Zeitschrift für
Ernährungswissenschaft Z Ernährungswiss 26:219–225 (1987)

Dietary cottonseed protein and cholesterol metabolism

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Summary: Low-gossypol, glandless cottonseed protein may become a major product for human consumption. In this presentation, the influence of cottonseed protein on several aspects of cholesterol metabolism will be described. Studies with rabbits and rats have shown that dietary cottonseed protein very effectively lowers the concentration of plasma cholesterol when compared to the animal protein casein. No such effect has been found in hamsters, but cottonseed protein may slightly elevate the percentage of serum total cholesterol carried by the high density lipoproteins. There is suggestive evidence that in humans the replacement of animal proteins in the diet by cottonseed protein causes a small decrease in serum cholesterol levels, but more studies are required to substantiate this. In hamsters dietary cottonseed protein effectively suppresses the formation of gallstones when compared to casein. On diets containing cottonseed protein the concentrations of both cholesterol and phospholipids in bile fluid are significantly decreased. We tentatively suggest that the consumption of cottonseed protein will have a favorable effect on cholesterol metabolism.

Zusammenfassung: Eiweiß aus Baumwollsamen, das wenig Gossypol enthält, könnte für die Humanernährung ein wichtiges Produkt werden. In diesem Artikel wird der Einfluß von Eiweiß aus Baumwollsamen auf den Cholesterinstoffwechsel beschrieben. Versuche mit Kaninchen und Ratten zeigten, daß Baumwollsameneiweiß die Serumcholesterinkonzentration im Vergleich zum tierischen Eiweiß, Kasein, sehr stark erniedrigt. Dieser Effekt wurde bei Hamstern nicht beobachtet, aber Baumwollsameneiweiß erhöhte den Anteil des Gesamtserumcholesterins in der HDL-Lipoproteingruppe ein wenig. Es gibt Hinweise dafür, daß bei Menschen der Ersatz von tierischem Eiweiß durch Baumwollsameneiweiß in der Nahrung eine geringe Abnahme des Serumcholesterinspiegels verursacht, jedoch sind noch Studien erforderlich, um dies zu untermauern. Bei Hamstern unterdrückt Baumwollsameneiweiß, im Vergleich mit Kasein, die Bildung von Gallensteinen. Bei Diäten, die Baumwollsameneiweiß enthalten, sind sowohl die Konzentration von Cholesterin sowie auch von Phospholipiden in der Gallenflüssigkeit signifikant erniedrigt. Wir vermuten, daß der Konsum von Baumwollsameneiweiß einen günstigen Effekt auf den Cholesterinmetabolismus hat.

Key words: dietary cottonseed protein, serum cholesterol, gallstones

Introduction

Cottonseeds may be higher in protein than components of any other type of plant. These seeds contain approximately 22 % protein, which is of

relatively good nutritional quality compared to that of other major oilseeds. However, the traditional varieties of cottonseed contain gossypol, a polyphenolic compound that is toxic to man. With the development of a low gossypol, glandless cottonseed variety, the protein has become available for human consumption (2). Researchers and food producers are enthusiastic about foods containing cottonseed protein (4), and it is anticipated that glandless cottonseed protein will become a major product on the protein food market.

Epidemiologic data from different countries have shown a strong positive correlation between coronary heart disease and consumption of animal protein (15). Since the intake of animal protein is strongly associated with that of total dietary fat and cholesterol, it is difficult to ascertain the etiological significance of this correlation. Despite this, there is some evidence from studies with hypercholesterolemic human subjects that replacement of animal protein in the diet by soybean protein lowers the level of serum cholesterol (14, 22), which is a major risk factor for coronary heart disease. Other studies (5, 19) however, failed to demonstrate a clear effect of the type of dietary protein on serum cholesterol in humans.

Animal studies have been more conclusive. It has been repeatedly shown that rabbits fed on the animal protein casein, develop a marked degree of both hypercholesterolemia and atherosclerosis, when compared to rabbits fed soybean protein (21). There are marked differences between animal species in the level of their serum cholesterol response to the nature of the protein in the diet (20, 21). However, under appropriate conditions dietary casein, when compared to soybean protein, induces hypercholesterolemia in many animal species (20).

Like coronary heart disease, gallstone disease has also become a major health and economic problem in many westernized countries. The gallstones typically found in individuals from these countries are composed of cholesterol. Amongst various dietary components, the nature of the protein has been implicated in gallstone formation. Kritchevsky and Klurfeld (7) have demonstrated in hamsters that casein induces a high incidence of gallstones, whereas when soybean protein is the protein source in the diet no such effect is seen. Replacement of casein by soybean protein was shown to cause dissolution of the casein-induced gallstones (7).

In this communication we describe the effects of dietary cottonseed protein on cholesterol metabolism. We will focus on serum cholesterol concentrations, the levels of biliary lipids, and the formation of gallstones.

Cottonseed protein and serum cholesterol

Most studies which have looked at the effects of dietary proteins on serum cholesterol concentration have used relatively pure preparations of casein and soybean protein for comparison. A study in which delipidated proteins from various sources were fed as part of cholesterol-free, semi-purified diets to rabbits showed that various plant proteins led to lower concentrations of plasma cholesterol than did casein (6). In these experiments (6) it was thus found that feeding of cottonseed protein resulted in low concentrations of plasma cholesterol, similar to those produced when soy proteins are fed (Table 1).

Table 1. Total plasma cholesterol concentrations in rabbits fed various proteins.

Dietary protein	Plasma cholesterol (mmol/l)
Casein	5.28 ± 1.14
Rapeseed protein	2.56 ± 0.28
Oat protein	1.99 ± 0.36
Cottonseed protein	1.97 ± 0.36
Sesame protein	1.81 ± 0.13
Soy protein isolate	1.74 ± 0.18
Sunflower protein	1.37 ± 0.31

Means ± SE for six animals per dietary group. The diets contained 25 % protein by weight, and they were fed for 28 days. Date are taken from Huff et al. (6).

Lower concentrations of serum cholesterol were also shown to occur in rats fed diets containing cottonseed protein as compared to animals fed diets containing casein (12). In this study arginine and lysine were added to casein and cottonseed protein, respectively, so as to duplicate the arginine to lysine ratio normally found in these proteins (lysine:arginine ratio in cottonseed protein is 0.36 and in casein it is 1.58). As the lysine:arginine ratio of the casein diet was altered to resemble cottonseed protein, it became less hypercholesterolemic. When the ratio was altered so that the cottonseed protein resembled casein, the plant protein became somewhat less hypocholesterolemic.

Diets containing cottonseed protein have been shown to enhance the excretion of neutral steroids in feces of rats when compared with a casein diet (11). Possibly, cottonseed protein reduces the absorption of intestinal cholesterol, which is of exogenous and/or endogenous origin. This may tend to lower liver cholesterol concentrations. The liver responds by an increase in the number of lipoprotein receptors and by enhancing de novo cholesterol synthesis. Indeed, in rats fed cottonseed protein cholesterol turnover is much faster than in their counterparts fed casein (11). The increased number of lipoprotein receptors induced by cottonseed protein is responsible for the fall in serum cholesterol. The lipoprotein cholesterol taken up by the liver can be used for bile acid synthesis. However, in order to prevent the body from depletion of cholesterol, de novo synthesis apparently has to be activated. A new steady-state will be reached, at which hepatic cholesterol synthesis is increased and fecal excretion of bile acids is also increased. Thus, cholesterol turnover is enhanced. At this new steady-state, serum cholesterol is low and the number of lipoprotein receptors high. Experimental evidence is needed to prove or disprove this description of the metabolic basis of the cholesterol lowering activity of cottonseed protein.

Table 2 shows the effects of dietary casein, soybean protein and cottonseed protein on the concentrations of serum total and high-density lipoprotein (HDL) cholesterol in golden Syrian hamsters. The diets used were essentially fat- and cholesterol-free, and contained 20 % (w/w) of protein and 74.3 % of sucrose. It is clear that the plant proteins from soybeans and

Table 2. Serum total and HDL cholesterol concentrations in hamsters fed various proteins.

	Duration of exp. (days)	Dietary protein	Total serum cholesterol (mmol/l)	HDL cholesterol (mmol/l)
Exp. 1	45	Casein (n = 16)	5.51 ± 0.28	
		Soybean (n = 19)	5.13 ± 0.13	
		Cottonseed (n = 16)	4.68 ± 0.23	
Exp. 2	35	Casein (n = 10)	2.71 ± 0.43	2.07 ± 0.35
		Cottonseed (n = 10)	2.95 ± 0.32	2.58 ± 0.29
Exp. 3	63	Casein (n = 12)	3.56 ± 0.20	2.53 ± 0.14
		Soybean (n = 10)	3.21 ± 0.16	2.63 ± 0.15
		Cottonseed (n = 11)	3.56 ± 0.18	2.77 ± 0.14

Results are expressed as means ± SE. Data are taken from Mahfouz-Cercone et al. (9), Richmond et al. (13), and Sullivan et al. (17).

cottonseeds do not significantly lower serum total cholesterol when compared to casein. Cottonseed protein versus casein however, may cause a slight increase in the percentage of total cholesterol carried in the HDL particles: 87 % versus 76 % in exp. 2, and 78 % versus 71 % in exp. 3 (Table 2). This may be important. In humans relatively high levels of HDL cholesterol protect against the development of atherosclerosis (10).

The data in Table 2 should be interpreted with caution. Non-protein components in the protein preparations may have influenced the concentration of cholesterol in the serum. Purity of the protein preparations varied from about 57 % (cottonseed protein in exp. 1) to about 85 % crude protein (all other protein sources). The protein preparations contained 0.5 to 3 % fat, and this may have been crucial, since no other source of fat was added to these diets. The hamsters, especially those on the casein diets, may have had suboptimal diets with regard to the provision of essential fatty acids.

The ultimate aim of such nutrition research with experimental animals is to provide further insight into practical means of decreasing the concentration of serum cholesterol in man through changes in dietary habits. We know of only two published studies in which serum cholesterol levels have been measured for human subjects consuming cottonseed protein. In one study (3), glandless cottonseed protein was substituted for 25 % of the conventional proteins in the diet of older subjects (n = 17) living in a nursing home. Residents (n = 14) of another nursing home served as a control group. Serum total cholesterol levels were found to be approximately 10 % lower in the experimental group (5.74 ± 0.33 versus 6.47 ± 0.38 mmol/l, means ± SE), but the difference failed to reach statistical significance. Thomas et al. (18) studied seven women, aged 18 to 23 years, on a mixed, natural-ingredient diet, containing glandless cottonseed protein as the sole source of protein. When compared to the habitual diet, the diet containing cottonseed protein caused an 11% decrease in serum total cholesterol (4.79 ± 0.29 versus 5.41 ± 0.34 mmol/l, means ± SE). The habitual diet probably differed from the experimental diet as to various

components. Thus, this study does not provide conclusive data about the effect of cottonseed protein per se.

It can be concluded that rabbits and rats fed cottonseed protein maintain lower levels of plasma cholesterol than those fed casein. In studies using hamsters, serum total cholesterol was not decreased on a diet containing cottonseed protein, when compared to a casein diet. The ratio of HDL to total cholesterol may be somewhat elevated in hamsters fed cottonseed protein. It is possible that the hamster is relatively insensitive to the type of dietary protein with respect to serum cholesterol. It is also conceivable that other components of the diet also determine the susceptibility to proteins in the diet. For instance, diets with a cholesterol base may enhance the differential cholesterolemic effects of dietary casein and soybean protein in hamsters (1). In humans, cottonseed protein in the diet may slightly decrease serum cholesterol levels, but more work is needed to substantiate this, and also to establish whether such an effect could really contribute to the prevention of coronary heart disease.

Cottonseed protein and gallstones

As far as we know, the effect of cottonseed protein on gallstone formation and biliary lipid composition has only been studied using hamsters. Table 3 summarizes these effects. Although the absolute incidences of gallstones are not reproducible from one experiment to another, cottonseed protein was consistently very effective in the prevention of gallstone formation when compared to casein. Cottonseed protein also consistently produced low concentrations of both phospholipids and cholesterol in bile fluid. It would appear that cottonseed protein, when compared to soybean protein, has a specific effect on biliary phospholipids. The concentration of biliary bile acids is not systematically influenced by cottonseed protein (Table 3).

Table 3. Gallstone formation and biliary lipid composition of hamsters fed various proteins.

			Biliary lipids (mmol/l)		
	Dietary protein	Gallstone incidence (%)	Bile acid	Phospho-lipids	Cholesterol
Exp. 1	Casein (n = 16)	100	140 ± 15	17 ± 2	6.3 ± 0.5
	Soybean (n = 19)	32	141 ± 7	17 ± 1	5.2 ± 0.03
	Cottonseed (n = 16)	0	117 ± 8	12 ± 1	3.0 ± 0.2
Exp. 2	Casein (n = 10)	63	126 ± 16	26 ± 4	3.8 ± 0.5
	Cottonseed (n = 10)	0	137 ± 12	15 ± 2	1.7 ± 0.3
Exp. 3	Casein (n = 12)	25	152 ± 10	17 ± 0.2	6.0 ± 0.9
	Soybean (n = 10)	0	233 ± 18	17 ± 0.4	4.3 ± 0.5
	Cottonseed (n = 11)	8	241 ± 14	14 ± 0.9	4.7 ± 0.4

See legend to Table 2.

From the data presented in Table 3 it would appear that dietary cottonseed protein effectively suppresses the formation of gallstones in hamsters. Again, it should be stressed that the protein preparations were not completely pure, and that components other than the protein could have influenced the outcome. Kritchevsky and coworkers (8) have recently shown, using the hamster model, that the addition of lysine to the diet containing soybean protein, and the addition of arginine to the casein diet, caused a reduction and increase in the incidence of gallstones, respectively, when compared to the proteins alone. Since the ratio of lysine to arginine in casein is higher than in soy protein, these amino acid supplements made this ratio in the casein diet more comparable to that in the soy protein diet, and vice versa. Thus, intact proteins may determine the incidence of gallstones because of their specific amino acid composition.

Practical considerations

There is considerable evidence that high serum cholesterol levels cause atherosclerotic diseases. In order to lower serum cholesterol, it is widely recommended to limit cholesterol intake and to increase the intake of polyunsaturated fats at the expense of saturated fats. Perhaps, the latter intervention may at the same time enhance the risk of gallstone formation (16). We realize that caution is warranted in extrapolating data from animals to man, but we feel that cottonseed protein could have a slight, advantageous effect on the concentration of serum total cholesterol, and perhaps also on the distribution of cholesterol between serum lipoprotein fractions. In addition, dietary cottonseed protein might help to prevent gallstone formation and/or dissolve existing stones. From the practical point of view, partial substitution of the vegetable protein from cottonseeds for animal protein will also have indirect, favorable effects on cholesterol metabolism, as such diets will automatically be low in saturated fat. The encouragement of the consumption of glandless cottonseed protein is reinforced by the fact that this protein has been shown to be of relatively high nutritional quality (2–4, 18).

Acknowledgements

This work was supported by grants from the Natural Fibers and Food Protein Commission of Texas, Dallas, TX, and Cotton Inc., Raleigh, NC, USA. We thank Ineke Zaalmink for typing this manuscript.

References

1. Beynen AC, Schouten JA (1983) Nutr Rep Int 28:835
2. Blankenship DC, Alford BB (1983) Cottonseed: The New Staff of Life. TWU Press, Denton, Texas
3. Cater CM, Mattil KF, Meinke WW, Taranto MV, Lawhon JT, Alford BB (1977) J Am Oil Chem Soc 54:90A
4. El-Shaarawy MI, Mesallam AS (1987) Z Ernährungswiss 26:100
5. Grundy SM, Abrams JJ (1983) Am J Clin Nutr 38:245
6. Huff MW, Hamilton RMG, Carroll KK (1977) Atherosclerosis 28:187
7. Kritchevsky D, Klurfeld DM (1979) Am J Clin Nutr 32:2174

8. Kritchevsky D, Weber MM, Klurfeld DM (1984) Nutr Rep Int 29:117
9. Mahfouz-Cercone S, Johnson JE, Liepa GU (1984) Lipids 19:5
10. Miller NE (1978) Lipids 13:914
11. Park M-SC, Kudchodkar BJ, Liepa GU (1987) J Nutr 117:30
12. Park M-SC, Liepa GU (1982) J Nutr 112:1892
13. Richmond AS, DiMarco NM, Stroebel MG, Liepa GU (1984) Nutr Rep Int 30:1069
14. Sirtori CR, Agradi E, Conti F, Mantero O, Gatti E (1977) Lancet 1:275
15. Stamler J (1979) In: Levy R, Rifkind B, Dennis B, Ernst N (eds) Nutrition, Lipids, and Coronary Heart Disease. Raven Press, New York, p 25
16. Sturdevant RAL, Pearce ML, Dayton S (1973) New Engl J Med 288:24
17. Sullivan MA, Duffy A, DiMarco N, Liepa GU (1985) Lipids 20:1
18. Thomas MR, Ashby J, Sneed SM, O'Rear LM (1979) J Nutr 109:397
19. Van Raaij JMA, Katan MB, West CE, Hautvast JGAJ (1982) Am J Clin Nutr 35:925
20. West CE, Beynen AC (1985) In: Galesloot TE, Tinbergen BJ (eds) Milk Proteins '84. Pudoc, Wageningen, p 80
21. West CE, Beynen AC, Terpstra AHM, Scholz KE, Woodward CJH, Carroll KK (1983) Atherosclerosis 46:253
22. Wolfe BM, Giovanetti PM, Cheng DCH, Roberts DCK, Carroll KK (1981) Nutr Rep Int 24:1187

Received August 3, 1987

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