The effect of graded inclusion of baked beans (*Phaseolus vulgaris*) on plasma and liver lipids in hypercholesterolaemic pigs given a Western-type diet

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The aim of the present study was to measure the effect of graded inclusion of baked beans (*Phaseolus vulgaris*) on plasma and liver lipids in hypercholesterolaemic pigs fed on a Western-type diet. Twenty-four Large White × Landrace pigs of about 30 kg body weight were made hypercholesterolaemic by feeding a semi-purified diet, high in saturated fat and supplemented with 10 g cholesterol/kg, for 14 d. After that, six pigs were randomly assigned to one of the four experimental groups. They received their respective diets, containing 0, 100, 200 or 300 g baked beans/kg, on a dry-matter basis, for a further 28 d. Fasting blood samples were taken and analysed for total plasma cholesterol, lipoproteins and triacylglycerols. After the pigs were slaughtered at the end of the study, livers were analysed for their cholesterol content. Consumption of baked beans at 100, 200 and 300 g/kg reduced plasma total cholesterol by 5·3, 20·2 and 35·6% respectively. However, only the diet with 300 g baked beans/kg showed a significant reduction ($P < 0.05$) compared with the control (without baked beans). The level of low-density-lipoprotein-cholesterol was also significantly ($P < 0.05$) reduced by 48% at 300 g baked beans/kg. Plasma very-low-density-lipoprotein-cholesterol, high-density-lipoprotein-cholesterol and triacylglycerol contents were not affected by bean consumption. The supplements of 200 and 300 g baked beans/kg promoted a significant ($P < 0.05$) reduction of about 50% in cholesterol deposition in the liver, compared with the control.

Pig: Baked beans: Cholesterol: Lipoproteins: Liver lipid

As raised plasma cholesterol levels are associated with an increased risk of coronary heart disease (CHD), much attention has been placed on manipulation of the diet to restore normal levels of cholesterol in plasma. Some foods, such as oats and legumes, have been extensively studied in this context and reported to reduce plasma cholesterol levels in experimental animals (Cho *et al.* 1985; Kozuharov *et al.* 1986; Sautier *et al.* 1986; Rigotti *et al.* 1989) and in humans (Zavoral *et al.* 1983; Anderson *et al.* 1984; Behall *et al.* 1984). Although the mechanism responsible for the hypocholesterolaemic property of legumes is not completely understood, soluble forms of dietary fibre (Nuovo, 1989; Anderson *et al.* 1990), saponins (Oakenfull *et al.* 1984) and the composition of the protein (Sirtori *et al.* 1979; Gibney, 1982) have all been suggested as the causative factors. It is possible that this effect is due not to a single constituent of the beans but to a number of factors working together. Recent reviews (Shutler *et al.* 1987*a, b*; Kingman, 1991) have pointed out some possible mechanisms responsible for the hypocholesterolaemic property of these legumes.

Haricot beans (*Phaseolus vulgaris*) processed as canned baked beans in tomato sauce are widely consumed in the UK diet. This product has been found to reduce plasma cholesterol when included in the diet of hypercholesterolaemic pigs (Shutler *et al.* 1988) and healthy young normolipidaemic subjects (Shutler *et al.* 1989). In the pig study, baked beans given

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at a level of 300 g/kg (dry-matter basis) in the diet for 4 weeks reduced plasma cholesterol levels by 39 % and increased significantly HDL:total cholesterol.

These findings have stimulated us to look in more detail at the hypocholesterolaemic property of baked beans. A point not yet investigated is whether lower levels of baked beans, such as 200 or 100 g/kg diet, have a similar capacity to lower plasma cholesterol as 300 g/kg substitution, or whether the effect is dose-dependent. In the present study we have measured the dose–response relationship of baked beans in tomato sauce on plasma and hepatic cholesterol levels in hypercholesterolaemic pigs. For reasons of comparison the experimental conditions were kept as close as possible to those adopted by Shutler *et al.* (1988) in their work with pigs.

METHODS

Diets

The composition of the semi-purified diets is given in Table 1. Diets C, B1, B2 and B3 were designed to provide the same composition in terms of total protein, fat and carbohydrate when baked beans (*Phaseolus vulgaris*) in tomato sauce were substituted in the diet at 0, 100, 200 and 300 g/kg on a dry-matter basis respectively. The diets provided about 12 % of the energy from protein, 48 % from carbohydrate and 40 % from fat, with polyunsaturated:saturated fatty acids (P:S) ratio of approximately 0.3, which is similar to that of a typical UK human diet.

Crystalline cholesterol was added at 10 g/kg in the diets. It was thoroughly mixed with soya-bean oil before addition to the other ingredients. The diets were fed at 30 g/kg body weight per d in two equal meals at 08.30 and 15.30 hours. Tap water (2.5 l/kg air-dry diet) and baked beans were added to the semi-purified diets immediately before feeding. Additional water was available on an *ad lib.* basis.

Experimental design

Twenty-four Large White × Landrace male pigs of about 30 kg live weight were fed on the semi-purified diet C for 14 d. They were then divided equally into six blocks in accordance with their responsiveness to dietary cholesterol, in such a way that block 1 contained the highest mean levels of plasma cholesterol and block 6 the lowest ones. Six pigs comprising one from each block were randomly assigned to one of the four experimental diets (C, B1, B2 or B3), as shown in Table 2. The mean values of plasma total cholesterol were as similar as possible between groups on day 14. Groups C, B1, B2 and B3 received their experimental diet for a further 28 d.

The animals were accommodated in individual floor pens in a temperature-controlled room, maintained at $23 \pm 3°$ throughout the experiment. They were weighed at weekly intervals in order to monitor growth and to calculate diet requirement. At 14 d intervals fasting blood samples were taken by venepuncture into heparinized tubes and centrifuged at $1500 g$ for 15 min. Plasma was stored at $-20°$ before measurement of circulating metabolites. At the end of the trial the animals were slaughtered and the livers stored at $-20°$ before lipid analysis.

Chemical analyses

The analysis of plasma metabolites were carried out in an automated analyser using appropriate enzymic reagent kits (Baker Instruments Ltd, Egham, Surrey). Total plasma cholesterol estimation was based on the method of Allain *et al.* (1974). Plasma lipoproteins were fractionated by differential ultracentrifugation, on the basis of their hydrated density, into very-low-density (VLDL), low-density (LDL) and high-density (HDL) components according to the procedure described in this paragraph. Then total cholesterol content of

Table 1. *Composition of the experimental diets (g/kg, on a dry-matter basis)*

	Diet			
Ingredient	C	B1	B2	B3
Baked beans (*Phaseolus vulgaris*)*	—	100	200	300
Maize starch	478	427	394	358
Sucrose	84	78	70	62
Soya-bean oil	30	30	27	25
Silkido (beef tallow)	145	145	145	145
Casein	157	130	105	76
Solka-floc (cellulose)	57	46	20	—
Mineral mix†	10	9	8	7
Vitamin mix‡	2	2	2	1
Dicalcium phosphate	31	28	24	22
Choline chloride	1	1	1	1
Sodium chloride	5	4	4	3
Total	1000	1000	1000	1000
Cholesterol	10	10	10	10

* Composition of baked beans in accordance with Paul & Southgate (1978).

† Composition of the mineral mix (g/kg mixture): K_2CO_3 447, $MgCO_3 . 3H_2O$ 173, $FeSO_4 . 7H_2O$ 33, $ZnCO_3$ 10, $MnSO_4 . 4H_2O$ 8, $CuSO_4 . 5H_2O$ 1·7, NaF 0·8, $CoCl_2$ 0·6, maize starch 325·8.

‡ Composition of the vitamin mix (g/kg mixture): retinol 6·25, thiamin hydrochloride 1, riboflavin 1·625, pyridoxine 1·625, cyanocobalamin 0·015, ascorbic acid 15, cholecalciferol 0·3, DL-α-tocopheryl acetate 4, biotin 2·5, menadione sodium bisulphite 1, nicotinic acid 7·875, pantothenic acid 8, folic acid 0·5, *p*-aminobenzoic acid 10, inositol 97·5, maize starch 842·81.

Table 2. *Assignment of pigs to dietary groups based on plasma cholesterol level (mmol/l) on day 14*

Dietary group*	Block						Mean
	1	2	3	4	5	6	
C	8·30	6·53	5·65	5·41	4·97	4·56	5·90
B1	8·58	7·92	5·86	4·71	4·27	1·99	5·55
B2	8·27	6·24	5·62	4·43	4·01	3·05	5·27
B3	7·28	6·34	6·34	5·45	4·09	3·99	5·83
Mean	8·11	6·76	5·87	5·00	4·33	3·40	

C, control; B1, 100 g baked beans (*Phaseolus vulgaris*)/kg; B2, 200 g baked beans/kg; B3, 300 g baked beans/kg.

* For details, see Table 1 and p. 516.

each fraction was analysed by the method of Allain *et al.* (1974). The ultracentrifugation was carried out using a TL-100 Tabletop ultracentrifuge (Beckman Instruments, Palo Alto, CA, USA) fitted with a titanium TLA-100.2 rotor, at 500000 *g*, 16° for 2·5 h. Plasma samples (0·5 ml) were mixed with 0·5 ml NaCl solution (9 g/l), to achieve a combined density of 1·006 g/ml, and centrifuged. The top 0·5 ml fraction was removed using a modified Pasteur pipette curved through 90° at the tip. The fraction was taken from the meniscus, by rotating the pipette, and was then analysed for VLDL-cholesterol. The remaining 0·5 ml sample was mixed with 0·5 ml NaCl solution (167 g/l; combined density of 1·063 g/ml) and centrifuged at 500000 *g* for 2·5 h. The top 0·5 ml fraction was removed

and analysed for LDL-cholesterol. The bottom fraction (0·5 ml) was analysed for HDL-cholesterol. The saline solutions were prepared with anhydrous NaCl, augmented by EDTA (1 mg/ml) as a preservative. Plasma triacylglycerol content was estimated by the method of Fossati & Prencipe (1982).

Liver lipids were extracted in Soxhlet thimbles by the method of Folch *et al.* (1957). Samples (about 1 g) were previously minced in a domestic grinder and weighed accurately. A volume of 0·5 ml of the lipid extract was taken into a glass ampoule, mixed with 50 μl 5α-cholestane (1 mg/ml ethanol) and dried under N_2. Methanoic KOH (1 ml, 1 M) was added, the ampoule was sealed and then boiled for 1 h. After cooling, 1 ml hexane was added, followed by approximately 10 ml saturated NaCl and mixed. Samples were left to stand until complete phase separation. The top layer, containing cholesterol, was taken into small vials for GLC, which was carried out using a Pye Unicam series 204 chromatograph (Pye Unicam Ltd, Cambridge), fitted with a flame-ionization detector. A 2 m × 4 mm i.d. glass column packed with Supelcoport (100–120 mesh) coated with 3 % SP 2100 (Supelchem Ltd, Saffron Walden, Essex) was used. The carrier gas flow-rate was 40 ml N_2/min. The injection and detection temperatures were 260° and 270° respectively. Cholesterol concentration of the samples was determined against a calibration curve obtained from cholesterol standards.

Statistical analysis

Results were analysed by one-way analysis of variance (ANOVA), according to a random block design. Results obtained for plasma metabolites on day 14 were taken as covariate for the results of day 28 and day 42. The analyses were carried out on Genstat package (Rothamsted Experimental Station). Mean values of treatments were compared with those for the control group (without baked beans) by Student's *t* test. All resulting significance levels refer to the two-sided alternative.

RESULTS

Animals

The pigs readily adapted to the experimental conditions. Some of them, however, presented transitory signs of vomiting and diarrhoea for a period of 1 or 2 d, maybe due to the amount of fat and baked beans in the diet. No drug treatment was prescribed for them during the experimental period. Diets were given at 30 g/kg body weight, on a dry-matter basis, and the animals consumed all the food offered to them. At the end of the trial pigs fed at 0, 100, 200 and 300 g baked beans/kg consumed respective amounts of 50·9, 51·8, 49·8 and 48·6 kg diet. Body weight is shown in Fig. 1. No statistical difference was observed between pigs fed on baked beans and the control group throughout the experiment, although at the levels of 200 and 300 g baked beans/kg there was a slight reduction in body weight.

Total plasma cholesterol

The effect of the diet on plasma cholesterol levels is shown in Fig. 2. The pigs developed hypercholesterolaemia after eating the control diet (C) for 14 d. Nevertheless, the individual response to the diet was highly variable. For this reason hyper- and hyporesponders were allocated equally in the experimental groups, on day 14, in such a way that mean values of plasma cholesterol were as similar as possible between groups. The high variability in responsiveness to dietary cholesterol is reflected by the high standard deviation of the means of the group (Fig. 2).

The effect of baked beans on plasma cholesterol levels can be seen by comparing the results of day 14 with those of day 28 and day 42. The addition of baked beans to the diets reduced plasma cholesterol levels in proportion to the amount of beans eaten. At the end
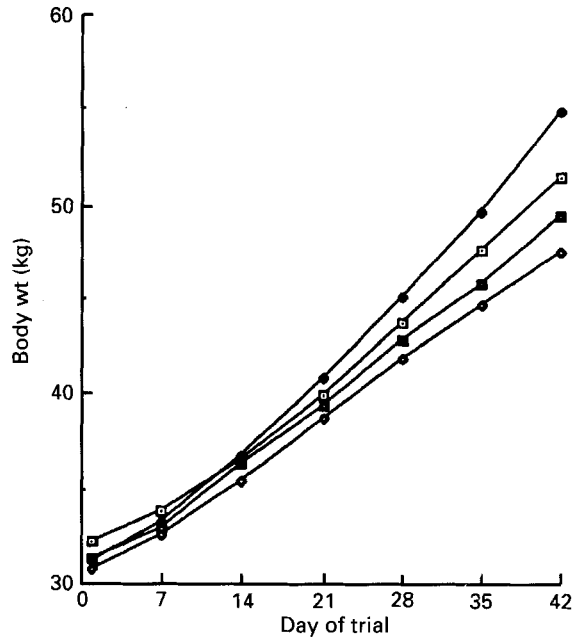
Fig. 1. Body weight (kg) of pigs fed on control diet (without baked beans (*Phaseolus vulgaris*)) for the first 14 d of the trial and at different levels of baked beans (0 (▣), 100 (◆), 200 (■) or 300 (◇) g/kg) from day 14 to day 42. For details of diets and procedures, see Table 1 and p. 516.
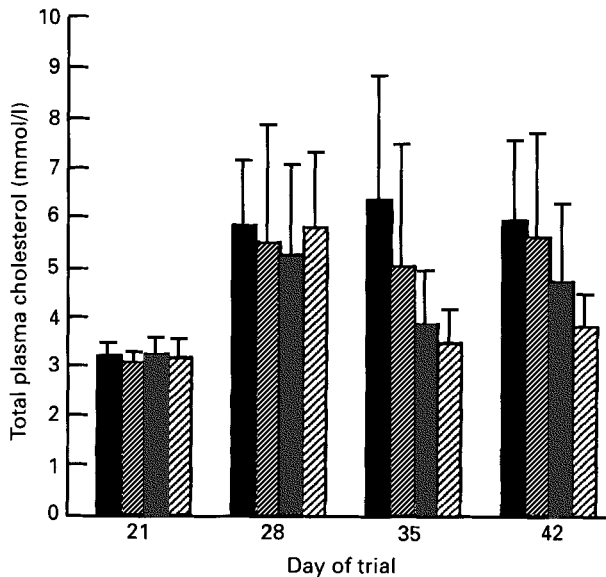

Fig. 2. Total plasma cholesterol (mmol/l) of pigs fed on control diet (without baked beans (*Phaseolus vulgaris*)) for the first 14 d of the trial and at different levels of baked beans (0 (■), 100 (▨), 200 (▩) or 300 (▨) g/kg) from day 14 to day 42. For details of diets and procedures, see Table 1 and p. 516. Values are means and standard deviations represented by vertical bars. Mean values were significantly different from those for control group (Student's *t* test): $P < 0.05$.
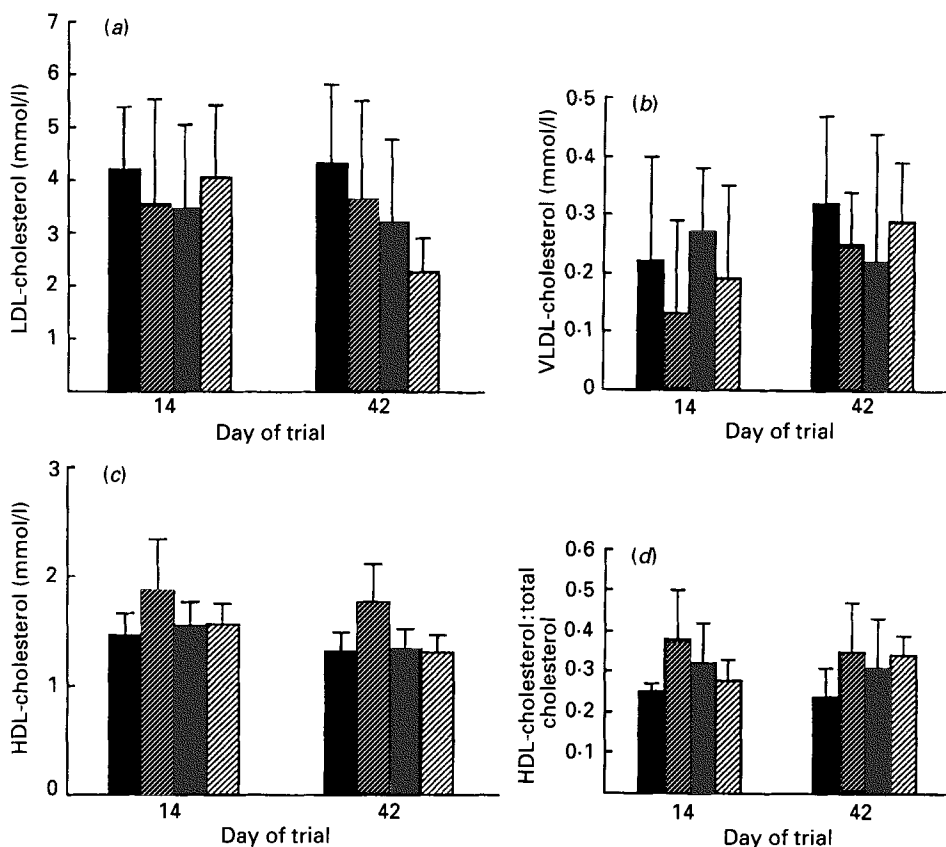
Fig. 3. Plasma lipoprotein fractions of pigs fed on control diet (without baked beans (*Phaseolus vulgaris*)) for the first 14 d of the trial and at different levels of baked beans (0 (■), 100 (▨), 200 (▤) or 300 (▧) g/kg) from day 14 to day 42. For details of diets and procedures, see Table 1 and pp. 516–518. (*a*), low-density-lipoprotein (LDL)-cholesterol; (*b*), very-low-density-lipoprotein (VLDL)-cholesterol; (*c*) high-density-lipoprotein (HDL)-cholesterol; (*d*) HDL-cholesterol:total cholesterol. Values are means and standard deviations represented by vertical bars. Mean values were significantly different from those for control group (Student's *t* test): $P < 0.05$.

of the trial (day 42) pigs on diets with 100, 200 and 300 g baked beans/kg respectively showed reductions of 5·3, 20·2 and 35·6 % in their total plasma cholesterol; only the diet with 300 g baked beans/kg showed a significant reduction ($P < 0.05$) compared with the control group (without baked beans).

Plasma lipoproteins

Fig. 3 shows the plasma lipoprotein levels when pigs were fed on control (day 14) and experimental (day 42) diets.

LDL-cholesterol (Fig. 3(*a*)) was the lipoprotein fraction most affected by the bean consumption. Although the diets containing 100 and 200 g beans/kg lowered plasma cholesterol by 16 and 20 % respectively, only the diet containing 300 g/kg promoted a statistically significant reduction ($P < 0.05$) of 48 %.

In relation to VLDL-cholesterol (Fig. 3(*b*)), there was no significant difference between treatments throughout the experiment. Although there was a trend towards a general increase in VLDL-cholesterol on day 42, this does not seem to be related to the bean intake since the level was also increased in the control group.
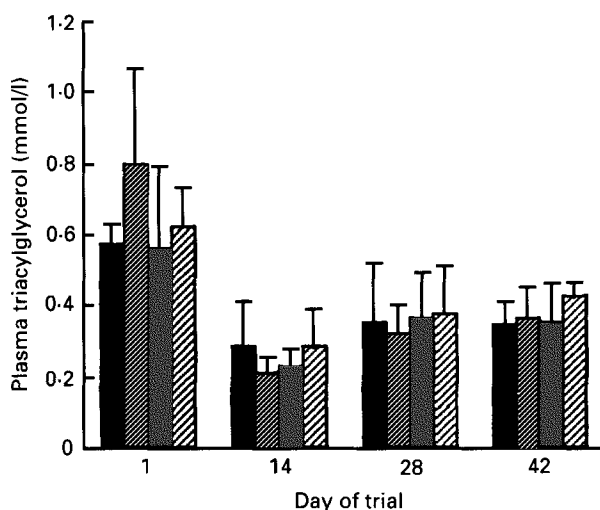
Fig. 4. Plasma triacylglycerol (mmol/l) of pigs fed on control diet (without baked beans (*Phaseolus vulgaris*)) for the first 14 d of the trial and at different levels of baked beans (0, (■), 100 (▨), 200 (▧) or 300 (▨) g/kg) from day 14 to day 42. For details of diets and procedures, see Table 1 and pp. 516–518. Values are means and standard deviations represented by vertical bars.

Table 3. *Fresh weight, lipid and cholesterol composition of the livers of pigs fed on different levels of baked beans* (Phaseolus vulgaris; *0, 100, 200 or 300 g/kg*)

Diet†	Liver wt (g)	Total lipid (mg/g)	Cholesterol (mg/g)
C	824·4	53·8	4·4
B1	939·4	54·0	4·6
B2	757·4	50·9	2·3*
B3	794·3	49·5	2·2*

C, control; B1, 100 g/kg; B2, 200 g/kg; B3, 300 g/kg.
Mean values were significantly different from those for the control group (Student's *t* test): * $P < 0.05$.
† For details of diets and procedures, see Table 1, and pp. 516–518.

The mean levels of HDL-cholesterol (Fig. 3(c)) of the group receiving 100 g baked beans/kg were higher in both periods of analysis, i.e. before and after bean intake. When the values of day 14 were used as covariate, no statistically significant difference was obtained between treatments on day 42. Therefore, any change in HDL-cholesterol cannot be attributed to the bean intake but was due to a non-homogeneity between groups when treatment was applied. The values for HDL:total cholesterol were highly variable. There was a significant ($P < 0.05$) increase in the ratio of pigs receiving 100 and 300 g baked beans/kg on day 42. For the pigs on 100 g beans/kg the increase does not seem to be related to the bean intake, since the level was also significantly higher on day 14. The increased ratio in the group receiving 300 g baked beans/kg reflects the reduction observed in the level of LDL-cholesterol of that group in the same period.

Triacylglycerols
The results obtained for triacylglycerols are shown in Fig. 4. In contrast to the results for plasma total cholesterol, mean triacylglycerol levels were reduced when pigs were fed on the

control diet, rich in fat (day 14). From day 14 to day 42 pigs showed an increase in their plasma triacylglycerol levels, but the effect was not significant. These findings are consistent with those obtained for VLDL-cholesterol, the triacylglycerol-rich lipoprotein.

Liver lipid

Table 3 shows the effect of baked bean intake on total liver lipid and liver cholesterol. No statistical difference was observed between groups in terms of total liver lipid. Nevertheless, the diets containing 200 and 300 g baked beans/kg promoted a significant reduction ($P < 0.05$) of about 50 % in cholesterol deposition in the liver, compared with the control group. The addition of 100 g baked beans/kg had no effect on total lipid and cholesterol in the liver compared with the control group.

DISCUSSION

The consumption of a high-saturated-fat diet, supplemented with 10 g cholesterol/kg for 14 d, raised plasma cholesterol levels of the pigs by 76 %. A similar result was obtained by Shutler *et al.* (1988) when pigs were fed on a diet of the same composition. Kunnert *et al.* (1982) also reported a large increase in serum cholesterol in pigs fed on a diet with 150 g lard plus 15 g cholesterol/kg. This indicates that dietary cholesterol can promote hypercholesterolaemia in pigs fed on a diet with a high saturated fat content. In our study the responsiveness to dietary cholesterol was highly variable and accounted for the high standard deviation of the groups. Indeed, hyper- and hyporesponders to dietary cholesterol were identified in a similar study with pigs (Shutler *et al.* 1988) as well as humans (Katan *et al.* 1986) and rats (Beynen *et al.* 1984).

The addition of baked beans to the diets reduced plasma total cholesterol levels in proportion to the amount of beans added. The level of 100 g baked beans/kg reduced plasma cholesterol by 5·3 %, 200 g baked beans/kg by 20·2 % and 300 g baked beans/kg showed a significant reduction of 35·6 % compared with the control group. This is of interest in relation to the dose–response relationship of soluble fibre on plasma cholesterol reported by Nuovo (1989) in humans. These findings suggest that the greater the fibre intake the greater is the cholesterol-lowering effect. Baked beans contain 134 g non-starch polysaccharide/kg, on a dry-matter basis, of which 8·2 % is soluble (Englyst *et al.* 1988). Foods rich in soluble fibre, such as bean products, have frequently been reported to lower plasma cholesterol in pigs (Cho *et al.* 1985; Shutler *et al.* 1988), rats (Kozuharov *et al.* 1986; Rigotti *et al.* 1989) and humans (Anderson *et al.* 1984; Lo *et al.* 1986; Ullrich, 1987; Shutler *et al.* 1989; Anderson *et al.* 1990). Based on these findings, it seems that the soluble fibre of baked beans contributed substantially to the reduced plasma cholesterol levels obtained in the present study. Although a significant reduction on plasma total cholesterol was obtained only at 300 g bean/kg substitution in pigs, others (Shutler *et al.* 1989; Anderson *et al.* 1990) have shown that in man plasma total cholesterol was significantly reduced when beans were consumed at lower levels in the diet (approximately 100 g/d, on a dry-weight basis). The difference between pig and human protocols may have contributed to these results. The high intakes of saturated fat and cholesterol in the pig diet may have accounted for more resistance of the animals to reduce significantly their plasma cholesterol levels when fed at lower levels of baked beans, such as 100 and 200 g/kg.

The cholesterol-lowering effect of baked beans in the present study was found to be exerted in the LDL-cholesterol fraction. Although the levels of 100 and 200 g baked beans/kg showed a reduction in LDL-cholesterol of 16 and 26 % respectively, only 300 g/kg substitution promoted a statistically significant reduction of 48 %. LDL-cholesterol was also reported to be reduced in pigs fed on soya-bean fibre (Cho *et al.* 1985).

The same effect was observed in humans receiving either soya-bean fibre (Lo *et al.* 1986) or gums (Behall *et al.* 1984) in their diets. Therefore, there seems to be a direct relationship between the intake of soluble fibre and the selective reduction of LDL-cholesterol levels. The other lipoproteins were not affected by bean consumption in the present study. In addition, plasma triacylglycerol level was neither affected by intake of dietary cholesterol nor by baked beans. In their work with pigs, Shutler *et al.* (1988) also found no change in triacylglycerol levels when 300 g baked beans/kg were given. Working with hyper-cholesterolaemic rats, Sharma (1986) found no effect on plamsa triacylglycerol level when a legume (fenugreek (*T. foenum graecum*) seed) was given for 4 weeks, although plasma cholesterol levels were reduced. A number of authors have reported that soluble fibre also has no triacylglycerol-lowering property in humans (Behall *et al.* 1984; Tuomilehto *et al.* 1988; Behall, 1990).

A similar effect of bean diet on body weight has also been reported in studies with rats (Chang *et al.* 1986) and humans (Anderson *et al.* 1990). This may be due to the poorer quality of the bean protein compared with casein (the source of protein in control diet), and possibly due to a lower digestibility and/or antinutritional factors present in the beans even after the canning process. Nevertheless, Yadav & Liener (1977) reported that there does not appear to be any obvious relationship between growth response to the diet and their ability to influence the cholesterol levels of the body. Therefore, the somewhat reduced body weight of the pigs fed at 200 and 300 g baked beans/kg in the present study was not considered to be a responsible factor for any effect on body cholesterol of these animals.

Hypercholesterolaemic diets have been shown in the past to develop lipid infiltration and high cholesterol deposition in the livers of pigs (Baldner-Shank *et al.* 1987) and rats (Jaya & Venkataraman, 1979). For this reason we measured the effect of baked beans on liver lipid. Our results showed no significant reduction in total liver lipid of pigs fed on different levels of baked beans. Nevertheless, a significant reduction in liver cholesterol was observed in the pigs fed on 200 and 300 g baked beans/kg compared with the control group. These findings are in agreement with those found in rats fed on either legumes (Jaya & Venkataraman, 1979) or pectin (Ershoff & Wells, 1962).

The fact that both plasma and liver cholesterol levels were reduced in pigs fed on 300 g baked beans/kg suggests that baked beans may act either by increasing cholesterol excretion and/or turnover or by inhibiting cholesterol synthesis. Further studies, however, are necessary to elucidate the mechanism by which baked beans reduce cholesterol levels.

REFERENCES

Allain, C. C., Poon, L. S., Chan, C. S. G., Richmond, W. & Fu, P. C. (1974). Enzymatic determination of total serum cholesterol. *Cinical Chemistry* **20**, 470–475.

Anderson, J. W., Gustafson, N. J., Spencer, D. B., Tietyen, J. & Bryant, C. A. (1990). Serum lipid response of hypercholesterolemic men to single and divided doses of canned beans. *American Journal of Clinical Nutrition* **51**, 1013–1019.

Anderson, J. W., Story, L., Sieling, B., Chen, W. J. L., Petro, M. S. & Story, J. A. (1984). Hypocholesterolemic effects of oat bran or bean intake for hypercholesterolemic men. *American Journal of Clinical Nutrition* **40**, 1146–1155.

Baldner-Shank, G. L., Richard, M. J., Beitz, D. C. & Jacobson, N. L. (1987). Effect of animal and vegetable fats and proteins on distribution of cholesterol in plasma and organs of young growing pigs. *Journal of Nutrition* **117**, 1727–1733.

Behall, K. M. (1990). Effect of soluble fibers on plasma lipids, glucose tolerance and mineral balance. *Advances in Experimental Medicine and Biology* **270**, 7–16.

Behall, K. M., Lee, K. H. & Moser, P. B. (1984). Blood lipids and lipoproteins in adult men fed four refined fibers. *American Journal of Clinical Nutrition* **39**, 209–214.

Beynen, A. C., West, C. E., Van Raaij, J. M. A. & Katan, M. B. (1984). Dietary soybean protein and serum cholesterol. *American Journal of Clinical Nutrition* **39**, 840–841.

Chang, K. C., Ethen, S., Harrold, R. & Brown, G. (1986). Effect of feeding dry beans on rat plasma cholesterol. *Nutrition Reports International* **33**, 659–664.

Cho, B. H. S., Egwim, P. O. & Fahey, G. C. (1985). Plasma lipid and lipoprotein cholesterol levels in swine-modification of protein-induced response by added cholesterol and soy fiber. *Atherosclerosis* **56**, 39–49.

Englyst, H. N., Bingham, S. A., Runswick, S. A., Collinson, E. & Cummings, J. H. (1988). Dietary fibre (non-starch polysaccharides) in fruit, vegetables and nuts. *Journal of Human Nutrition and Dietetics* **1**, 247–286.

Ershoff, B. H. & Wells, A. F. (1962). Effects of gum guar, locust bean gum and carrageenan on liver cholesterol of cholesterol-fed rats. *Proceedings of the Society for Experimental Biology and Medicine* **110**, 580–582.

Folch, J., Lees, M. & Sloane-Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry* **226**, 497–509.

Fossati, P. & Prencipe, L. (1982). Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clinical Chemistry* **28**, 2077–2080.

Gibney, M. J. (1982). Hypocholesterolaemic effect of soya-bean proteins. *Proceedings of the Nutrition Society* **41**, 19–26.

Jaya, T. V. & Venkataraman, L. V. (1979). Germinated legumes and their influence on liver and serum cholesterol levels in rats: influence of different components of chickpea and greengram on tissue cholesterol levels in rats. *Nutrition Reports International* **20**, 383–392.

Katan, M. B., Beynen, A. C., De Vries, J. H. M. & Nobels, A. (1986). Existence of hypo- and hyperresponders to dietary cholesterol in man. *American Journal of Epidemiology* **123**, 221–234.

Kingman, S. M. (1991). The influence of legume seeds on human plasma lipid concentrations. *Nutrition Research Reviews* **4**, 97–123.

Kozuharov, S., Oakenfull, D. G. & Sidhu, G. S. (1986). Navy beans and navy bean saponins lower plasma cholesterol concentrations in rats. *Proceedings of the Nutrition Society of Australia* **11**, 162.

Kunnert, B., Massmann, J., Wagner, J., Wujanz, G. & Krug, H. (1982). Cholesterol esters in pig liver during atherogenic diets and foreign serum protein infusion. *Experimental Pathology* **22**, 95–102.

Lo, G. S., Goldberg, A. P., Lim, A., Grundhauser, J. J., Anderson, C. & Schonfeld, G. (1986). Soy fiber improves lipid and carbohydrate metabolism in primary hyperlipidemic subjects. *Atherosclerois* **62**, 239–248.

Nuovo, J. (1989). Use of dietary fiber to lower cholesterol. *American Family Physician* **39**, 137–140.

Oakenfull, D. G., Topping, D. L., Illman, R. J. & Fenwick, D. E. (1984). Prevention of dietary hyper-cholesterolaemia in the rat by soya bean and quillaja saponins. *Nutrition Reports International* **29**, 1039–1046.

Paul, A. A. & Southgate, D. A. T., eds (1978). *McCance and Widdowson's The Composition of Foods*. 4th ed. London: H.M. Stationery Office.

Rigotti, A., Marzolo, M. P., Ulloa, N., Gonzalez, O. & Nervi, F. (1989). Effect of bean intake on biliary lipid secretion and on hepatic cholesterol metabolism in the rat. *Journal of Lipid Research* **30**, 1041–1048.

Sautier, C., Flament, C., Doucet, C. & Suquet, J. P. (1986). Effects of eight dietary proteins and their amino acid content on serum, hepatic and faecal steroids in the rat. *Nutrition Reports International* **34**, 1051–1061.

Sharma, R. D. (1986). An evaluation of hypocholesterolemic factor of Fenugreek seeds (*T. foenum-graecum*) in rats. *Nutrition Reports International* **33**, 669–677.

Shutler, S. M., Bircher, G. M., Tredger, J. A., Morgan, L. M., Walker, A. F. & Low, A. G. (1989). The effect of daily baked bean (*Phaseolus vulgaris*) consumption on the plasma lipid levels of young, normo-cholesterolaemic men. *British Journal of Nutrition* **61**, 257–265.

Shutler, S. M., Low, A. G. & Walker, A. F. (1988). Influence of baked beans on plasma lipids in pigs fed on a hypercholesterolaemic diet. *Proceedings of the Nutrition Society* **47**, 97A.

Shutler, S. M., Walker, A. F. & Low, A. G. (1987a). The cholesterol-lowering effects of legumes. I. Effects of the major nutrients. *Human Nutrition: Food Science and Nutrition* **41**F, 71–86.

Shutler, S. M., Walker, A. F. & Low, A. G. (1987b). The cholesterol-lowering effects of legumes. II. Effects of fibre, sterols, saponins and isoflavones. *Human Nutrition: Food Science and Nutrition* **41**F, 87–102.

Sirtori, C. R., Gatti, E., Mantero, O., Conti, F., Agradi, E., Tremori, E., Sirtori, M., Fraterrigo, L., Tavazzi, L. & Kritchevsky, D. (1979). Clinical experience with the soybean protein diet in the treatment of hypercholesterolemia. *American Journal of Clinical Nutrition* **32**, 1645–1658.

Tuomilehto, J., Silvasti, M., Aro, A., Koistinen, A., Karttunem, P., Gref, C. G., Ehnholm, C. & Uusitupa, M. (1988). Long term treatment of severe hypercholesterolaemia with guar gum. *Atherosclerosis* **72**, 157–162.

Ullrich, I. H. (1987). Evaluation of a high-fiber diet in hyperlipidemia: a review. *Journal of the American College of Nutrition* **6**, 19–25.

Yadav, N. R. & Liener, I. E. (1977). Reduction of serum cholesterol in rats fed vegetable protein or an equivalent amino acid mixture. *Nutrition Reports International* **16**, 385–389.

Zavoral, J. H., Hannan, P., Fields, D. J., Hanson, M. N., Frantz, I. D., Kuba, K., Elmer, P. & Jacobs, D. R. Jr (1983). The hypolipidemic effect of locust bean gum food products in familial hypercholesterolemic adults and children. *American Journal of Clinical Nutrition* **38**, 285–294.