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Review Article

Hypocholesterolaemic effects of soya proteins: results of recent studies are predictable from the Anderson meta-analysis data

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In 1995, Anderson *et al.* published a meta-analysis, derived from most of the clinical studies on soya proteins given to individuals with varying levels of cholesterolaemia that had been reported up to that time. The meta-analysis clearly indicated that cholesterolaemias were generally reduced by diets with soya given as a partial or total substitution of animal proteins, with final mean total and LDL-cholesterol reductions of 23·2 mg/dl and 21·7 mg/dl, respectively. These findings were recently strongly criticised, based on the evaluation of later studies, frequently involving individuals with normal or moderately elevated cholesterolaemias. In the present paper, these more recent studies were re-evaluated using a 'nomogram' prepared on the basis of the quartiles of initial cholesterol concentrations in the Anderson meta-analysis and their corresponding CI for net cholesterol change. The five studies belonging to the first quartile and thirteen out of the fourteen belonging to the second quartile gave results perfectly in line with the nomogram. Out of the fourteen studies belonging to the third quartile, ten agreed with the nomogram and two gave lower cholesterol reductions, whereas two gave higher reductions. Unfortunately, none of the recent studies belonged to the fourth quartile as treatment with statins or other lipid-lowering drugs is now mandatory in the presence of very high cholesterol levels. The re-evaluation thus shows that the thirty-three studies published in the past 10 years are in agreement with the Anderson meta-analysis and confirm its validity.

Soya proteins: Hypercholesterolaemia: Meta-analysis: Lipoproteins

The dietary intake of soya proteins in experimental animals, as well as in humans, may lead to significant reductions in plasma cholesterol level. A number of studies, starting from those of our group in the 1970s on human subjects (Sirtori *et al.* 1977, 1979; Descovich *et al.* 1980), and from those of Carroll's group on animals (Carroll, 1982), had pointed out that the substitution of animal proteins with vegetable proteins, mainly from soya, was associated with a hypocholesterolaemic response.

These initial studies clearly indicated that the hypocholesterolaemic activity of soya proteins, given as a total protein substitution, was detected mainly in severely hypercholesterolaemic individuals. This activity is of a lesser degree, and frequently absent, in individuals with total and LDL-cholesterol levels below 240 and 150 mg/dl, respectively (Sirtori *et al.* 1979; Gaddi *et al.* 1991). These early studies, also in subjects of childhood age (Gaddi *et al.* 1987), provided an extreme dietary approach, although one accepted by patients who were not at that time offered effective cholesterol-lowering medications. Even though resin therapy (Levy *et al.* 1973), and in specific cases LDL apheresis (Franceschini *et al.* 1988), could achieve biochemical results not too different from those obtained today with statins, personal and organizational complexities did not allow a wider use of these alternative procedures.

In more recent years, the availability of effective medications and the improved organolectic features of soya protein-based food items suggested that these could be given as partial substitutes in the daily diet, eventually attempting to improve the response to medication. Evaluation of the findings from a large number of studies on soya proteins, carried out up to 1995, was provided by a widely quoted meta-analysis (Anderson *et al.* 1995). This investigated thirty-eight papers evaluating cholesterol changes following soya protein intake, twenty-nine of which were used for the final analysis, for a total of thirty-one studies. A variety of approaches and patient characteristics were considered, ranging from studies with a complete substitution of soya in the diet to studies with just additions, from studies in severely hypercholesterolaemic patients to studies in those with a normal range of cholesterol level.

The mean change in plasma total cholesterol was −23·2 mg/dl (−9·3 %) and that in LDL-cholesterol− 21·7 mg/dl (−12·9 %). The reduction in cholesterol level,

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however, appeared to be strongly dependent upon the baseline cholesterol. In particular, subjects with cholesterol values below 200 mg/dl showed essentially no reduction, whereas more significant changes were noted with high starting cholesterolaemias. A regression model predicting net changes in serum cholesterol concentration as a function of the characteristics of the studies (see Table 3 in Anderson *et al.*'s meta-analysis) showed that 77·3 % of the variance between studies was explained by the square value of the initial plasma cholesterol level, whereas other characteristics, such as type of soya protein, amount of soya protein per day, type of diet and age of the subjects, provided much smaller or negligible contributions.

This 10-year-old meta-analysis was recently criticised on the grounds that some more recent studies, again encompassing individuals with a variety of cholesterol levels and different durations and types of product, failed clearly to indicate a sizable cholesterol reduction following soya protein-based diets (Sacks *et al.* 2006). A major criticism was also that, in the Anderson meta-analysis, the authors put great emphasis on the presence of phytoestrogens in the soya products as a possible hypocholesterolaemic mechanism. Interestingly, no phytoestrogens were present in the initial studies on soya proteins in severely hypercholesterolaemic patients (Sirtori *et al.* 1997), and most of the studies from this group used soya products without phytoestrogens. In addition, the possibility that phytoestrogens may play a real role in cholesterol reduction caused by soya has recently been ruled out (Sirtori *et al.* 2005; Hall *et al.* 2006).

It thus seemed of value to assess whether or not a nomogram prepared from the data of the Anderson meta-analysis, i.e. a predictive model based on the final results of the thirty-one studies, could still be used in order to predict changes in cholesterolaemia in later studies. Some studies in the meta-analysis involved total substitutions, others just a partial substitution of soya proteins, but the overall results still seemed to be relatively independent of these variables.

Materials and methods

Selection of the studies

Studies on soya proteins and serum cholesterol were searched using MEDLINE and also using published material, in particular the recent American Heart Association Science Advisory on Soy Proteins (Sacks *et al.* 2006). A selection was made from all studies published after 1995, using the following criteria: a well-defined study with clear definitions of total and LDL-cholesterol levels, and a duration of at least 3 weeks, in order to allow meaningful changes of cholesterol over time (Keys, 1967).

A total of twenty-seven papers examined the effect of soya proteins on cholesterolaemia after the Anderson meta-analysis and satisfied our selected criteria. Of these, twenty-three papers were examined in the review article by the American Heart Association Science Advisory (Sacks *et al.* 2006). Among the papers considered in that review, one was excluded because it was based on a mixed diet including both soya and oats (van Horn *et al.* 2001) and one because changes were presented only in very small, unreadable graphs (Dent *et al.* 2001).

Conversely, we included a paper (not considered in the American Heart Association Science Advisory) by our group on severely hypercholesterolaemic patients poorly responsive to or intolerant of statins (Sirtori *et al.* 1999). In addition, three new papers were included, one from Denmark (Hermansen *et al.* 2005) and two from Taiwan (Chen *et al.* 2005, 2006). These last two, involving renal patients using 30 g/d soya protein, reported highly significant reductions in cholesterol level. Thus, in total, we report here the results from twenty-five different publications.

Results

The characteristics and results of the selected papers are shown in Table 1. Some of these reported two studies: in four papers, cholesterol changes observed both in the complete population and, separately, in hypercholesterolaemic patients are reported (Wong *et al.* 1998; Crouse *et al.* 1999; Lichtenstein *et al.* 2002; Chen *et al.* 2005). Two papers investigated different daily intakes of soya protein (Teixeira *et al.* 2000; Tonstad *et al.* 2002), and two papers considered the same amount of soya protein but with varying isoflavone contents (Gardner *et al.* 2001; Jenkins *et al.* 2002).

Unlike other studies dealing with the same topic (Sacks *et al.* 2006), net changes in total and LDL-cholesterol levels, obtained by subtracting the cholesterol changes after the reference diet, considered as a 'control', are not given as percentage changes in Table 1 but as absolute changes (mg/dl). This allows a more direct comparison with the CI of the Anderson meta-analysis. This same criterion was used historically in the calculation of plasma cholesterol reductions well before Keys' equations, predicting cholesterolaemia following modifications in the polyunsaturated:saturated fat ratio or in the cholesterol content of the diet (Keys *et al.* 1965*a*,*b*).

In the studies shown in Table 1, the ingestion of a diet containing soy protein, compared with a control diet, was accompanied by a moderate reduction in total (mean −11·7 mg/dl) and LDL-cholesterol (mean −8·6 mg/dl). Apparently, these values are significantly smaller than those reported in the Anderson meta-analysis (−23 mg/dl in total cholesterol and −21·7 mg/dl in LDL-cholesterol).

Since the detailed correlation analysis presented in the Anderson study clearly demonstrated that the square of the initial plasma cholesterol level was the main significant predictor of the change in cholesterol concentration (i.e. it explained 77·3 % of the variance *v.* 12·6 % for type of diet, and only 1·0 % for type of soya protein), it was decided to divide the studies into quartiles. This is presented in Table 2, also reporting the data from the original Table 4 of the Anderson meta-analysis.

In the meta-analysis, subjects who had initial cholesterol values below 200 mg/dl had non-significant mean reductions of 5·2 mg/dl; those with mild hypercholesterolaemia (initial values of 200–255 mg/dl) had mean reductions of 10·1 mg/dl; those with moderate hypercholesterolaemia (initial values of 259–333 mg/dl) showed a mean reduction of 22·2 mg/dl; finally, those with severe hypercholesterolaemia (initial cholesterol level of 335–410 mg/dl) had a mean reduction of 71·5 mg/dl. Classifying the new studies with the same criteria, five belonged to the first, fourteen to the second and fourteen to the third quartile. No recent study

Table 1. Effects of soya protein diets on plasma total cholesterol (TC) and LDL-cholesterol (LDL-C) levels in recent studies

Reference	Number of patients	Types of patient	Mean age (years)	Design	Daily dose and control diet	Duration	Baseline TC (mg/dl)	Net change in TC (mg/dl)	Baseline LDL-C (mg/dl)	Net change in LDL-C (mg/dl)
Baum *et al.* (1998)	21	F, postmen.	61	Para, DB	ISP 40 g + IF 90 mg *v.* casein	24 weeks	250	−13	Non-HDL 196	−11
Blum *et al.* (2003)	24	F, HC	55	X, DB	ISP 25 g + IF 85 mg *v.* milk proteins	6 weeks	270	+2	178	+5
Chen *et al.* (2005)	10	HC dialysis	61	Para, DB	30 g as soya drink with 36 mg isoflavone *v.* milk	12 weeks	266	−49	151	−31
Chen *et al.* (2005)	10	LC dialysis	61	Para, DB	30 g as soya drink with 36 mg isoflavone *v.* milk	12 weeks	170	−3	106	0
Chen *et al.* (2006)	13	HC dialysis	59	Para, DB	30 g as soya drink with 36 mg isoflavone *v.* milk	12 weeks	271	−49	166	−-25
Crouse *et al.* (1999)	15	M, F	52	Para, DB	ISP 25 g + IF 62 mg *v.* casein	9 weeks	226	+1	147	0
Crouse *et al.* (19 990	15	M, F	52	Para, DB	ISP 25 g + IF 62 mg *v.* casein	9 weeks	261	−24	185	−21
Cuevas *et al.* (2003)	18	F, HC, menop.	59	X, DB	ISP 40 g + IF 80 mg *v.* casein	4 weeks	286	−3	195	−1 NS
Dalais *et al.* (2003)	38	F, menop.	60	Para, DB	ISP 40 g + IF 118 mg *v.* casein	3 months	236	−11	154	−12
Gardner *et al.* (2001)	33	F, menop.	58	Para, DB	ISP 42 g + IF 3 mg *v.* milk proteins	12 weeks	228	+8 NS	151	+8 NS
Gardner *et al.* (2001)	31	F, menop.	63	Para, DB	ISP 42 g + IF 80 mg *v.* milk proteins	12 weeks	228	0 NS	151	−4 NS
Hermansen *et al.* (2001)	20	diabetes	64	X, DB	ISP 50 g + IF 165 mg *v.* casein	6 weeks	220	−6	140	−13
Hermansen *et al.* (2005)	100	M, F, HC	60	Para, DB	ISP 30 g, 9 g fibre + IF 100 mg *v.* 30 g casein	24 weeks	266	−12	178	−8
Jenkins *et al.* (2000)	66	M, F, HC	25	X	ISP 36 g + IF 168 mg *v.* wheat protein	3 weeks	270	−12	187	−-8
Jenkins *et al.* (2002)	41	M, F, HC	62	X	ISP 50 g + IF 10 mg *v.* dairy and egg proteins	1 months	258	−18	175	−7
Jenkins *et al.* (2002)	41	M, F, HC	62	X	ISP 50 g + IF 73 mg *v.* dairy and egg proteins	1 months	261	−17	176	−10
Kreijkamp-Kaspers *et al.* (2004)	88	F, menop.	67	X, DB	ISP 26 g *v.* milk proteins	12 months	240	−2 NS	161	−1 NS
Lichtenstein *et al.* (2002)	22	M, F	63	X	ISP 25 g *v.* mixed animal proteins	6 weeks	220	+1	145	0
Lichtenstein *et al.* (2002)	22	M, F, HC	63	X	ISP 25 g *v.* mixed animal proteins	6 weeks	278	−10	196	−10
Meinertz *et al.* (2002)	24	F, M	30	X	ISP 133 g + IF 318 mg *v.* casein	32 d	161	−3 NS	84	−3 NS
Puska *et al.* (2002)	30	HC	56	Para, DB	ISP 52 g + IF 192 mg *v.* casein	6 weeks	290	−24	199	−10
Sirtori *et al.* (1999)	21	M, F, HC	52	X	36 g as soya drink *v.* milk	4 weeks	337	−22	246	−19
Sirtori *et al.* (2002)	20	M, F, HC	60	X, DB	ISP 25 g + IF 77 mg *v.* milk	4 weeks	318	−12	230	−10
Steinberg *et al.* (2003)	28	F	55	X, DB	ISP 25 g + IF 107 mg *v.* milk proteins	6 weeks	190	−4 NS	111	−1 NS
Teede *et al.* (2001)	90	M, F	61	X, DB	ISP 40 g + IF 80 mg *v.* casein	3 months	228	−6	151	−5
Teixeira *et al.* (2000)	16	M, HC	45	Para, DB	ISP 20 g + IF 38 mg *v.* casein 50 g	6 weeks	231	−5	Non-HDL 190	−5
Teixeira *et al.* (2000)	16	M, HC	45	Para, DB	ISP 50 g + IF 95 mg *v.* casein 50 g	6 weeks	243	−8	Non-HDL 199	−9
Tonstad *et al.* 92 002)	31	M, F, HC	54	Para, DB	ISP 50 g + IF 185 mg *v.* casein	16 weeks	251	−9	186	−7
Tonstad *et al.* (2002)	34	M, F, HC	54	Para, DB	ISP 30 g + IF 111 mg *v.* casein	16 weeks	265	−12	189	−12
Vigna *et al.* (2000)	40	F, menop.	53	X, DB	ISP 60 g + IF 76 mg *v.* casein	12 weeks	160	−2 NS	106	−7
West *et al.* (2005)	26	M, F menop	58	X, DB	ISP 25 g + IF 90 mg *v.* milk protein	3 weeks	210	0	140	0

Table 1. *Continued*

Reference	Number of patients	Types of patient	Mean age (years)	Design	Daily dose and control diet	Duration	Baseline TC (mg/dl)	Net change in TC (mg/dl)	Baseline LDL-C (mg/dl)	Net change in LDL-C (mg/dl)
Wong *et al.* (1998)	13	M, HC	36	X	ISP 50 g *v.* mixed animal proteins	5 weeks	262	−15	181	−9
Wong *et al.* (1998)	13	M, LC	41	X	ISP 50 g *v.* mixed animal proteins	5 weeks	170	−6	111	−8

F, female; menop., menopausal; HC, hypercholesterolaemia; LC, low cholesterol; M, male; Para, parallel design; DB, double blind; X, cross-over design; ISP, isolated soya proteins; IF, isoflavones.

belonged to the fourth quartile as treatment with statins or other lipid-lowering drugs is now mandatory in these patients. Mean changes in serum cholesterol and LDL-cholesterol concentrations of the studies grouped according to the Anderson quartiles are reported in Table 2. Subjects with normal cholesterol levels (first quartile) had mean reductions of 3·6 mg/dl; those with mild hypercholesterolaemia (second quartile) had mean reductions of 5·0 mg/dl, and those with moderate hypercholesterolaemia (third quartile) had mean reductions of 18·5 mg/dl. These values are just 3–5 mg/dl less than the reductions reported in the Anderson meta-analysis but always well inside ranges defined by the 95 % CI.

Figure 1 provides a chart that we propose calling the 'Anderson nomogram'. In this, each box indicates the range of initial total cholesterol (*y*-axis) and the 95 % CI of cholesterol change (*x*-axis) for each quartile. This nomogram allows checking immediately whether or not any particular study falls within the prediction of the Anderson meta-analysis. Each point on the graph represents one recent study examined in the present paper. The five studies that belonged to the first quartile and thirteen out of fourteen studies that belonged to the second quartile gave results perfectly in line with the Anderson prediction. Out of the fourteen studies belonging to the third quartile, ten agreed with the nomogram, whereas two gave lower cholesterol reductions (Blum *et al.* 2003; Cuevas *et al.* 2003) and two higher reductions (Chen *et al.* 2005, 2006). Thus, these thirty-three recent studies are well in agreement with the Anderson meta-analysis and confirm its validity.

Discussion

We investigated whether, by applying the same criteria used in the Anderson meta-analysis, i.e. evaluation of the net cholesterol change compared with baseline plasma cholesterol level, we could detect a prediction model allowing future studies on soya products to be evaluated in terms of their cholesterol-reducing potential. This allowed us to prepare a nomogram that clearly and visually confirmed what was already distinctly stated in the Anderson meta-analysis: that the plasma cholesterol response to soya protein is not linear, but rather correlates to the square of baseline cholesterol level.

There is apparently a threshold level that needs to be reached before a significant reduction in plasma cholesterol occurs, and the cholesterol response is far more dramatic in individuals with the highest cholesterolaemias. In brief, any study carried out on individuals with cholesterol levels below 240–250 mg/dl will most likely lead to minimal (probably clinically insignificant) cholesterol reductions. This was already evident from the earliest clinical study (Sirtori *et al.* 1977), carried out on inpatients given a complete substitution of animal proteins with soya proteins. In this, in spite of the similarity and obligatory adherence to the dietary regimen for all patients, individuals with cholesterol levels in the range 240–250 mg/dl (Fig. 2 in the original paper) showed only minimal reductions. A somewhat similar finding was reported by Bakhit *et al.* (1994), when evaluating the effects of adding 25 g/d soya proteins (the final daily amount recommended by the US Food and Drug Administration) to the diet of individuals with varying baseline plasma cholesterol levels. In this series, the threshold for cholesterol reduction

Table 2. Comparison of the net changes in total and LDL-cholesterol concentrations in early and recent studies according to quartiles of the study group for initial plasma cholesterol levels in the Anderson meta-analysis (Anderson *et al.* 1995)

Anderson meta-analysis: characteristics of quartiles and changes in total and LDL-cholesterol	Q1	Q2	Q3	Q4
Total cholesterol (mg/dl)				
Initial range	127·1–197·8	201·2–255·4	259·3–332·8	335–410
Net change	−5·2	−10·1	−22·2	−71·5
95 % CI	−17·1 to +6·7	−21·8 to +1·7	−37·3 to −7·1	−86·6 to −56·5
% Change	−3·3	−4·4	−7·4	−19·7
LDL-cholesterol (mg/dl)				
Net change	−7·1	−10·7	−18·3	−68·1
95 % CI	−20·0 to +6·0	−24·3 to +2·9	−35·3 to −1·3	−90·2 to −45·9
% Change	−7·7	−6·8	−9·8	−24·0
Data from recent studies divided according to Anderson's quartiles				
Number of studies per quartile	5	14	14	
Total cholesterol (mg/dl)				
Net change	−3·6 (SD 1·36)	−5·0 (SD 6·8)	−18·5 (SD 14·2)	
% Change	−2·11	−2·2	−6·7	
LDL-cholesterol (mg/dl)				
Net change	−3·8 (SD 3·18)	−4·7 (SD 5·8)	−12·1 (SD 9·0)	
% Change	−3·7	−3·4	−6·4	

compared with no reduction, or possibly a cholesterol increase, was indicated at approximately 220 mg/dl. In this study too, definite reductions occurred in subjects with cholesterolaemias of around 240 mg/dl. It may be worthy of note that, in spite of publication of the Anderson meta-analysis, more than one third of the recent studies quoted (thirteen out of thirty-three) were based on patients with initial cholesterol levels below 240 mg/dl.

As pertains to the more marked cholesterol reductions occurring in hypercholesterolaemic individuals, the 'threshold' seems to be around 280–300 mg/dl (Anderson *et al.* 1995; Sirtori *et al.* 1998). If the basic mechanism of cholesterol reduction, i.e. LDL-receptor upregulation, the object of a series of reports by our group (Lovati *et al.* 1987, Duranti *et al.* 2004) and confirmed by other investigators (Baum *et al.* 1998), is accepted, it then becomes reasonable to conclude that this mechanism is most likely to be effective in carriers of an LDL-receptor deficiency status (Brown & Goldstein, 2004), i.e. those with more severe hypercholesterolaemias, compared with moderately hypercholesterolaemic individuals. In the past 10 years, only two studies, by our group, have examined individuals with initial cholesterol levels above 300 mg/dl (Sirtori *et al.* 1999, 2002), both belonging to the third quartile of the Anderson nomogram.

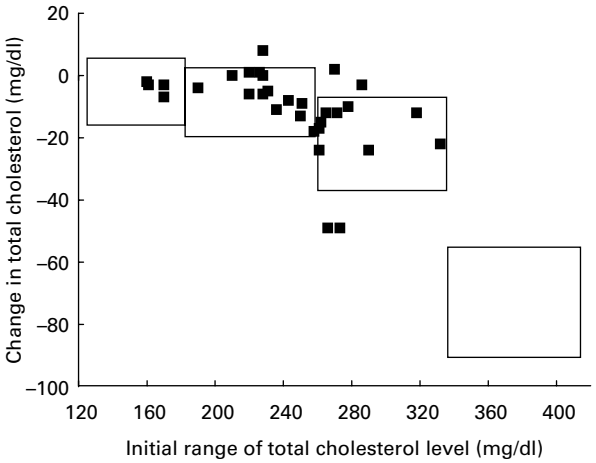

Fig. 1. Initial cholesterol levels and net cholesterol changes after soya protein-based diets in the thirty-three studies that were the object of this report are plotted together with the 'Anderson nomogram', in which boxes indicate initial ranges of cholesterolaemias and 95 % CI of cholesterol changes for each quartile considered in the Anderson meta-analysis.

A surprising finding, when comparing recent with earlier studies using soya protein, given as a total dietary substitute, is that even with partial substitution or just addition, remarkable cholesterol reductions occurred in severely hypercholesterolaemic individuals. This was particularly noticeable in two papers on patients on dialysis, in which 30 g/d soya protein were included in the diet as a drink (Chen *et al.* 2005, 2006). A paper that has compared different daily soya protein intakes in hypercholesterolaemic individuals seems to indicate that increasing the intake above 30 g/d does not improve the response (Tonstad *et al.* 2002). It is possible that the absorption of intact soya peptides (Duranti *et al.* 2004) after dietary addition may be associated with activation of the liver LDL-receptor system in a similar way to that found after total soya substitution (Lovati *et al.* 1987).

In conclusion, the majority of studies on hypercholesterolaemic individuals treated with a soya protein-based regimen fall within the 95 % CI indicated in the Anderson nomogram. Only three studies showed a lesser effectiveness, whereas in two a higher effectiveness was observed (Fig. 1). Parallel evaluations of reductions in LDL-cholesterol lead to the same conclusions. These findings clearly indicate that the nomogram of cholesterol reduction based on individual plasma cholesterol levels is a good predictor of cholesterol changes in studies carried out after the Anderson meta-analysis. The meta-analysis was based on a wide variety of studies, some with a total substitution of dietary proteins, others with only a partial substitution, whereas all the recent studies involved partial substitutions/additions. This observation clearly suggests that the beneficial effects of soya proteins

are probably not consequent on gross dietary changes, for example in lipid composition, fibre or other substances, as suggested by some authors (Lichtenstein *et al.* 2002; Sacks *et al.* 2006), but most likely depend on some components that elicit a 'pharmacological effect' on the LDL-receptor system (Anderson, 2003). This conclusion would thus be no different from that relating to the hypotensive activity of milk peptides, antagonists of the angiotensin-converting enzyme system (Vermeirssen *et al.* 2004), and clearly effective only in hypertensive individuals.

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