THE INFLUENCE OF LEGUME SEEDS ON HUMAN PLASMA LIPID CONCENTRATIONS

SUSAN M. KINGMAN

Dunn Clinical Nutrition Centre, 100 Tennis Court Road, Cambridge CB2 1QL

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INTRODUCTION

Early studies of the effects of dietary carbohydrates on plasma lipid levels identified leguminous seeds as potential hypocholesterolaemic agents (Luyken *et al.* 1962; Grande *et al.* 1965). As interest in the relationship between diet and lipid metabolism has developed, many investigations into the influence of legumes on cholesterol and triacylglycerol levels in the blood have been made. Particular interest has been shown in two legume derivatives, guar gum and soya-bean protein.

Several investigators have studied the effects of a leguminous diet on human subjects, but many more have utilized animal models, particularly the rat. Whilst it is necessary to use animals for certain experimental manipulations, inherent differences in the metabolism of blood lipids in different animal species may sometimes make the results of such studies difficult to interpret. For example, in the rabbit, low-density-lipoprotein (LDL)-receptor activity is markedly down-regulated by dietary cholesterol, resulting in a rapid hypercholesterolaemia in response to a cholesterol-containing diet. In the rat, however, dietary cholesterol does not regulate LDL-receptor activity and does not lead to large increases in plasma cholesterol unless a further manipulation, such as the feeding of cholic acid, is made (Goldstein & Brown, 1984). In between these two extremes animals exhibit a whole spectrum of different responses to dietary cholesterol. Some species, such as the pig, exhibit within-species variation which has been described as hyper- or hypo-response to dietary cholesterol. A less consistent hyper- hypo-response phenomenon occurs in humans consuming cholesterol-rich diets (Beynen *et al.* 1987).

The hyperlipidaemias observed in man may have their origins in quite different metabolic defects from those deliberately induced in animals, and it is by no means certain that the effects of dietary components will be the same in both cases. For this reason, the present review will focus specifically on the influence of legumes and legume isolates on lipid metabolism in humans. Recourse to animal studies will only be made where no information is otherwise available.

In order to understand better the following discussion, first it is necessary to define and examine the nutritional characteristics of legumes, and second to review normal lipid metabolism in humans. The effects of whole pulses, legume polysaccharides and soya-bean-protein preparations on human lipid metabolism will then be discussed.

DEFINITION AND NUTRITIONAL CHARACTERISTICS OF LEGUMES

Legumes may be defined as the dried seeds of plants from the *Leguminosae* family, the members of which are characterized by having their fruit in a pod. Many different *Leguminosae* species are grown as food or feed crops (Duke, 1981). Their seeds usually fall into one of two main classes: pulses, in which the principal storage material is a polysaccharide, usually starch, and oilseeds, in which the principal storage material is fat. Legume seeds are typically rich in protein and non-starch polysaccharides (NSP), and contain significant levels of oligosaccharides which contribute to the flatulence often experienced following their consumption (Price *et al.* 1988). Table 1 shows the major components of some common pulses and oilseeds.

The amount of protein in legume seeds ranges from 200 to 400 g/kg dry matter (Mosse & Pernollet, 1983), one of the richest sources of legume protein being the soya bean (*Glycine max*). The proteins of legumes are relatively good sources of lysine, aspartic acid, glutamic acid and leucine, but are generally deficient in the sulphur-containing amino acids methionine and cystine (Kanamori *et al.* 1982; Mosse & Pernollet, 1983).

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Table 1. *Typical composition of some common legumes (g/kg dry matter)*
(Compiled from Paul & Southgate, 1978; Duke, 1981; Englyst *et al.* 1988)

Legume	Protein	Fat	Starch	Sugars	Non-starch polysaccharides		
					Total	Soluble	Insoluble
Butter beans (*Phaseolus lunatus*)	241	10	530	51	178	74	104
Haricot beans (*Phaseolus vulgaris*)	219	17	525	27	183	91	112
Dried peas (*Pisum sativum*)	232	13	613	30	151	44	107
Red lentils (*Lens culinaris*)	272	18	581	29	59	18	41
Soya beans (*Glycine max*)	391	197	132	120	166	72	94
Peanuts (*Arachis hypogaea*)	254	513	57	32	66	20	46

With the exception of oilseeds, legume seeds tend to contain only small amounts of lipid (g/kg), for example, lentils (*Lens culinaris*) 6 (Duke, 1981), *Phaseolus* beans 10–20 (Tobin & Carpenter, 1978) and chick peas (*Cicer arietinum*) 50–60 (Duke, 1981). However, the lipid component is highly unsaturated, consisting of 55–78 mol unsaturated fatty acids/100 mol (Salunkhe *et al.* 1983), and often contains relatively high levels of other constituents such as plant sterols, isoflavones and saponins which may be physiologically active (Shutler *et al.* 1987*b*).

The starch content of legumes is notoriously difficult to measure, but it is estimated that they contain between 2 and 565 g/kg dry matter (Reddy *et al.* 1984). Legume starches are particularly rich in the unbranched moiety amylose, which is found at levels in the range 100–600 g/kg total starch (Reddy *et al.* 1984). Thus, they are particularly prone to retrogradation and the development of resistant starch after heat treatment (Englyst & Kingman, 1990). Legume starch may also resist digestion due to entrapment within thick-walled cells (Wursch *et al.* 1986). These, and possibly other aspects of legume starches (O'Dea & Wong, 1983), undoubtedly contribute to the exceptionally low rate at which they are digested in vivo, and the low blood glucose and insulin responses that follow their ingestion (Jenkins *et al.* 1980*b*).

The NSP of legumes is present in both the hull and the cotyledon, but the hull tends to contain more cellulose and other insoluble NSP fractions than the cotyledon which contains more soluble NSP (Champ *et al.* 1986). Generally, legumes are considered to be rich sources of soluble NSP, which ranges from 18 g/kg dry matter in split, red lentils to 91 g/kg in haricot beans (*Phaseolus vulgaris*) (Englyst *et al.* 1988). Also present, at varying levels, are oligosaccharides of the sucrose family, particularly raffinose, stachyose and verbascose (Reddy *et al.* 1984). The amount and type of oligosaccharide present is dependent on the species of legume.

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LIPID METABOLISM IN HUMANS

BIOSYNTHESIS OF CHOLESTEROL

Almost every cell in the body is potentially capable of *de novo* cholesterol synthesis, and sufficient is synthesized every day to meet body requirements (Sabine, 1977). Synthesis is balanced, and to a certain extent regulated by the absorption of cholesterol from the diet, and under normal circumstances cholesterol homeostasis is maintained within fairly narrow limits.

The major sites for biosynthesis of cholesterol are the liver and intestines (Brown & Goldstein, 1976), the liver probably being responsible for the majority of production (Dietschy & Siperstein, 1967; Dietschy & Wilson, 1970). The precursor of cholesterol is acetate, a small, ubiquitous molecule produced during the metabolism of protein, carbohydrate and fat. This undergoes a stepwise conversion involving twenty-five enzymes into squalene and, thence, to cholesterol. The first stage in the conversion is a series of reactions by which acetyl-CoA is built up into mevalonic acid. The series is catalysed by microsomal enzymes of which the most important is 3-hydroxy 3-methylglutaryl-CoA reductase (*EC* 1.1.1.88; HMG-CoA reductase), which is the rate-limiting enzyme of cholesterol biosynthesis and, therefore, a target for intervention (Goldstein & Brown, 1984). Recently, pharmacological agents which inhibit the action of HMG-CoA reductase have become available for the treatment of hypercholesterolaemia (Gordon & Rifkind, 1987).

ABSORPTION

An average of 400–500 mg cholesterol enters the intestines daily from the Western human diet, mainly from animal products. In addition 800–1200 mg biliary cholesterol/d is presented to the gut (Grundy & Metzger, 1972). In order to be absorbed, cholesterol is solubilized by incorporation into the hydrophobic centre of micelles comprising bile acids, fatty acids, monoacylglycerols and lysolecithin (Hofmann & Bergstrom, 1962, 1964). Transport of cholesterol across the mucosal membrane occurs by simple, passive diffusion (Grundy, 1983). This seems to be the rate-limiting step for cholesterol absorption and is another point at which intervention may take place.

Once absorbed into the mucosal cell the cholesterol undergoes esterification with long-chain fatty acids. The esters thus formed combine with free cholesterol and small amounts of specific apoproteins to form chylomicrons which are released into the lymphatics.

TRANSPORT

Lipoproteins are the vehicles used to transport cholesterol and triaclyglycerols in the bloodstream. The behaviour of lipoproteins during analysis provides the means for their classification into four broad categories. Using the ultracentrifuge it is possible to separate chylomicrons (the lightest fraction with a density less than plasma), very-low density lipoproteins (VLDL), LDL and high-density lipoproteins (HDL). Each of these fractions contains protein, phospholipid, triacylglycerol and cholesterol, but in varying proportions (Levy, 1981). The major cholesterol carrying particle is LDL, but HDL also plays an important role in cholesterol flux.

Chylomicrons

Lipid transport may be considered to start in the intestine with the assembly of chylomicrons from triacylglycerols and cholesterol which become available during the absorption of dietary fat (see Fig. 1). Chylomicrons (CM) are large, light, triacylglycerol-

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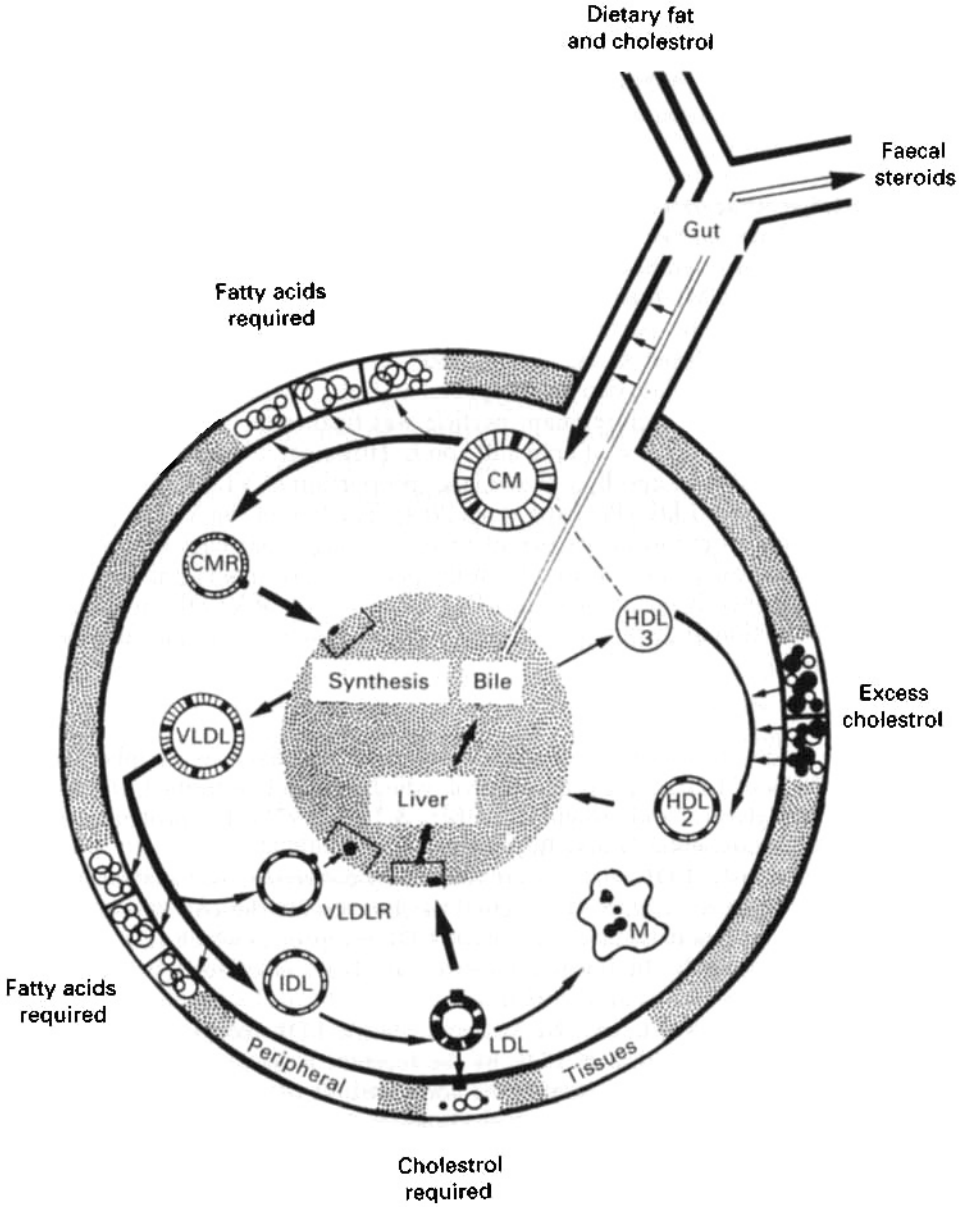

Fig. 1. Lipid metabolism in humans. Dietary fat and cholesterol enter the bloodstream as chylomicrons (CM) and after disseminating their lipid content are assimilated by the liver as CM remnants (CMR). The liver secretes very low-density lipoproteins (VLDL) which carry lipid to the tissues and become VLDL remnants (VLDLR) or intermediate density lipoproteins (IDL) which are subsequently metabolized to low-density lipoproteins (LDL). LDL are taken up by the tissues or scavenged by macrophages (M). High-density lipoproteins (HDL) receive lipid from tissues and other lipoproteins, and return it to the liver.

rich particles containing a characteristic apoprotein known as apo B_{48}. Their function is to transport triacylglycerols to sites of combustion or the adipose tissue store. Thus, as they travel around the bloodstream they are rapidly depleted of triacylglycerol and become relatively enriched with cholesterol. At this stage CM receive apo E molecules from HDL

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(Imaizumi *et al.* 1978) and become what are known as CM remnants. These are rapidly cleared from the plasma by avid binding to a hepatic receptor called the apo E (remnant) receptor (Innerarity *et al.* 1982) which recognizes the combination of apo E and apo B_{48} (Van Berkel *et al.* 1983). Lipid transport resumes with the hepatic synthesis of VLDL.

VLDL

VLDL are large, triacylglycerol-rich lipoproteins which are secreted by the liver at a relatively constant rate. Unlike CM their triaclyglycerol load is derived from endogenous supplies, either *de novo* synthesis or uptake of circulating free fatty acids (Mayes & Topping, 1974). Like CM, their primary function is to transport triacylglycerols to extrahepatic tissues. As the particles become depleted of triacylglycerol they become relatively enriched in cholesterol and two apoproteins, apo B_{100} and apo E, becoming VLDL remnants or intermediate density lipoproteins (IDL; Sparks & Sparks, 1985). Until recently it was thought that each remnant particle was further metabolized to become an LDL particle, with the further loss of lipid and apo E. However, it is now clear from studies of the metabolism of labelled apo B_{100}, that a large proportion of VLDL apo B_{100} does not become incorporated into LDL (Packard *et al.* 1984). The fate of the VLDL particle after metabolism appears to depend on its size at secretion, larger particles becoming VLDL remnants which are subsequently cleared from the plasma, and smaller particles becoming IDL which are subsequently metabolized to LDL. Since the size of VLDL on secretion may depend on the interaction of dietary, genetic and hormonal factors, this may also be an area open to intervention.

LDL

LDL are the major transporters of cholesterol from the liver to peripheral tissues. Approximately 50% of LDL mass is cholesterol, which is mainly esterified, the rest being phospholipid, triacylglycerol and protein (Eisenberg & Levy, 1975). The protein component of human LDL is almost entirely apo B_{100} (Schaefer *et al.* 1978).

As indicated previously, LDL particles are formed by conversion of hepatically secreted small VLDL via IDL intermediates. The actual mechanism for the conversion of IDL to LDL is not known, but it may involve the hepatic LDL receptor to which IDL binds avidly (Brown & Goldstein, 1983). In normal humans, all LDL is derived from VLDL by intravascular conversion (Janus *et al.* 1980).

LDL delivers its cholesterol to cells by way of a specific LDL receptor which is present in the cell membrane. Recognition of LDL by the receptor is by the apo B_{100} component (Goldstein & Brown, 1977). Most tissues in the body are able to express the LDL receptor, but it is thought that many cells satisfy their cholesterol needs by endogenous synthesis (Dietschy, 1984). The liver clears at least two-thirds of the LDL in plasma, mostly via the hepatic LDL receptor (Brown & Goldstein, 1983). Binding of LDL to the receptor results in internalization of the particle with consequent suppression of further receptor expression and down-regulation of cholesterol synthesis.

HDL

HDL are small, dense particles, consisting mainly of protein, but containing small amounts of phospholipid, free cholesterol, triacylglycerol and cholesteryl ester (Eisenberg, 1984). The characteristic apoproteins of HDL are apo AI (70%) and apo AII (20%; Tall & Small, 1978). The HDL fraction typically contains two subfractions, HDL_2 and HDL_3. HDL_2 has a lower density than HDL_3 and contains more cholesteryl esters which are derived from the lipolysis of CM and VLDL (Taskinen *et al.* 1982), from the surface coat of intact lipoproteins or from cell membranes.

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Nearly all the variation in plasma HDL concentration may be explained by variation in the HDL_2 subfraction (Anderson *et al.* 1978). This, in turn, is strongly related to the activity of lipoprotein lipase (*EC* 3.1.1.34; Kekki, 1980) and, hence, to rates of triacylglycerol transport (Huttunen *et al.* 1976). Thus, certain dietary perturbations which increase the production or turnover of CM and VLDL will also raise HDL_2 levels.

The best known hypothetical role of HDL is in 'reverse cholesterol transport', that is, the net efflux of free cholesterol from cells. Most tissues appear to possess binding sites for HDL (Eisenberg, 1984), and it has been demonstrated to act as an effective acceptor of cholesterol from cells. Having accepted the cholesterol it may redistribute it to other peripheral cells, transfer it to other lipoprotein carriers or deliver it to the liver.

DEGRADATION AND EXCRETION OF CHOLESTEROL

The major pathway for the removal of cholesterol is its conversion to bile acids and excretion in the faeces. It may also be converted to steroid hormones and excreted from the skin surface. Bile acid synthesis takes place entirely in the liver and accounts for approximately one-third of the daily production of cholesterol (Grundy, 1978).

The two primary bile acids formed directly in man are cholic acid and chenodeoxycholic acid. These acids are conjugated with either glycine or taurine to yield four primary bile salts which are secreted into the bile and pass through the biliary tract into the duodenum. The bile acid pool is small (2–3 g) but there is efficient hepatic recycling whereby 98% of bile acids are reabsorbed in the small intestine and returned directly to the liver for resecretion (Grundy, 1978). Binding, or sequestration of bile acids within the intestine by dietary components or pharmacological agents, may interrupt the recycling process, forcing new bile acids to be made from cholesterol to replete the pool.

HYPERLIPIDAEMIC STATES

Abnormally high levels of plasma lipids (hyperlipidaemias) may occur in response to a number of genetic and environmental influences and manifest in a number of different forms. At present, knowledge of the genetic factors governing the hyperlipidaemias is fragmentary, so a phenotypic classification of these conditions is used for their definition. Fredrickson *et al.* (1967) described five distinct abnormalities of lipoprotein metabolism, of which the most significant are types II, III and IV.

Type II hyperlipidaemia, or hypercholesterolaemia, is the most common of the lipid disorders and is characterized by elevated levels of plasma cholesterol, most of which is found in the LDL fraction. If the raised cholesterol levels are accompanied by raised triacylglycerol levels, the disease is designated type IIb; if triacylglycerol levels are normal, the disease is type IIa. The prevalence of type II hyperlipidaemia within the population, and the relative strength of the association between elevated LDL levels and atherosclerosis compared with that of triacylglycerols, makes this hyperlipidaemia by far the most important in terms of risk from heart disease.

Grundy (1987) has suggested the existence of three divisions of hypercholesterolaemia dependent on the degree of genetic influence. These are 'dietary hypercholesterolaemia' which is represented by cholesterol levels within the range 5·2–6·2 mmol/l, 'primary hypercholesterolaemia' which constitutes cholesterol levels above 6·2 mmol/l, and 'familial hypercholesterolaemia' (FH) which is a specific, genetically determined form of primary hypercholesterolaemia. FH arises out of a genetic deficiency of LDL receptors. Subjects homozygous for FH have no LDL receptors, whilst heterozygous subjects have about half the normal number.

The major cause of cholesterol levels within the range 5·2–6·2 mmol/l is a diet rich in

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saturated fat and cholesterol (Grundy, 1987). The extent to which cholesterol levels are raised depends on the responsiveness of the recipient (Beynen *et al.* 1987). Dietary treatment is usually sufficient to reduce plasma cholesterol levels within this range.

Type III hyperlipidaemia is characterized by accumulations of CM remnants and abnormal VLDL in the plasma, leading to elevated levels of plasma triacylglycerols. The metabolic defect responsible for this disease has not yet been identified. Type IV hyperlipidaemia is associated with accumulation of normal VLDL particles in the plasma and seems to represent an inability to convert these particles to LDL. Plasma levels of LDL and HDL are commonly below normal.

INFLUENCE OF LEGUMES ON LIPID METABOLISM

STUDIES USING WHOLE PULSES

Since 1965 at least fifteen studies of the effects of whole pulses on plasma lipid levels have been carried out. Of these, eight have involved hyperlipidaemic subjects (mainly types IIa, IIb, and IV) and seven have used normolipidaemic subjects, either healthy or diabetic. Despite major differences in the protocols of the studies reviewed, all but one showed a significant reduction in the total plasma cholesterol level of the study group by the end of the study period. On average legumes produced a fall in total cholesterol of 16% of the initial level of the study group, but in individual studies reductions of from 7 to 26% have been reported. The observed differences in the magnitude of cholesterol reduction between the studies are undoubtedly related to the experimental protocols followed, which have differed in such aspects as the amount of legume consumed, species eaten, background diet, initial lipid level of the group, and the time-course of the study.

Quantity

The quantity of legume seeds that must be consumed in order to achieve a significant reduction in plasma cholesterol has not been clearly defined. In the long term it may be possible to sustain a hypocholesterolaemic effect by habitual consumption of relatively small quantities of beans. Bingwen *et al.* (1981) observed that the minimum quantity of mixed legumes to have an effect on plasma cholesterol levels in free-living Chinese subjects was 1 kg dry weight/month (about 33 g/d). Over 60% of subjects consuming legumes at this rate experienced reductions in plasma cholesterol, compared with only 16·7% of subjects consuming half this quantity per month over a period of 3 months. In shorter experiments there seems to be a lower limit of between 60 and 100 g at which inclusion of beans in an otherwise uncontrolled diet can influence the cholesterol levels of normolipidaemic people. Shutler *et al.* (1989) showed that the consumption of one 450 g can of baked beans in tomato sauce daily (approximately 120 g dry weight of beans) significantly reduced the plasma cholesterol level of male students eating a self-selected diet over a period of 2 weeks. However, a subsequent experiment following a similar protocol but using one 225 g can of baked beans (approximately 60 g dry weight) daily failed to show any reduction in plasma cholesterol in normal subjects (J. A. Tredger & L. M. Morgan, personal communication). In controlled studies using hyperlipidaemic subjects smaller quantities of beans have been shown to have a hypocholesterolaemic effect (Anderson *et al.* 1990).

It is not clear whether consumption of legumes in excess of about 100 g dry weight/d results in any further benefit with regard to plasma cholesterol. In an uncontrolled experiment with free-living hyperlipidaemic subjects who were already stabilized on a lipid-lowering diet, Jenkins *et al.* (1983) found that the individual changes in serum cholesterol experienced by their subjects were largely unrelated to the quantity of mixed legumes

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consumed, within the range 95–190 g/d. All subjects had reduced serum cholesterol levels but there was a broad spread of individual response that was not attributable to adherence to the diet. However, Anderson *et al.* (1984*a*) observed that subjects who adhered conscientiously to a long-term bean diet did experience greater cholesterol reductions than those whose compliance was only fair or poor. In this case, good compliance required the consumption of 100 g (dry weight) beans/d or an equivalent amount of oat bran.

Very few studies have addressed the question of a dose-response to legume seeds. Anderson *et al.* (1990) were the first to study the effects of different doses of the same legume species on cholesterol levels in a controlled situation. They compared diets containing either 120 or 162 g wet weight of *Phaseolus* beans (as canned 'pork and beans') given to hypercholesterolaemic subjects either in a single dose or at two separate meals. Although marked reductions in serum cholesterol were observed, there were no significant differences between the cholesterol levels of the groups consuming the higher and lower doses, whether these were eaten at a single meal or at two meals. However, the two doses were not very different from each other, and the quantities eaten were small compared with other studies, making a differential response difficult to detect. A dose-response to different levels of *Phaseolus* beans has recently been demonstrated in pigs (A. F. Walker, personal communication).

Species

The level of inclusion of legumes required to have an effect on plasma cholesterol may depend upon the type of legume consumed. However, species specific differences are difficult to assess, as there have been no direct comparisons of single legume species using the same study group or protocol. In the majority of cases the need for variety in the diet, particularly in long-term studies, has prompted investigators to use mixtures of different legume seeds, usually varieties of *Phaseolus* beans, peas, lentils and chick peas (Grande *et al.* 1965; Bingwen *et al.* 1981; Simpson *et al.* 1981; Jenkins & Jepson 1982; Jenkins *et al.* 1983; Nervi *et al.* 1989). However, several different types of legume, including various cultivars of *Phaseolus vulgaris* (Anderson *et al.* 1984*a*, *b*: Shutler *et al.* 1989), chick peas (Mathur *et al.* 1968), roasted lucerne (*Medicago sativa*) seeds (Molgaard *et al.* 1987) and fenugreek (*Trigonella foenum graecum*) seeds (Sharma & Raghuram, 1990) have been studied in short-term single-species experiments. Because of the unpredictability of individual responses to legumes and differences in protocols, it is difficult to compare species efficacy across studies. However, there is some evidence to suggest that some legumes are more effective than others. For example, Molgaard *et al.* (1987) observed a significant reduction in serum cholesterol of their hypercholesterolaemic subjects following consumption of only 40 g roasted lucerne seeds/d, when other researchers have had to use more than twice this quantity of *Phaseolus* beans or fenugreek flour in order to achieve the same effect (Anderson *et al.* 1984*b*; Sharma & Raghuram, 1990). The greater apparent potency of lucerne may be related to the chemical structure of its saponins which, when isolated, bind cholesterol in vitro and have been shown to have a hypocholesterolaemic effect in animals (Malinow *et al.* 1981; Story *et al.* 1984). Other legume saponins such as those from soya bean do not interact with cholesterol in vitro, and are apparently ineffective at reducing plasma cholesterol in vivo (Malinow, 1984). For legumes with a significant content of saponins, their concentration and structure may go some way to explaining species-specific differences in effectiveness. Further experiments in humans are needed to determine to what extent differences occur between other legumes. Using pigs it has been shown that legume seeds from the species *Phaseolus vulgaris*, *Phaseolus lunatus*, *Pisum sativum*, and *Lens culinaris* reduce cholesterol levels to different extents when substituted at the same concentration into a diet based on casein and maize starch (Shutler

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et al. 1988). Thus, it is likely that legumes from different sources will also have different potencies as part of a human diet.

Background diet

In general, the background diet against which a legume diet has been studied has not been reported in detail. Where hyperlipidaemic subjects have been used, some investigators have chosen to use a standard lipid-lowering diet as a control situation (Jenkins *et al.* 1983; Molgaard *et al.* 1987), whilst others have preferred a 'traditional' high-fat background diet (Anderson *et al.* 1984*a*, *b*, 1990). However, the actual sources of nutrients replaced by the beans usually remain unspecified, even in controlled studies. In an exceptional case, Grande *et al.* (1965) showed that the carbohydrate and protein components of mixed pulses were hypocholesterolaemic when substituted isoenergetically for sucrose and soya-bean protein, but in a later study were unable to demonstrate a hypocholesterolaemic effect of mixed pulses or chick peas substituted isoenergetically for combinations of sucrose, wheat flour, gluten and egg white (Grande *et al.* 1974). In the study of Sharma & Raghuram (1990) chapatis made with defatted fenugreek flour reduced cholesterol levels compared with chapatis made with wheat flour and groundnut (*Arachis hypogaea*) protein. It is interesting to note that in two of these three studies pulses lowered cholesterol compared with products containing a legume protein and a non-legume carbohydrate source.

The fat content of the background diet against which legumes are studied appears to have a major influence on the outcome of the study. The greatest reductions in plasma cholesterol are observed when the normal diet is high in fat. For example, Anderson *et al.* (1984*b*) introduced 100 g *Phaseolus* beans into daily regimen of hyperlipidaemic subjects consuming a controlled metabolic diet containing 37% energy as fat, and reported a mean reduction in serum cholesterol of 18% after 3 weeks. In contrast, Jenkins *et al.* (1983) introduced a mean of 140 g mixed legumes into the daily diets of hyperlipidaemic subjects who were already adhering to a standard lipid-lowering diet (25% energy as fat) and observed a reduction of only 7% in total serum cholesterol. It is possible that the action of legumes is particularly effective against elevations in plasma cholesterol caused by consumption of fat. Certainly, in the study of Mathur *et al.* (1968), chick peas were highly effective at reducing the cholesterol levels of men in whom mild hypercholesterolaemia had been induced artificially by feeding 50% of energy as butterfat.

It has recently been suggested that certain legumes do not reduce plasma cholesterol unless they also displace fat in the diet (Cobiac *et al.* 1990). In uncontrolled studies, displacement of fat and reduction in energy intakes cannot be ruled out as contributory factors in the hypocholesterolaemic effect attributed to legumes. However, there is good evidence from closely controlled studies in humans (e.g. Anderson *et al.* 1984*a*) and in animals (e.g. Kingman *et al.* 1992) to indicate that legume seeds do offer a benefit that is independent of changes in dietary fat or energy intake.

Initial lipid level

Whilst the plasma cholesterol levels of both hyperlipidaemic and normolipidaemic subjects have been reduced in response to a leguminous diet, the greatest reductions have generally been seen when starting cholesterol levels are high. Bingwen *et al.* (1981) reported that 77% of hypercholesterolaemic subjects experienced a reduction in cholesterol while consuming approximately 33 g mixed legumes daily, whereas only 48% of subjects with cholesterol levels within the normal range had lower cholesterol levels as a result of the same diet. Similarly, Molgaard *et al.* (1987) observed that administration of 40 g lucerne seeds daily to hyperlipidaemic subjects affected the cholesterol levels of type II subjects, but not those of the type IV subjects who tended to have lower initial cholesterol levels.

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However, Jenkins *et al.* (1983) did not find a consistent relationship between the initial lipid levels of their seven subjects (three type II and four type IV) and the reduction in cholesterol seen as a result of a legume-rich diet. In fact, in this study, the greatest reduction was seen in a type IV subject with a relatively low starting cholesterol level.

Individual responses to a legume diet may be quite diverse. Some researchers have reported both highly responsive and unresponsive subjects (Jenkins & Jepson, 1982; Molgaard *et al.* 1987; Shutler *et al.* 1989). In uncontrolled studies it is possible that differences in response are related to compliance with the diet (Anderson *et al.* 1984*a*), but under controlled conditions they are more likely to be related to the metabolic origin of the hyperlipidaemic state exhibited by the subject.

Time-course

Short-term studies have shown that the initial phase of cholesterol reduction on commencement of a leguminous diet is very rapid, being evident within 1 week. Shutler *et al.* (1989) observed a significant reduction in the mean total cholesterol level of thirteen male students after only 3 d of daily baked bean consumption. Subsequently a progressive decline was observed over the 14 d of the study. A similar pattern was described by Anderson *et al.* (1990) who made daily measurements of plasma cholesterol in their subjects over a period of 3 weeks on a diet of canned beans. After an initial rapid decline, levels continued to fall slowly until they reached a lowest point at between 11 and 17 d. After this they tended to rise slightly, and were higher at the end of the study than at the lowest point. Thus, studies of the cholesterol-lowering effect of legumes that last less than 3–4 weeks may overestimate the effect potentially achievable in the long term.

During sustained consumption of legume seeds a new steady-state for plasma cholesterol appears to be reached after 4–8 weeks, after which no additional benefit is observed if the diet is continued unchanged. Bingwen *et al.* (1981) found that, for those subjects consuming more than 1 kg legumes/month, the significant reduction in plasma cholesterol evident after 1 month was maintained for the duration of the study (at least 3 months). Other long-term studies (Jenkins *et al.* 1983; Anderson *et al.* 1984*a*) confirm that the reductions in plasma cholesterol caused by legumes persist as long as the diet is followed.

Lipoprotein fractions

In more recent studies where the distribution of cholesterol between the lipoprotein fractions has been reported, the LDL fraction has been the most responsive to intervention with legumes, with changes in HDL being less pronounced. The LDL cholesterol is usually reduced in parallel with the reduction in total cholesterol. Of the fifteen studies reviewed, ten reported measurements of the LDL cholesterol fraction, and this was significantly reduced in all but one by a mean of 18% (range 8–28%). Molgaard *et al.* (1987) measured the concentrations of apolipoproteins B and A-1 in the plasma of hyperlipidaemic subjects consuming 40 g roasted lucerne seeds daily. They found that apo B was reduced in proportion to the reduction in LDL-cholesterol, suggesting a decrease in the number rather than the size of circulating LDL particles. Apo A-1 and its HDL counterpart were unchanged.

In the three studies where VLDL-cholesterol has been measured (Simpson *et al.* 1981; Molgaard *et al.* 1987; Nervi *et al.* 1989), no significant change in the blood concentration of this fraction has been observed. This suggests that the reduced LDL concentration is the result of either a change in the proportion of VLDL converted to LDL, or a specific increase in the catabolism of LDL particles. Since changes in LDL turnover in response to a leguminous diet have not been studied in humans, the metabolic events occurring are open to speculation. From work in pigs (Shutler, 1988) there is some evidence to suggest

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that the fractional catabolism of LDL is not increased by *Phaseolus vulgaris* beans, and that, by inference, it is the rate of synthesis of LDL from VLDL that is affected. Further metabolic studies, preferably in humans, are much needed if progress is to be made in this area.

HDL-cholesterol levels are usually unchanged by a legume diet. In a few cases, significant reductions in HDL-cholesterol have contributed to the overall decline (Anderson *et al.* 1984*a*, 1990; Shutler *et al.* 1989), but in these cases the HDL:LDL or HDL:total cholesterol ratio has always been maintained at initial levels. Anderson *et al.* (1984*a*) reported that HDL-cholesterol levels fell significantly while their subjects were consuming a controlled diet containing beans. However, when the same subjects were subsequently maintained on a high-fibre diet containing oat bran and beans for a further 24 weeks in a free-living environment, their HDL-cholesterol levels gradually rose to starting levels whereas LDL-cholesterol remained at a lower level. Thus, in the long term the HDL-:LDL-cholesterol ratio of these subjects increased. Occasionally the HDL-cholesterol is found to be raised by legumes (Simpson *et al.* 1981).

Opinions are divided as to the effects of a legume diet on plasma triacylglycerol levels. Approximately half the studies reviewed reported no significant change in triacylglycerol levels while the other half detected significant reductions, particularly in hypertriglyceridaemic subjects (types IIb and IV). Simpson *et al.* (1981) showed that triacylglycerol levels were not affected by a high-legume diet in patients with insulin-dependent diabetes mellitus, but that they were reduced in non-insulin-dependent diabetics. Triacylglycerol levels were also significantly reduced in non-insulin-dependent diabetics in response to fenugreek chapatis (Sharma & Raghuram, 1990). It is possible that this effect is related to the reduced glycaemic and insulinaemic responses occurring after legume consumption.

Steroid excretion

The excretion of steroids in faecal matter has been measured in only two studies using whole pulses. In the first study (Mathur *et al.* 1968), twenty subjects consumed a very-high-fat diet containing an unspecified quantity of chick peas for a period of 55 weeks. During this period the authors observed a significant increase in the excretion of total bile acids, but there was no change in the output of neutral sterols in comparison with the high-fat diet alone. In the second study, Anderson *et al.* (1984*b*) discovered that men consuming a controlled diet containing approximately 115 g dry weight of *Phaseolus* beans/d excreted significantly less bile acid in their faeces than they had while they were consuming a control diet. As in the chick-pea study, neutral sterol excretion was unaffected. An additional observation from this study was that faecal weight and faecal dry weight were not significantly increased by eating beans. These results were in contrast to those obtained for oat bran in the same study, which increased both faecal weight and steroid output, and seem to be in conflict with those normally obtained for diets containing rich sources of NSP (Stasse-Wolthuis, 1981). However, the results are supported by a study of the effects of four legume species on plasma cholesterol levels in cholesterol-fed pigs in which neither faecal weight nor faecal steroid excretion (neutral or acid) was increased as a result of legume consumption, compared with a control group (Kingman *et al.* 1992). It is clear that further studies of the effects of legume seeds on faecal weight and composition in humans are required before any reliable conclusions can be drawn on this point.

Biliary saturation

In epidemiological studies the consumption of large quantities of beans has been associated with increased prevalence of cholesterol gallstones, particularly in South America (Covarrubias *et al.* 1984), suggesting that legumes somehow alter the cholesterol

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saturation characteristics of the bile. In a study especially designed to investigate the effect of legumes on biliary cholesterol saturation, Nervi *et al.* (1989) showed that, under controlled conditions, a diet containing beans, peas and lentils (120 g dry weight/d) increased biliary cholesterol and decreased biliary phospholipid in such a way that the biliary cholesterol saturation index rose from 110 to 169%. At the same time plasma total and LDL-cholesterol levels were significantly reduced. Whilst other authors have also noted an epidemiological association between cholesterol-reducing diets and the prevalence of cholesterol gallstones (Sturdevant *et al.* 1973), the metabolic connection between the two is unclear.

STUDIES USING LEGUME POLYSACCHARIDES

The most frequently studied legume polysaccharide is guar gum, the storage polysaccharide of the Indian Cluster Bean (*Cyamopsis tetragonoloba*). Guar gum can be readily isolated in a purified power form and is principally a galactomannan. In solution guar gum is highly viscous, and it is its effect on viscosity within the small intestine that is thought to be at the heart of its physiological effects, particularly its attenuation of the rate of post-prandial glucose absorption (Blackburn *et al.* 1984). It has been investigated as a potentially useful therapeutic measure in both diabetes and hyperlipidaemia.

Another legume polysaccharide that has been studied for its effects on plasma lipids is locust bean gum (Zavoral *et al.* 1983). This gum, the storage polysaccharide of the species *Ceratonia siliqua*, is almost identical to guar gum in its chemical and physical properties, but appears to be easier to incorporate into palatable food products.

The only leguminous cell-wall polysaccharide that has been concentrated and fed to humans is that from the soya bean (*Glycine max*). Soya-bean polysaccharides have been concentrated in various forms. For example, Lo *et al.* (1986) isolated a product which they named soya-bean fibre and which consisted of cell-wall material from the soya-bean cotyledon, whereas Sasaki *et al.* (1985) isolated a residue (probably representing both cotyledon and hull) which they called soya-bean crude fibre. Yet another group compared two different soya-bean-fibre preparations, one of which had been purified and dried, the other of which was less pure and had never been dried (Schweizer *et al.* 1983). Since the storage polysaccharide gums represent a different fraction of the legume seed from the cell-wall polysaccharides, and have peculiar physicochemical properties, their effects on plasma lipids will be considered separately from those of the soya-bean preparations.

Guar and locust bean gums

There have been numerous studies of the effects of guar gum on plasma lipids in man (Jenkins *et al.* 1975, 1976*a*, *b*, 1979, 1980*a*; Aro *et al.* 1981, 1984; Khan *et al.* 1981; Simons *et al.* 1982; Tuomilehto *et al.* 1983; Uusitupa *et al.* 1984; McIvor *et al.* 1986; Penagini *et al.* 1986; Turner *et al.* 1990). In all these studies reductions in total and/or LDL-cholesterol have been observed, although in two studies the change was not significant (Jenkins *et al.* 1976*b*; McIvor *et al.* 1986). The magnitude of the reduction has been in the range 8·5–16% of the total plasma cholesterol level.

Guar gum on its own is an unpalatable product and researchers have sought different ways of introducing it into the diet of volunteers. The most popular mode of administration has been to disperse the dry power in a fluid, usually soup or fruit juice, which is taken at the start of each meal. However, some investigators have used other vehicles such as crispbread (Jenkins *et al.* 1980*a*; Penagini *et al.* 1986), capsules (Khan *et al.* 1981), or granola bars (McIvor *et al.* 1986). Simons *et al.* (1982) reported the use of a modified guar gum formulation which remained fluid before ingestion, but developed its viscosity on

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contact with gastric acid. This formulation proved to be more palatable than the unmodified version. The mode of administration of guar gum does not appear to influence its effectiveness greatly. Jenkins *et al.* (1980*a*) showed that, on an equal weight basis, guar gum hydrated in milk, soup or fruit juice and guar gum incorporated into crispbread were equally effective at reducing cholesterol in hyperlipidaemic subjects. Semi-hydrated guar gum given in the form of bread was not so effective, but this may have been related to its lower palatability and the small size of the subject group used.

To my knowledge, the relationship between the amount of guar gum consumed and its effect on blood lipids has not been studied. Comparisons between studies are strained by differences in the protocols used, but it may be acceptable to compare the studies of Jenkins *et al.* (1975) and Khan *et al.* (1981) who both measured the effect of including guar gum in the normal diets of normolipidaemic subjects over a 2-week period. In the earlier study, 36 g guar gum powder/d induced a mean fall of 16% in the plasma cholesterol level of the group, whereas in the later study a daily dose of 9 g guar gum in capsule form induced a mean fall of 10%. These results suggest that there is diminished benefit to be obtained by increasing the consumption of guar gum above a certain dose. An optimum dose of guar gum has not yet been proposed. However, Tuomilehto *et al.* (1983) have suggested that doses of guar gum in excess of 15 g/d are required in order to achieve a significant long-term effect. This value was suggested in an attempt to explain the conflicting results obtained by various groups for the long-term effectiveness of guar gum as a treatment for hypercholesterolaemia. Aro *et al.* (1984) using 15 g guar gum/d observed an attenuation of its effect after 12 weeks compared with that seen at 6 weeks. However, other groups (Simons *et al.* 1982; Uusitupa *et al.* 1984) using slightly larger doses have claimed that the effect is sustainable for several months, even up to 1 year.

The hypocholesterolaemic effect of guar gum is evident within 2 weeks of daily consumption (Jenkins *et al.* 1975; Khan *et al.* 1981; Penagini *et al.* 1986). After this there may be further small decrements for another 2 weeks, but then the effect seems to stabilize (Simons *et al.* 1982). This is the case for both hypercholesterolaemic and normolipidaemic subjects. Unlike whole pulses, guar gum appears to reduce the cholesterol levels of both groups to similar extents when expressed on a mg/l basis. In the studies reviewed, the mean reduction in plasma cholesterol experienced by the subjects was approximately 300 mg/l (range 230–460 mg/l), regardless of the starting lipid level of the group or the protocol followed. Thus, when expressed as a percentage of the starting lipid level, normolipidaemic subjects experienced greater reductions than hypercholesterolaemic subjects. This was not the case for locust-bean gum, which affected the plasma lipid levels of familial hypercholesterolaemic subjects to a greater extent than those of normals (Zavoral *et al.* 1983).

As in the case of whole pulses, authors have observed considerable disparity in individual responses to guar gum which may be attributable to differences in lipid metabolism between subjects (Turner *et al.* 1990).

In studies where lipoprotein fractions have been measured, the reduction in plasma cholesterol seen as a result of guar gum administration is almost always due to a reduction in LDL-cholesterol. No significant changes in HDL- or VLDL-cholesterol have been reported. Locust-bean gum reduced HDL-cholesterol significantly in the study of Zavoral *et al.* (1983) but, as in the case of whole pulses. the HDL:LDL ratio was maintained unchanged. Triacylglycerol levels have also remained unchanged by treatment with these viscous gums, only being reduced in one study (Gatti *et al.* 1984).

In a recent paper, Turner *et al.* (1990) reported the results of a metabolic study using ^{125}I-labelled LDL to investigate the cholesterol-lowering effect of guar gum. Patients with primary hypercholesterolaemia received 30 g guar gum/d for 6 weeks, after which their

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LDL and IDL levels were reduced by 11·5 and 10·7% respectively, due to a 39% increase in the fractional catabolism of apolipoprotein B. There was also a slight increase in the production rate of apo B. These changes are similar to those seen with the bile acid-binding resin cholestyramine, and the authors suggest that guar gum may act in a similar way. By reducing the reabsorption of bile acids in the small intestine such resins stimulate a compensatory synthesis of hepatic LDL receptors in order that the liver can assimilate sufficient cholesterol for the production of new bile acids.

The idea that guar gum binds steroids in the small intestine of man appears to rely heavily on extrapolation from studies of pectin, which, although similar in some ways to guar gum, is a chemically distinct polymer. Jenkins *et al.* (1976*a*) have provided some evidence that both substances can enhance faecal steroid excretion quite considerably, but there have been few other studies on this phenomenon in humans.

Soya-bean polysaccharides

Unlike guar gum, soya-bean polysaccharide concentrates have not been shown to reduce the cholesterol levels of normolipidaemic subjects, and may even raise them (Schweizer *et al.* 1983; Tsai *et al.* 1983). Nevertheless, some forms may be of benefit in the management of hyperlipidaemia. Shorey *et al.* (1985) showed that concentrated cell-wall material from soya-bean cotyledons (25 g/d) given to mildly hyperlipidaemic subjects caused a 5–11% decrease in total cholesterol compared with a starch placebo. When given at the same level to primary hyperlipidaemic subjects as an adjunct to a standard lipid-lowering diet, this material reduced the mean total cholesterol by an additional 130 mg/l over that seen with the standard diet alone (Lo *et al.* 1986). In both cases LDL-cholesterol levels were reduced in proportion to the reduction in total cholesterol. Triacylglycerol levels were unaffected. In contrast, Sasaki *et al.* (1985) using a slightly different soya-bean-polysaccharide concentrate, observed significant reductions in serum and VLDL-triglycerides and VLDL-cholesterol in the absence of any effect on LDL- or HDL-cholesterol levels, when this preparation was fed to hyperlipidaemic subjects at a level of 11·4 or 15 g/d. The reasons for the discrepancies between the two studies are not known.

Steroid excretion in relation to soya-bean polysaccharide consumption has been studied, but only in experiments where plasma lipids have been unaffected. Tsai *et al.* (1983) saw no change in faecal steroid excretion resulting from their dietary supplementation which, nevertheless, increased faecal wet weight. Schweizer *et al.* (1983) observed a 21% increase in the excretion of bile acids by a group of normal subjects consuming a never-dried soya-bean pulp for 3 weeks. Faecal wet weight was also increased. However, there was no effect on total, LDL- or HDL-cholesterol levels. In the same study, a second group of normal volunteers showed a reduction in their excretion of bile acids by 11% compared with the control period in response to consumption of a dried, purified soya-bean-polysaccharide preparation. In these subjects both LDL- and HDL-cholesterol levels were raised.

It seems that the effects of soya-bean polysaccharides on plasma lipids in humans are inconsistent, and that the inconsistencies may depend on the chemical and physical characteristics of the particular concentrate used.

STUDIES USING SOYA-BEAN-PROTEIN PREPARATIONS

Epidemiological studies have suggested that the incidence of coronary heart disease is correlated positively with consumption of animal protein (Yudkin, 1957) and negatively with that of vegetable protein (Connor & Connor, 1972). Consequently the effects of animal and plant proteins on lipid metabolism have been studied widely. The ready availability and versatility of soya-bean protein had made this the obvious choice in clinical

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studies, for which it has been used almost exclusively as the archetypal plant protein. When considering the outcome of such studies it is important to recognize that soya-bean protein is available in various forms of different composition. In particular, the protein content of soya-bean-'protein' preparations ranges from as little as 400 g/kg to as much as 920 g/kg.

Many investigators have used soya-bean-protein isolates which are produced by isoelectric precipitation. These are the purest products, comprising at least 900 g protein/kg, the remainder being mainly ash. Others have used soya-bean-protein concentrates, containing approximately 700 g protein/kg, which are produced from defatted soya-bean flakes by removing much of the sugar content. Both isolates and concentrates may be texturized to imitate meat, but such analogues should be distinguished from 'textured soya-bean-protein' which is made directly from the defatted flake and contains only 400–600 g protein/kg. Some investigators have chosen to use textured products containing a large proportion of non-protein constituents, mainly carbohydrate in origin, as their source of plant protein in clinical studies (Sirtori *et al.* 1979, 1985; Holmes *et al.* 1980; Vessby *et al.* 1982). Indeed, such products have been used to great effect in the management of hypercholesterolaemia. However, because many soya-bean-protein preparations are so impure it has been difficult for researchers to identify the contribution of soya-bean protein *per se* to cholesterol reduction in humans.

It has been shown unequivocally that proteins of differing origin can have marked effects on cholesterolaemia in laboratory animals, notably rabbits (e.g. Lovati *et al.* 1990). However, the experiments of van Raaij *et al.* (1979) have exposed considerable differences in the responses of rabbits and humans to identical diets, so that extrapolation from such studies is of little value. Following a critical review of early human experiments in this area, Sacks *et al.* (1983) concluded that very little change in plasma cholesterol occurred as a direct result of substituting vegetable proteins (mainly soya bean) for animal proteins in human diets. Indeed several closely controlled studies have shown that low-lipid diets containing casein or meat as their principal protein source can be just as effective at reducing cholesterol levels as those containing soya-bean protein (Holmes *et al.* 1980; Shorey *et al.* 1981).

Attempting to resolve the question of whether plant and animal proteins affect cholesterol metabolism differently in humans, Grundy & Abrams (1983) and Meinertz *et al.* (1988) fed volunteers for 1 month on liquid-formula diets containing either soya-bean-protein isolate or casein. Both groups showed that these two proteins did not differ in their effect on plasma cholesterol in normolipidaemic people when the diet was low in cholesterol. However, this is not altogether surprising. Studies in normolipidaemic subjects have generally demonstrated little, if any, change in plasma total or LDL-cholesterol in response to soya-bean-protein preparations of all types (Carroll *et al.* 1978; van Raaij *et al.* 1981, 1982; Noseda *et al.* 1982; Giovannetti *et al.* 1986). Studies showing major reductions in human plasma cholesterol attributable to soya-bean-protein diets have, almost exclusively, involved hyperlipidaemic subjects. In fact the initial lipid level of the subject and the amount of dietary cholesterol consumed appear to be major determinants of the efficacy of a soya-bean-protein diet with regard to plasma cholesterol reduction. The effects of soya-bean-protein and casein-based liquid-formula diets on the cholesterol levels of primary hypercholesterolaemic subjects have not yet been studied: however, normocholesterolaemic subjects receiving a dietary cholesterol supplement of 500 mg/d have been shown to respond differently to the two proteins (Meinertz *et al.* 1989). In contrast to their previous observations with low-cholesterol diets, it was found that when the diet was cholesterol-rich, the LDL-cholesterol level of subjects consuming soya-bean protein was 16% lower than that of subjects consuming casein.

In studies using solid foods, soya-bean-protein isolates have only been shown to be

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hypocholesterolaemic in cases of moderate to severe hyperlipidaemia. Wolfe *et al.* (1981) reported a 13% reduction in plasma cholesterol of subjects with a mean starting level of 3210 mg/l, after 7 weeks on a soya-bean-protein diet. Similarly, Goldberg *et al.* (1982) demonstrated that a diet containing a soya-bean-protein isolate reduced the cholesterol concentrations of primary hyperlipidaemics with moderate hypercholesterolaemia to a small but significant extent, whereas it did not affect plasma lipids in normolipidaemic subjects. Mildly hyperlipidaemic subjects were not responsive to soya-bean-protein isolate in the study of Shorey *et al.* (1981).

Impure soya-bean-protein preparations are demonstrably more effective than soya-bean-protein isolates at reducing cholesterol. Two products in particular, Temptein (Miles Laboratories, Elkhart, Ind, USA) and Cholsoy (Gipharmex, Milan, Italy) containing 600 and 520 g protein/kg respectively, have repeatedly been shown to reduce cholesterol in hyperlipidaemic subjects, both in controlled and uncontrolled situations (Sirtori *et al.* 1977, 1979; Descovich *et al.* 1980; Verrillo *et al.* 1985; Lovati *et al.* 1987). Subjects consuming these products have experienced reductions in total cholesterol of up to 35% over several months. A lecithinated version of Cholsoy, Cholsoy-L, has also been used to great effect (Verrillo *et al.* 1983).

The effectiveness of these impure soya-bean preparations in comparison with purified protein isolates suggests that the protein element is not the only, or even perhaps the principal, determinant of the cholesterol-reducing effect attributed to soya-bean-protein preparations. Cholsoy contains approximately 300 g carbohydrates/kg of which (g/kg) 40 is raffinose, 50 stachyose and 160 NSP (Verrillo *et al.* 1985), all of which could conceivably influence cholesterol metabolism.

Not surprisingly, the effects of soya-bean-protein preparations on the metabolism of lipoprotein fractions are very similar to those of whole pulses. Reductions in total cholesterol are largely due to reductions in LDL-cholesterol, and there is a concomitant decrease in the amount of circulating apoprotein B (Goldberg *et al.* 1982). Meinertz *et al.* (1989) showed that the reduction in LDL seen in response to soya-bean-protein isolate was specifically in the LDL_2 subfraction.

Most investigators have reported no change in the concentration of VLDL in the plasma, although isolated studies have identified small changes in the composition or turnover of this fraction when subjects are consuming a soya-bean-protein preparation. In the liquid-formula-diet study of Grundy & Abrams (1983), soya-bean protein led to reductions in VLDL-cholesterol and triacylglycerols in comparison with casein, but only in subjects with initial hypertriglyceridaemia. Conversely, Wolfe & Giovannetti (1985) observed a significant increase in the concentration of VLDL-cholesterol in five hyperlipidaemic subjects consuming a textured soya-bean-protein diet, in the absence of any effect on triacylglycerols. In a study using dishes prepared from whole soya beans, Huff *et al.* (1984) noted increases in both the synthesis and the fractional catabolism of VLDL, but these caused no overall change in plasma concentrations of total, LDL- or VLDL-cholesterol.

HDL-cholesterol is generally unaffected by a soya-bean-protein diet. Increased HDL-cholesterol has only been reported in two studies, both of which have been in normolipidaemic subjects receiving a cholesterol-rich diet (van Raaij *et al.* 1981; Meinertz *et al.* 1989). The increase, specifically identified in the HDL_2 subfraction, was not associated with a reduction in triacylglycerols. Indeed, reductions in triacylglycerols attributable to a soya-bean-protein diet appear to be independent of changes in cholesterol level, and are confined to those subjects with initial hypertriglyceridaemia (Grundy & Abrams, 1983; Verrillo *et al.* 1985).

Despite the abundance of research into the effects of soya-bean-protein preparations on cholesterol metabolism, their effect on the excretion of steroids in the faeces has received

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little attention. In two of the three studies that have included this aspect, the diet used did not result in a reduction in plasma total, LDL- or VLDL-cholesterol. Thus, it is not surprising that no change in the output of steroid molecules was observed (Calvert *et al.* 1981; Grundy & Abrams, 1983). In the third study, Fumagalli *et al.* (1982) reported reductions in total cholesterol of 10–28% in six of seven type II hyperlipidaemic subjects receiving a diet containing Temptein for 3 weeks. In this case there was no change in the excretion of either neutral or acidic steroids in the faeces. As a result of their observations the authors suggested that the non-digestible (i.e. carbohydrate) fraction of Temptein was probably not involved in the hypocholesterolaemic effect. However, as discussed later, this might not necessarily be the case.

POSSIBLE MECHANISMS FOR THE HYPOCHOLESTEROLAEMIC EFFECT OF LEGUMES

Clinical investigations into the cholesterol-lowering effect of legumes have usually been approached from a therapeutic, rather than a mechanistic angle. Thus, human studies have sought and found answers to such questions as 'How much?', 'How long?' and 'Who will benefit?', but not to questions such as 'How?', and 'Why?'. The answers to these latter questions have been sought rather through animal experimentation. Many individual components of legume seeds, once isolated and fed to experimental animals, have been shown to reduce cholesterol levels in the plasma. These components (protein, lipid, NSP, plant sterols, saponins, isoflavones) and the mechanisms by which they are postulated to exert their effects have been reviewed in detail elsewhere (Price *et al.* 1987; Shutler *et al.* 1987*a*, *b*) and they will not be discussed in any depth here. Animal experiments suggest that the effect of legume seeds on lipid metabolism represents the sum of a number of different events acting in concert to reduce plasma cholesterol. It is likely that this is also the case in humans. However, as discussed previously, the results of animal experiments are not always applicable to the human situation, and it is probably true to say that the mechanisms involved in the hypocholesterolaemic effect of legumes in humans are still not known for sure. It is the intention, here, to draw together the few strands of mechanistic information available from human studies of whole pulses, legume polysaccharides and soya-bean-protein preparations in order to try to discern the metabolic events that occur when legumes are eaten. In this way it may be possible to identify the likely routes for the action of legumes, and to identify target areas for further research.

EFFECTS ON LIPOPROTEIN METABOLISM

The principal change that occurs in response to a diet rich in legumes or their derivatives is that of reduced LDL in the absence of any change in plasma VLDL. Specific LDL reduction may be the result of one of two metabolic changes, i.e. (a) increased LDL-receptor activity or (b) reduced synthesis of LDL, either of which may be secondary to events occurring in the gut lumen.

Increased LDL-receptor activity

LDL receptors are synthesized in response to a need for cholesterol, and are down-regulated by a sufficiency, either supplied from the circulation or available from *de novo* synthesis. An increase in LDL-receptor activity, therefore, indicates that the tissues require

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cholesterol, and this may be because they are unable to synthesize adequate cholesterol for their needs, or because they have been depleted; for example, if they have been required to convert cholesterol into other steroid molecules for export.

The most popular hypothesis to explain the cholesterol-reducing effect of legumes, and indeed many other foods containing NSP, is that of steroid binding. According to this hypothesis several components of the legume seed, notably NSP and saponins, bind biliary steroids in the small intestine and inhibit their reabsorption during the enterohepatic cycle. New bile acids are thus required and these are synthesized from cholesterol. In this manner, cells are depleted of their cholesterol and synthesize LDL receptors in order to obtain more. The net result is an increase in LDL-receptor activity. As discussed earlier, recent evidence suggests that this mechanism, which is responsible for the effects of steroid-binding resins like cholestyramine, may also be responsible for the cholesterol-reducing effect of guar gum (Turner *et al.* 1990). It is conceivable that whole pulses and other legume polysaccharides with sufficient affinity for bile acids could influence lipid metabolism in a similar way. However, at present there is not enough evidence from human studies of lipid turnover or steroid excretion to conclude that this is so.

Anderson *et al.* (1984*b*) dismissed the steroid-binding hypothesis as an explanation for the hypocholesterolaemic effect of navy beans (*Phaseolus vulgaris*) following their observation that the bean diet resulted in a 30% decrease in their subjects' output of acidic faecal steroids. They proposed instead that the soluble NSP present in the beans was fermented in the colon producing short-chain fatty acids, notably propionic acid. This molecule is absorbed and taken to the liver where it has an inhibitory effect on the enzyme HMG-CoA reductase, thus reducing *de novo* cholesterol synthesis. According to reports of the effects of proprietary HMG-CoA reductase inhibitors, reducing cholesterol synthesis results in a compensatory increase in LDL-receptor activity and, hence, a reduction in plasma cholesterol (Ma *et al.* 1986).

There is strong evidence in rats to suggest that propionic acid can inhibit cholesterol synthesis (Bush & Milligan, 1971; Chen *et al.* 1984). It has also been shown that dietary propionate reduces total plasma cholesterol in pigs (Thacker *et al.* 1981), although in this case the reduction has been associated with reduced HDL-cholesterol as well as LDL-cholesterol. The outstanding question is whether or not the NSP present in legumes is fermented to propionate by human gut flora in sufficient quantity to affect plasma cholesterol level. Englyst *et al.* (1987) have demonstrated that the molar ratios of acetic, propionic and butyric acids produced by cultures of human faecal bacteria are dependent on the substrate used. For example, the main end-product of pectin fermentation is acetate, whereas starch is fermented more readily to butyrate. Using porcine faecal flora, Goodlad & Mathers (1988) have shown that both raffinose and pea fibre are relatively good substrates for the production of propionate. This lends weight to the idea that the end-products of the fermentation of legume carbohydrates may influence lipid metabolism in vivo. If this were the case, species-specific differences in effectiveness could be due to the differing compositions of their oligo- and polysaccharide components. So far the individual carbohydrate fractions of legumes have received little attention in human experimental work.

There is recent evidence to suggest that soya-bean-protein preparations can also increase LDL-receptor activity in hyperlipidaemic subjects, possibly by inhibiting the down-regulation of LDL receptors (Lovati *et al.* 1987). This hypothesis is supported by the consistent observation that soya-bean-protein preparations are only effective in situations where plasma cholesterol levels are already raised, i.e. where there is already some down-regulation of LDL receptors. At present the specific amino acid or peptide components responsible for the effect have not been identified.

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Reduced synthesis of LDL

The turnover of LDL in response to a diet rich in pulses has not yet been studied in man. However, in pigs, contrary to our expectations, we found that the fractional catabolism of ^{125}I-labelled LDL was not increased by a hypocholesterolaemic dose of baked beans in tomato sauce (Shutler, 1988). The finding suggested that legumes were influencing the synthesis, rather than the clearance of LDL from the plasma. Under normal circumstances all LDL is derived from VLDL via IDL. However, not all VLDL particles become LDL particles. As described earlier, the metabolic fate of a newly secreted VLDL particle is decided by its size. Large VLDL particles tend to become VLDL remnants, whereas small VLDL are metabolized to IDL which have the potential to become LDL. Thus, any manipulation that alters the size of VLDL particles on secretion could also influence the number of circulating LDL particles without affecting the absolute amount of VLDL in the plasma.

The factors that determine the size of VLDL on secretion are not well understood, but it is known that dietary manipulations can influence plasma LDL levels in this way. For example, Cortese *et al.* (1983) found that, in comparison with a high-fat diet, a low-fat diet reduced the proportion of VLDL converted to LDL without affecting the turnover of VLDL itself and, thus, resulted in a lower plasma cholesterol level. In contrast, a diet rich in polyunsaturated fatty acids was found to reduce synthesis of both VLDL and LDL. The former observation suggests that a diet high in fat will favour the production of small (i.e. LDL-forming) VLDL, and it is interesting to note that legumes are particularly effective at reducing cholesterol levels when the background diet is high in fat (Mathur *et al.* 1968). If there is a component in legumes that can alter the secretion of VLDL in favour of large particles it has not yet been identified. Careful studies of the kinetics of VLDL and LDL metabolism in response to leguminous diets are required in order to determine the role, if any, of VLDL partitioning in their cholesterol-reducing effect.

EFFECTS ON INSULIN SECRETION

In addition to their effects on plasma lipids, diets rich in leguminous seeds are associated with improved control of carbohydrate metabolism, particularly in diabetes. Following a meal containing legumes or certain legume derivatives, post-prandial glucose absorption and insulin secretion are reduced and delayed in comparison with other starchy foods (Jenkins *et al.* 1980*b*). The attenuated glucose response to legumes may occur for several reasons. In the case of substances such as guar gum, the mixing of lumen fluid is inhibited by the presence of a viscous material (Blackburn *et al.* 1984), whereas for starchy legumes the phenomenon is probably related to the nature of the starch and its entrapment within thick cell walls (Wursch *et al.* 1986). Reduced insulin secretion occurs as a natural consequence of the delayed glucose absorption. Insulin is known to stimulate the activity of HMG-CoA reductase and, hence, the *de novo* synthesis of cholesterol (Lakshmanan *et al.* 1973). Thus, reducing the absolute amount of insulin circulating in the plasma could potentially reduce cholesterol synthesis and plasma cholesterol. It has recently been shown that reductions in post-prandial plasma glucose and insulin, achieved simply by increasing the frequency of meals (nibbling rather than gorging) are associated with significant reductions in LDL-cholesterol and apolipoprotein B in human subjects (Jenkins *et al.* 1989), but it is uncertain whether the magnitude of the reduction in plasma insulin as a consequence of legumes in the diet is sufficient to effect a change in plasma cholesterol.

In diabetes, elevated plasma cholesterol is a common complication which is thought to be secondary to accumulation of triacylglycerol-rich lipoproteins due to impaired activity

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of lipoprotein lipase. It is possible that, under certain circumstances, delayed glucose absorption could lead to improved clearance of triacylglycerol-rich lipoproteins and, thus, reduce plasma cholesterol concentrations. Jenkins *et al.* (1987) observed that a low-glycaemic-index diet was associated with significant reductions in plasma total and LDL-cholesterol and triacylglycerol levels in patients with hypertriglyceridaemic conditions, but not in those with normal triacylglycerol levels. These results suggest that the effect of a low-glycaemic-index diet on cholesterol metabolism is indeed linked to its effect on triacylglycerol-carrying lipoproteins. However, legumes have also been shown to reduce plasma cholesterol independently of changes in triacylglycerols.

In addition to the effects of absolute insulin levels on lipid metabolism, it has been suggested that the insulin: glucagon ratio is instrumental in determining plasma lipid levels. There is some evidence to suggest that plasma cholesterol and triacylglycerols are reduced when glucagon is present in excess of insulin (Lakshmanan *et al.* 1973). Recently, Jenkins *et al.* (1990) reported that a reduced rate of glucose ingestion was associated with reductions in the secretion of both glucagon and insulin. Although the ratio of the two hormones was not reported, the change in secretion of glucagon appeared to be less marked than that of insulin, and may have resulted in an increased glucagon:insulin ratio.

In relation to the influence of legume proteins on cholesterol metabolism, it has been suggested that glucagon secretion may be stimulated by arginine, an amino acid in which soya-bean protein is rich, and that this might be the means by which legume proteins affect plasma cholesterol. However, in testing this hypothesis, Noseda *et al.* (1982) found that, although the peak glucagon secretion following arginine infusion was increased by soya-bean protein, it was increased even further by casein. Thus, there seems to be little basis for this particular hypothesis for the cholesterol-lowering property of soya-bean protein.

CONCLUSION

The present review shows that legume seeds and certain legume derivatives, eaten frequently, are able to reduce the level of LDL-cholesterol in the plasma, particularly when this is abnormally high for genetic or dietary reasons. However, the mechanisms responsible for the effect are still not fully understood. Although the specific reduction of LDL-cholesterol is common to whole pulses, legume polysaccharides and soya-bean-protein preparations, it seems that the metabolic processes underlying this reduction may be different for all three products.

The compositional complexity of legumes has been a major obstacle to researchers, making it difficult to control for all potentially active components in experimental diets, and to predict metabolic areas for specific consideration. In addition, studies have been hampered by the use of genetically heterogeneous groups of hyperlipidaemic subjects, whose responses to the diets have sometimes been highly variable and, therefore, difficult to interpret. In studies using animal models, progress has been made by isolating potentially hypocholesterolaemic components and incorporating these into formula diets. This approach is impractical in humans because of such considerations as palatability and monotony. Nevertheless, such difficulties should not have prevented research of a fundamental nature; for example, determination of the effects of leguminous diets on steroid balance, of which there is a dearth. If the metabolic sequelae of eating a leguminous diet can be determined, some indication of the component responsible for the effect might be obtained.

At present, direct mechanistic information relating to the effects of whole pulses, legume polysaccharides and soya-bean protein preparations on human plasma lipid metabolism is

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derived from only a handful of carefully planned and executed studies. Much more information about the specific effects of leguminous diets on sterol balance, lipoprotein kinetics and hormone secretion is required before the mechanism responsible for the hypocholesterolaemic effect of legumes and their derivatives can be elucidated.

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