

Alimentary proteins, amino acids and cholesterolemia

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Abstract Numerous data from both epidemiological and experimental origins indicate that some alimentary proteins and amino acids in supplements can modify the blood LDL cholesterol, HDL cholesterol and total cholesterol. After an initial approval of the health claim for soy protein consumption for the prevention of coronary heart disease, more recently it has been concluded from an overall analysis of literature that isolated soy protein with isoflavones only slightly decrease LDL and total cholesterol. Other plant extracts and also some proteins from animal origin have been reported to exert a lowering effect on blood cholesterol when compared with a reference protein (often casein). The underlying mechanisms are still little understood. Individual amino acids and mixture of amino acids have also been tested (mostly in animal studies) for their effects on cholesterol parameters and on cholesterol metabolism. Methionine, lysine, cystine, leucine, aspartate and glutamate have been tested individually and in combination in different models of either normo or hypercholesterolemic animals and found to be able to modify blood cholesterol and/or LDL cholesterol and/or HDL cholesterol. It is however not known if these results are relevant to human nutrition.

Keywords Alimentary proteins · Amino acid supplementation · Cholesterol

Introduction

In the course of studies related to metabolic interactions between nutrients, there have been several studies beginning in the late 1960s, which have examined the influence of dietary proteins from various origins and also of amino acids tested individually or as mixtures on the blood concentrations of total cholesterol, low-density lipoproteins and high-density lipoproteins. These studies have been conducted both in human volunteers and in different experimental animal models in order to gain knowledge on the type of dietary nitrogenous compounds, which may contribute to lower the risk of coronary heart diseases. Briefly, elevated plasma levels of low-density lipoproteins (LDL) and triglycerides present a risk for cardiovascular disease. In contrast, high-density lipoproteins (HDL) are believed to be beneficial. A low ratio of LDL to HDL and low plasma triglyceride levels decrease the risk of cardiovascular disease (Friedman and Brandon 2001). Overall, the studies related to protein and amino acid ingestion and cholesterolemia have led mostly to descriptive results with little mechanistic data. In this review paper, we will examine firstly the effects of various alimentary proteins on cholesterol-related parameters and secondly the effects of several amino acids on such parameters.

Alimentary proteins and cholesterol

There have been several reports on the effects of dietary plant protein extracts on the cholesterolemia in both human

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and animal studies. Most studies were performed using soy protein extracts. This is probably because according to epidemiological investigations (Anderson et al. 1995), consumption of soy foods has been proposed as being able to contribute in lowering the risk of coronary heart disease. Animal (Lin et al. 2004; Moriyama et al. 2004) and human (Anthony et al. 1998) studies have reported that consumption of soy protein and/or isoflavones has beneficial impacts on the risk factors associated with cardiovascular disease, including the lowering of total and LDL cholesterol levels and increasing the HDL cholesterol and/or the HDL/LDL cholesterol ratio. In 1999, the food-labeling health claim for soy proteins in the prevention of coronary heart disease was approved by the US Food and Drug Administration (1999) (FDA). Similar health claims for soy protein have also been approved in various countries including the UK, Brazil, Japan, South Africa, Philippines, Malaysia, Korea, etc. (Xiao 2008). However, the studies on the effects of soy protein extracts have led to rather heterogeneous results, as will be described later in this review. The Nutrition Committee of the American Heart Association has assessed 22 randomized trials and concluded that, overall, isolated soy protein with isoflavones slightly decreased LDL cholesterol, but has no significant effect on HDL cholesterol (Sacks et al. 2006). The decrease in blood cholesterol concentration related to soy-based plant protein represents approximately between 6 and 12% of total cholesterol (Friedman and Brandon 2001). Although the individual factors that could explain the discrepancies in the reported results are not clear, it is possible that the source of soybeans and processing of the protein extracts are important parameters because of their consequences on the concentrations of bioactive peptides and other minor components in the soy extracts, which may likely play a role in the effect of soy-based protein extracts.

An early human study on the cholesterol-lowering effect was reported in 1967 (Hodges et al. 1967). This study reported that replacement of mixed proteins by isolated soy protein extracts at a dose of 100 g/day reduced cholesterol levels in hypercholesterolemic men. On the basis of a food-frequency questionnaire given to 4,800 Japanese men and women, Nagata et al. (1998) noted decreasing total cholesterol plasma concentrations with increasing consumption of soy products. In another epidemiological study, it was found that consumption of soy-based diet induced a decrease in the plasma level of LDL cholesterol of both normocholesterolemic and hypercholesterolemic men (Wong et al. 1998). The extent of LDL cholesterol-lowering by soy-based diets was dependent on the concentrations of naturally occurring estrogenic isoflavones (Crouse et al. 1999). Potter et al. (1998) and Washburn et al. (1999) investigated the role of soy isoflavones on the risk of cardiovascular disease and menopausal symptoms in women

consuming soy-based diets. They reported improvement in lipid and lipoprotein levels. A double-blind study suggests that even partial replacement of animal protein with soy protein was effective in lowering plasma cholesterol in 21 severely hypercholesterolemic patients (Sirtori et al. 1999). Two studies have shown that in hypercholesterolemic subjects following a diet with a poor content in fat and a relatively high content in fibers, dietary plant protein extracts obtained mainly from soya exerted no significant beneficial effect in term of hypercholesterolemia (Cuevas et al. 2003; West et al. 2005). Inversely, other studies clearly showed a significant reduction of LDL cholesterol in hypercholesterolemic subjects, who consumed a diet with low lipid content and receiving dietary plant protein extracts that originated mainly from soya (Tonstad et al. 2002; Teixeira et al. 2000). The dose effect of these proteic preparations was detectable only after 3 weeks of consumption and was no more detectable after 6 weeks (Teixeira et al. 2000) or after 8–16 weeks (Tonstad et al. 2002). The efficiency of the protein extracts on hypercholesterolemia was similar using 30 or 50 g proteins (Tonstad et al. 2002; Teixeira et al. 2000). In their publication, Teixeira et al. (2000) concluded that a 20 g dose is able to reduce the non-HDL cholesterol by 2.6% after 6 weeks of treatment.

Studies on animal models

In the work reported by Ni et al. (1998), mice were fed a purified diet containing 1 g/100 g cholesterol and cholate (0.25 g/100 g) for 6 weeks together with soy protein isolate or casein as the protein source. In this study, although serum total cholesterol was similar in both groups, the lesion area in the thoracic area of the soy protein group was lower than that of the casein group. In a study published in 2002 (Kern et al. 2002), the effects of soy protein isolate versus casein on blood lipids and adiposity were investigated in rats fed with diets supplemented with L-methionine. As a result, total blood cholesterol concentration was higher for the casein-fed rats than for the soy protein isolate-fed rats suggesting that methionine supplementation did not abolish the hypocholesterolemic effect of soy. In the paper by Horigome and Cho (1992), a diet containing 22.5–23.5 g/100 g of soybean protein was hypocholesterolemic in rats when compared with a diet containing 20.0 g casein/100 g diet. A comparison of serum amino acids in soybean protein-fed versus casein-fed rats showed that whereas concentrations of many amino acids were lower in the soybean protein-fed rats compared with the casein-fed groups, glycine was the only amino acid having a higher concentration. However, this study did not demonstrate a causal link between hypocholesterolemia and modified glycine concentration.

In the study by Potter et al. (1996), the objective was to determine if serum cholesterol concentration differed in the hamster model feeding soy protein from different sources in comparison with caseins. The authors found that serum total cholesterol concentrations were lower in animals fed on isolated soy protein when compared with animals fed on soy protein concentrate. Both of the diets containing soy proteins were able to induce a decrease in blood cholesterol concentration when compared with caseins.

Taken as a whole, it appears that the effects on cholesterolemia attributed to soy-based plant protein extract are most likely complex and may be relevant to a synergistic effect of various soy compounds (Anderson et al. 1995; Francis et al. 2002) including nonprotein compounds such as saponins, fiber, phytic acid, minerals and, last but not least, isoflavones (Potter 1995). But in the case of soy-based plant protein extracts depleted in isoflavones, several authors suggest that the relatively low content of methionine in soy protein would play a role in the hypocholesterolemic effect measured (Morita et al. 1997; Kern et al. 2002).

From a general point of view, the mechanisms underlying the effects of soy-based plant protein extracts may be a decrease in intestinal cholesterol absorption and/or biliary salts, an increase of cholesterol clearance by hepatic LDL receptors and/or changes in hepatic cholesterol metabolism and/or lastly endocrine modifications (Potter 1995; Potter et al. 1998; Belleville 2002; Adams et al. 2002; Baum et al. 1998).

In addition, the situation is further complicated by the fact that interaction between the protein fraction and other compounds of diet may modify the effects of the protein fraction on cholesterol metabolism. For instance, in the work presented by Nagaoka et al. (1999), the authors have studied in rats the effects of casein, soy protein, soy protein with bound phospholipids (SP), soy protein peptic hydrolysate (SPH) or soy protein peptic hydrolysate with bound phospholipids (SPHP) on the plasma and liver cholesterol. Serum total cholesterol was lower in rats fed with SPHP than in those fed with casein. The concentration of cholesterol in liver was lower in the SPHP-fed group compared with all other groups.

Several works strongly suggest that the beta-conglycinin fraction in soya proteins would be implicated in the atheromatous process in mice. However, and in contrast with previous results, this effect would not be explained by an action upon the LDL receptors and also not by an effect through the plasma lipoproteins (Adams et al. 2002; Adams et al. 2004).

In addition, it is worth noting that other plant protein extracts than soy-based protein extract and also protein from animal origin have been reported to exert a lowering effect on blood cholesterol. This includes meat proteins, fish

proteins and whey proteins (Nagaoka et al. 1991, 1992; Zhang and Beynen 1993; Anderson et al. 1999; McCarty 1999; Washburn et al. 1999; Tomatoko et al. 2000; Wang et al. 2004; Wergedahl et al. 2004; Zhan and Ho 2005; Choi et al. 2005; Mayilvaganan et al. 2004; Debry 2004). In a recent study by Shukla et al. (2006), rats were fed with diets containing fish protein or casein as control and cholesterol in the HDL fraction was determined. This parameter was decreased after fish protein consumption when compared with casein consumption. In contrast, in postmenopausal women, lean white fish compared with other animal protein products induced higher concentration of plasma cholesterol and HDL cholesterol (Jacques et al. 1995). In the study by Zhang and Beynen (1993), the effect of dietary whey protein versus casein on plasma and liver cholesterol concentrations was determined in rats. At a low dietary protein level, whey protein versus caseins did not affect plasma total cholesterol, but lowered the concentration of liver cholesterol. At the high dietary protein level, whey protein lowered plasma and liver cholesterol. This indicates that the effects of soy protein extract versus a reference protein (often casein) on cholesterol blood concentration depend on the amount of proteins in the diet. Therefore, once again, if it appears that the nature of proteins can exert an effect on the blood cholesterol level (at least modestly), the underlying mechanisms remain unclear.

Amino acids and cholesterol

Individual amino acids and mixture of amino acids have been tested for their effects on cholesterol concentrations in blood and tissues as well as on cholesterol metabolism, mostly in animal studies.

Methionine and lysine

From early studies performed using the rabbit model, it was found that high levels of casein or casein amino acids increased serum total and LDL cholesterol. However, this was not observed for soy protein and soy amino acids (Huff et al. 1977; Kurowska et al. 1989). The hypercholesterolemic potential of casein was presumed to be due to its high content in the essential amino acids, rather than nonessential amino acids (Kurowska and Carroll 1990). A working hypothesis was built presuming that alterations in plasma cholesterol concentration could be mediated by differences in the amino acid patterns of the dietary proteins. Indeed, methionine is one of the amino acids that is believed to contribute to hypercholesterolemia in animal models due to the fact that this latter amino acid is more abundant in casein than in soya protein.

Further experiments reported that lysine in combination with methionine, but not lysine or methionine used separately, were the most hypercholesterolemic (Kurowska and Carroll 1994; Giroux et al. 1999). In accordance with these results, once again relatively high levels of lysine and methionine were found in dietary casein and other animal proteins, which are found to be hypercholesterolemic in animal models (Carroll 1981). In a study, Kurowska and Carroll (1994) using the rabbit model reported that a high level of dietary lysine, leucine and methionine produced substantial hypercholesterolemia. In the same study, a combination of lysine and methionine produced a greater effect than either a mixture of lysine and leucine or a mixture of leucine and methionine. The mechanisms by which lysine and methionine modulate cholesterolemia in the rabbit model was studied with special emphasis on the liver metabolism. The results suggested that these amino acids can act directly in the liver by regulating both synthesis and catabolism of LDL protein (Kurowska and Carroll 1992, 1996). Using another animal model i.e., the gerbil model, Hidioglou et al. (2004) reported that a combination of dietary lipids (saturated and unsaturated fatty acids) as well as methionine supplementation increased both serum total cholesterol and LDL cholesterol. In their study, Hirche et al. (2006) have examined the effect of dietary methionine on plasma and liver cholesterol concentrations using the rat model. They found that rats fed with 3.5 g/kg (or more) methionine had higher cholesterol concentrations in plasma and in liver than rats fed with lower (i.e., 2.6 g/kg) methionine. Interestingly, isolated hepatocytes incubated in media supplemented with 100 or 200 $\mu\text{mol/L}$ methionine had a higher cholesterol synthesis than hepatocytes incubated in a medium supplemented with 50 $\mu\text{mol/L}$ methionine. However, the LDL uptake in hepatocytes was independent of the methionine concentration in the medium. This study is a good indication that in the rat model, dietary methionine induces hypercholesterolemia, in part at least via an enhanced hepatic cholesterol synthesis. In the mouse model, Velez-Carrasco et al. (2008) reported that when comparing two solid food diets; one containing 2% added methionine and the other containing 2% added glycine, the methionine diet provoked a decrease in HDL-cholesterol level. Furthermore, these authors determined that using an HDL turnover study, the HDL-cholesterol production rate was reduced in mice fed on a methionine diet.

Leucine

A study by Zhang et al. (2007) reported that in the mouse model, a leucine supplementation in a high fat diet provided ad libitum decreases markedly in the plasma levels of

total and LDL cholesterol when compared with the control experiments, i.e., mice fed on the same diet without leucine. Although the mechanisms involved in such an effect remain unclear, the study determined that the effect of leucine on plasma cholesterol coincided with a reduction in weight gain (without changes in food intake), a decrease in adiposity, and an increase in energy expenditure together with an increase in the expression of uncoupling protein 3 (UCP3). UCP3 mediates mitochondrial uncoupling of oxidative phosphorylation in thermogenic tissues, therefore likely accounting for increased energy expenditure with reduced fuel efficiency in skeletal muscle and adipose tissues.

Some attention was given to the effects of isolated amino acid supplementation on cholesterolemia by (Ohtani et al. 2001). In this latter study, an amino acid mixture containing L-leucine, L-isoleucine, L-valine, L-arginine and L-glutamine was given to 23 rugby players twice a day for 90 days (3.6 g of total amino acids per dose). After this supplementation period, an increase in total cholesterol and LDL in blood was detected. However, as noted by the authors, the blood cholesterol concentrations were maintained in the normal range, i.e., less than 200 mg/dl (Ohtani et al. 2001). Importantly, Ohtani et al. reported that 8 among the 23 volunteers noted an increase in appetite. This points to possible changes in the food intake for some individuals in the course of experiments, changes which may explain (in part at least) some of the reported data. Unfortunately, food intakes were not reported in this publication. Also, the amino acid supplementation gave rise to an increase in hemoglobin and iron blood concentration. This was interpreted by the authors as an enhancement of hematopoiesis capacity due to the amino acid mixture supplementation (Ohtani et al. 2001). However, in our opinion, an increased hematopoiesis would have presumably led to an increased iron requirement. Finally, it is tempting to speculate that the amino acid supplementation performed by Ohtani et al. may have modified the food intake in some individuals and, as a consequence, may have participated in the changes observed in several biochemical and hematological parameters including total cholesterol and LDL cholesterol.

Recently, Zanchi et al. (2008) have published a review on the antiproteolytic effects of leucine emphasizing the inhibitory effect of leucine supplementation on skeletal muscle proteolysis. Several data presented suggest that the concentration of leucine, which is able to diminish protein degradation may be higher than the one required in order to maximally stimulates protein synthesis. This proposition is likely mostly relevant in atrophic conditions. Leucine catabolism is regulated by either of the first degradative steps: transamination to the keto acid alpha-ketoisocaproate (KIC) or subsequent

decarboxylation. In an elegant and classical study, Matthews et al. (1981) using in volunteers L-leucine with ^{15}N and ^{13}C double labeling, demonstrated that leucine transamination was found to operate several times faster than the keto acid decarboxylation. Furthermore, it was determined in this latter study that whatever the dietary conditions, i.e., postabsorptive state and fed conditions; the keto acid decarboxylation was of similar magnitude (Matthews et al. 1981). Overall, these findings indicate that leucine metabolism is more related to the transamination pathway than to the decarboxylation pathway. In the course of leucine catabolism, beta-hydroxy-beta-methylbutyrate (HMB) can be formed from leucine. HMB is produced from KIC by the cytosolic enzyme KIC-dioxygenase (Sabourin and Bieber 1983). Numerous studies have reported that HMB is a precursor for cholesterol biosynthesis (Bloch 1944; Rabinowitz 1955; Zabin and Bloch 1951). HMB in the liver and muscle cytosol is converted to beta-hydroxy-beta-methylglutarate CoA (HMGCoA), which can then be utilized for cholesterol biosynthesis (Rudney 1957). In stressed or damaged muscles, it has been proposed that an insufficient synthesis of HMGCoA may limit adequate cholesterol synthesis, thus impairing the proper functioning of cell membranes (Nissen et al. 2000). In the work of Nissen et al. (2000), human volunteers received HMB supplementation (3 g/day) for 3–8 weeks. The authors found that when compared with the control group, HMB supplementation resulted in a 6% decrease in total cholesterol, 7% decrease of LDL cholesterol and a decrease in systolic blood pressure. It thus appears that the role of leucine on cholesterol metabolism is associated with different effects on cell and blood cholesterol. These effects of HMB on surrogate markers of cardiovascular functions lead the authors to propose a possible effect of HMB in decreasing the risk of heart attack and stroke (Nissen et al. 2000).

Cystine

Several works reported on the effect of cystine on cholesterol metabolism. In the study by Aoyama et al. (1999), it was determined that hypercholesterolemia caused in rats by feeding a high-cystine diet was probably due to the stimulation of hepatic cholesterol synthesis. Similar findings were reported in the study by Sérougne et al. (1995), which described hypercholesterolemia in rats induced by dietary cholesterol or by increasing cholesterogenesis in cystine-fed rats. This hypercholesterolemia was characterized by different plasma lipoprotein and apolipoprotein concentration and was associated with different apolipoprotein gene expression in the liver. In a study by Sérougne

et al. (1987), rats were fed a cystine-enriched diet and it was determined that this diet increased hepatic cholesterogenesis.

Taurine

In a study by Yokogushi and Oda (2002), it was determined that dietary taurine enhances cholesterol degradation and reduces serum and liver concentration in rats fed on a high-cholesterol diet. The results presented are compatible with the hypothesis that taurine exerts a decreasing effect on cholesterolemia, partly via excretion of bile acid. Similar results were found by Choi et al. (2006) also in the rat model. In this latter study, rats in the control group received a 1.5% cholesterol diet and those in the experimental group received a diet containing 1.5% cholesterol and 1.5% taurine-supplemented diet. In the taurine group, it was found that total cholesterol as well as LDL-cholesterol were reduced and HDL-cholesterol was increased when compared with the data obtained in the control group. Also, the rats receiving taurine showed decreased hepatic content in cholesterol. Overall, these data suggest a role for taurine in rats receiving a high-cholesterol diet. A third study performed on rats (Yamori et al. 2004) reported an effect of dietary taurine in hypercholesterolemic animals. Indeed, taurine was able to lower blood cholesterol concentration in these experiments.

Aspartate and glutamate

In the work by Yanni et al. (2005), the authors examined the effect of L-aspartate and L-glutamate on serum lipoprotein cholesterol in rabbits fed with high-cholesterol diet using the rabbit model. The animals received a cholesterol/corn oil diet in the control group and the same diet supplemented with aspartate and glutamate for 4 weeks. The animals receiving the amino acid supplement were characterized by an increase in serum HDL-cholesterol. However, no differences were found between the two groups of animals for the serum total cholesterol, LDL-cholesterol and triglycerides.

Conclusion

1. Based on epidemiological studies and some experimental data, in 1999, the US FDA has approved the food-labeling health claim of soy protein in the prevention of coronary heart disease.
2. However later on, the Nutrition Committee of the American Heart Association released a review of

22 randomized trials and concluded that, overall, isolated soy protein with isoflavones slightly decrease LDL cholesterol and total cholesterol. The decrease in blood cholesterol concentration following soy-based protein extracts does not represent more than 6–12% of total cholesterol.

3. It is likely that the source of soybeans and processing of the protein extracts are important for explaining the heterogeneity of the data obtained, since the concentrations of bioactive peptides and other minor components in the soy extracts are different according to the process used.
4. Taken as a whole, it appears that the effect of soy extracts on cholesterolemia is complex and relevant to a synergistic effect of various soy compounds including amino acid composition and nonprotein compounds like isoflavones, fiber, minerals, etc.
5. Other plant extracts than soy-based protein extracts and also protein from animal origin have been reported to exert a lowering effect on blood cholesterol when compared with a reference protein (often casein). The underlying mechanisms are little understood at the present time.
6. Individual amino acid and a mixture of amino acids have been tested (mostly in animal studies) on cholesterol blood and liver concentrations and metabolism. Methionine and lysine have been used in the rabbit model and found to provoke hypercholesterolemia. Although this effect was also found in other experimental animals (rat, gerbil), the relevance of these results to human nutrition remains putative.
7. In the mouse model, leucine supplementation is able to markedly reduce the plasma level of total and LDL cholesterol, but the underlying mechanisms remain to be determined in a causative perspective.
8. In experimental animal models, high dietary supplementation with cystine is associated with hypercholesterolemia. In contrast, taurine supplementation is able to decrease blood cholesterol concentration in hypercholesterolemic animals
9. Aspartate and glutamate supplementation is able to increase HDL-cholesterol in the rabbit model.

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