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New dietary proteins for cholesterol control: lupin and hempseed

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ABSTRACT

The consumption of dietary proteins from vegetable sources can be very helpful in the dietary management of hypercholesterolemia. While it is well established that soy proteins can reduce LDL-cholesterol, particularly in patients with high cholesterolemia, novel protein sources have lately attracted much interest. Recent studies have clearly established the nutraceutical properties of eggs and egg peptides, which have significant effects on both cholesterolemia and blood pressure. In the last few years, extensive studies have shown that lupin and hempseed proteins have good cholesterol-lowering properties. Lupin, particularly *Lupinus angustifolius*, is characterized by peptides that can significantly raise LDL-receptor activity and also reduce PCSK9 levels, thus providing very effective treatment for hypercholesterolemic patients when used as substitutes or additives for standard drugs. More recently, hempseed, a protein source rarely evaluated clinically, has been shown to reduce cholesterolemia in animal models, by inhibiting HMG-CoA reductase activity, upregulating LDL receptors and, surprisingly, also increasing PCSK9 levels, with an overall profile similar to that of statins. These novel additions to the nutraceutical armamentarium for treating raised cholesterol may lead to exciting progress in the management of hypercholesterolemic patients.

Keywords

Nutraceutical proteins
Soy
Lupin
Hempseed
Cholesterolemia
LDL receptors
PCK9 activity
Atherosclerosis
Peptides

Introduction

Changes in the types of dietary protein consumed have significantly influenced the treatment of hypercholesterolemia over the last 40 years. In the earliest study [1], it was shown that in severely hypercholesterolemic in-patients (mean cholesterol 322 mg/dl), total substitution of animal proteins with a soy protein-based diet over a period of 6 weeks (3-week crossover from animal to soy proteins and vice versa) lowered total cholesterol by a mean of 21% and LDL-cholesterol by a mean of 23% compared with an animal protein diet. The study was appropriately designed, since compliance was 100% guaranteed, and also evaluated the addition of cholesterol (given as 500 mg/day in lyophilized egg yolk) which was found to not modify the cholesterol-lowering effect of soy proteins.

At that time, soy proteins were the best available means for reducing cholesterolemia, as drug alternatives consisted of anion exchange resins (cholestyramine, colestipol) with poor palatability and very low compliance. In this earliest study, there was clear evidence of a correlation between the severity of hypercholesterolemia and plasma cholesterol reduction by soy. A later meta-analysis by Anderson *et al* [2] showed that a mean daily intake of 48 g of soy proteins was associated with a mean total cholesterol reduction of –13 to –32.9 mg/dl (–9.3%) and an LDL-cholesterol reduction of 12.9% with modest changes in triglycerides and HDL.

The Anderson meta-analysis was performed when statins were starting to be prescribed in daily practice. This dramatic change in drug approach made it increasingly difficult to evaluate the cholesterol-lowering activity of soy or other proteins in severe hypercholesterolemia as determined in the earlier studies. However, the Anderson study [2] provided a nomogram based on quartiles of initial cholesterol and

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corresponding confidence intervals (CI) for plasma cholesterol changes. This nomogram allowed evaluation of whether the soy protein-associated cholesterol reduction was maximal in hypercholesterolemic individuals and minimal to non-existent in normolipidemics. This was indeed found to be the case [3] (Fig. 1), clearly indicating that other proteins might also have the same therapeutic potential when given in appropriate conditions.

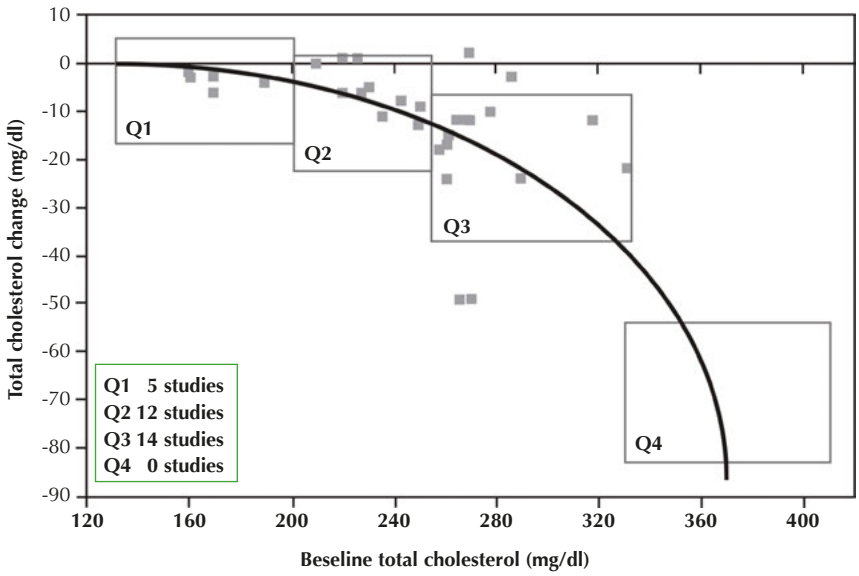


Figure 1 - Application of the Anderson nomogram to clinical studies on soy proteins. In the meta-analyses by Anderson *et al* [2], a correlation was reported between baseline cholesterolemia and cholesterol reduction. This nomogram was applied to more recent clinical studies, confirming that patients in the highest quartile of cholesterolemia show the best response to soy proteins [3]

Mechanism of soy protein effect on cholesterol reduction

The mechanism(s) by which soy proteins lower cholesterol levels have been extensively investigated. Since there is no evidence that these proteins cause sterol loss in the gastrointestinal tract [4], a possible mechanism inducing cholesterol reduction could be regulation of low-density lipoprotein (LDL) receptors [5]. Studies by our group and other investigators concluded that changing dietary proteins from animal to soy could lead to an increase in the expression of LDL receptors, mainly isolated in liver cells [6]. A study in familial severely hypercholesterolemic subjects found a dramatic cholesterol reduction when all animal protein in the diet was replaced with soy protein. The results clearly showed increased LDL receptors in lymphomonocytes, evaluated as parallel expression of liver LDL receptors [7].

Newer protein sources for hypercholesterolemia: from eggs to lupin to hempseed proteins

Interest in lowering cholesterolemia by additions to or modifications of the diet was highlighted in a previous issue of this journal [8] where the possible use of egg proteins was described. More recently, up-to-date clinical studies have focused on the possibility of reducing cholesterolemia by administering egg white fermented with lactic acid. This dietary addition reduced LDL cholesterolemia by 15% after 8 weeks [9], and interestingly, at 8 g/day for 12 weeks, also reduced visceral fat by 20% [10].

These positive results with egg white were accompanied by growing interest in other, mainly vegetable, dietary proteins. Foremost among these are lupin and hempseed proteins which may be clinically useful additions to the diet.

Lupin proteins

There are four main species of lupin: *Lupinus albus, L. angustifolius, L. luteus* and *L. mutabilis*. *L. albus* and *L. luteus* mainly grow in the Mediterranean area, *L. angustifolius* mainly in Australia and South America, and *L. mutabilis* in the Andes.

A number of studies have been conducted in animals, particularly on *L. angustifolius*. Significant reductions in cholesterolemia and triglyceridemia were found in apoE-deficient mice fed 10% *L. angustifolius* protein for 16 weeks [11]. Studies in lactating rats fed a diet containing 20% *L. angustifolius* protein also showed markedly reduced cholesterol and triglyceride levels [12].

Finally, a study by our group [13] in hypercholesterolemic rats on Nath's diet, reported a 53% reduction in total cholesterol at 14 days and a 55.3% reduction at 28 days. In these animals, higher hepatic mRNA levels of SREBP-2, a major transcriptional regulator of intracellular cholesterol levels, as well as of CYP7A1, the rate-limiting enzyme in bile acid biosynthesis, were observed, suggesting a definite mechanism underlying the cholesterol reduction. Cholesterolemia and aortic plaque were both significantly reduced in rabbits fed lupin protein compared to casein [14]. Carotid plaques were reduced by 37.4% ($p<0.05$) after 90 days of this dietary treatment.

Finally, clinical studies on moderately hypercholesterolemic individuals compared the administration of bars containing lupin proteins plus cellulose with bars containing casein plus cellulose or casein plus oat fibre or apple pectin, or, finally, combination bars containing pea protein plus oat fibre or apple pectin [15]. Significant reductions in cholesterolemia were observed in subjects receiving bars containing lupin protein plus cellulose or pea protein plus oat fibre or apple pectin.

These experimental and clinical findings led to more detailed evaluation of the lupin protein components responsible for these effects. It should be noted that lupin seeds contain negligible amounts of phytoestrogens [16].

All these investigations clearly indicated that lupin protein contains peptides that can be released by pepsin (P) or trypsin (T). These proteins can interfere with the activity of hydroxylmethylglutaryl CoA (HMG-CoA) reductase, thus upregulating LDL receptors (by 106% and 84% vs controls for P and T peptides, respectively, at 1 mg/ml) as well as SREBP2 proteins (148% and 73% vs controls, respectively). LDL uptake in HepG2 cell lines was raised by 40% and 50% for P and T peptides, respectively, at 1 mg/ml [17] (Fig. 2). Using differentiated human Caco-2 cells, these authors also investigated whether lupin peptides in addition to modulating metabolism in human HepG2 cells, could also be absorbed in the small intestine [18]. In a co-culture system, it was shown that not only did absorbed peptides maintain their bioactivity in HepG2 cells, but that this activity was improved by the crosstalk between the two cell systems.

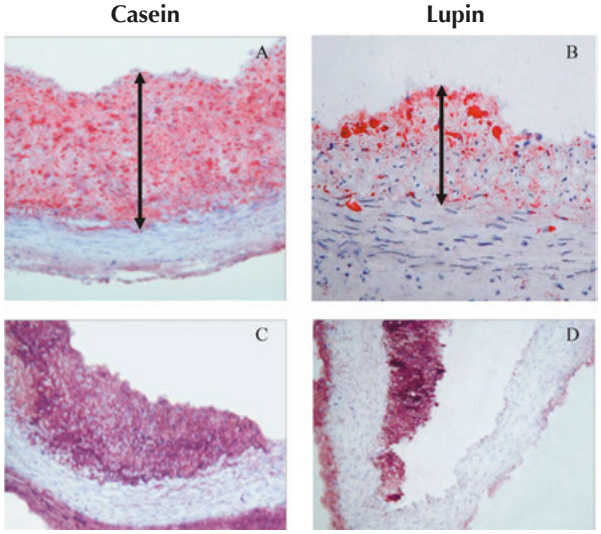


Figure 2 - Reduction in carotid neo-intimal injury of cholesterol-fed rabbits after either a casein (A,C) or lupin protein diet (B,D) [14]. (A,B) Oil red O staining for lipid deposition; (C,D) RAM11 immunostaining for macrophages

Interestingly, P and T peptides also appeared to reduce pro-protein convertase subtilisin/keksin type 9 (PCSK9) secretion in Caco-2 cells [19]. These findings confirm the powerful cholesterol-lowering potential of lupin proteins in addition to their activity on PCSK9, somewhat similar to that exerted by the novel selective antagonists (evolocumab, alirocumab). The authors further investigated the influence of PCSK9 reduction on the binding of LDL to its receptors. The two best candidate peptides were selected by simulating docking molecular dynamics and estimating peptide binding energy. In addition to inhibiting PCSK9–LDLR binding (IC_{50} value equal to 1.6±0.33±0.3 μM), the most active peptide (P5) increased the ability of HepG2 cells to take up LDL from the extra-cellular environment by 66±21.4% [19].

Hempseed

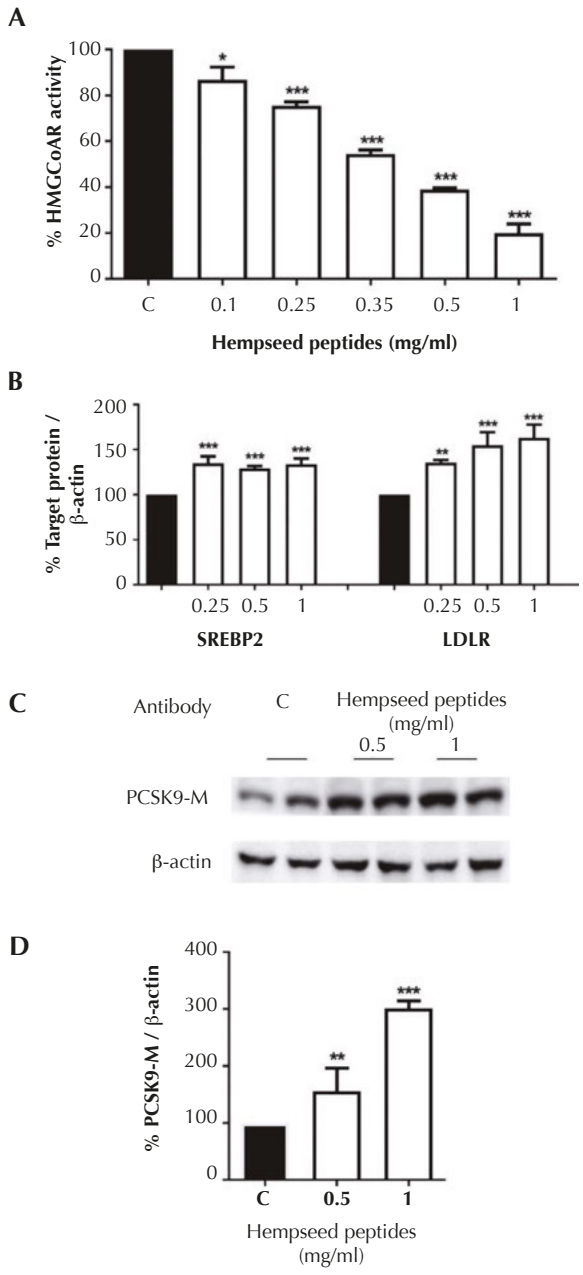
The seed of the non-drug cultivar of industrial hemp is an underexplored source of protein. The use of hemp (cannabis) as a human food and the use of hemp fibre as a textile may date back to pre-history. However, cultivation of this plant has been banned for decades in many developed countries because of its morphological similarity to marijuana: hemp and marijuana are both strains of *Cannabis sativa*, although they contain very different amounts of Δ^9-tetrahydrocannabinoid (THC). Marijuana is a slang term for *C. sativa* strains bred for their potent resinous glands, trichomes, flowers and leaves (buds). Cultivation of low-THC cultivars (THC content <200 mg/kg) has become legal again in recent years.

The nutritional content of hemp seed is of great interest as it contains 35.5% oil, 24.8% protein, 20–30% carbohydrate and 27.6% total fibre (5.4% digestible and 22.2% indigestible) with minimal amounts of antinutritional factors [20].

Hemp seed proteins mainly consist of a storage protein, edestin, which accounts for 60–80% of total protein content, with albumin accounting for the rest [21]. Zanoni *et al* hydrolyzed hempseed proteins and identified a number of peptides, mainly belonging to edestin 1, 2 and 3, and also to other protein families.

Zanoni *et al* used HPLC ESI-MS/MS analyses to identify 90 peptides from 33 hempseed proteins [21]. The hydrolysates showed that the highest number of active peptides came from six isoforms of edestin 1 and the other peptides from well-known plant proteins. Hempseed peptides did not impair HepG2 cell viability and, at concentrations between 0.1 and 1 mg/ml, showed powerful activity on HMG-CoA reductase. Exposure to these peptides at concentrations above 0.5 mg/ml raised LDL-R activity and LDL uptake (Fig. 3). Uptake

was increased 300% by hempseed peptides at a concentration of 0.5 mg/ml [21]. Interestingly, hempseed peptides at concentrations of 0.5 mg/ml or higher significantly raised PCSK9 protein levels by up to 300%, thus resembling the activity of statins, which reduce cholesterol synthesis and increase LDL-receptor activity and PCSK9 protein levels. More recently, detailed exploration of the potential of active peptides obtained by enzymatic hydrolysis of hempseed proteins [22] showed production of a large number of peptides in the 1.5–2.1 kDa range. Trypsin hydrolysis products especially inhibited HMG-CoA reductase activity to a similar extent. A number of peptides showed bioactivity, with edestin 2 in particular also showing significant antagonist activity against angiotensin converting enzyme (ACE) as well as stimulating glucose uptake.



*p<0.05; **p<0.001; ***p<0.0001 *vs* control

Figure 3 - Hempseed peptides affect cholesterolemia by reducing hydroxymethylglutaryl CoA reductase (**A**), increasing LDL receptor activity (**B**) and, similar to statins, increasing PCSK9 activity (**C**,**D**) [21]

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