Effects of dietary protein source on plasma lipids, HMG CoA reductase activity, and hepatic glutathione levels in gerbils

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It has recently been postulated that soy protein-induced hypocholesterolemia is induced by increased production of reduced glutathione (GSH). The objective of the current study was to determine the influences of dietary proteins and amino acids on plasma lipid concentrations, hepatic GSH, and HMG CoA reductase activity. Twenty-eight adult male, inbred gerbils were fed diets similar in all respects except that dietary protein source was from either soy protein isolate (SOY), casein (CAS), L-amino acid patterned after SOY (SAA), or L-amino acid patterned after CAS (CAA). Results indicated that gerbils fed SOY had lower plasma total cholesterol concentrations than gerbils fed CAS or CAA. Low density lipoprotein-cholesterol was also lowest in SOY-fed animals compared with all other groups. High density lipoprotein (HDL)-cholesterol concentrations were highest in CAA-fed animals. Plasma triglycerides were unaffected by dietary treatment. Hepatic GSH concentrations were lowest in SOY-fed gerbils, but were not different between SAA- and CAA-fed groups. HMG CoA reductase activity was highest in gerbils fed SOY compared with CAS; however, when amino acids were fed the opposite occurred–reductase activity was higher in gerbils fed CAA compared with animals fed SAA. Correlation analysis showed no association between GSH and HMG CoA reductase activity. Positive correlations were present between GSH and total and HDL-cholesterol concentrations. These results indicate that lipid metabolism is influenced by dietary protein source, but these changes are not likely to be mediated by a GHS-mediated modification of cholesterol biosynthesis.

Keywords: soy protein; casein; cholesterol; GHS; HMG CoA reductase

Introduction

The hypocholesterolemic effect of soy protein compared with animal protein has been demonstrated in humans and a variety of animal models.[1-3] In rabbits, feeding L-amino acids patterned after casein produces hypercholesterolemia similar to that produced by feeding intact casein. Feeding L-amino acids patterned after soy protein isolate produces hypocholesterolemia; however, values were not as low as those produced by feeding the intact protein.[4,5] This indicates that the cholesterolemic changes induced by dietary proteins are partially due to their amino acid composition.

One of the main differences in amino acid composition between casein and soy protein is the proportionality of sulfur-containing amino acids. The ratio of cysteine to methionine in casein is 0.34:2.9, while in soy protein isolate, it is 1.3:1.3. It has been postulated that the higher cysteine content of soy protein may contribute to the resultant hypocholesterolemia by enhancing glutathione production.[6] There are indications that reduced glutathione (GSH) is a cofactor in cholesterol metabolism.[7,8] In addition, we have reported that adding cysteine (0.33%) to soy protein isolate lowers serum cholesterol levels, while adding methionine (0.33%) in-

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creases levels in rats.[9] The current investigation was designed to examine the effects of dietary proteins and amino acids on plasma lipid concentrations, hepatic GSH concentration, and HMG CoA reductase activity; and to examine if there are associations between hepatic GSH content and activity of HMG CoA reductase.

Methods and materials

Experimental design

Gerbils were chosen as the animal model for this study due to reported lipemic responses to dietary fat and cholesterol.[10,11] Male, inbred gerbils (*Meriones unguiculates*, strain designation Mon/Tum; Tumblebrook Farms, Brent Lake, NY USA) were fed diets similar in all respects with the exception of dietary protein source coming from soy protein isolate (SOY), casein (CAS), L-amino acid patterned after SOY (SAA), or L-amino acids patterned after CAS (CAA).

General procedures

Gerbils weighing approximately 60 g each were individually housed in an environmentally controlled room (23° C) with an alternating 12-hr light/12-hr dark cycle. Upon arrival, all animals were fed a pulverized commercial pelleted diet (Purina Rat Chow, Ralston Purina, St. Louis, MO USA) for 1 week. Animals were then randomly assigned to dietary treatment groups (eight animals/group). Composition of basal diet is provided in *Table 1*. Amino acid composition of soy protein isolate and casein is provided in *Table 2*. Feed and water were provided ad libitum, and feed intake and weight gain were monitored throughout the project.

After a 28-day experimental period, animals were sacrificed via decapitation starting 2 hours after initiation of the dark cycle and completed 4 hours after initiation to allow for peak HMG CoA reductase activity.[12] Blood was collected into heparinized tubes and plasma was separated. High density lipoprotein (HDL) and low density lipoprotein + very low density lipoprotein (LDL + VLDL) (LDL) lipoprotein fractions were separated, and plasma and fractions were frozen at −20° C for subsequent analysis. Portions of livers were prepared for GSH analysis, while microsomes were isolated from the remainder of the livers and stored in liquid nitrogen until microsomal HMG CoA reductase activity was assayed.

Blood lipid analysis

HDL and LDL lipoprotein fractions were separated by heparin-bound agarose columns (Liposep, Isolabs, Inc., Akron, OH USA). Total, HDL, and LDL cholesterol were measured enzymatically by a modification of the method described by Allain et al.[13] using a commercially available reagent (Sigma Diagnostics, St. Louis, MO USA). Total triglyceride concentrations were measured enzymatically, based on the method described by McGowen et al.[14], also using a commercially available reagent (Sigma Diagnostics).

Hepatic GSH concentration

Livers were removed from animals and minced. From this, 0.2 g samples were taken and homogenized in 800 μL of ice-cold 1.15% KCl. Aliquots of the homogenate were deproteinized with 10% sulfosalicylic acid, and reduced GSH was analyzed according to methods described by Asaoka and Takahashi.[15] Briefly, conjugated GSH and 0-dinitrobenzene, upon being acted on by glutathione S-aryltransferase, release nitrite ions that diazo-couple with sulfanilamide and N-(1-

Table 1 Basal diet composition

Component	% by weight
Protein[a]	18.00
Dextrose	53.90
Lard	16.00
Safflower oil	1.00
Cellulose	5.00
Vitamin mix[b]	1.00
Mineral mix[c]	5.00
Cholesterol	0.10

[a]Soy protein isolate, PP610 (91.5% protein based on manufacturer's analysis, calculated as gNx6.25 (30); Protein Technologies Int., St. Louis, MO USA); Casein, vitamin-free test (91% protein based on manufacturer's analysis, calculated as gNx6.38 (30); Teklad, Madison, WI USA); L-amino acid mixture patterned after soy protein isolate PP610 based on manufacturer's analysis *(Table 2)*; L-amino acid mixture patterned after vitamin-free test casein based on manufacturer's analysis *(Table 2)*.

[b]Teklad vitamin mix. Vitamin mix supplied the following vitamins in mg/kg of diet: vitamin B-12 (0.1% trituration in mannitol), 30; calcium pantothenate, 66; dihydrogen citrate, 3500; folic acid, 2; inositol, 110; menadione (vitamin K), 50; niacin, 99; pyridoxine HCl, 22; riboflavin, 22; thiamin HCl, 22; dry retinyl palmitate (500,000 IU/g), 40; dry ergocalciferol (500,000 IU/g), 4.4; dry tocopherol acetate (500 IU/g), 2423.

[c]Mineral mix, Hegsted IV (Teklad). Mineral mix supplied the following minerals in g/kg of diet: calcium carbonate, 15.0; potassium phosphate, diabasic, 16.1; calcium phosphate, diabasic, 3.7; magnesium sulfate, 5.1; sodium chloride, 8.4; ferric citrate (USP 16.7% Fe), 1.4; potassium iodide, 0.034; manganese sulfate, 0.19; zinc chloride, 0.012; cupric sulfate, 0.015; nonnutritive fiber, 0.061.

Table 2 Composition of amino acid mixtures[a]

	SAA[b]	CAA[c]
L-Alanine	4.3	2.8
L-Arginine HCl	7.6	3.4
L-Aspartate	11.6	6.3
L-Cysteine[d]	1.3	0.3
L-Methionine	1.3	2.9
Total Sulfur Amino Acids	2.6	3.2
L-Glutamate	19.1	20.5
Glycine	4.2	1.6
L-Histidine HCl H_2O	2.6	2.5
L-Isoleucine	4.9	4.7
L-Leucine	8.2	8.2
L-Lysine HCl	6.3	7.2
L-Phenylalanine	5.2	9.5
L-Serine	5.2	5.0
L-Threonine	3.8	3.8
L-Tryptophan	1.3	1.6
L-Tyrosine	3.8	4.7
L-Valine	5.0	6.0

[a]g/100 g protein.

[b]Based on amino acid composition of soy protein isolate, PP610 (Protein Technologies, Int., St. Louis, MO USA).

[c]Based on amino acid composition of vitamin-free test casein (Teklad, Madison, WI USA).

[d]Half cystine.

naphthyl) ethylene diamine dihydrochloride. The resulting diazo product was then measured at 540 nm.

HMG CoA reductase activity

Microsomes were prepared from the remaining liver as described by Shapiro et al.[16] HMG CoA reductase activity was assessed using ^{14}C HMG CoA as a substrate and ^{3}H mevalonate (New England Nuclear, Boston, MA USA) as an internal standard.[17] The reaction was carried out at 37° C for 20 minutes, stopped by the addition of 1O N HCl, and allowed to lactonize for 30 min at 37° C. ^{14}C-mevalonate produced was separated by thin-layer chromatography (Silica Gel G, Alltech, Deerfield, IL USA), counted, and quantified. Microsomal protein was quantified using the bicinchoninic acid (BCA) copper reagent (Pierce, Rockford, IL USA) method for use in the presence of sulfhydryl reagents.[18]

Statistical analysis

Data were analyzed using the Statistical Analysis System (SAS, Inc. Cary, NC USA) by a one-way analysis of variance. Differences between treatments were determined using contrast analysis, and an alpha level of 0.05 was used to indicate statistical significance. Pearson's correlation coefficients between hepatic GSH and HMG CoA reductase activity, plasma lipids, feed intake, and weight gain were also computed.[19]

Results

Feed intake and weight gain

Mean weight gain and feed intake are summarized in *Table 3*. Weight gain was unaffected by dietary treatment; however, feed intake was lower in CAA-fed animals compared with those fed CAS ($P < 0.05$).

Plasma lipid concentrations

Mean plasma lipid concentrations are summarized in *Table 4*. Plasma total cholesterol concentrations were lower in gerbils fed SOY compared with those fed CAS or CAA ($P < 0.05$), or SAA ($P = 0.06$). LDL-cholesterol concentrations were lower in the SOY-fed group compared with all other groups ($P < 0.05$). When the L-amino acid mixtures simulating SOY and CAS were fed, no differences in plasma lipid concentrations were observed, with the exception of CAA-fed gerbils having highest HDL-cholesterol values ($P < 0.05$). Total triglycerides were unaffected.

Hepatic GSH and HMG CoA reductase activity

Mean hepatic GSH concentrations and HMG CoA reductase activities are summarized in *Table 5*. Concentrations of hepatic GSH were lowest in animals fed SOY compared with all other groups ($P < 0.05$). When L-amino acid mixtures were fed, there were no differences in hepatic GSH concentrations.

With regard to HMG CoA reductase, animals fed SOY exhibited highest activities compared with those fed CAS and SAA ($P < 0.01$). When L-amino acid mixtures were fed, reductase activity was higher in the CAA group compared to the SAA group ($P < 0.05$).

Correlations

Significant correlations were found between GSH and plasma total (R = 0.44, $P = 0.03$) and HDL-cholesterol concentrations (R = 0.42, $P = 0.03$). There were no statistically significant correlations between GSH and HMG CoA reductase activity, feed intake, or weight gain.

Discussion

Data from the present study indicate that variations in dietary protein source influence lipid metabolism in

Table 3 Weight gain and feed intake (g/28 days)[a]

Treatment	Weight gain	Feed intake	n
SOY	9.9 ± 2.2	159 ± 16[a]	8
CAS	8.6 ± 2.3	174 ± 43[a]	8
SAA	8.7 ± 1.3	153 ± 14[a]	7
CAA	8.5 ± 2.1	141 ± 10[b]	8

Values represent mean ± SD. Means in a column with a different superscript are significantly different at $P < 0.05$.

Table 5 Hepatic GSH concentrations and HMG CoA reductase activities[1]

	GSH[2]	HMG CoA[3]
SOY	5.7 ± 0.8[a]	147.8 ± 17.2[a]
CAS	7.4 ± 1.5[b]	81.2 ± 26.1[b]
SAA	7.7 ± 1.9[b]	83.8 ± 25.3[b]
CAA	7.2 ± 0.8[b]	118.3 ± 32.3[a]

[1]Values represent mean ± SD. Means in a column with a different superscript are significantly different at $P < 0.05$.
[2]SOY n = 6; CAS n = 7; SAA n = 7; CAA n = 7.
[3]SOY n = 4; CAS n = 6; SAA n = 6; CAA n = 7.

Table 4 Plasma lipid concentrations

Treatment	n	T-CHOL (mmol/L)	LDL-CHOL (mmol/L)	HDL-CHOL (mmol/L)	Triglyceride (mmol/L)
SOY	7	3.15 ± 0.52[b]	1.24 ± 0.28[b]	1.94 ± 0.31[b]	0.79 ± 0.30
CAS	7	3.95 ± 0.24[a]	1.79 ± 0.20[a]	2.08 ± 0.36[b]	0.88 ± 0.46
SAA	6	3.75 ± 0.83[a*]	1.58 ± 0.67[a]	2.09 ± 0.52[b]	0.68 ± 0.24
CAA	8	4.09 ± 0.53[a]	1.59 ± 0.53[a]	2.48 ± 0.23[a]	0.69 ± 0.15

Values represent mean ± SD. Means in a column with a different superscript are significantly different at $P < 0.05$.
*SOY versus SAA ($P = 0.06$).

gerbils. Consumption of soy protein isolate resulted in significant decreases in plasma total and LDL-cholesterol concentrations in comparison with values for other treatment groups. When L-amino acids patterned after SOY or CAS were fed, neither plasma total nor LDL-cholesterol concentrations were demonstrated to be significantly different, although total cholesterol values were numerically lower in the SAA-fed animals in comparison with those fed CAA. HDL-cholesterol, however, was found to be highest in CAA-fed animals, which may represent a beneficial partitioning of lipoproteins even though total cholesterol was numerically highest in this group. Both total and LDL-cholesterol values were higher in gerbils fed the L-amino acids patterned after soy than when they were fed soy protein isolate. This indicates that the hypocholesterolemic effect of soy protein in gerbils may be mediated by a nonprotein constituent, possibly a phytochemical.

The mechanism for soy protein-induced hypocholesterolemia is not known; however, it has been postulated that GSH concentrations could be increased by feeding adequate amounts of soy protein due to the higher content of cysteine.[6] Hepatic GSH level is closely dependent on diet, especially the amount of ingested cysteine, because this amino acid is rate limiting in GSH biosynthesis.[20] In addition to HMG CoA reductase, cholesterol 7-α-hydroxylase,[21] the rate-limiting enzyme in bile acid synthesis,[22] has been reported to be influenced by GSH.

GSH is also believed to be a cofactor in conversion of thyroxine (tetraiodothyronine, T_4) to triiodothyronine (T_3, the active form of thyroid hormones).[23] In vitro data indicate that the release of T_3 and T_4 is dependent on thyroid GSH breaking disulfide bonds of thyroglobulin.[24] Forsythe[10] reported increases in T_4 concentrations in gerbils fed isolated soy protein compared with casein, and we have noted an increase in plasma T_4 (without concurrent increases in plasma thyroid-stimulating hormone) in humans consuming isolated soy protein compared to a basal mixed protein diet.[25] Furthermore, hepatic GSH concentrations in hyperthyroid rats have been reported to be lower and plasma GSH higher than in euthyroid rats.[26] This may indicate that hepatic GSH is being depleted by increases in extrahepatic transport, possibly to the thyroid, facilitating thyroid hormone release. The actions of thyroid hormones on cholesterol metabolism are to increase biosynthesis, bile acid synthesis, and LDL receptor activity.[24] These same responses in cholesterol homeostasis are seen upon consumption of soy protein.[27]

Our findings do not support the hypothesis that feeding soy protein increases hepatic GSH concentrations, thereby increasing activity of cholesterol metabolic enzymes. In fact, hepatic GSH concentrations were lowest while HMG CoA reductase activities were highest, and correlation analysis failed to show a significant association between these two parameters. Perhaps concentrations of GSH in the thyroid and periphery are more reflective of the possible role of GSH in the hypocholesterolemic effect of soy protein than hepatic concentrations.

Others have reported increased growth rates and hepatic total glutathione in rats fed diets containing 16% protein from casein versus soy protein isolate.[28] However, when level of protein intake increased to 24%, growth rates and GSH concentrations were similar. In the current investigation, older animals were used, producing minimal growth rates that were similar between treatment groups. It is not clear why gerbils fed only the SOY diet and not the SAA diet had depressed hepatic GSH concentrations if sulfur-amino acid intake alone is the determining factor for hepatic GSH concentrations. The reason for this divergence in response may be a result of differential absorption of amino acids between the intact proteins and their amino acid counterparts or, possibly, the action of an unidentified phytochemical present in soy protein isolate that either inhibits protein absorption or affects synthesis of GSH directly.

Increased HMG CoA reductase activity in soy protein-fed rats has been reported by Nagata et al.,[29] but when amino acids replaced intact proteins, higher reductase activities were observed in rats fed amino acids patterned after casein. In our study, when the amino acid mixtures simulating SOY (SAA) or CAS (CAA) were fed reductase activity was also higher in CAA-fed gerbils compared with those fed SAA. Nagata et al.[29] propose that this variation in response between the intact and simulated proteins is a function of diurnal variation. However, in our experiments animals were sacrificed randomly, at specific time points corresponding to peak reductase activity. It is possible that slight differences in time points and/or stress involved in sacrificing could have caused the observed differences between gerbils fed the intact versus simulated proteins. This does not explain why both the Nagata group and our group observed higher HMG CoA reductase activities with intact soy protein in comparison with casein and lower HMG CoA reductase activities with SAA in comparison with CAA. These observations again may be more reflective of differences in absorption of the amino acids or the action of a nonprotein constituent of soy protein isolate resulting in variations in HMG CoA reductase activity.

In conclusion, data from the present study show that dietary protein source influenced lipid metabolism in gerbils. Our data also indicate that hepatic GSH concentration and HMG CoA reductase are influenced by dietary protein source. It is unlikely that changes in plasma lipids associated with feeding soy protein are a result of changes in hepatic GSH concentrations causing an effect on activity of HMG CoA reductase. However, it is clear that feeding intact soy protein isolate produces lower hepatic GSH concentrations and hypocholesterolemia–responses that are not present when L-amino acids patterned after soy protein are fed. Further studies looking into GSH concentrations in the plasma, thyroid, and possibly skeletal muscle along with thyroid hormone activation and release warrant further investigation.

References

1 Erdman, J.W. Jr. and Fordyce, E.J. (1989). Soy protein and the human diet. *Am. J. Clin. Nutr.* **49,** 725–737

2 Carroll, K.K. (1991). Review of clinical studies on cholesterol-lowering response to soy protein. *J. Am Diet. Assoc.* **91,** 820–827

3 Forsythe, W.A., Green, M.S. and Anderson, J.J.B. (1986). Dietary protein effects on cholesterol and lipoprotein concentration: a review. *J. Am. Coll. Nutr.* **5,** 533–549

4 Huff, M.W., Hamilton, R.M.G., and Carroll, K.K. (1977). Plasma cholesterol levels in rabbits fed low fat, cholesterol-free, semipurified diets: effects of dietary proteins, protein hydrolosates, and amino acid mixtures. *Atherosclerosis* **28,** 187–195

5 Huff, M.W. and Carroll, K.K. (1980). Effects of dietary protein and amino acid mixtures on plasma cholesterol levels in rabbits. *J. Nutr.* **110,** 1676–1685

6 Hagemeister, H., Scholz-Ahrens, K.E., Schulte-Coerne, H., and Barth, C.A. (1990). Plasma amino acids and cholesterol following consumption of dietary casein or soy protein in minipigs. *J. Nutr.* **120,** 1305–1311

7 Doton, I. and Schechter, I. (1982). Thiol-disulfide-dependent interconversion of active and latent forms of rat hepatic HMG CoA reductase. *Biochim. Biophys. Acta* **713,** 427–434

8 Roitleman, J. and Schechter, I. (1984). Regulations of rat liver 3-hydroxy-3 methyl glutaryl coenzyme. A reductase. Evidence for reduced thiol dependent allosteric modulation of enzyme activity. *J. Biol. Chem.* **259,** 870–877

9 Potter, S.M. and Kies, C.V. (1990). Influence of sulfur-amino acid content variation in plant vs animal protein on serum and tissue lipids in rats. *Plant Foods for Human Nutrition* **40,** 297–308

10 Forsythe, W.A. III. (1986). Comparison of dietary casein or soy protein: effects on plasma lipids and hormone concentrations in the gerbil (meriones unguiculates). *J. Nutr.* **116,** 1165–1171

11 DiFrancesco, L., Allen, O.B., and Mercer, N.H. (1990). Long-term feeding of casein or soy protein with or without cholesterol in Mongolian gerbils II. Plasma lipid and liver cholesterol responses. *Acta Cardiologica* **XLV,** 273–290

12 Mercer, N.J.H. and Holub, B.J. (1981). Measurement of hepatic sterol synthesis in the Mongolian gerbil in vivo using [^{3}H] water: diurnal variations and effect of type of dietary fat. *J. Lipid Res.* **22,** 792–799

13 Allain, C.A., Poon, L.S., Chang, C.S.G., Richmond, W., and Wu, P.C. (1974). Enzymatic determination of total serum cholesterol. *Clin. Chem.* **20,** 470–475

14 McGowen, M.W., Artiss, J.D., Strandbergh, D.R., and Zak, B. (1983). A peroxidase-coupled method for the colorimetric determination of serum triglycerides. *Clin. Chem.* **29,** 538–542

15 Asaoka, K. and Takahashi, K. (1981). An enzymatic assay of reduced glutathione using glutathione S-aryltransferase with o-dinitrobenzene as a substrate. *J. Biochem.* **90,** 1232

16 Shapiro, D., Imblum, R., and Rodwell, V. (1969). Thin-layer chromatographic assay for HMG CoA reductase and mevalonic acid. *Anal. Biochem.* **31,** 383–390

17 Shapiro, D., Nordstrom, J., Mitschelen, J., Rodwell, V., and Schimke, R. (1974). Micro assay for 3-hydroxy-3-methyl glutaryl CoA reductase in rat liver and L-cell fibroblasts. *Biochim. Biophys. Acta* **370,** 369–377

18 Hill, M. and Straka, J. (1988). Protein determination using bicinchoninic acid in the presence of sulfhydryl reagents. *Anal. Biochem.* **170,** 203–208

19 SAS Institute, Inc. (1985). *SAS User's Guide: Statistics,* SAS Institute, Inc., Cary, NC USA

20 Tatieshi, N., Higashi, T., Naruse, A., Nakashima, K., Shiozaki, H., and Sakamoto, Y. (1977). Rat liver glutathione: possible role as a reservoir of cysteine. *J. Nutr.* **107,** 51–60

21 Hassan, A., Hackley, J., and Jeffrey, P. (1984). Role of glutathione in the regulation of hepatic 7-α-hydroxylase, the rate-limiting enzyme in bile acid synthesis. *Steroids* **44,** 373–380

22 Myant, N. and Mitropoulos, R. (1977). Cholesterol 7-α-hydroxylase. *J. Lipid Res.* **18,** 135–153

23 Visser, T.J. (1991). Role of glutathione and other thiols in thyroid hormone metabolism. In *Glutathione: Chemical, Biochemical, and Medical Aspects, Part B,* (D. Dolphin, O. Avramovic, and R. Poulson, eds.), pp. 571–612, John Wiley & Sons, New York, NY USA

24 Larson, P.R. and Ingbar, S.H. (1992). The thyroid gland. In *Williams Textbook of Endocrinology,* (J.D. Wilson and D.W. Foster, eds.), 8th Ed., p. 355–487, W.B. Saunders & Co., Philadelphia, PA USA

25 Ham, J.O., Chapman, K.M., Essex-Sorlie, D., and Bakhit, R.M. (1993). Endocrinological response to soy protein and fiber in mildly hypercholesterolemic men. *Nutrition Research* (in press)

26 Morini, P., Casalino, E., Sblano, C., and Landriscina, C. (1991). The response of rat liver lipid peroxidation, antioxidant enzyme activities and glutathione concentration to the thyroid hormone. *Int. J. Biochem.* **23,** 1025–1030

27 Scholz-Ahrens, K.E., Hagemeister, H., Unshelm, J., Agergaard, N., and Barth, C.A. (1990). Response of hormones modulating plasma cholesterol to dietary casein or soy protein in minipigs. *J. Nutr.* **120,** 1387–1392

28 Hosokawa, Y., Niizeki, S., Tojo, H., Sato, I., and Yamaguchi, K. (1988). Hepatic cysteine dioxygenase activity and sulfur amino acid metabolism in rats: possible indicators in the evaluation of protein quality. *J. Nutr.* **118,** 456–461

29 Nagata, Y., Ishiwaki, N., and Sugano, M. (1982). Studies on the mechanism of the antihypercholesterolemia action of soy protein and soy protein-type amino acid mixtures in relation to their casein counterparts in rats. *J. Nutr.* **112,** 1614–1625