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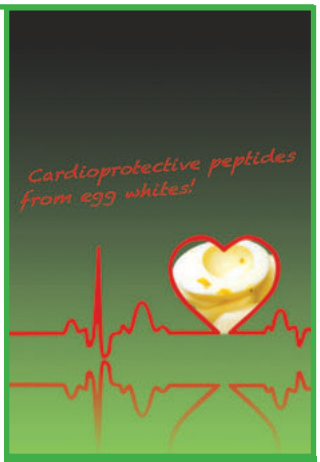
From hypertension to hyperlipidaemia: the nutraceutical properties of egg proteins



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Abstract

Atherosclerosis is the main cause of cardiovascular disease (CVD), the leading cause of death worldwide, and is characterized by the thickening and loss of elasticity of the arterial wall as well as compromised blood flow. Risk factors for the development of atherosclerosis include elevated blood pressure, high levels of LDL-cholesterol, diabetes mellitus, age, cigarette smoking and family history. While some of these risk factors cannot be modified, food components, such as fats, proteins, peptides, fibre and polyphenols, are likely to be important in the prevention of CVD. Recently extensive studies have evaluated the nutraceutical properties of eggs, particularly egg peptides (cholesterol in eggs is no longer considered a risk factor in the development of CVD). Egg contains antihypertensive peptides, in particular ovokinin, antidiabetic peptides as well as anti-obesity peptides and proteins. In addition, egg components may reduce cholesterol absorption from the intestine, and some egg proteins can possibly raise LDL-receptor activity in different tissues. The consumption of egg whites may decrease cholesterol levels in humans similar to the effect of soybean proteins. Well-defined egg components could thus act as nutraceuticals and could be used as functional food ingredients to prevent cardiovascular disease.

Introduction

Eggs constantly attract interest, particularly because of the dramatic change in views on the atherogenicity of their elevated cholesterol content [1]. In addition, the presence of different components with significant beneficial effects on major cardiovascular risk factors has recently received substantial attention.

Egg white contains 10–12% protein, including ovalbumin, ovotransferrin, ovomucoid, globulins and lysozyme [2]. These proteins may have significant biological activity, including antihypertensive, anti-oxidant, hypolipidaemic, anti-adhesive, antimicrobial and immunomodulatory properties [3].

Antihypertensive peptides from egg

Bioactive peptides, inhibiting angiotensin I-converting enzymes (ACE), have been isolated from enzymatic digests of animal and plant origin [4, 5]. A vasorelaxing peptide was isolated from a peptic digest of ovalbumin [6].

This peptide was called ovokinin, and has the following structure: Phe-Arg-Ala-Asp-His-Pro-Phe-

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Leu (FRADHPFL), corresponding to residues 358–365 of ovalbumin.

Ovokinin can relax canine mesenteric arteries, an activity blocked by a bradykinin B1 antagonist, thereby suggesting that ovokinin is a weak bradykinin B1 agonist peptide [6]. At 25 mg/kg, ovokinin can significantly lower the systolic blood pressure (SBP) of spontaneously hypertensive rats (SHR) when given as an emulsion in 30% egg yolk [7]. The most effective egg white-derived peptide is a hexapeptide with the sequence RADHPF, characterized as fragment 1-7 of ovokinin and given the name ovokinin(2-7) [6]. Purification of this peptide was carried out by Matoba *et al.* [8] from an egg albumin hydrolysate. Doses of ovokinin(2-7) 10 times smaller than the effective doses of ovokinin markedly reduced BP in SHR [8].

More complete hydrolysis of ovalbumin with pepsin, carried out by Fujita *et al.* [4], produced six ACE-inhibitory peptides: FGRCVSP, ERKIKVYL, FFGRCVSP, LW, FCF and NIFYCP. These ACE-inhibitory peptides have IC_{50} values between 0.4 and 15 µmol/l, but only the dipeptide Leu-Trp (LW) has a clear antihypertensive action in SHR.

Hydrolysis of egg white proteins (EWP) with different enzymes can also produce other hydrolysates with significant ACE-inhibitory activity [9]. These hydrolysates and the peptide sequences YAEERYPIL, RADHPFL and IVF exhibit antihypertensive effects in SHR [9, 10]; other peptides such as RADHP or YPI derived from ovalbumin also exhibit antihypertensive activity in SHR, but are not likely to act through an ACE-inhibitory mechanism. RADHP may actually be the active end product of gastrointestinal digestion of the antihypertensive peptides RADHPFL (ovokinin) and FRADHPFL (ovokinin2-7) [11].

Particular interest has been directed at novokinin (RPLKPW), whose design was based on the structure of ovokinin(2-7) but which has a greater reducing activity on SBP. A significant contribution was made by the group of Matoba *et al.*, who produced a transgenic soybean novokinin, markedly effective on SBP after a single oral administration of 0.15 g/kg in SHR [12] (Figs. 1 and 2).

Another source of ACE-inhibitory peptides is ovotransferrin from which the peptide RVPSL

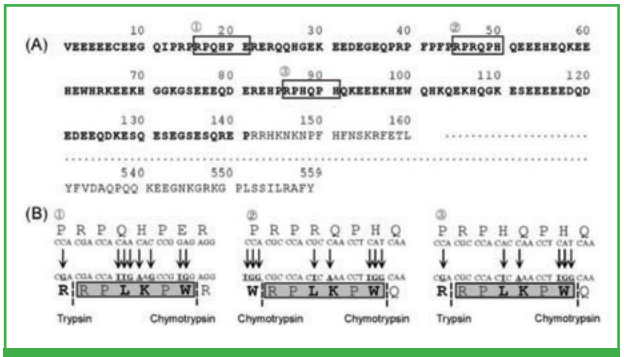

Figure 1 - Detection of three RPLKPW-like sequences in a soybean β-conglycinin α′ subunit (**A**) and design of site-directed mutagenesis (**B**). Bold letters in (**A**) correspond to the extension region [12]

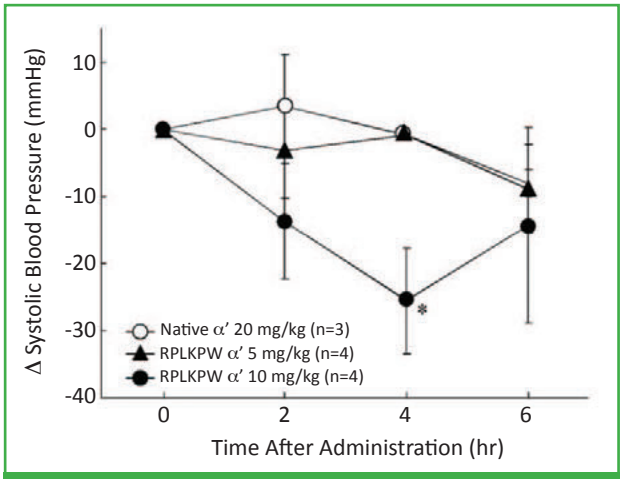

Figure 2 - Anti-hypertensive activity of the RPLKPW-containing β-conglycinin α′ subunit after oral administration in spontaneously hypertensive rats. Numbers of experiments are shown in parentheses. Changes in systolic blood pressure from zero time are expressed as means±SEM. *Significantly different from the native α′ group (*p<0.05) [12]

(ovotransferrin 328-332) is derived. Administered for 4 weeks at a dose of 50 mg/kg, RVPSL significantly reduced SBP in SHR [13]; mRNA levels of renin, ACE and AT1 receptors in the kidney as well serum Ang2, renin and aldosterone levels were also reduced [13]. The egg ovotransferrin-derived ACE inhibitory peptide, IRW, also lowered BP in SHR through reduced vascular inflammation and increased nitric oxide (NO)-mediated vasorelaxation [14]. It also significantly increased the expression of ACE-2, ABCB-1, IRF-8 and CDH-1, while significantly decreasing the expression of the pro-inflammatory ICAM-1 and VCAM-1 in mesenteric arteries [14].

In recent years a number of reports have focused on the direct cellular mechanisms of novokinin. It was found to inhibit vasocontractility induced by different agonists (KCl and $CaCl_2$) as well as by

PGF2α in precontracted porcine coronary arterial ring segments. These relaxing effects were reduced in endothelial-free arteries as well as after administration of an NO antagonist (L-NNA) [15].
Finally, the use of a proteinase from fig-leaf gourd fruit (*Cucurbita ficifolia*) was proposed as a more effective enzyme, particularly for producing antihypertensive peptides from EWP [16]. With this new tool, two novel ovalbumin-derived tetrapeptides (SWVE and DJLN), both with powerful ACE-inhibitory activity, were obtained. SWVE showed the stronger ACE-inhibitory activity. These different peptides, of varying length, can pass from the intestine into the general circulation, as described by Oseguera-Toledo *et al.* [17].

Egg peptides for diabetes and obesity

A number of peptide components in egg white have been described as potentially affecting glucose levels and animal correlates of the human metabolic syndrome. Dietary egg white hydrolysate (EWH) peptide mixtures have been shown to decrease hyperglycaemia in diabetic rodent models [18]. Active peptide sequences have not yet been fully identified. The likely candidates include an α-glucosidase inhibitory peptide, RVPSLM [19].
The peptide KLPGF, an α-glucosidase and α-amylase inhibitor with antidiabetic activity, is derived from EWH and ovalbumin [20].
Another potential effect of egg protein on diabetes could be by way of combined dipeptidyl peptidase 4 (DPP-4) and ACE inhibition, important target enzymes in glycaemic control and renovascular protection. Long-term supplementation with EWH NWT-03, a hydrolysate with both DPP-4 and ACE-inhibitory activities, markedly attenuated renovascular damage in hyperinsulinaemic Zucker rats [21]. Interestingly, this hydrolysate did not affect insulinaemia but reduced inflammatory markers, BP and ACE activity. Since the metabolic syndrome is mainly characterized by insulin resistance and hypertension, the activity of NW3 appears to be of potential clinical interest.
In contrast to the hydrolysate NWT-03, the pepsin egg white hydrolysate HEW1 improved complications related to the metabolic syndrome in Zucker fatty rats [22]. Consumption of HEW significantly reduced body weight gain and abdominal circumference, improving the quantitative indices of insulin resistance related to obesity-related complications. In addition, it improved allodynia, a sign of peripheral neuropathy [22].
Few pharmacological agents have an effect on obesity, so interest in natural agents has grown significantly [23]. Included among these natural agents are synthetic and natural lipase inhibitors which are effective in obesity management, likely due to their inhibition of intestinal lipid absorption [24]. Orlistat, a synthetic pancreatic inhibitor, is being used clinically to treat obesity [25] but has considerable and disturbing side effects.
Oral administration of an immunoglobulin from hen egg yolk (IgY) can reduce the activity of pancreatic lipase. Oral administration of IgY blocked porcine lipase activity with an IC_{50} of 0.49 μM, similar to Orlistat [26]. Supplementing a high fat diet with only 0.2% (w/w) of antilipase IgY for 35 days significantly decreased the weights of intraperitoneal, epididymal and perirenal adipose tissues, while reducing liver total lipid, triglycerides and cholesterol [26]. These dramatic changes were accompanied by a significant increase in faecal excretion of triglycerides without diarrhoea. Antilipase IgY, by inhibiting pancreatic lipase, could be an effective treatment because of the increased faecal excretion of triglycerides without clear-cut diarrhoea. Clinical data on this nutraceutical are awaited with interest.

Egg proteins for hyperlipidaemia

Hyperlipidaemia is an important risk factors for ischaemic heart disease [27–29]. The prevention and treatment of hypercholesterolaemia with specific nutrients is of growing importance. Dietary proteins can regulate cholesterol concentrations [30]: diets low in saturated fat that include 25 g soy protein per day can lower serum cholesterol concentrations and may reduce the risk of heart disease as concluded by the Food and Drug Administration [31].

The total world sales of soy products exceeds $14 billion a year. The use of soy products varies in

different countries. In Japan, for instance, soy peptides are bound to soy phospholipids to produce c-SPHP which has shown significant cholesterol-lowering properties in mildly hypercholesterolaemic individuals [32]. Most studies on the effects of dietary proteins have focused on soybean protein versus casein [33–35]. Other animal proteins such as milk whey proteins [35, 36] and their peptides [36], beef and milk proteins [37, 38], and fish proteins [39, 40] have aIso provided significant experimental data.

Egg proteins [41] have been studied to a more limited extent. Egg proteins and peptides may be of great interest in hypercholesterolaemia treatment. Low cholesterol diets including ovalbumin do not raise cholesterolaemia in rabbits compared to casein-containing diets [34]. Conversely, male rats and mice fed a diet containing EWP together with cholesterol/cholic acid show a clear hypocholesterolaemic effect compared to those fed a casein-containing diet; in contrast to soybean protein, EWP consumption results in lower HDL-cholesterol levels [41].

In Golden Syrian hamsters [42], ovalbumin consumption lowers total and HDL-cholesterol compared to casein, bovine albumin, soybean, cottonseed and peanut. In addition, triglycerides and peroxidation products are reduced [43]. An opposite effect was found in rabbits, in which dietary egg yolk protein and amino acid mixtures resulted in hypercholesterolaemia compared to dietary sunflower or soybean protein, with a similar effect to proteins or amino acid mixtures corresponding to casein or egg yolk [44]. Carroll and Kurowska suggested that these effects were mainly due to essential amino acids, especially lysine and methionine [45]. Indeed down-regulation of liver LDL receptors in rabbits fed a cholesterol-free, casein semipurified diet was the major finding of several authors [46–48]. Further, Khosla *et al.* reported that LDL apolipoprotein B (apoB) synthesis is stimulated by a casein semipurified diet and downregulation of LDL-receptors may precede the increased cholesterolaemia [49].
The primary effects of dietary proteins and amino acids on cholesterol metabolism are thus likely to be exerted in the liver. Effects on LDL/apoB synthesis and secretion in liver cells reported by Zhang *et al.* [50] may thus be relevant in the handling of some clinical dyslipidaemias.
Interestingly, there is as yet no convincing evidence that egg proteins have a direct effect on LDL-receptor expression. The major hypothesis concerning the cholesterol lowering consequent to dietary amino acid changes was based on the evaluation of Wistar rats fed a high cholesterol diet containing 25% of either ovalbumin, casein, pork protein, cod protein, corn gluten, wheat gluten or soybean protein. Plasma cholesterol levels were lower with vegetable proteins and ovalbumin, and a significant negative correlation ($p<0.05$) was noted between cholesterolaemia and the cysteine content of the protein source [51]. This molecular mechanism is likely not linked to the regulation of cholesterol synthesis but rather to changes in excretion. In fact, consumption of EWP decreased cholesterol concentrations in serum, liver and intestinal mucosa, and raised excretion of faecal neutral sterols and bile acids versus casein [52]. The authors suggested that the ratio of cholesterol and bile acids in the micellar phase compared to that in the solid phase was lower in the intestinal contents from EWP-fed rats versus those fed casein, potentially resulting from altered intestinal micellar formation.
In a recent study, rats were subjected to permanent lymph duct cannulation in order to investigate the effects of dietary EWP on lipid transport. Dietary EWP reduced lymphatic cholesterol transport by 20% compared to casein [53]. These authors also evaluated pepsin-hydrolysed EWP and casein in order to eliminate any exclusion effect of cholesterol from the micelles. EWP and, particularly, ovalbumin hydrolysates significantly reduced micellar solubility and transfer rate and increased water-holding capacity and relative viscosity compared to the casein pepsin hydrolysate. This will lead to inhibition of cholesterol absorption. The authors thus postulate that the mechanism of cholesterol reduction by EWP and ovalbumin is consequent to reduced absorption exerted by a combination of physicochemical effects in the gut.
In view of the significant negative correlation between cholesterolaemia levels and the cysteine

content of intact dietary proteins, interest in ovomucin (OV), a protein with hypocholesterolaemic properties [54], has increased. In fact, OV contains significantly higher levels of glycine and cysteine compared to casein, and differences in amino acid content may be related to differences in cholesterolaemia [55]. Approximately 3.5% (w/w) of EWP is OV, which is a highly glycosylated protein composed of two subunits: a 'protein-rich' subunit (220 kDa) and 'carbohydrate-rich' subunit (400 kDa) [54]. The higher faecal excretion of cholesterol and acidic steroids in OV-fed rats appears to be induced by cholesterol binding by OV in the intestine [55]. This is further supported by the raised bile-acid-binding capacity of mucin [56].

Data indicative of increased bile acid metabolites in the faeces, particularly by EWP hydrolysate (EP-1) [55], led to studies on cholesterol 7α-hydroxylase (CYP7A1) activity by peptide screening. It was found that CYP7A1 mRNA is significantly raised by the peptide GLWEK (1 mM: 135.7±7.3%) compared to control (100±8.0%) (p<0.01). GLWEK, named ovocholestin, was identified as a CYP7A1-activating peptide derived from ovalbumin in HepG2 cells [57]. GLWEK resembles the novel hypocholesterolaemic peptide IIAEK derived from bovine β-lactoglobulin in the C-terminal EK sequence [38, 58]. The suppression of cholesterol absorption, possibly by direct interaction between cholesterol mixed micelles and OV is postulated. Reduction of cholesterol absorption by, for example, dietary fibre, is not, however, always successful [59].

However, the mechanism of the cholesterol-lowering activity of egg peptides, supported by a number of in vitro studies, is still disputed. Indeed, there are no data on the LDL-receptor activation characteristic of essentially all peptides of legume origin. The observation by Yamamoto *et al.* [41] of favourable effects of egg white on serum lipids in rats and mice led to a clinical study in men and women with moderate hypercholesterolaemia. Asato *et al.* evaluated young women with moderate hypercholesterolaemia [60] receiving about 30% of total protein from egg white, tofu or cheese (about 30% of total daily energy). The egg white group showed decreased cholesterolaemia and a larger increase in HDL-C compared to the tofu group. This indicates that EWP can help to control hypercholesterolaemia. Unfortunately, no further, more recent studies supporting this indication have been conducted.

Conclusions

Egg proteins are a new target for the clinical evaluation of major preventive dietary approaches for CVD prevention. While there are abundant data in support of a hypotensive effect and, to a lesser extent, anti-obesity/antidiabetic indications, the mechanisms of cholesterol reduction by egg protein require more extensive studies. Recognition of different mechanisms involved in a variety of vascular/metabolic consequences supports more in-depth studies on the identification of active components of egg proteins.

Conflict of Interest

The author declares that there is no conflict of interest regarding the publication of this article

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