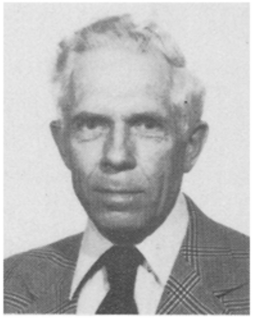

Soya Protein and Athersclerosis

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ABSTRACT

Cholesterol-free, semipurified diets produce hypercholesterolemia and atherosclerosis in rabbits when casein is used as dietary protein, but not when the casein is replaced by soya protein. In general, animal proteins produce higher levels of plasma cholesterol in rabbits than plant proteins. At least part of this difference appears to be related to differences in the amino acid composition of the proteins. Rabbits fed soya protein had a faster turnover of plasma cholesterol, absorbed cholesterol from the intestine less readily, and excreted more fecal neutral sterols and bile acids than rabbits fed casein. Differences in composition and turnover of plasma lipoproteins were also observed. Some, but not all, studies on humans with either normal or elevated levels of plasma cholesterol have shown that these levels can be lowered by replacing animal protein in the diet by soya protein. Epidemiological data have also provided evidence of an association between coronary heart disease and amount of animal protein in the diet. Relatively small changes in the diet can substantially decrease the ratio of animal to plant protein, and this may offer a means of lowering serum cholesterol levels and decreasing the high incidence of coronary heart disease in industrialized countries. The use of soya protein for this purpose minimizes the risk of essential amino acid deficiency, because its amino acid composition tends to complement that of cereal proteins, which are the major source of plant protein in most human diets.

When Ignatowski reported more than 70 years ago that atherosclerosis could be produced in rabbits by feeding diets containing meat, milk and eggs, he thought the effect was due to protein components of these foodstuffs. Later experiments in other laboratories provided further evidence that dietary protein can produce hypercholesterolemia and atherosclerosis in rabbits and other animals as well. A number of studies have also indicated that dietary protein can influence serum cholesterol levels in humans. This earlier work has been reviewed on previous occasions (1,2). Although this evidence has been gradually accumulating over the years, dietary protein has, until recently, been generally considered to be of little significance in the etiology of atherosclerosis and cardiovascular disease.

Dietary Protein in Relation to Hypercholesterolemia and Atherosclerosis in Animals Fed Cholesterol-Free, Semipurified Diets

In attempting to explain the hypercholesterolemia and atherosclerosis produced by feeding cholesterol-free, semipurified diets to rabbits (3,4), we found that these effects could be prevented by replacing the casein in such diets by soya protein (5,6). Further studies (1,7) showed that diets containing proteins from animal sources generally gave a hypercholesterolemic response whereas plasma cholesterol levels remained low when diets containing plant proteins were fed (Fig. 1).

Our experiments (5) confirmed the reports of earlier workers (3,4) that the hypercholesterolemia could be largely prevented by including relatively large amounts of polyunsaturated fat in the diet. Our studies also showed that the hypercholesterolemic effect of dietary protein could be modified by the type of carbohydrate in the diet (5,6) and experiments by Kritchevsky et al. (2,9) and ourselves (10) showed that dietary fiber could influence the results as well.

Still unclear is why diets containing animal proteins give higher levels of plasma cholesterol than diets containing plant proteins. Experiments in our laboratory showed that enzymatic digests of casein and soya protein gave results similar to those obtained with intact proteins (11). A mixture of L-amino acids corresponding to casein also produced a hypercholesterolemia in rabbits similar to that obtained by feeding the intact protein, but a mixture of amino acids corresponding to soya protein failed to give consistently low levels of plasma cholesterol like those observed in animals fed intact soya protein (11).

Kritchevsky and his colleagues (2,12,13) have suggested that the differing effects of casein and soya protein on serum cholesterol and lipoprotein levels and on atherosclerosis in rabbits may be related to the higher ratio of lysine to arginine in casein compared to soya protein. This hypothesis was based on the observation that lysine inhibits arginase activity and it was postulated (2) that the extra arginine in casein might contribute to the formation of larger amounts of arginine-rich apoprotein in the blood, which is thought to be atherogenic. These workers found that addition of lysine to soya protein enhanced its atherogenicity but addition of arginine to casein gave equivocal results (2).

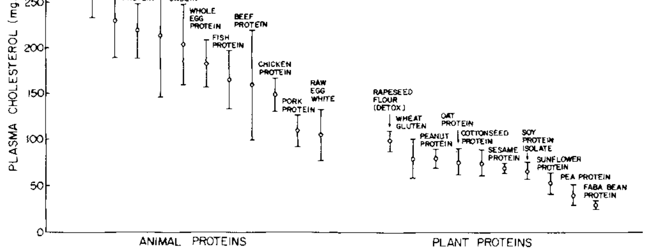

FIG. 1. Effects on plasma cholesterol levels of feeding low-fat, cholesterol-free, semipurified diets containing different delipidated protein preparations. The diets were fed to groups of 5-6 rabbits for 28 days and the results are shown as mean ± SE. (Reproduced from ref. 8 by permission of the publisher.)

The results of our feeding trials with amino acid mixtures have failed to localize the effect to any specific amino acids although it seems clear that plasma cholesterol levels in rabbits can be influenced by feeding different combinations of amino acids (14). In our experiments, there was possibly a trend toward higher plasma cholesterol levels with increasing lysine to arginine ratios but the results were very scattered (Fig. 2).

Plant proteins tend to have lower levels of essential amino acids than animal proteins (15,16) and this may be the reason for their differeing effects on plasma cholesterol levels, even though no specific amino acid is consistently low in all plant proteins. The best protein preparations available for dietary studies contain appreciable amounts of nonprotein constituents, including carbohydrates, inorganic salts and other compounds, such as phytic acid. However, there is no reason at present to think that these minor components are responsible for the observed differences in effects of dietary proteins on plasma cholesterol levels. In our experiments (6), diets containing soya protein concentrate and soya protein isolate gave similar levels of plasma cholesterol, although the protein isolate is a much more highly purified protein preparation.

Mechanism of Action of Dietary Protein

Possible mechanisms by which dietary protein may affect plasma cholesterol levels have been investigated in our own and other laboratories. In our studies, it was found that the turnover of plasma cholesterol and the excretion of fecal neutral sterols and bile acids were increased in rabbits fed soya protein compared with those fed casein (17). These increased rates of turnover and excretion were associated with an apparent decrease in the ability of soya protein-fed rabbits to absorb cholesterol from the gut. The diets used for these experiments contained no cholesterol and the lower plasma cholesterol levels in rabbits fed the soya protein diet may have been at least partly due to inability of cholesterol biosynthesis to compensate for the extra cholesterol lost by fecal excretion.

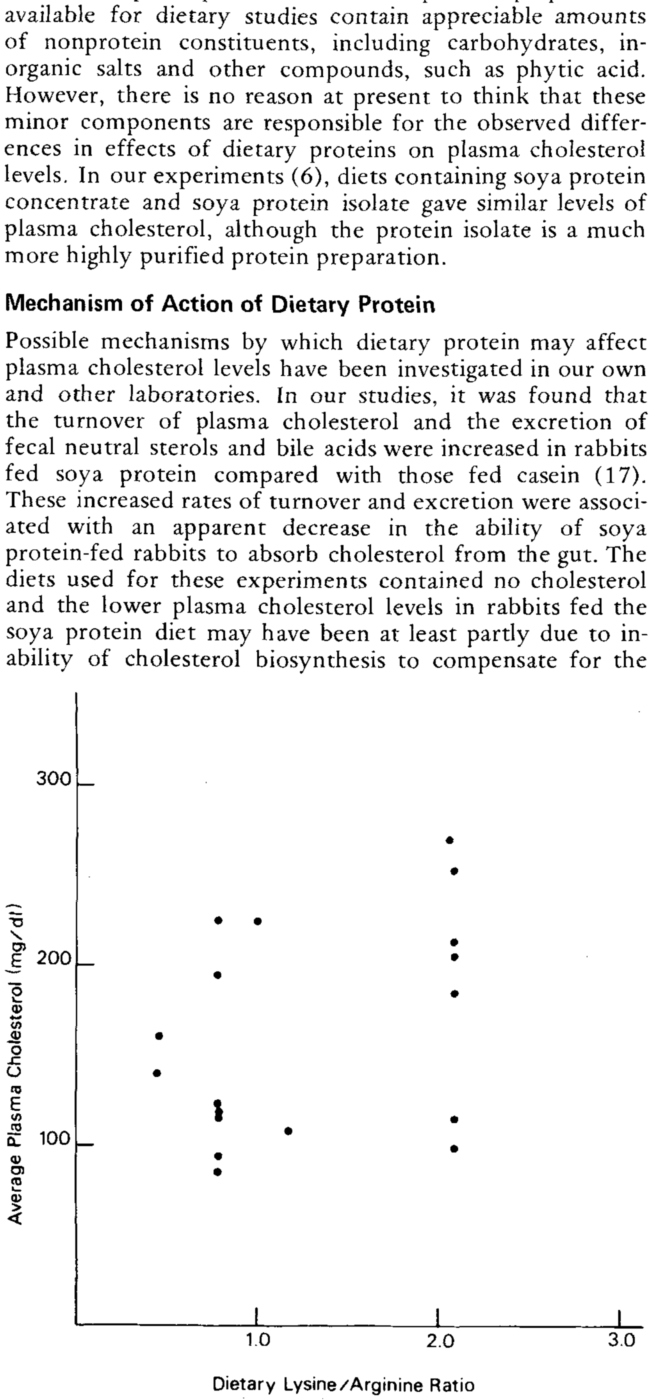

FIG. 2. Relationship between plasma cholesterol level and dietary lysine/arginine ratio in groups of 6 or more rabbits fed for 28 days on low-fat, cholesterol-free, semipurified diets containing different mixtures of L-amino acids. Data from Huff and Carroll (14).

This explanation is perhaps too simplistic, however, and still leaves the question of how dietary protein can alter the rates of absorption and excretion of cholesterol. One possibility is that products of partial digestion of soya protein may sequester cholesterol and/or bile acids to a greater extent than those of casein and thus decrease their reabsorption from the small intestine. Another possibility is that dietary protein may influence levels of plasma lipoproteins by altering the synthesis or metabolism of some of the apoproteins.

Comparison of the plasma lipoproteins of rabbits fed casein and soya protein showed that the excess cholesterol in the plasma of casein-fed rabbits was carried largely in the intermediate density lipoproteins, although some of the excess was also found in the very low and low density lipoproteins (7). Examination of the apoproteins by isoelectric focusing has indicated that the levels of apoE are also higher in these lipoprotein classes of the casein-fed rabbits (M.W. Khalil, J.C. Hutchinson and K.K. Carroll, unpublished data). Other experiments have indicated that the rate of turnover of plasma apoproteins is influenced by the type of protein fed (18). Such studies may help to clarify the mechanisms by which dietary protein affects plasma cholesterol levels.

Dietary Protein in Relation to Hypercholesterolemia and Cardiovascular Disease in Humans

The ultimate aim of these studies is to provide further insights into practical means of decreasing the level of serum cholesterol and the incidence of cardiovascular disease in humans by means of dietary alterations. The protein component of the diet may be more important in this regard than previously thought.

In the past few years, experiments (19-22) have shown that the level of serum cholesterol can be reduced in human subjects by substituting soya protein for animal protein in the diet. Other workers (23,24) have failed to observe any decrease in serum cholesterol as a result of such treatment, but Liebermeister and Toluipur (25) recently reported a 40% reduction in serum cholesterol levels of obese subjects fed a reducing diet containing soybean proteins and pectins.

Epidemiological data have shown a strong positive correlation between the amount of animal protein in the diet and the incidence of coronary heart disease in different countries (1). Data collected by the U.S. Department of Agriculture (26) show that the ratio of animal to plant protein in the American diet increased from 1.04:1 in 1910 to 2.36:1 in 1974 (Fig. 3) and this may have been a factor in the apparent rise in cardiovascular disease during this period of time (8,27). Over the same period, the increase in dietary fat intake was proportionately less (Fig. 3), and most of the change was due to increased consumption of the more unsaturated vegetable oils (27). The continuing rise in the ratio of animal to plant protein up to 1975 is not in accord with the decrease in cardiovascular disease which began in the U.S. about 1965, but other factors such as the decline in cigarette smoking, improved control of hypertension and increased intake of polyunsaturated fat may have combined to produce this decrease (28,29). Studies on vegetarians have provided clear evidence that they have lower levels of plasma lipids and lipoproteins (30-32) and are at lower risk from cardiovascular disease (33) than nonvegetarians living under similar environmental conditions. Unfortunately, it is not possible from such studies to say how much of the difference can be attributed to dietary protein.

Our experiments with rabbits, in which mixtures of casein and soya protein were fed, showed that the level

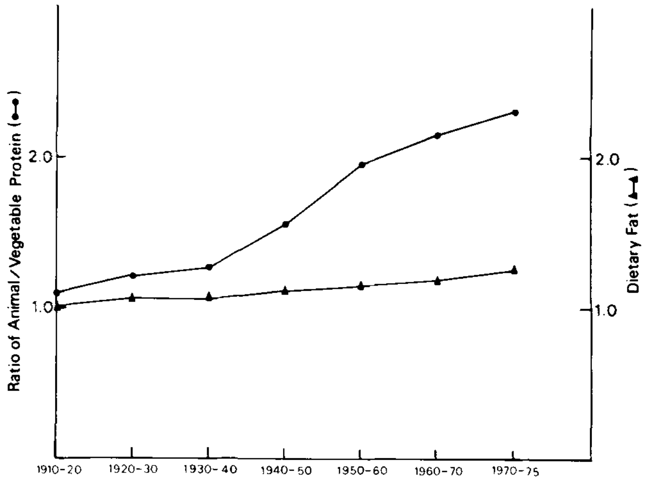

FIG. 3. Changes in ratio of animal to vegetable protein and in fat content of the American diet from 1910 to 1975 (26). The points are averages for each decade. Dietary fat is expressed as a ratio, in which the average per capita consumption of 126 g/day during the decade 1910-20 is assigned a value of 1.0.

of plasma cholesterol decreased as the proportion of soya protein increased, so that partial replacement of casein was sufficient to prevent its hypercholesterolemic effect (7,11). From a practical point of view, it is also of interest to note that a substantial change in the ratio of animal to plant protein in the human diet can result from relatively small changes in dietary habits. For example, the average daily amount of dietary protein available for consumption in the U.S. in 1970 was 100 g, consisting of 70 g from animal and 30 g from vegetable sources, giving a ratio of 2.33:1 (26). An individual drinking 3 cups of milk per day would obtain ca. 24 g of animal protein from that source (34). Simple calculation shows that if this were replaced by an equivalent amount of soya milk with the same protein content, the ratio of animal to plant protein would be reduced to 0.85:1.

$$\frac{(\text{Animal protein}}{(\text{Plant protein}} = \frac{70 - 24}{30 + 24} = \frac{46}{54} = \frac{0.85)}{1\ )}$$

It is not certain that such a reduction in the ratio of animal to plant protein would lower the level of serum cholesterol in humans. Even if this were effective, it might be undesirable to achieve such a reduction exclusively at the expense of milk, which is a valuable source of a number

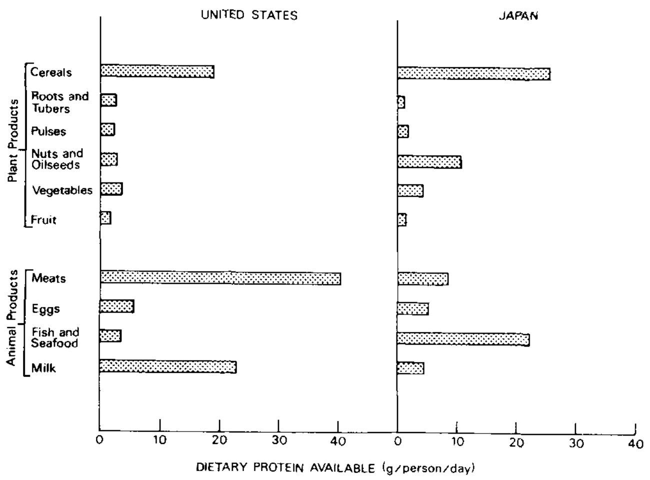

FIG. 4. Sources of available protein in the U.S. and Japan, based on data published by FAO (38).

of different nutrients. Furthermore, there have recently been suggestions that milk or some of its constituents may themselves have a hypocholesterolemic effect in humans (35,36). A similar decrease in the ratio of animal to plant protein in the diet could be achieved by replacing ca. 1/4 lb of cooked meat (37) by an equivalent amount of soya protein.

Sources of dietary protein in the U.S. are indicated in Figure 4. Corresponding data are also shown for Japan, which has a much lower incidence of cardiovascular disease. It can be seen that meats and milk are the major sources of animal protein in the U.S. and that cereal grains are the main sources of plant protein in both countries. Cereal proteins tend to be deficient in lysine, whereas proteins from soybeans and other legumes are higher in lysine but contain relatively less methionine (15,16). The two types of proteins thus complement one another, and increased use of soya protein in human diets provides an option for increasing the ratio of plant to animal protein while minimizing the risk of incurring a deficiency of essential amino acids. Much additional work will be required to assess the possible value of such a change in dietary habits as a means of reducing serum cholesterol levels and risk of atherosclerotic heart disease in human populations.

ACKNOWLEDGMENT

The author is greatly indebted to the Ontario Heart Foundation for continuing support of this research program.

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Nutritional Qualities of Soya Protein As Affected by Processing

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ABSTRACT

The nutritional qualities of soybean proteins are basically determined by amino acid patterns, amino acid availabilities (digestibility) and contents of biologically active components. Of these factors, the last two are most affected by processing conditions, whereas amino acid analysis is least affected, although it too may be modified in those cases in which soybean proteins are fractionated. In the preparation of the large variety of soya products presently available, soybeans are subjected to many different processes, all of which are discussed. Heat treatment appears to be the process which most affects protein nutritional quality; generally, quality first increases with heat treatment due to inactivation of biologically active factors, passes through a maximum and then decreases due to destruction and/or inactivation of essential amino acids such as cystine and lysine. Other processes affect protein nutritional quality to different degrees, inasmuch as they affect amino acid analysis, digestibility and content of biologically active components. When soybean proteins are used to extend animal proteins, supplement other vegetable proteins, or in vegetable protein mixtures, nutritional quality of the combined proteins appears to be affected in the same manner as that of soya proteins alone.

NUTRITIONAL QUALITY OF PROTEINS

Various means for determining and comparing protein quality have been developed, including chemical score, essential amino acid index, biological value, net protein utilization, protein efficiency ratio and nitrogen balance methods, all of which have been defined and discussed previously (1-4). Generally, these indices are classified into four groups, i.e., those based on (a) protein amino acid composition (chemical score and essential amino acid index); (b) nitrogen absorption and/or retention (biological value, net protein utilization and digestibility); (c) ability of the protein to produce growth in test animals, usually rats (protein efficiency ratio); and (d) ability of the protein to provide amino acids for the synthesis or replacement of body tissue protein (nitrogen balance methods). Of these, perhaps most limited are the chemical indices, because they depend solely on amino acid composition and not amino acid availability, and thus may give misleading information. For example, it is known that soya protein quality is significantly affected by processing under conditions that have little or no effect on its amino acid composition. The remaining indices, which all are of a biological nature, do provide a reasonable measurement of functional protein quality, and each is useful in comparing proteins from different points of view, according to the nature of each index.

Although all indices have been used in protein quality work, three have been especially useful: protein efficiency ratio (PER), nitrogen balance methods and digestibility.

The PER, first proposed by Osborne and Mendel (1), remains the most widely used technique today for biological evaluation of proteins. It is defined as the weight gain of a growing animal, usually the rat, divided by its protein intake, and when conducted under standard conditions, is capable of yielding fairly accurate and reproducible results (5). By including casein as a control and relating the observed PER of the experimental groups to casein (whose PER is usually standardized at 2.5), meaningful comparisons between laboratories can be made. Table I shows typical PER values of different proteins.

The nitrogen balance method evaluates the ability of a protein to provide amino acids for the synthesis or replacement of body tissue protein. This can be done in the intact animal by comparing the quantity of nitrogen ingested with the amount which is excreted in the urine and feces. The