

## Invited commentary

### Cholesterol-lowering effects of high-protein soya milk

In this month's issue, Sirtori *et al.* (1999) demonstrate the efficacy of high-protein soya milk *v.* cows' milk in lowering serum cholesterol in patients with severe hypercholesterolaemia and either resistance to or intolerance of the most commonly used and potent group of cholesterol-lowering drugs known as statins. Dietary soyabean and statins have been previously shown to produce additive effects in reducing serum cholesterol in experimental animals (Giroux *et al.* 1997). This current paper extends these observations by providing evidence to suggest that soyabean may exert its hypocholesterolaemic effects through its protein moiety via a mechanism that is independent of the classic LDL-receptor pathway.

In a double-blind, crossover study design, partial supplementation with high-protein soya milk (500 ml soya or cows' milk/4 weeks) was associated with significant reductions in total serum cholesterol (–6.5% when given before cows' milk, –7.4% when given after cows' milk) and LDL-cholesterol (–7.8% when normalized into a single sequence; cows' milk *v.* soya milk,  $P < 0.01$ ). Although these changes in serum and LDL-cholesterol are quite small by comparison with what can be achieved with statins, in terms of a dietary effect they are of a similar magnitude to that recently reported by Weststrate & Meijer (1998) for soyabean and the new Benecol margarines in normo-lipidaemic and mildly hypercholesterolaemic subjects (30 g/d for 3.5 weeks: –8.3% and –7.3% decrease in total serum cholesterol with soyabean and Benecol margarines respectively). Whilst the latter study did show a greater decrease in LDL-cholesterol (–13%) in response to both margarines, a direct comparison between this and the effects of soya milk described in the present study is unfortunately impossible due to the extreme differences in study design and choice of experimental subjects. A possible explanation for the response to soya milk in these statin resistant/intolerant patients lies in the different mechanisms of action of the two agents. Statins work essentially by inhibiting hydroxymethylglutaryl-CoA reductase (*EC* 1.1.1.34), the rate-limiting enzyme in the synthesis of cholesterol, chiefly in the liver. The depletion of intracellular pools of free cholesterol activates a sensitive feedback mechanism which increases the synthesis of cell surface receptors that are specific for the binding and uptake of the principal cholesterol-carrying lipoprotein LDL. The subsequent fall in serum cholesterol, by as much as 30 or 40% in some patients, results from the removal of these LDL particles from the circulation via this activated LDL-receptor pathway. In contrast, it is postulated that soyabean may upregulate LDL receptors directly and, by short-circuiting the feedback mechanism, actually by-pass possible causes of drug resistance. This effect is presumably in addition to

the action of soyabean phytosterols such as sitosterol which inhibit the uptake of dietary and biliary cholesterol in the gut, thereby interrupting the enterohepatic circulation and upregulating LDL receptors via the classical pathway described earlier. Whatever the mechanism, these data provide further support for the potential role of soyabean-based food products as a valuable adjunct to lipid-lowering drug therapy in the prevention of CHD.

While the cholesterol-lowering action of soyabean is well beyond dispute, the nature of the active constituents responsible for this effect and the molecular mechanisms by which LDL receptors may be stimulated directly are still unclear. Many hold the view that the lipid-lowering action of soyabean derives from the non-steroidal phyto-oestrogens known as isoflavones. Alternatively, the lipid lowering properties of soyabean may be attributed to the protein component itself. The authors of this paper acknowledge that their study was not designed to address the role of specific soyabean constituents in lipid lowering. This was clearly untestable in this situation as the soya-milk supplement delivered both soyabean protein (35 g/d) and isoflavones (32 mg/d). Since the cardiovascular benefits associated with isoflavones have, in the main, been reported to occur in human subjects at doses in excess of 50 mg/d, it seems reasonable to contest that lower levels of exposure will have no biological effects. However, stepwise reductions in serum cholesterol have been reported in a dose–response fashion at intakes of isoflavones ranging from 4 to 58 mg/d (Anderson *et al.* 1999). In spite of this, there is persuasive evidence from studies with isoflavone-free supplements to implicate soyabean protein in the hypocholesterolaemic action of soyabeans. This is supported by evidence presented in a recent review to show that specific soyabean proteins such as 7S globulin, a major soyabean storage protein, can increase LDL-receptor mRNA levels in cell culture by activating transcription of the LDL receptor gene (Sirtori *et al.* 1998). The same review article cites evidence to suggest that the principal soyabean isoflavone genistein may actually suppress the activity of LDL receptors by inhibiting the enzyme tyrosine kinase (*EC* 2.7.1.112), a major regulatory element of many cell-membrane receptors. The idea that a dietary isoflavone decreases the activity of LDL receptors, whilst plausible, is inconsistent with an abundance of evidence *in vivo* to support the cholesterol-lowering effects of these compounds in various animal models, non-human primates and human subjects. Moreover, the extraction of isoflavones from soyabean has actually been shown to attenuate the lipid-lowering potential of the remaining soyabean protein (Anderson *et al.* 1999).

Phyto-oestrogens are believed to exert their metabolic effects by binding to tissue-specific oestrogen receptors

where they elicit weak oestrogenic effects (for review see Cassidy, 1999), and/or by interacting directly with nuclear transcription proteins within the cell. Phyto-oestrogens also undergo bacterially induced bio-transformations in the gut into what are potentially more metabolically active compounds such as equol. Consideration of these pathways may help to explain some of the inconsistencies in the biological response to dietary isoflavones but more importantly within the context of this commentary, may complicate the interpretation of findings from *in vitro* experiments. Interestingly, highly purified preparations of isoflavones in the form of pills have been shown to have no cholesterol-lowering capacity whatsoever (Nestel *et al.* 1997), suggesting that there is some factor associated with soyabean protein which facilitates the lipid-lowering effects of isoflavones. The possibility that the protein and isoflavone constituents within soyabean work together to produce additive or even synergistic effects on serum lipids via the same or independent mechanisms seems reasonable and is favoured by the weight of evidence for each case.

The paper by Sirtori *et al.* (1999) succeeds in highlighting the importance of establishing the nature of the active constituents and mechanisms by which they exert their effects, an increased knowledge of which will be essential for the commercial development of soya-based foods with enhanced biological activity. Nevertheless, it is well recognized that the cardiovascular benefits associated with soyabeans extend beyond their influence on serum lipids. Non-lipid-mediated cardiovascular risk factors associated with endothelial dysfunction, the inflammatory response to injury and haemostasis have long been of interest to the pharmaceutical industry. Dietary phyto-oestrogens in soyabeans score highly in this respect having been shown to produce favourable effects on vascular reactivity, thrombin generation and platelet function (Cassidy & Griffin, 1999). These effects will undoubtedly have an impact on the more acute and frequently fatal manifestations of coronary atherosclerosis, and as such warrant further attention.

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