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Hypocholesterolaemic effects of lupin protein and pea protein/fibre combinations in moderately hypercholesterolaemic individuals

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Abstract

The present study was aimed to evaluate the effect of plant proteins (lupin protein or pea protein) and their combinations with soluble fibres (oat fibre or apple pectin) on plasma total and LDL-cholesterol levels. A randomised, double-blind, parallel group design was followed: after a 4-week run-in period, participants were randomised into seven treatment groups, each consisting of twenty-five participants. Each group consumed two bars containing specific protein/fibre combinations: the reference group consumed casein + cellulose; the second and third groups consumed bars containing lupin or pea proteins + cellulose; the fourth and fifth groups consumed bars containing casein and oat fibre or apple pectin; the sixth group and seventh group received bars containing combinations of pea protein and oat fibre or apple pectin, respectively. Bars containing lupin protein + cellulose (−116 mg/l, −4·2 %), casein + apple pectin (−152 mg/l, −5·3 %), pea protein + oat fibre (−135 mg/l, −4·7 %) or pea protein + apple pectin (−168 mg/l, −6·4 %) resulted in significant reductions of total cholesterol levels ($P<0·05$), whereas no cholesterol changes were observed in the subjects consuming the bars containing casein + cellulose, casein + oat fibre or pea protein + cellulose. The present study shows the hypocholesterolaemic activity and potential clinical benefits of consuming lupin protein or combinations of pea protein and a soluble fibre, such as oat fibre or apple pectin.

Key words: Apple pectin: β-Glucan: Cholesterol: Functional foods: Hypercholesterolaemia: Lupin protein: Pea protein

The management of individuals with moderate hypercholesterolaemia in primary prevention constantly poses the question whether it is more acceptable to treat with drugs or with an appropriate diet[1,2]. In fact, although low-risk individuals with accompanying features, such as elevated inflammatory markers, could possibly benefit from statins, as shown, for example, in the JUPITER (Justification for the Use of Statins in Prevention: An Intervention Trial Evaluating Rosuvastatin) trial[3], the evidence is less clear for individuals with a baseline Framingham risk score between 5 and 10 % over 10 years and with LDL-cholesterol (LDL-C) levels below 1600 mg/l (ASCOT-LLA[4], ASPEN[5] and FIELD (Fenofibrate Intervention and Event Lowering in Diabetes)[6] studies). In these cases, concern might be raised by the potential muscular side effects of statins[7] as well as by the costs[8].

A well-planned diet may be quite effective: the most extreme example is provided by the 'portfolio diet' approach developed by Jenkins *et al.*[9]. In severe hypercholesterolaemic individuals, this diet, containing vegetable proteins, viscous fibres, phytosterols and tree nuts, was as effective as a mid-dose statin[10]. However, the long-term compliance of such a diet may be problematic.

Thus, besides a 'healthy diet' regimen, generally resulting in LDL-C reductions of $<5\%$[11], it may be useful to advise patients on functional foods or dietary supplements[12]. In this field, soya protein has attracted considerable interest, since the early demonstration in 1977 of its cholesterol-reducing properties[13]. This initial report was followed by numerous investigations summarised in a meta-analysis[14], indicating a particular sensitivity of hypercholesterolaemic individuals to this protein. However, in the following years, these results were confirmed by other groups[15], with the limitation that patients with severe hypercholesterolaemia could not be involved, as the treatment of these subjects

Abbreviation: LDL-C, LDL-cholesterol.

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with drugs has become compulsory[16]. A recent review[17] has confirmed that consuming 25 g of soya protein instead of animal proteins in the diet of adults with normal or mild hypercholesterolaemia results in significant reductions in total and LDL-C, equivalent to 6 % LDL-C reduction.

Some recent papers suggest that the proteins of other grain legumes may possess similar activities[18,19]. For example, investigations on a rat model of hyperlipidaemia have shown that pea proteins[20,21] and lupin proteins[22,23] are effective in reducing total cholesterol and LDL-C. In addition, a recent study on a rabbit model of atherosclerosis has shown that the latter is also able to slow the formation of atherosclerotic plaques[24]. Clinical studies on these innovative plant proteins are, instead, still scarce[25–28].

The main objectives of the present work were twofold: (1) to investigate the potential cholesterol-lowering activity of lupin or pea proteins in hypercholesterolaemic subjects; (2) to verify whether combinations of one plant protein and one soluble fibre in the same food product may provide an improved cholesterol-lowering activity. The selected soluble fibres were oat fibre[29,30] and apple pectin[31], two fibres with different structural characteristics. Owing to fund limitation, it was possible to test combinations including only one plant protein.

A double-blind clinical trial was thus planned, with a parallel design: the participants were treated with dietary bars containing the protein/fibre combinations for 4 weeks, casein being the control protein and cellulose the control fibre. The present study was carried out in a single centre with a large input of hypercholesterolaemic participants.

Subjects, materials and methods

Participants

The present study was conducted according to the guidelines laid down in the Declaration of Helsinki. Participants were recruited among those attending the University Centre for Dyslipidaemias of the Niguarda Hospital (Milano, Italy), according to the following criteria: (1) males and postmenopausal females; (2) participants in primary prevention, non-diabetics; (3) total cholesterol >2200 mg/l and TAG <2000 mg/l. Only participants free from any lipid-lowering drug and not affected by any kind of food allergy were enrolled. All procedures were in accordance with the ethical standards of the institution on human experimentation and, before starting, the study was approved by the Ethics Committee of the Niguarda Hospital. The participants were carefully informed about the modalities and end points of the present study and, after receiving all necessary information, they signed their written informed consent.

Design

A randomised, double-blind, parallel group design was followed, with total cholesterol as the main end point. All the candidates underwent a stabilisation period on a hypolipidaemic dietary regimen for 4 weeks (Table 1). Dietary intakes were assessed using a 3 d food diary designed by a qualified dietitian. After this period, a baseline blood sample was drawn. All participants showing cholesterol changes exceeding ± 10 % were excluded from the present study. The selected subjects were randomised into seven dietary treatment groups: each group consumed two bars per day containing the specific protein/fibre combination for 4 weeks. The first group consumed casein as the protein and cellulose as the fibre (control); in the second and the third groups lupin or pea protein replaced casein and the fibre was cellulose; the fourth and fifth groups consumed casein as the protein and oat fibre and apple pectin as the fibre, respectively; the sixth and seventh group received combinations of pea protein with oat fibre and apple pectin, respectively. At the starting visit (time 0), all randomised subjects received their complete bar supplies for the treatment period. The composition of the total diet during the present study is reported in Table 1. At the end of this, participants were examined by an attending physician, recording body weight and measuring blood pressure with the subject in sitting position. A final blood sample was recovered for the evaluation of the lipid, metabolic and inflammatory changes after treatment.

Composition of the dietary bars

The dietary bars, whose composition is given in Table 2, were prepared by the Fraunhofer Institute (IVV, Freising, Germany). The main ingredients were a protein (casein, pea protein or lupin protein) and a fibre (cellulose, oat fibre or apple pectin), whereas the other ingredients listed in the table were added to obtain suitable texture and taste; the bars with casein contained a larger amount of water in order to allow an easier chewing. Sodium sorbate was added as a preserving agent and, immediately after production, the packed bars were sealed under a modified atmosphere.

Pea protein was a commercial protein isolate from seeds of *Pisum sativus* (Pisane C9; Provital Industrie SA, Pecq, Belgium), constituted by 90·8 % pea protein on DM and low amounts of fat (1·2 %) and fibre (0·9 %). The lupin protein isolate was prepared by the Fraunhofer Institute starting from deoiled *Lupinus angustifolius* cultivar Boregine, consisting of 91·7 % lupin protein on DM and low amounts of fat (1·16 %) and ash (3·39 %). The production procedure is described elsewhere[32]. The oat fibre was an experimental ingredient containing 25–28 % β-glucan provided by CreaNutrition

Table 1. Nutrient intake during the study
(Medians, and minimum and maximum values)

	Median	Minimum–maximum
Energy (MJ)	6·7	5·8–8·6
Protein (% of energy)	17·0	15·8–18·2
Carbohydrate (% of energy)	54·2	52·3–56·8
Fat (% of energy)	24·1	21·8–25·3
Saturated fat (g)	9·1	6·3–11·6
Unsaturated fat (g)	7·3	6·2–8·8
Fibre (g)	33·8	29·7–37·7

Table 2. Total components of the two bars that each participant consumed per day

	Casein/ cellulose	Lupin protein/ cellulose	Pea protein/ cellulose	Casein/ oat fibre	Casein/ pectin	Pea protein/ oat fibre	Pea protein/ pectin
Carbohydrate content (g)	54·0	54·0	54·0	53·8	54·0	53·8	54·0
Lipid content (g)	3·5	3·5	3·5	3·5	3·5	3·5	3·5
Protein content (g)	40·6	34·6	34·6	40·6	40·6	34·6	34·6
Fibre (g)	10	10	10	10·5	10	10·5	10
Water (g)	35	10	10	35	35	10	10
Total mass (g)	152	121	121	152	152	121	121
Energy (MJ)	1·56	1·46	1·46	1·52	1·53	1·52	1·53

AG (Zurich, Switzerland), whereas apple pectin was a high methylester pectin from apple peels (degree of esterification 68–78%, Pectine Classic AU201 ESP; Herbstreith & Fox, Neuenbürg, Germany).

Laboratory procedures

Blood samples were collected after an overnight fast. Both serum and EDTA plasma were prepared by low-speed centrifugation at 4°C and stored at −80°C. Plasma total cholesterol, TAG, HDL-cholesterol and glucose concentrations were determined with standard enzymatic techniques on a Roche Diagnostics Cobas 400 Analyser (Roche Diagnostics GmbH, Mannheim, Germany). Plasma LDL-C was calculated with Friedewald's formula. High-sensitivity C-reactive protein concentrations were measured by immunoturbidimetry on a Roche Diagnostics Cobas 400 Analyser (Roche Diagnostics GmbH). Plasma levels of the soluble forms of intracellular cell adhesion molecule-1, IL-6 and adiponectin were measured by using commercially available monoclonal antibody-based ELISA kits (R&D Systems, Minneapolis, MN, USA). Serum insulin levels were determined by a solid-phase enzyme-amplified sensitivity immunoassay performed on breakable microtiter plates (Biosource International, Camarillo, CA, USA). The assays were performed in duplicate for each sample. The homeostasis model assessment of insulin resistance index was calculated as proposed by Matthews *et al.*[33].

Statistical analysis

Results were expressed as mean values and standard deviations, if not otherwise stated. Data with skewed distributions (TAG) were log-transformed before analyses. Changes caused by each treatment were analysed by using the paired *t* test. Multiple comparison analysis was performed by ANOVA with Dunnett's test, followed by adjustment for baseline values, for comparisons of the six treated groups *v.* the control group (casein/cellulose). Group differences with $P<0{\cdot}05$ were considered as statistically significant.

Results

The participant flow diagram is shown in Fig. 1. In total, 271 subjects were evaluated in order to find the most appropriate candidates. At the end, 193 subjects were selected and evaluated before and after a run-in period on the standard dietary regimen for 4 weeks. After the stabilisation period, two were non-compliant and sixteen subjects had cholesterol changes exceeding ±10% and were therefore excluded. A total of 175 participants were thus randomised into seven dietary treatment groups. The demographic and anthropometric characteristics and the lipid values measured at randomisation in participants participating in the present study are shown in Table 3. The comparison of baseline values by ANOVA revealed a borderline significant difference among groups. In total, seventeen subjects dropped out from the present study: three because of health problems (one because of vomiting, one because of nausea and one because of minor gastrointestinal side effects) and fourteen because they considered the bars unsatisfactory when consumed daily. The vast majority were, however, satisfied with the product they had received. There were no significant body weight changes during the treatments and, in particular, no subject reported any relevant body weight reduction (data not shown).

The plasma total cholesterol changes are reported in Table 4. There were no cholesterol changes in the control diet (casein + cellulose), a fact that strongly supports the reliability of the present study. Statistically significant total cholesterol reductions were observed in the case of casein + apple pectin (−152 mg/l, −5·3%), lupin protein + cellulose (−116 mg/l, −4·2%), pea protein + oat fibre (−135 mg/l, −4·7%) and pea protein + apple pectin (−168 mg/l, −6·4%). When all the groups were compared by ANOVA with the Dunnett's test, followed by adjustment for baseline values, the association of pea protein + apple pectin showed the most statistically significant reduction ($P=0{\cdot}0098$ *v.* control).

Data on LDL-C were mainly confirmatory: the combinations lupin protein + cellulose and casein + apple pectin were slightly active, but the changes were not statistically significant, whereas the combinations of pea protein + oat fibre and pea protein + apple pectin determined a statistically significant decrease (−118 mg/l, −5·8% and −106 mg/l, −9·2%, respectively). When all the groups were compared by ANOVA with the Dunnett's test, followed by adjustment for baseline values, the association of pea protein + apple pectin showed the most statistically significant reduction ($P=0{\cdot}004$ *v.* control).

HDL-cholesterol changes (Table 4) were negligible; in general, there were small reductions (mean −12 mg/l) randomly distributed among the different treatment groups. Similarly, TAG changes were again minimal, some treatments being

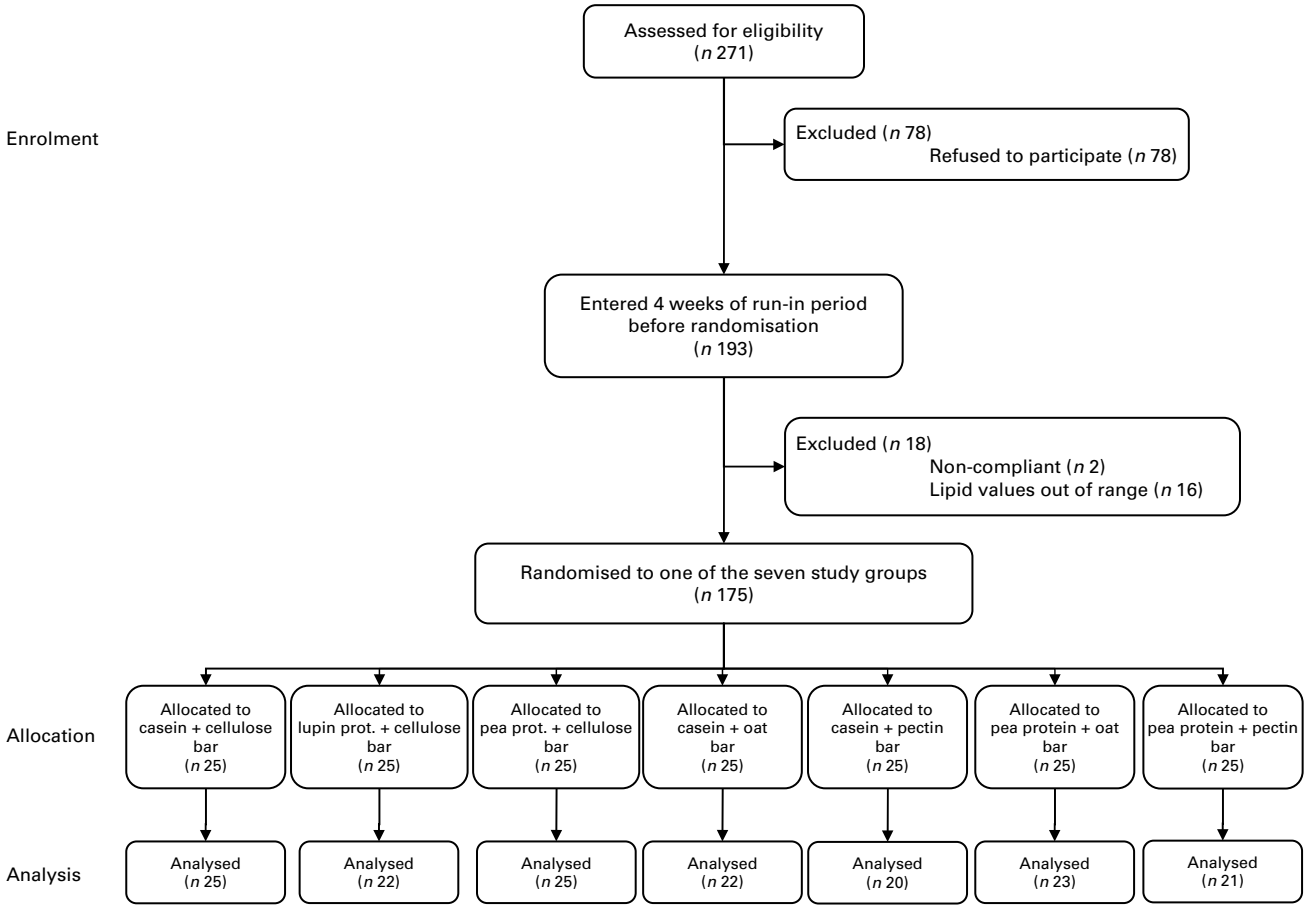

Fig. 1. Participation flow throughout the trial. prot., protein.

followed by modest increases (e.g. pea protein + apple pectin) or reductions (e.g. lupin protein + cellulose).

The homeostasis model assessment of insulin resistance index values were significantly reduced after consumption of casein + cellulose, casein + pectin and pea protein + oat fibre. This was mainly dependent on the insulin level reductions, whereas only the last combination was able to decrease glucose concentrations also (Table 5). On the whole, the most promising combination was pea protein + oat fibre, as the participants showed 4% glucose reduction, 57% insulin reduction and 25% decrease of the homeostasis model assessment of insulin resistance index. Adiponectin and the inflammatory markers remained essentially constant with all the treatments (Table 5).

Table 3. Baseline characteristics of the participants
(Mean values, standard deviations and number of participants)

	Casein/ cellulose		Lupin protein/ cellulose		Pea protein/ cellulose		Casein/ oat fibre		Casein/ pectin		Pea protein/ oat fibre		Pea protein/ pectin	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Sex (*n*)														
Female	15		12		11		11		14		17		13	
Male	10		13		14		14		11		8		12	
Age (years)	54·7	10·5	52·3	12·4	52·5	12·7	54·3	12·8	54·6	15·5	55·3	14·6	53·9	15·9
BMI (kg/m^2)	25·4	4·2	24·0	2·0	25·0	2·1	24·9	3·4	25·1	3·0	25·6	3·2	25·3	3·6
SBP (mmHg)	113·2	16·9	110·5	10·8	111·4	10·9	113·2	9·9	110·0	16·5	111·7	9·8	113·8	19·7
DBP (mmHg)	73·0	9·4	75·2	8·2	74·2	8·1	75·5	9·1	72·1	7·8	78·3	7·2	75·0	9·1
Total cholesterol (mg/l)	2724	344	2740	400	2711	263	2619	326	2912	521	2913	403	2641	353
TAG (mg/l)	1261	454	1456	709	1498	796	1253	469	1246	456	1563	861	1438	783
LDL-cholesterol (mg/l)	1888	365	1882	354	1824	276	1787	299	2003	560	2018	413	1740	350
HDL-cholesterol (mg/l)	570	124	560	139	569	151	581	141	628	131	590	134	570	135

SBP, systolic blood pressure; DBP, diastolic blood pressure.

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Table 4. Effects of treatments on lipid parameters
(Mean values and standard deviations)

		Casein/ cellulose (*n* 25)		Lupin protein/ cellulose (*n* 22)		Pea protein/ cellulose (*n* 25)		Casein/ oat fibre (*n* 22)		Casein/ pectin (*n* 20)		Pea protein/ oat fibre (*n* 23)		Pea protein/ pectin (*n* 21)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Total cholesterol (mg/l)	Baseline	2724	344	2740	400	2711	263	2619	326	2870	448	2913	403	2641	353
	4 weeks	2752	269	2624*	408	2684	255	2634	360	2718*	408	2775*	362	2473*	290
LDL-cholesterol (mg/l)	Baseline	1888	365	1882	354	1824	276	1787	299	1973	476	2018	413	1740	350
	4 weeks	1937	267	1826	406	1908	361	1813	327	1885	453	1900*	294	1581*	292
HDL-cholesterol (mg/l)	Baseline	570	124	560	139	569	151	581	141	644	130	590	134	570	135
	4 weeks	576	128	547	157	584	153	560	142	618	122	572	141	555	120
TAG (mg/l)	Baseline	1261	454	1456	709	1498	796	1253	469	1185	454	1563	861	1438	783
	4 weeks	1152	346	1265	596	1316	452	1286	562	1102	376	1470	816	1520	931

* Mean values were significantly different from those of baseline ($P<0·05$).

Discussion

In the present clinical trial, dietary bars containing different mixtures of plant proteins and soluble fibres were tested with the objective of developing new strategies for the prevention and the long-term control of mild hypercholesterolaemia based on innovative ingredients and combinations. The present study was carried out in a single clinical centre and included participants with a low cardiovascular risk and those willing to undergo a non-drug treatment. The present study was conducted in favourable conditions, as most participants were well known by the personnel of the centre; the diet of each individual was planned in detail by an expert dietitian and, finally, all participants who, after the run-in period, showed cholesterol changes exceeding ±10 % were excluded. In addition, although the lower limit of cholesterolaemia for patient selection was 2200 mg/l, the actual baseline averages of all groups were >2600 mg/l. The reliability of the present study is confirmed by the fact that the lipid parameters of the reference group at the end of the study were essentially equal to the baseline values.

The objectives of the work were twofold: the former was to determine the cholesterol-lowering activity of lupin and pea protein, never investigated before in hypercholesterolaemic human subjects; the latter was to assess the potential efficacy of combinations of a plant protein and a soluble fibre.

The efficacy of each plant protein may be evaluated from the results of the group consuming plant proteins + cellulose combinations. There is a difference between pea protein, which was inactive, and lupin protein, which appeared to be moderately active. This difference was unexpected on the basis of the outcomes of studies based on the rat model of hyperlipidaemia, which gave equivalent results for pea protein isolates[20,21] and lupin protein isolates[22,23]. The observed decrease in total cholesterol levels (−116 mg/l, −4·2 % change) in the group consuming the lupin protein/cellulose

Table 5. Effects of bioactive fibres and proteins on metabolic and inflammatory parameters
(Mean values and standard deviations)

		Casein/ cellulose (*n* 25)		Lupin protein/ cellulose (*n* 22)		Pea protein/ cellulose (*n* 25)		Casein/ oat fibre (*n* 22)		Casein/ pectin (*n* 20)		Pea protein/ oat fibre (*n* 23)		Pea protein/ pectin (*n* 21)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Glucose (mg/l)	Baseline	807	96	806	96	807	86	796	83	815	76	841	113	824	102
	4 weeks	814	72	820	92	791	80	836	82	826	79	806*	95	830	100
Insulin (μU/ml)†	Baseline	5·2	3·3	5·3	3·4	7·8	11·2	8·6	14·5	6·0	3·4	9·7	13·6	9·5	12·5
	4 weeks	3·7*	3·1	5·6	9·5	4·2	2·0	7·3	14·0	4·0*	2·5	4·2*	3·0	8·7	14·8
HOMA-IR	Baseline	1·0	0·6	1·1	0·7	1·2	0·8	1·1	0·8	1·2	0·7	1·2	0·8	1·9	2·3
	4 weeks	0·8*	0·6	1·3	2·4	0·9	0·4	0·9	0·8	0·9*	0·6	0·9*	0·7	1·7	2·6
Adiponectin (μg/ml)	Baseline	12·2	10·3	11·0	7·1	10·0	6·3	10·2	5·4	12·2	5·1	11·7	10·0	12·0	7·7
	4 weeks	12·5	9·5	10·7	7·3	9·6	6·0	10·7	6·3	12·1	4·7	11·1	9·7	11·9	8·1
sICAM-1 (ng/ml)	Baseline	187·0	79·9	152·6	42·8	163·3	44·2	165·9	93·5	173·8	39·7	160·3	53·1	167·0	74·4
	4 weeks	191·4	79·1	153·1	45·9	163·9	42·3	180·5	99·9	168·4	40·5	159·2	55·4	172·9	67·7
IL-6 (pg/ml)	Baseline	0·2	0·2	0·3	0·4	0·3	0·3	0·2	0·1	0·2	0·1	0·3	0·3	0·2	0·2
	4 weeks	0·3	0·3	0·4	0·4	0·3	0·5	0·2	0·1	0·2	0·1	0·3	0·3	0·2	0·2
Hs-CRP (mg/l)	Baseline	2	2	3	5	2	1	2	1	2	2	2	3	2	2
	4 weeks	2	2	3	3	2	1	2	1	2	3	3	3	2	2

HOMA-IR, homeostasis model assessment of insulin resistance; sICAM-1, soluble intracellular cell adhesion molecule-1; Hs-CRP, high-sensitivity C-reactive protein.
* Mean values were significantly different from those of baseline ($P<0·05$).
† 1 μU/ml = 6·945 pmol/l.

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bar is in the same range of the cholesterol change observed when treating participants with comparable baseline cholesterolaemia with 25 g/d of soya protein[15,17]. On the contrary, in a previous study, where normocholesterolaemic subjects consumed a lupin-enriched diet for 12 months, lupin did not appear to decrease cholesterol[27]. As in the case of soya, legume proteins are effective mainly in hypercholesterolaemics[14].

As a first step to evaluate the efficacy of plant proteins and soluble fibres, it was also necessary to test casein + soluble fibre combinations. In our conditions, only pectin produced a statistically significant decrease of total cholesterol (−5·3 %), accompanied by a non-statistically significant decrease of LDL-C, whereas oat fibre resulted to be inactive. This outcome may be possibly explained by the moderate amount of β-glucan consumed by the subjects (only 2·8 g/d), as, in general, at least 3 g of this ingredient is needed to observe a clear effect[34]. There is still debate on the actual efficacy of soluble fibres in cholesterol reduction, and the reported variability is in any case very large: the range of effects on total cholesterol varies, in fact, from 0 to −18 % for oat products, from −5 to −16 % for pectin, from 3 to −17 % for psyllium and from 4 to −17 % for guar gum[35]. Several reasons may explain such variability and certainly some fibres are more effective than others; for example, psyllium-enriched cereals lower cholesterol more effectively than pectin-enriched ones[36]. In addition, the response depends on the baseline cholesterol value, hypercholesterolaemic individuals being much more sensitive than normolipidaemic individuals[29,37].

The strategy of combining a plant protein and a soluble fibre appeared to be successful, as the cholesterol reductions observed with pea protein + oat fibre (total cholesterol change −4·7 %, LDL-C change −5·8 %) or pea protein + apple pectin (total cholesterol change −6·4 %, LDL-C change −9·2 %) were larger than those of each single ingredient. The potential health benefits of the latter combination also were confirmed by the observed decreases of glucose, insulin and homeostasis model assessment index. The observed LDL-C change falls in the high range of recent trials on soya protein[38] and is comparable to the activity of phytosterols/stanols in individuals with similar hypercholesterolaemia[1].

As the data on pea and lupin are scarce, the mechanism of the hypocholesterolaemic activity of plant protein may be deduced from what is known on soyabeans. Rodent and *in vitro* studies have established a link between the hypocholesterolaemic effects of soya and the activation/depression of liver LDL receptors[39,40]. Animals on cholesterol/cholic acid dietary regimens with casein have a dramatic down-regulation of liver LDL receptors, and this effect is reversed in the presence of soya proteins. Two clinical studies have confirmed these experimental outcomes. In the former[41], familial hypercholesterolaemia patients were treated with animal protein or textured soya protein (with the addition of cholesterol to balance the two diets) and both plasma lipids and LDL degradation by circulating lymphomonocytes (used as mirror images of hepatocytes) were monitored. After the animal protein diet, there were minimal changes in LDL-C levels or LDL receptor activity, whereas during the soya protein diet, in addition to a marked LDL-C reduction, an increase of approximately eightfold in LDL degradation was observed. This study was subsequently confirmed in individuals with lesser cholesterol elevations[42]. The bioactive soya components are probably peptides released during protein digestions[43].

The main mechanism of action of soluble fibres, instead, is linked to impaired cholesterol and bile acid absorption from the gut. A study[44] on β-glucan from oat, for example, has shown a significant reduction of two markers of cholesterol absorption (serum plant sterol concentrations) and synthesis (serum concentrations of lathosterol) by 13 and 11 %, respectively. Other mechanisms are related to the fermentation of dietary fiber in the colon by intestinal bacteria[45]. This fermentation produces, among other products, short-chain acids (mainly acetate and propionate), which are adsorbed in the colon mucosa together with water and enter liver, where they affect lipid metabolism, including cholesterol[46].

The main mechanisms at the base of the fibre and plant protein activities being so different, it is at present difficult to hypothesise how they participate to the observed activity.

In conclusion, although this is only a first study carried out in a single centre, its successful outcomes, particularly in relation to the combination of pea and apple pectin, encourage further investigations with the participation of more centres from different nations and the use of well-designed foods with improved sensory properties for an optimal compliance. Although the market of functional foods and dietary supplements already offers numerous products for controlling cholesterol, the use of bioactive ingredient combinations opens new possibilities of choice for patients compelled to follow restricted diets for a very long period.

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