used by those groups was not the same as the one used by Williams et al.[5] and Antonio et al.[6] Further, the use of untrained subjects[5,6] may present an important confounding variable. The training status (or lack thereof) may have masked any potential gains in lean body mass or strength. Changes in strength may be due in large part to neural adaptation rather than to skeletal muscle hypertrophy.[7]

Another interesting concept in the protein and amino acid metabolism field is "slow" versus "fast" proteins.[8] Beaufrere et al. found that, in addition to the amount of protein consumed and the amino acid composition, the speed at which a protein is digested significantly affects the ensuing net protein balance. That is, slowly digesting proteins such as casein increase total protein synthesis more than swiftly digesting proteins such as whey or an equivalent amount in free-form amino acids.

Future studies should examine how the absorption of different dietary proteins and single amino acids and amino acid combinations affect total and skeletal muscle protein synthesis. Also, future studies should examine the effect of exercise training coupled with exogenous protein and/or amino acid supplementation on the "real-world" indicators of efficacy (i.e., body composition and increased lean body mass).

For now, it is apparent that the use of amino acids to improve body composition via an effect on GH is largely ineffectual. However, there are other mechanisms in which proteins and/or amino acids might positively influence body composition.

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REFERENCES

1. Chromiak JA, Antonio J. Use of amino acids as growth hormone-releasing agents by athletes. Nutrition 2002;18:657
2 Tipton KD, Ferrando AA, Phillips SM, Doyle D, Wolfe RR. Postexercise net protein synthesis in human muscle from orally administered amino acids. Am J Physiol 1999;276:E628
3. Rasmussen B, Tipton KD, Miller SL, Wolfe SE, Wolfe RR. An oral essential amino acid–carbohydrate supplement enhances muscle protein anabolism after resistance exercise. J Appl Physiol 2000;88:386
4. Tipton KD, Rasmussen BB, Miller SL, et al. Timing of amino acid-carbohydrate ingestion alters anabolic response of muscle to resistance exercise. Am J Physiol 2001;89:E197
5. Williams AG, van den Oord M, Sharma A, Jones DA. Is glucose/amino acid supplementation after exercise an aid to strength training? Br J Sports Med 2001;35:109
6. Antonio J, Sanders MS, Ehler LA, et al. Effects of exercise training and amino acid supplementation on body composition and physical performance in untrained women. Nutrition 2000;16:1043
7. Fleck SJ, Kraemer WJ. *Designing resistance training programs.* Champaign, IL: Human Kinetics, 1997
8. Beaufrere B, Dangin M, Boirie Y. The 'fast' and 'slow' protein concept. In: Furst P, Young V, eds. *Proteins, peptides and amino acids in enteral nutrition. Nestle Nutrition Workshop Series Clinical and Performance Program, Vol 3:121–133*

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Hypocholesterolemic Effect of Soy Protein

The importance of dietary protein in the regulation of cholesterol metabolism has been well established in various species including humans and rats (reviewed by Huang et al.[1]). Soybean protein compared with casein with or without dietary cholesterol lowers plasma cholesterol and triacylglycerol concentrations in rats.[2,3] In humans the cholesterol-lowering effect of soybean protein is achieved only when cholesterol is included in the diet.[4]

In this issue of *Nutrition*, Kern et al.[5] compared two diets consisting of 20% by weight of protein with soy protein (92% protein) or casein (95% protein) with 1% cholesterol for 28 d in Sprague-Dawley rats. L-methionine was adjusted to be equivalent between the diets. Soy protein isolate versus casein did not significantly modify food intake, weight gain, food efficiency ratio, epididymal fat pad weight, serum triacylglycerol or high-density lipoprotein (HDL) cholesterol concentrations. However, total cholesterol was lower with the soy protein diet (−25%) than with the casein diet. Their results indicated that methionine supplementation may eliminate the decreased fat deposition previously ascribed[6] to soy protein but that methionine does not abolish the hypocholesterolemic effect of soy protein.

The hypocholesterolemic effect of soy is largely attributable to the differences in the amino acid profile of soybean protein and casein. Indeed, a major difference in the amino acid profile of soy protein versus that of casein is the methionine content, which barely represents half the amount in casein; in addition, methionine has been demonstrated to elevate serum cholesterol concentration.[7,8]

However, because methionine supplementation in the study by Kern et al. did not abolish the commonly observed hypocholesterolemic effects of soy protein relative to casein, some factor other than methionine must be at least partly responsible for the cholesterol-lowering effect of soy.

Moreover, differences between casein and soy other than their methionine content have been reported. Glycine is present at almost twice the concentration in soy protein isolate. The higher methionine:glycine ratio in casein may be responsible for elevation of serum cholesterol.[8] Glycine added to a casein diet tested on rats has been demonstrated to lower serum cholesterol concentration.[7] Although the methionine levels were equal in the diets in the study by Kern et al., the glycine difference between the sources of protein may have been responsible for the differences in serum cholesterol concentrations.

Several investigators[8,9] have suggested that the lysine:arginine ratio also may be a candidate for causing greater serum cholesterol level that occurs with casein feeding, potentially due to increased insulin sensitivity. This ratio is two-fold higher in casein than in soy protein. Lavigne et al.[10] demonstrated that soy protein as opposed to casein improves glucose tolerance and insulin sensitivity in rats. In addition, postprandial insulinemia decreased in humans who consumed soy protein versus casein.[11]

However, studies in which animals were fed amino acid mixtures simulating soybean protein or casein have suggested that some non-protein components in partly purified soybean protein may be responsible for the hypocholesterolemic effect.[12–14] Indeed, a mixture of amino acids corresponding to soybean protein exhibited higher plasma cholesterol concentrations than did the protein itself.[12] In contrast, Morita et al.[8] suggested that the pattern of amino acid composition in soy versus that in casein is responsible for the differences in cholesterol metabolism. Thus, no consensus regarding this issue has been reached.

However, when the patterns were fed as individual amino acids rather than as whole proteins, the results differed from what occurs with whole protein feeding, particularly at the digestion and intestinal absorption levels. Indeed, no significant differences in cholesterol absorption and excretion were observed between rats fed amino acid mixtures equivalent to either protein.[13]

Several studies have suggested that the hypocholesterolemic effect of vegetable proteins, in particular soybean protein, is largely attributable to higher fecal steroid excretion as a consequence of the reduction in intestinal absorption.[13,15] Iwami et al.[16] reported that soybean isolate is inferior to casein in digestibility

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and suggested that the hydrophobic peptides of soybean protein that remain after digestion may bind well to bile acids and serve as a cholesterol-lowering factor. Greater fecal steroid excretion may lead to increased bile acid production from cholesterol, thus reducing serum cholesterol concentration. However, some research has suggested that increased fecal bile acid secretion does not necessarily result in decreased serum cholesterol concentration.[17] Indeed, in compensation for the fecal loss of steroids, soybean protein may stimulate hepatic activities of hydroxy methyl glutaryl coenzyme A reductase, the rate-limiting enzyme in the biosynthesis of cholesterol[13] and cholesterol 7α-hydroxylase, the key enzyme that converts cholesterol to bile acids.[18]

The hypocholesterolemic effect of soy also may be due to non-protein components in soy protein (such as fiber, phytic acid, minerals, and isoflavones), and the variable purity of vegetable protein used is questionable.[19,20] Soybean proteins are generally used as protein isolate, which contains non-protein components associated with soybean protein, and thus may be responsible for the cholesterol-lowering action.

Madani et al.[21] carried out a study with dietary soybean protein purified to the utmost (98%) to eliminate any additional action of non-protein components on plasma cholesterol concentrations. The effects of a 20% soybean protein diet and a 20% casein (95% protein) diet consumed for 28 d by growing Wistar rats were compared.[21] The soy protein diet involves less weight gain and final body and liver weights but similar food intake and energy intake by weight unit. The impaired amino acid contents (especially lysine and methionine) in soy protein versus those in casein may be responsible for this slow growth.

The most important result was that plasma total cholesterol concentrations are not affected by the origin of protein. These data indicated that the cholesterol-lowering effect often observed with soybean protein as opposed to casein is attributable to large amounts of non-proteinous components in these proteins. These findings are inconsistent with those of Sjöblom et al.[22] who found a cholesterol-lowering effect with soybean protein (97% crude protein) compared with casein in male Sprague-Dawley rats. A possible explanation for this discrepancy is that the diets in the study by Madani et al. were free of cholesterol, whereas the diets in the study by Sjöblom et al. contained 1% cholesterol, as did the diets composed by Kern et al. Despite unchanged total cholesterol concentration in plasma in the study by Madani et al.,[21] a highly purified soybean protein diet as opposed to a casein diet reduced plasma low-density lipoprotein and very low-density lipoprotein (VLDL) masses, decreased the number of VLDL particles, raised the amounts of HDL2-3 and HDL2-3–cholesterol, and increased the number of HDL2-3 particles. Eklund and Sjöblom[23] also reported a lower amount of VLDL when soybean protein rather than casein was fed. The lowered plasma VLDL level after soybean protein consumption might have been the result of enhanced VLDL and remnant VLDL uptake by the liver. Consumption of soybean protein by rat was associated with an increase in hepatic apolipoprotein B/E receptor activity,[24] and this receptor is involved in VLDL, intermediate-density lipoprotein, and low-density lipoprotein uptake.[25] These results in part may account for the enhanced cholesteryl ester contents in liver from rats fed the highly purified soybean protein compared with rats fed the casein diet.

In another experiment, Madani et al.[26] used the same sources of protein but the diets were fed for 2 mo with 0.1% or no dietary cholesterol. However, Eklund and Sjöblom[23] found a hypocholesterolemic effect of soybean protein compared with that of casein and the hypercholesterolemic effect of exogenous cholesterol when rats were fed a higher level ($>$0.25% cholesterol). But these amounts are higher than the quantities consumed by humans (usually $<$0.05%). In the study by Madani et al., neither dietary protein nor cholesterol supplementation affected plasma cholesterol or triacylglycerol concentrations. Fecal neutral and acidic steroid excretions were higher in the rats fed soybean protein than in those fed casein and greater in both groups fed the cholesterol-enriched diets. The increased excretion of fecal steroids was not associated with a lower plasma cholesterol level. Madani et al.[21,26] probably minimized fecal steroid excretion by using highly purified soybean protein, which might be responsible for the absence of the cholesterol-lowering effect of soybean protein compared with casein. Moreover, hydroxy methyl glutaryl coenzyme A reductase activity was 1.7-fold higher in the soy protein group than in the casein group, but the difference was not significant due to large individual variations. This higher hydroxy methyl glutaryl coenzyme A reductase activity may compensate for the loss of fecal steroids without modifying plasma cholesterol concentration. Dietary cholesterol supplementation lowered hydroxy methyl glutaryl coenzyme A reductase activity and this decrease was more marked in rats fed the soybean protein diet. In the absence of dietary cholesterol, highly purified soybean protein lowered cholesterol 7α-hydroxylase activity. In the presence of 0.1% cholesterol, this activity was higher in rats fed a highly purified soybean protein diet than in those fed casein. However, Choi et al.[4] found no dietary protein-dependent difference in cholesterol 7α-hydroxylase activity among rats fed soy protein or casein supplemented or not supplemented with cholesterol.

Isoflavones from soybean have been hypothesized as the cause of the cholesterol-lowering effect.[27] Anthony et al.[28] found that the isoflavone-intact protein, but not the alcohol-extracted soybean protein, reduces plasma cholesterol in peripubertal rhesus monkeys. Madani et al.[21,26] did not use aqueous alcohol extraction to obtain soybean protein, so the highly purified soybean protein used might have contained isoflavones in small quantities. Nonetheless, feeding rats these proteins did not produce any hypocholesterolemic effect in comparison with rats fed casein.

In conclusion, soy protein may trigger various effects on lipid and cholesterol metabolism according to the species, but the effects are due mostly to the presence or absence of non-protein components and, hence, the purity of the selected protein and the concomitant addition or non-addition of cholesterol. The effects observed also may depend on whether dietary cholesterol is added to the diet and on its intake level. These points may account for some divergent results and explain the hypocholesterolemic effect or absence of soybean protein.

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REFERENCES

1. Huang YS, Koba K, Horrobin DF, Sugano M. Interrelationship between dietary protein, cholesterol and n-6 polyunsaturated fatty acid metabolism. Prog Lipid Res 1993;32:123
2. Park MS, Kudchodkar BJ, Liepa GU. Effects of dietary animal and plant proteins on the cholesterol metabolism in immature and mature rats. J Nutr 1987;117:1892
3. Choi YS, Goto S, Ikeda I, Sugano M. Interaction of dietary protein, cholesterol and age on lipid metabolism of the rat. Br J Nutr 1989;61:531
4. Meinertz H, Nilausen K, Faergement O. Effects of dietary protein on plasma lipoprotein levels in normal subjects:interaction with dietary cholesterol. J Nutr Sci Vitaminol 1990;36:S157
5. Kern M, Ellison D, Marroquin Y, Ambrose M, Mosier K. Effects of soy protein supplemented with methionine on blood lipids and adiposity of rats. Nutrition 2002;18:654
6. Baba N, Radwan H, Itallie T. Effects of casein versus soy protein on body composition and serum lipid levels in adult rats. Nutr Res 1992;12:279
7. Sugiyama K, Ohkawa S, Muramatsu K. Relationship between aminoacid composition of diet and plasma cholesterol level in growing rats fed a high cholesterol diet. J Nutr Sci Vitaminol 1986;32:413
8. Morita T, Oh-hashi A, Taeki K, et al. Cholesterol lowering effects of soybean, potato, and rice proteins depend on their low methionine contents in rats fed a cholesterol-free purified diet. J Nutr 1997;127:470
9. Kritchevsky D, Tepper SA, Czarnecki SK, Klurfeld DM. Atherogenicity of

animal and vegetable protein. Influence of the arginine/lysine ratio. Atherosclerosis 1982;41:429
10. Lavigne C, Marette A, Jacques H. Cod and soy proteins compared with casein improve glucose tolerance and insulin sensitivity in rats. Am J Physiol Endocrinol Metab 2000;278:E491
11. Hubbard RW, Sanchez A. Dietary protein control of serum cholesterol by insulin and glucagon. Monogr Atheroscler 1990;16:139
12. Huff MW, Hamilton RMG, Carroll KK. Plasma cholesterol in rabbits fed low fat, cholesterol free, semi-purified diet:effects of dietary protein, protein hydrolysates and aminoacid mixtures. Atherosclerosis 1977;28:187
13. Nagata Y, Ishiwaki N, Sugano M. Studies on the mechanism of antihypercholesterolemic action of soy protein and soy protein-type amino acid mixtures in relation to the casein counterparts in rats. J Nutr 1982;112:1614
14. Tasker TE, Potter SM. Effects of dietary protein source on plasma lipids, HMGCoA reductase activity, and hepatic glutathione levels in gerbils. J Nutr Biochem 1993;4:458
15. Huff MW, Carroll KK. Effects of dietary protein on turn-over, oxidation and absorption of cholesterol, and on steroid excretion in rabbits. J Lipid Res 1980;21:546
16. Iwami K, Sakakibara K, Ibuki F. Involvement of post-digestion hydrophobic peptides in plasma cholesterol-lowering effect of dietary plant protein. Agric Biol Chem 1986;50:1217
17. Younes H, Levrat MA, Deigne C, Remesy C. Resistant starch is more effective than cholestyramine as a lipid-lowering agent in the rat. Lipids 1995;30:847
18. Beynen A. Comparison of the mechanims proposed to explain the hypocholesterolemic effect of soybean protein versus casein in experimental animals. J Nutr Sci Vitaminol 1990;36:S87
19. Potter SM. Overview of proposed mechanisms for the hypocholesterolemic effect of soy. J Nutr 1995;125:606S
20. Balmir F, Staack R, Jeffrey E, et al. An extract of soy flour influences serum cholesterol and thyroid hormones in rats and hamsters. J Nutr 1996;126:304
21. Madani S, Prost J, Belleville J. Dietary protein level and origin (casein and highly purified soybean protein) affect hepatic storage, plasma lipid transport, and antioxidative defense status in the rat. Nutrition 2000;16:368
22. Sjöblom L, Eklund A, Humble L, Menschilk-Lundin A, Ostlund-Lindqvist AM. Plasma very low density lipoprotein from male rats fed casein or soybean protein diets: a comparison of fatty acid composition and influence on prostanoid production. J Nutr 1991;121:1705
23. Eklund A, Sjöblom I. Effect of dietary protein on hepatic and plasma lipids, and some properties of major plasma lipoprotein fractions during dietary-induced hypercholesterolemia in male rats. Biochem Biophys Acta 1986;877:127
24. Sirtori CR, Galli G, Lovati MR, et al. Effects of dietary proteins on the regulation of liver lipoprotein receptors in rats. J Nutr 1984;114:1493
25. Davis RA, Hyde PM, Kuan JC, Maline-McNeal M, Archaubault-Schexnayder J. Bile acid secretion by cultured rat hepatocytes. Regulation by cholesterol availability. J Biol Chem 1993;258:3661
26. Madani S, Lopez S, Blond JP, Prost J, Belleville J. Highly purified soybean protein is not hypocholesterolemic in rats but stimulates cholesterol synthesis and excretion and reduces polyunsaturated fatty acid biosynthesis. J Nutr 1998;128:1084
27. Tsai P, Huang P. Effects of isoflavones containing soy protein isolate compared with fish protein on serum lipids and susceptibility of low density lipoprotein and liver lipids to in vitro oxidation in hamsters. J Nutr Biochem 1999;10:631
28. Anthony MS, Clarkson TB, Hughes CL, Morgan TM, Burke GL. Soybean isoflavones improve cardiovascular risk factors without affecting the reproductive system of peripubertal rhesus monkeys. J Nutr 1996;126:43

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Intraportal Nutrition: Are There Indications for Clinical Relevance?

Bozzetti et al.[1] investigated the metabolic effects of intravenous nutrition in the portal (P) and systemic (S) circulations. They studied 20 patients undergoing colorectal surgery. The patients were randomized to receive P or S nutrition (N). They claimed a positive result. They concluded that short-term PN is safe and has several metabolic benefits: an accelerated recovery from postoperative hypoalbuminemia and hypoprealbuminemia, associated with a higher level of plasma glutamine, a closer to normal amino acid (AA) pattern in the PN group, and a blunted catabolic response of the muscle in PN patients.

In disease or trauma, amino acids are transported from peripheral tissues to the liver to accommodate the synthesis of visceral and acute-phase proteins.[2–8] Therefore, the idea of feeding intraportally has its base in the assumption that all nutrients are delivered directly to the liver. Thus, nitrogen use and hepatic protein synthesis are optimally supported. This method is in contrast to the intravenous route, where nutrition solutions pass directly into the systemic circulation, bypassing the gastrointestinal tract and the first circulation through the liver.[9]

Intravenous nutrition was designed specifically to maintain or improve the nutrition and metabolic status of patients who cannot be nourished adequately.[10,11] The lack of adequate food intake is associated with a significant deterioration of the protein-energy status.[12] An overall depleted protein-energy status leads to impaired function of vital organs and may exacerbate the disease and delay recovery.[13,14] Thus, intravenous nutrition would be expected to facilitate the stress response during trauma.

Bozzetti et al. assumed metabolic benefits based on their observations of accelerated recoveries from postoperative hypoalbuminemia and hypoprealbuminemia by postoperative day 7. This assumption was based entirely on the fact that only the SN group showed a significant difference on day 7 versus basal levels, so this conclusion is rather dubious. As they mentioned, the difference between basal and the 7-d levels was merely 0.3 g/dL and the same difference applied equally between groups. The reason that there was a significant difference in the SN group and not the PN group is probably due to the greater standard error in the PN group (0.5 versus 0.2 in the SN group). Hence, its significance can be debated.

Further, overall protein breakdown and skeletal muscle degradation were determined by and related to amino acid profiles. Although PN was claimed "to blunt the catabolic response of the muscle,"[1] neither the nitrogen balance nor the 3-methyl-histidine excretions showed a significant difference between groups in the study. In addition, the flux of 3-methyl-histidine showed no differences. Therefore, the only conclusion must be that there was no difference in protein muscle catabolism between the PN and SN groups. However, the release of tyrosine from muscle was higher in the SN group, supposedly indicating enhanced muscle degradation. Even so, this difference was present only on day 6. Usually, amino acid profiles are disturbed within 24 h after surgery and return to normal patterns after a couple of days.[15] The fact that the significant changes were observed on day 6 and not on day 3 suggests, in the perspective of the literature, that the 3-methyl-histidine and N results were a coincidence.

Nutritional depletion and different types of injury have been associated with low plasma levels of glutamine[14,16,17] and decreased muscle free-glutamine levels after surgery.[15,16,18] The efflux of amino acids from skeletal muscle increases at a rate corresponding to the deamination and metabolism of free intracellular amino acids. De novo synthetized alanine and glutamine constitute the major portion of the amino acid outflow from muscle.[19] In the report by Bozzetti et al., the plasma level of glutamine in the PN group was significantly increased, whereas no such rise was reported in the SN group. The physiologic importance of a higher glutamine level was not investigated and its pathophysiologic significance is not known. More important is the finding that neither glutamine efflux nor arginine efflux from peripheral muscle were decreased in the PN as opposed to the SN group. Therefore, the aim of PN, to provide sufficient substrate to prevent muscle catabolism, was not met as would have been demonstrated by a decreased efflux from the muscle.

Arterial amino acid patterns of the P and S groups were compared with those of malnourished patients. The investigators stated that the SN group has a "pattern midway that of malnourished and PN patients" and, hence, concluded that the results of the PN group were better. However, comparing amino acid patterns

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