidence that plant proteins, especially soy protein, have a cholesterol-lowering effect as compared with casein. However, dietary supply of sulfur amino acids may be deficient when soy protein is present in the diet at a suboptimal level, which could affect lipid metabolism. Accordingly, in rats fed 13% protein diets, soy protein feeding resulted in a cholesterol-increasing effect (+18%), which could be counteracted by methionine supplementation (0.4%). In contrast, soy protein was effective in decreasing plasma triglyceride, as compared with levels in rats fed casein; this triglyceride-lowering effect was entirely abolished by methionine supplementation. The hypercholesterolemic effect of soy protein was characterized by a higher cholesterol content in low-density lipoprotein (LDL) and high-density lipoprotein 1 ($HDL_1$) fractions, together with a marked induction of hepatic hydroxymethyl glutaryl coenzyme A (HMG CoA) reductase activity and to a lesser extent cholesterol 7α-hydroxylase. There was practically no induction of these enzymes, as compared with levels in rats fed casein diets, when the soy protein diet was supplemented with methionine. Very–low-density lipoprotein (VLDL) plus LDL susceptibility to peroxidation was higher in rats fed soy protein than in casein-fed rats, which could reflect in part the lack of sulfur amino acid availability, since methionine supplementation led to a partial recovery of lipoprotein resistance to peroxidation. These findings suggest that amino acid imbalance could be atherogenic by increasing circulating cholesterol and leading to a higher lipoprotein susceptibility to peroxidation.**


THE BIOLOGIC VALUE of various dietary protein sources is to a large extent governed by the availability of individual indispensable amino acids. Data about amino acid requirements for maintenance of adequate protein nutritional status are essentially derived from growth and nitrogen-balance studies. Whereas casein is considered well equilibrated (despite a slight deficiency of methionine), the sulfur amino acid content of soy protein may be insufficient to meet the nutritional requirements for optimal growth.[1]

Besides their influence on protein synthesis and growth, sulfur-containing amino acids are major precursors for the synthesis of intracellular molecules such as reduced glutathione (GSH) and taurine. GSH is known as the most prevalent cellular thiol that plays an important role in cellular metabolism[2] and in protection of cells against reactive oxygen species.[3,4] Furthermore, optimum concentrations of thiols and disulfides are essential for rapid and complete folding and secretion of many proteins,[5] as well as for maintenance of the redox state of their -SH groups.[6] Other sulfur-containing compounds such as cysteamine and hypotaurine have also been proposed as physiologic antioxidants.[7] Studies performed on sulfur amino acid supplementation have reported a hypercholesterolemic effect of methionine[8] and cyst(e)ine.[9] It has been reported that methionine or cysteine excess may induce profound modifications of lipid and lipoprotein metabolism,[10-12] but little information is available as to the effects of moderate supplementations with sulfur amino acids. Casein, as compared with soy protein, has been shown to be hypercholesterolemic, particularly in rabbits[13] and even in rats.[14] However, this response of cholesterol metabolism to dietary protein must be interpreted with caution, since it could be influenced by a number of factors such as age and sex of the animals, protein level in the diet (often 20% casein supplemented with methionine), or presence of cholesterol in the diet. Furthermore, the metabolic influence of methionine, when it has a limiting role for growth, could be of interest.

In this study, we have thus investigated the consequences of methionine deficiency on lipid metabolism in rats fed a moderate level (13%) of soy protein or casein. The study takes into consideration changes in circulating lipids and liver metabolism and, in parallel, parameters of in vitro susceptibility of lipoproteins to peroxidation.

## MATERIALS AND METHODS

### *Animals and Diets*

Male Wistar rats (IFFA-CREDO, L'Arbresle, France) weighing approximately 150 to 160 g were adapted for 21 days to semipurified diets containing either casein (Louis François, Paris, France) or soy protein isolate (ICN Biomedicals, Aurora, OH) at a level of 13% of the diets. Amino acid composition of the diets is listed in Table 1. Dietary carbohydrate was supplied as wheat starch (75% of the unsupplemented diets). L-Methionine (Sigma Chemicals, St Louis, MO) was added at the expense of wheat starch at a level of 0.4%. All diets contained 5% peanut oil, 6% salt mixture, and 1% vitamin mixture (UAR, Villemoisson/Orge, France). Animals were housed two per cage (wire-bottomed to limit coprophagy) and maintained in temperature-controlled rooms (22°C) with a dark period from 8 PM to 8 AM. Animals were maintained and handled according to recommendations of the Institutional Ethics Committee.

### *Sampling Procedures*

Rats were sampled at the end of the dark period. They were anesthetized with sodium pentobarbital 40 mg/kg and maintained on a hot plate at 37°C. Blood from the abdominal aorta was drawn into heparinized syringes, and plasma was obtained by centrifugation at 10,000 × *g* for 2 minutes. After centrifugation, plasma was

*From the Laboratoire des Maladies Métaboliques, Institute National de la Recherche Agronomique de Clermont-Ferrand/Theix, St Genès Champanelle, France.*

*Submitted August 18, 1994; accepted January 18, 1995.*

*Address reprint requests to Corinne Moundras, PhD, Laboratoire des Maladies Métaboliques, IRNA de Clermont-Ferrand/Theix, 63122 St Genès Champanelle, France.*



*0026-0495/95/4409-0008$03.00/0*

**Table 1. Amino Acid Composition of the Dietary Proteins**

| Protein | Casein | Soy Protein |
|---|---|---|
| Aspartic acid + asparagine | 6.6 | 11.9 |
| Glutamic acid + glutamine | 21.0 | 18.0 |
| Serine | 5.5 | 5.2 |
| Threonine | 4.1 | 3.7 |
| Glycine | 1.7 | 4.2 |
| Alanine | 2.8 | 4.1 |
| Proline | 10.1 | 5.5 |
| Valine | 5.9 | 4.5 |
| Isoleucine | 4.7 | 4.9 |
| Leucine | 8.4 | 8.1 |
| Tyrosine | 5.0 | 4.0 |
| Phenylalanine | 6.5 | 5.4 |
| Tryptophan | 1.3 | 1.5 |
| Histidine | 2.6 | 2.6 |
| Lysine | 7.2 | 6.3 |
| Arginine | 3.5 | 7.5 |
| Lysine/arginine ratio | 2.06 | 0.84 |
| Cysteine | 0.4 | 1.3 |
| Methionine | 2.9 | 1.3 |
| Methionine/cysteine ratio | 7.25 | 1.0 |
| Total sulfur amino acids | 3.4 | 2.6 |

NOTE. Values are given as percentage of total amino acids. Composition of proteins was determined by a standardized method, based on hydrolysis by HCl, measurement of amino acid levels on a cation-exchange resin, and detection by a ninhydrin reaction. Sulfur amino acids are determined after oxidation, and tryptophan after hydrolysis by $Ba(OH)_2$.[15]

removed and kept at +4°C for lipid and lipoprotein analysis. An aliquot of plasma was removed and kept at −20°C for amino acid analysis. A portion of the liver was freezed-clamped and stored at −80°C before GSH and amino acid analysis. In parallel, 2 g liver was quickly homogenized for microsome purification, as previously described.[16] The microsomal preparation was stored as 300-μL aliquots at −80°C until measurement of enzyme activities. Protein content of the preparation was determined using the Pierce BCA Reagent kit (Interchim, Montluçon, France).

### *Lipoprotein Separation and Oxidation Experiments*

Equal volumes of plasma samples were pooled for lipoprotein separation. Plasma lipoproteins were separated by ultracentrifugation on a density gradient, as described by Sérougne et al.[9] The gradient was then fractionated (500-μL fractions) and kept at 4°C for lipid analysis.

The very–low-density lipoprotein (VLDL) plus low-density lipoprotein (LDL) fraction used for oxidation experiments was obtained by sequential ultracentrifugation, as previously described.[17] The VLDL plus LDL fraction was then washed by a further period of ultracentrifugation at the same density. Before oxidation experiments, purified lipoprotein fractions were dialyzed against 0.01 mol/L phosphate buffer (pH 7.4) containing 0.15 mol/L NaCl. The final protein concentration of lipoprotein fractions was adjusted to 40 μg/mL. Oxidation experiments were performed as described by Esterbauer et al[18] and were initiated by addition of freshly prepared $CuSO_4$ (final concentration, 5 μmol/L) at 37°C. The kinetics of lipoprotein oxidation were determined by continuously monitoring the change in 234-nm absorbance at 37°C on a Uvikon 930 spectrophotometer (Kontron, St Quentin-Yoelines, France). The initial absorbance was set to zero, and the increase in conjugated-diene absorbance was recorded.

### *Analytic Procedures*

Triglycerides (Biotrol, Paris, France), cholesterol, and phospholipids (Biomérieux, Charbonnières-les-bains, France) were determined in plasma and lipoproteins by enzymatic procedures. Protein concentrations were determined with the Pierce BCA protein reagent kit (Montluçon, Interchim, France). The frozen liver samples were crushed in 5 vol 0.6-mol/L perchloric acid and the supernatant was neutralized with $K_2CO_3$ for measurement of amino acid levels. Plasma samples were deproteinized with 5% sulfosalicylic acid (1:1 vol/vol) immediately before analysis. All extracts were adjusted to pH 2.2 with lithium citrate buffer 0.2N. Amino acid analysis were performed on a Chromakon 500 autoanalyzer (Kontron, Zürich, Switzerland) using lithium citrate buffers (Pharmacia Biochrom, Cambridge, UK) and postcolumn ninhydrin detection. For GSH measurements, a portion of liver was disrupted in 20 vol 0.125-mol/L sodium phosphate buffer containing 1 mmol/L EDTA and 5% trichloroacetic acid. GSH concentration was determined colorimetrically on the 10,000 × *g* supernatant, using the method reported by Sedlak and Lindsay.[19]

Activity of hydroxymethylglutaryl coenzyme A reductase (HMG CoA reductase, EC 1.1.1.34) was measured on microsomal fractions as described by Wilce and Kroone.[20] Labeled mevalonolactone was separated from unreacted HMG CoA by column chromatography using AG1-X8 resin (200-400 mesh, formate form; Biorad, Paris, France). Specific radioactivity of the enzyme was expressed in picomoles of [3-$^{14}$C]HMG CoA transformed in [$^{14}$C]mevalonolactone per minute per milligram microsomal protein, after correcting for recovery of [$^3$H]mevalonolactone from the column. Cholesterol 7α-hydroxylase (EC 1.14.13.17) activity was determined as described by Chiang,[21] using 20α-hydroxycholesterol as internal standard; 3-keto derivatives of the sterols were analyzed by reverse-phase high-performance liquid chromatography on a 25-cm C18 Spherisorb ODS2 column (Interchim) and detected at 240 nm. Microsomal concentration of free cholesterol was determined by high-performance liquid chromatography using these conditions (but omitting the first incubation step in the presence of NADPH) on a shorter column (12.5 cm).

Bile acid analysis was performed after extraction from feces by 10 vol ethanolic KOH, using the reaction catalyzed by 3α-hydroxysteroid dehydrogenase (EC 1.1.1.50; Sigma).

### *Statistics*

Values are presented as the mean ± SEM, and where appropriate, significance of differences between mean values was determined by ANOVA and multiple-range comparison by Fisher's protected least-significant difference procedures (Staview 512+; Brain Power, Calabasas, CA).

## RESULTS

### *Animal Characteristics*

The influence of dietary protein source and methionine supplementation on animal growth is listed in Table 2. A marked improvement of animal growth was obtained with 0.4% methionine addition to the soy protein diet, whereas growth of casein-fed rats was unaltered by methionine supplementation. Liver weight was significantly less in rats fed the 13% soy protein diet than in those fed the 13% casein diet (4.0% and 4.6% of body weight, respectively). The liver was heavier in rats fed methionine-supplemented diets, especially when the protein source was soy protein (+37%, *v* +14% with casein). This is in keeping with data indicating that methionine supplementation improves effi-

**Table 2. Effects of Dietary Protein Source and Met Supplementation on Rat Growth**

| Diet | 13% Casein | 13% Casein + 0.4% Met | 13% Soy Protein | 13% Soy Protein + 0.4% Met |
|---|---|---|---|---|
| Food intake (g/d) | 25.5 ± 0.3 | 22.9 ± 0.6† | 25.5 ± 0.7 | 25.5 ± 0.5 |
| Daily weight gain (g/d) | 6.49 ± 0.41 | 7.79 ± 0.19 | 4.96 ± 0.65* | 7.90 ± 0.52† |
| Body weight (g) | 307 ± 7 | 322 ± 3 | 258 ± 5* | 319 ± 7† |
| Liver weight (g) | 14.2 ± 0.5 | 16.3 ± 0.7† | 10.3 ± 0.4* | 14.1 ± 0.4† |

NOTE. Values are the mean ± SEM of 8 animals per group.
*$P < .05$, 13% soy protein *v* 13% casein.
†$P < .05$, 0.4% Met *v* no supplementation.

ciency of diet utilization[22] and induces a certain degree of hyperplasia in the liver.[23]

*Changes in Amino Acids*

Soy protein feeding was characterized by a marked decrease of plasma methionine concentration as compared with levels in rats fed casein, which was completely counteracted by supplementation of this diet with 0.4% methionine (Table 3). Dietary supplementation with methionine produced a decrease of threonine concentration, which was particularly marked with the soy protein diet. In this group, plasma concentration of serine was also significantly decreased. Cysteine was present in low concentrations (5 to 7 μmol/L) in plasma of rats adapted to a 13% casein or soy protein diet. Methionine supplementation elicited a significant increase in plasma cysteine only in rats adapted to the casein diet.

**Table 3. Effect of Dietary Protein Source on Plasma and Liver Amino Acid Concentrations**

| Diet | 13% Casein | 13% Casein + 0.4% Met | 13% Soy Protein | 13% Soy Protein + 0.4% Met |
|---|---|---|---|---|
| Artery (mmol/L) | | | | |
| Thr | 0.63 ± 0.03 | 0.57 ± 0.02 | 0.51 ± 0.02* | 0.22 ± 0.01† |
| Ser | 0.45 ± 0.02 | 0.24 ± 0.01† | 0.57 ± 0.01* | 0.29 ± 0.01† |
| Glu | 0.10 ± 0.01 | 0.12 ± 0.01 | 0.10 ± 0.01 | 0.12 ± 0.01 |
| Gln | 0.73 ± 0.02 | 0.67 ± 0.02† | 0.56 ± 0.02* | 0.56 ± 0.01 |
| Gly | 0.32 ± 0.01 | 0.18 ± 0.01† | 0.38 ± 0.02* | 0.36 ± 0.02 |
| Ala | 0.70 ± 0.03 | 0.65 ± 0.03 | 0.53 ± 0.03* | 0.70 ± 0.03† |
| Met | 0.08 ± 0.01 | 0.10 ± 0.01 | 0.03 ± 0.01* | 0.09 ± 0.01† |
| Cys | 7 ± 2‡ | 15 ± 3†‡ | 5 ± 2‡ | 10 ± 3‡ |
| Tau | 0.02 ± 0.01 | 0.12 ± 0.01† | 0.02 ± 0.01 | 0.11 ± 0.01† |
| Liver (μmol/g) | | | | |
| Thr | 0.73 ± 0.04 | 0.53 ± 0.02† | 1.24 ± 0.12* | 0.16 ± 0.01† |
| Ser | 1.36 ± 0.06 | 0.42 ± 0.03† | 2.75 ± 0.08* | 0.85 ± 0.01† |
| Glu | 2.14 ± 0.10 | 1.78 ± 0.05† | 1.95 ± 0.07 | 2.56 ± 0.10† |
| Gln | 5.38 ± 0.43 | 6.00 ± 0.15 | 3.79 ± 0.23* | 5.14 ± 0.25† |
| Gly | 3.62 ± 0.13 | 2.31 ± 0.08† | 2.55 ± 0.12* | 3.21 ± 0.12† |
| Ala | 3.70 ± 0.28 | 3.28 ± 0.09 | 2.65 ± 0.21* | 3.66 ± 0.14† |
| Met | 0.05 ± 0.01 | 0.11 ± 0.01† | < 0.01 | 0.06 ± 0.01 |
| Tau | 1.25 ± 0.09 | 7.91 ± 0.40† | 0.59 ± 0.07* | 4.30 ± 0.36† |
| GSH | 2.15 ± 0.16 | 3.64 ± 0.27† | 1.20 ± 0.08* | 3.87 ± 0.25† |

NOTE. Values are the mean ± SEM of 8 animals per group.
*$P < .05$, 13% soy protein *v* 13% casein.
†$P < .05$, 0.4% Met *v* no supplementation.
‡$\times 10^{-3}$.

Liver concentration of methionine, undetectable in soy protein–fed rats, was nearly the same as in controls when this amino acid was added to the diet. Liver concentrations of threonine were dramatically decreased by methionine supplementation (78% less than control levels in soy protein–fed rats), even in rats fed casein (−27%). Liver concentrations of serine were consistently decreased by methionine supplementation, in keeping with data showing an induction of serine/threonine dehydratase activity by methionine.[22,24]

Methionine deficiency induced by soy protein feeding was accompanied by a marked decrease of both GSH and taurine concentrations in the liver, as compared with levels in casein-fed rats. Supplementation with methionine markedly improved GSH and taurine status, whatever the dietary protein.

*Lipid Metabolism*

Soy protein–fed rats exhibited a significant elevation of plasma cholesterol and a marked decrease of triglycerides as compared with casein-fed rats (Fig 1). Plasma lipid concentrations were unchanged by methionine supplementation with casein, whereas a reduction of cholesterol and an increase of triglyceride concentrations were observed in soy protein–fed rats.

Analysis of lipoprotein profiles isolated by gradient ultracentrifugation (Fig 2) shows that the elevation of plasma triglycerides induced by methionine supplementation of soy protein occurred principally in chylomicrons and VLDL fractions (triglyceride-rich lipoprotein [TGRLP]). Addition of methionine to the casein diet had little influence on triglyceride content of these lipoprotein fractions. Rats fed soy protein exhibited particularly low concentrations of cholesterol, triglycerides, and protein in TGRLP. In contrast, the hypercholesterolemic effect of soy protein as compared with casein was characterized by cholesterol enrichment in LDL and (high-density lipoprotein 1 ([$HDL_1$]

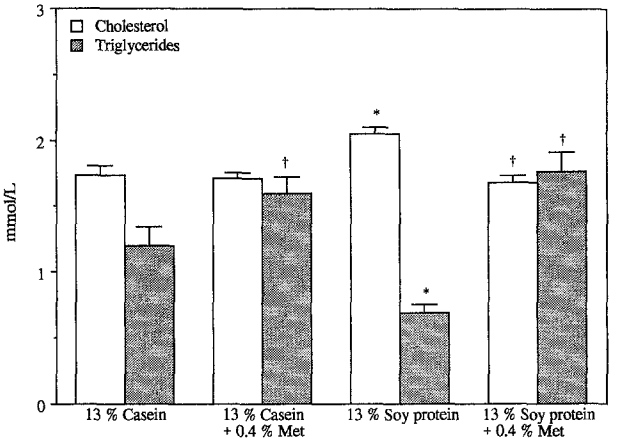


**Fig 1. Effects of dietary protein source and Met supplementation on plasma concentration of cholesterol and triglycerides. *$P < .05$, 13% soy protein *v* 13% casein; †$P < .05$, 0.4% Met *v* no supplementation.**

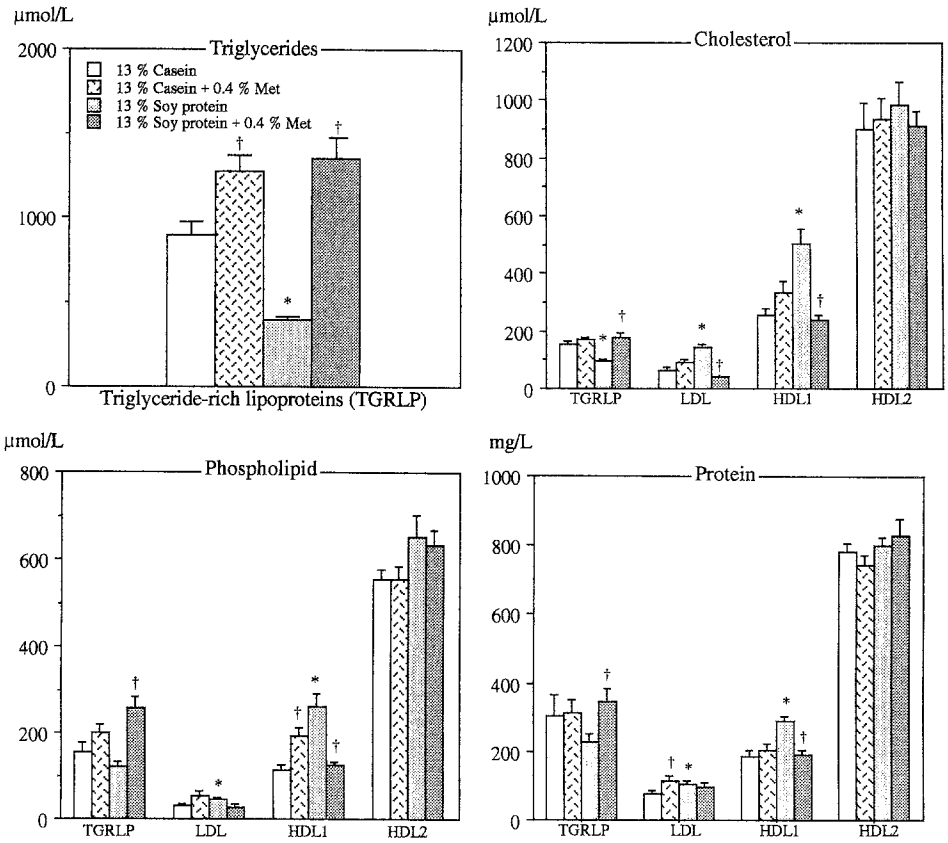


**Fig 2. Effects of dietary protein source and Met supplementation on triglycerides, cholesterol, phospholipids, and protein repartition in different plasma lipoprotein classes. TGRLPs (essentially VLDL) corresponded to fractions with d < 1.006. LDL, $HDL_1$, and $HDL_2$ corresponded to fractions with a density range from 1.006 to 1.040, 1.040 to 1.080, and 1.080 to 1.16, respectively. *P < .05, 13% soy protein v 13% casein; †P < .05, 0.4% Met v no supplementation.**

apolipoprotein E–rich) fractions. Phospholipid and protein contents of $HDL_1$ were also higher in this group, which reflects an accumulation of these particles in the plasma rather than compositional changes. Methionine supplementation of the soy protein diet caused a marked reduction of LDL and $HDL_1$ cholesterol and phospholipid, whereas no significant effect on lipoprotein composition was observed with casein diets supplemented with methionine. Lipid and protein contents of $HDL_2$ were not significantly altered by any dietary treatment. Elevated cholesterol concentrations observed in plasma of rats fed soy protein were accompanied by induction of HMG CoA reductase activity; this activity was not significantly different from the control value when methionine was added to this diet (Fig 3). Activity of

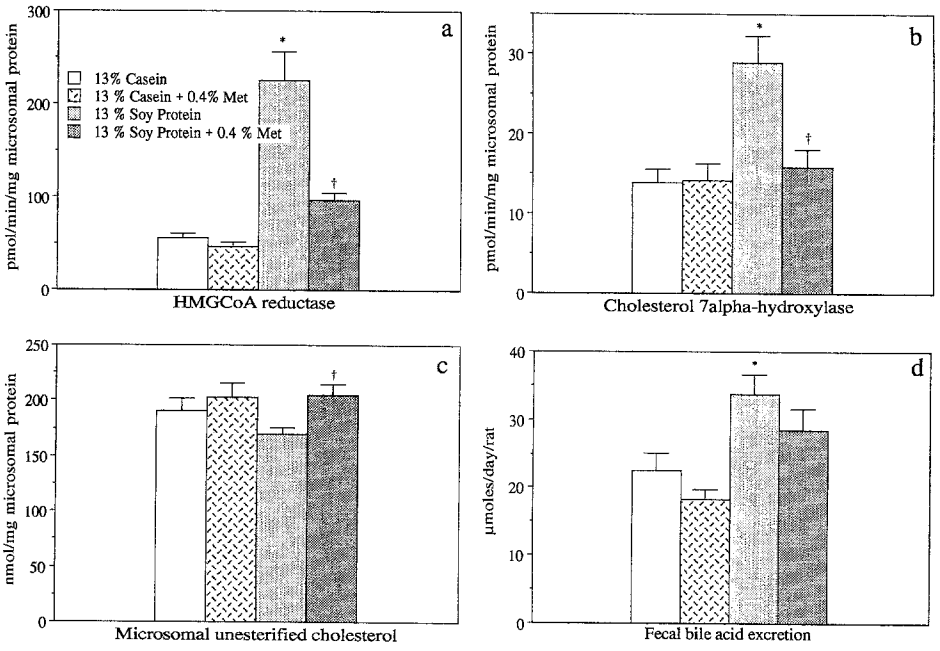


**Fig 3. Effects of dietary protein source and Met supplementation on activity of HMG CoA reductase (a), cholesterol 7α-hydroxylase (b), concentration of unesterified cholesterol in liver microsomes (c) and fecal excretion of bile acids (d). *P < .05, 13% soy protein v 13% casein; †P < .05, 0.4% Met v no supplementation.**

microsomal cholesterol 7α-hydroxylase was also induced in rats fed the soy protein diet (~twofold) as compared with rats fed the casein diet, and methionine supplementation resulted in activities that were not significantly different from basal conditions. Microsomal free cholesterol was not significantly decreased by adaptation to the soy protein diet, but methionine supplementation of this diet led to a significant increase of microsomal cholesterol. In parallel with the higher activity of HMG CoA reductase, soy protein–fed rats exhibited an enhanced excretion of bile acids (1.5-fold > controls). Fecal excretion of bile acids was not significantly modified by methionine supplementation (Fig 3).

*Susceptibility of Lipoproteins to Peroxidation*

The results obtained in this study indicate that sulfur amino acid deficiency or amino acid imbalance may induce profound changes in lipid metabolism. Thus, the question arises as to whether concomitant depletion of liver GSH influences the antioxidative defense of the organism, which could be reflected by an enhanced susceptibility of LDL to peroxidation. Since rats have a low level of LDL[25] and because both VLDL and LDL can undergo lipid peroxidation,[26] we have investigated in vitro oxidation of a lipoprotein fraction containing both VLDL and LDL.

In vitro measurement of lipoprotein copper-induced oxidation shows that the VLDL plus LDL fraction isolated from controls was more resistant to lipid peroxidation than that of soy protein–fed rats, as reflected by duration of the lag phase (Fig 4). The maximal rate of diene production, illustrated by the slope of the absorbance curve during the propagation phase, was markedly higher in soy protein–fed rats than in controls. Compared with the unsupplemented soy protein diet, addition of methionine prolonged the lag phase (which nevertheless remained shorter than in controls) without a noticeable effect on the propagation rate.

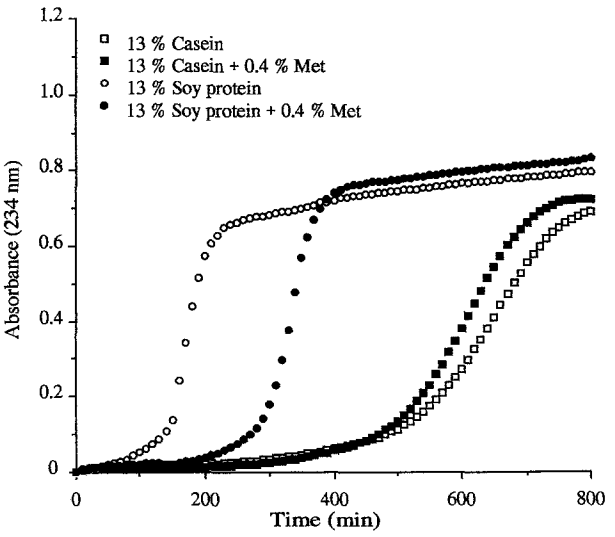


**Fig 4. Rate of formation of conjugated dienes in VLDL + LDL fractions (estimated using changes in absorbance at 234 nm) from rats fed casein or soy protein diets, with or without methionine supplementation.**

The total amount of dienes produced was also responsive to dietary conditions and was higher with soy protein, with practically no effect of methionine supplementation on this parameter. Supplementation of the casein diet with methionine did not noticeably alter the oxidizability of plasma lipoproteins.

## DISCUSSION

A number of studies have raised the hypothesis that plant proteins, especially soy protein isolate, are hypocholesterolemic relative to casein or other animal proteins (for review, see Forsythe et al[27]). This effect, more pronounced with high-cholesterol diets, has been evidenced in rabbits[28] and rats[14,29] when the dietary protein level was close to 20%. However, data from studies involving human subjects are less conclusive, and plant proteins appeared to be devoid of effect on plasma cholesterol levels in normocholesterolemic subjects.[30,31] The mechanisms by which dietary protein influences plasma cholesterol concentrations are not completely understood, since a number of parameters could be affected by the protein source. Casein feeding has often been shown to decrease fecal excretion of neutral and acidic steroids,[32] which could in turn influence the metabolism of lipoproteins. Furthermore, animal proteins have high lysine to arginine ratios, which may affect cholesterol metabolism directly (via nitric oxide, for example[33]) or by altering the hormonal status.[34,35] Accordingly, soy protein feeding results in higher plasma thyroxine levels,[36] which might explain the higher hepatic HMG CoA reductase activity,[37] increase of bile acid excretion, and lower triglyceride content of TGRLP. However, no decrease of plasma cholesterol has been observed in the present study, and there was even a slight hypercholesterolemic effect.

In this study, the main effect seems to arise from amino acid imbalance and its consequence on growth and sulfur amino acid status. Other studies have also shown that feeding growing rats low-protein diets may profoundly affect cholesterol metabolism, and to a larger extent when the protein quality is inadequate.[38,39] Protein malnutrition has various inhibitory effects on protein synthesis, as well as on other anabolic processes such as lipogenesis. A surprising result from this study was that a higher plasma cholesterol level was associated with low concentrations of triglycerides. This study clearly shows that when the dietary protein level is slightly less than required for optimal growth (here 13% of the diet), soy protein could be hypercholesterolemic as compared with casein. When the soy protein diet was supplemented with methionine, plasma cholesterol and triglycerides were not significantly different from control values. The elevated plasma cholesterol observed in rats fed soy protein was concomitant with a high activity of HMG CoA reductase. However, when this diet was supplemented with methionine, HMG CoA reductase activity was markedly decreased, but still higher than with the control diet. Curiously, no significant effect of methionine supplementation was observed with the casein diet, despite the slight deficiency of this protein in sulfur amino acid. Some studies have reported that liver GSH concentration may stimulate HMG CoA reductase[40] or cholesterol

7α-hydroxylase.[41] However, in the present study, GSH concentrations appear inversely related to these enzyme activities. It is noteworthy that there is an induction of HMG CoA reductase in rats fed soy protein despite elevated LDL and $HDL_1$ cholesterol, which raises the question as to whether these lipoproteins are effectively taken up (a process essentially dependent on the activity of apolipoprotein B/E receptors on plasma membrane).

Methionine supplementation markedly increased hepatic concentration of taurine, which could influence the proportion of tauro-conjugated bile acids.[42] Furthermore, these conjugated bile acids are more soluble and possibly more available for reabsorption than glycine-conjugates.[43] A preferential conjugation of bile acids with taurine, resulting in a greater proportion of bile acid returning to the liver, could downregulate activity of both HMG CoA reductase and cholesterol 7α-hydroxylase.[44] However, fecal loss of bile acids, moderately increased with soy protein (1.5-fold that of controls), was not modified by methionine addition, which suggests that the effect of methionine on plasma cholesterol is not associated with modifications of the amount of bile acid reabsorbed.

One important feature of this study is that soy protein–fed rats exhibited an increased in vitro susceptibility of plasma lipoproteins to peroxidation. It is now well established that LDL oxidation is an important event in the development of atherogenesis,[45] since oxidized LDL are efficiently absorbed by macrophages. Increased lipid peroxidation may originate from a decreased availability of sulfur-containing molecules, which are indispensable for maintenance of the antioxidative defense of the cells[46] and regeneration of antioxidant molecules such as ascorbic acid[4] and α-tocopherol. Another study, performed on growing rats, has shown that protein deficiency affects activities of scavenger enzymes and thiobarbituric acid–reactive substances accumulation.[47] Addition of methionine to the soy protein diet markedly improved resistance of lipoproteins to peroxidation; however, restoration of the sulfur amino acid status did not result in complete recovery of VLDL plus LDL resistance against peroxidation. It is noteworthy that methionine supplementation was accompanied by a decrease of plasma and liver threonine and serine concentrations. Thus, the question arises as to whether this amino acid imbalance could affect the effectiveness of systems involved in resistance against oxidative damage.

In conclusion, besides dietary fat and cholesterol, protein malnutrition (which is often associated with impaired protein synthesis due to amino acid imbalance) may also result in hypercholesterolemia. Curiously, despite high plasma cholesterol levels, activity of HMG CoA reductase was markedly induced; this suggests an impaired uptake of LDL and $HDL_1$ cholesterol, which should repress cholesterol endogenous synthesis, especially HMG CoA reductase activity. It is noteworthy that high plasma cholesterol and an elevated risk of cardiovascular disease have often been observed in humans exhibiting abnormal behavior related to weight control, such as prolonged periods of calorie-restricted dieting[48] or anorexia nervosa.[49] Furthermore, amino acid imbalance (particularly sulfur amino acids) potentiates the susceptibility of lipoprotein to peroxidation and thus tends to aggravate the consequences of elevated plasma lipids. However, it must be kept in mind that plant proteins are most frequently provided by plant foods generally rich in antioxidant compounds, which should counteract some untoward effects of unbalanced plant proteins. The fact that soy protein is particularly rich in arginine could also be of consequence, because of a higher availability of the nitrogen oxide precursor.[33]

## REFERENCES

1. Hosokawa Y, Niizeki S, Tojo H, et al: Hepatic cysteine dioxygenase activity and sulfur amino acid metabolism in rats: Possible indicators in the evaluation of protein quality. J Nutr 118:456-461, 1988

2. Meister A: Glutathione metabolism and its selective modification. J Biol Chem 263:17205-17208, 1988

3. Sies H, Wahlländer A, Waydhas C, et al: Functions of intracellular glutathione in hepatic hydroperoxide and drug metabolism and the role of extracellular glutathione. Adv Enzyme Regul 18:303-320, 1980

4. Meister A: Glutathione-ascorbic acid antioxidant system in animals. J Biol Chem 269:9397-9400, 1994

5. Hwang C, Sinskey AJ, Lodish HF: Oxidized redox state of glutathione in the endoplasmic reticulum. Science 257:1496-1502, 1992

6. Guilbert HF: Molecular and cellular aspects of thiol-disulfide exchange. Adv Enzymol 63:69-160, 1990

7. Aruoma OI, Halliwell B, Hoey BM, et al: The antioxidant action of taurine, hypotaurine and their metabolic precursors. Biochem J 256:251-255, 1988

8. Sugiyama K, Mizuno M, Muramatsu K: Effect of individual amino acids on plasma cholesterol level in rats fed a high cholesterol diet. J Nutr Sci Vitaminol 32:623-633, 1986

9. Sérougne C, Férézou J, Rukaj A: A new relationship between cholesterolemia and cholesterol synthesis determined in rats fed an excess of cystine. Biochim Biophys Acta 921:522-530, 1987

10. Fau D, Chanez M, Bois-Joyeux B, et al: Metabolic implications of methionine excess. Effects of methionine excess, dietary protein, fasting, cortisol and insulin on certain hepatic enzyme activities involved in transsulfuration, glycolysis, gluconeogenesis and lipogenesis in adapted adult rats. Ann Biol Anim Biochim Biophys 19:343-353, 1979

11. Sérougne C, Férézou J, Rukaj A: Effects of excess dietary L-cystine on the rat plasma lipoproteins. Ann Nutr Metab 28:311-320, 1984

12. Aoyama Y, Ishikawa T, Amano N, et al: Lipid accumulation in the liver of rats fed a soy protein isolate diet with excess cystine and its prevention by methionine or choline. Biosci Biotech Biochem 56:656-659, 1992

13. Terpstra AHM, Sanchez-Muniz FJ: Time course of the development of hypercholesterolemia in rabbits fed semipurified diets containing casein or soybean protein. Atherosclerosis 39:217-227, 1981

14. Sugano M, Ishiwaki N, Nakashima K: Dietary protein dependent modification of serum cholesterol level in rats. Annu Rev Metab 28:192-199, 1984

15. Prugnaud J, Pion R: Dosage des acides aminés dans les aliments. Paris, France, Beckman, 1976

16. Moundras C, Behr SR, Demigné C, et al: Fermentable

polysaccharides that enhance fecal bile acid excretion lower plasma cholesterol and apolipoprotein E–rich HDL in rats. J Nutr 124:2173-2188, 1994

17. Rayssiguier Y, Gueux E, Bussière L, et al: Copper deficiency increases the susceptibility of lipoproteins and tissues to peroxidation in rats. J Nutr 123:1343-1348, 1993

18. Esterbauer H, Striegl G, Puhl H, et al: Continuous monitoring of in vitro oxidation of human low density lipoprotein. Free Rad Res Commun 6:67-75, 1989

19. Sedlak J, Lindsay RH: Estimation of total protein-bound, and non-protein sulfhydryl groups in tissues with Ellman's reagent. Anal Biochem 25:192-205, 1968

20. Wilce P, Kroone PA: Assay of 3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase, in Converse CA, Skinner ER (eds): Lipoprotein Analysis. Oxford, UK, Oxford University Press, 1992, pp 203-214

21. Chiang JYL: Reversed-phase high-performance liquid chromatography assay of cholesterol 7α hydroxylase. Methods Enzymol 206:483-491, 1991

22. Girard-Globa A, Robin P, Forestier M: Long-term adaptation of weanling rats to high dietary levels of methionine and serine. J Nutr 102:209-218, 1972

23. Sanchez A, Swendseid M: Amino acid levels and enzyme activities in tissues of rats force-fed diets differing in methionine content. J Nutr 99:145-152, 1969

24. Mauron J, Mottu F, Spohr G: Reciprocal induction and repression of serine dehydratase and phosphoglycerate dehydrogenase by proteins and dietary-essential amino acids in rat liver. Eur J Biochem 32:331-342, 1973

25. Chapman MJ: Animal lipoproteins: Chemistry, structure and comparative aspects. J Lipid Res 21:789-853, 1980

26. Scaccini C, Nardini M, D'Aquino M, et al: Effect of dietary oils on lipid peroxydation and on antioxydant parameters of rat plasma and lipoprotein fractions. J Lipid Res 33:627-633, 1992

27. Forsythe WA, Green MS, Anderson JJB: Dietary protein effects on cholesterol and lipoprotein concentrations: A review. J Am Coll Nutr 5:553-549, 1986

28. Lovati MR, West CE, Sirtori CR, et al: Dietary animal proteins and cholesterol metabolism in rabbits. Br J Nutr 64:473-485, 1990

29. Muramatsu K, Sugiyama K: Relationship between amino acid composition of dietary protein and plasma cholesterol level in rats, in Sugano M, Beynen AC (eds): Dietary Proteins, Cholesterol Metabolism and Atherosclerosis, vol 16. Basel, Switzerland, Karger, 1990, pp 97-109

30. Van Raaij JMA, Katan MB, Hautvast JGAJ, et al: Effects of casein versus soy protein diets on serum cholesterol and lipoproteins in young healthy volunteers. Am J Clin Nutr 34:1261-1271, 1981

31. Meinertz H, Faergeman O, Nilausen K, et al: Effects of soy protein and casein in low cholesterol diets on plasma lipoproteins in normolipidemic subjects. Atherosclerosis 72:63-70, 1988

32. Nagata Y, Tanaka K, Sugano M: Further studies on hypocholesterolemic effect of soy protein in rats. Br J Nutr 45:233-241, 1981

33. Creager MA, Gallagher SJ, Girerd XJ, et al: L-Arginine improves endothelium-dependent vasodilatation in hypercholesterolemic humans. J Clin Invest 90:1248-1253, 1992

34. Kritchevsky D, Tepper SA, Czarnecki S, et al: Atherogenicity of animal and vegetable protein: Influence of the lysine to arginine ratio. Atherosclerosis 47:429-431, 1982

35. Sugano M, Ishiwaki N, Nagata Y, et al: Effects of arginine and lysine addition to casein and soya-bean protein on serum lipids, apolipoproteins, insulin and glucagon in rats. Br J Nutr 48:211-221, 1982

36. Barth CA, Pfeuffer M: Dietary protein and atherogenesis. Klin Wochenschr 66:135-143, 1988

37. Guder W, Nolte I, Wieland O: The influence of thyroid hormones on β-hydroxy-β-methylglutaryl coenzyme A reductase of rat liver. Eur J Biochem 4:273-278, 1968

38. Neves LB, Clifford CK, Kohler GO, et al: Effects of dietary protein from a variety of sources on plasma lipids and lipoproteins of rats. J Nutr 110:732-742, 1980

39. Lefevre M, Schneeman BO: Relationship between plasma cholesterol and protein quality in weanling rats. Nutr Rep Int 28:1369-1374, 1983

40. Kim S, Chao PY, Allen KGD: Inhibition of elevated hepatic glutathione abolishes copper deficiency cholesterolemia. FASEB J 6:2467-2471, 1992

41. Hassan AS, Bunick D, St Denis SH, et al: Glutathione and bile acid synthesis. II. Effect of hepatic glutathione content on the activity and mRNA levels of cholesterol 7α-hydroxylase in the rat. Biochem Pharmacol 46:555-556, 1993

42. Ide T, Horii M, Yamamoto T, et al: Contrasting effects of water-soluble and water-insoluble dietary fibers on bile acid conjugation and taurine metabolism in the rat. Lipids 25:335-340, 1990

43. Zhang R, Barnes S, Diasio RB: Differential intestinal deconjugation of taurine and glycine bile acid *N*-acyl amidates in rats. Am J Physiol 262:G351-G358, 1992

44. Björkhem I, Andersson U, Sudjama-Sugiaman E, et al: Studies on the link between HMG-CoA reductase and cholesterol 7α-hydroxylase in lymph fistula rats: Evidence for both transcriptional and post-transcriptional mechanism for down-regulation of the two enzymes by bile acids. J Lipid Res 34:1497-1503, 1993

45. Steinberg DS, Parthasarathy S, Carew TE, et al: Beyond cholesterol: Modifications of low-density lipoprotein that increase its atherogenicity. N Engl J Med 320:915-924, 1989

46. Gotoh N, Graham A, Niki E, et al: Inhibition of glutathione synthesis increases the toxicity of oxidized low-density lipoprotein to human monocytes and macrophages. Biochem J 296:151-154, 1993

47. Sambuichi Rumley EJ, Lai A, Hirakawa T, et al: Protein deficiency potentiates lipid peroxidation in growing rats exposed to hyperoxia. Nutr Res 12:1101-1112, 1992

48. Sanchez-Muniz FJ, Marcos A, Varela P: Serum lipids and apolipoprotein B values, blood pressure and pulse rate in anorexia nervosa. Eur J Clin Nutr 45:33-36, 1991

49. Mordasini R, Klose G, Greten H: Secondary type II hyperlipoproteinemia in patients with anorexia nervosa. Metabolism 27:71-76, 1978