THE UNIVERSITY OF MANITOBA

THE EFFECT OF ANIMAL PROTEIN AND PLANT PROTEIN DIETS ON BLOOD LIPIDS OF HEALTHY YOUNG MEN

by

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A B S T R A C T

A 42-day metabolic study was designed to compare the effect of dietary protein on blood lipids in healthy normolipidemic men by feeding either a diet containing plant protein from mixed sources (PPD) or a similar diet with 55% of the plant protein substituted by beef protein (APD). The subjects were eight young adult males, aged 18 to 27 years, each of which consumed both the PPD and the APD in a crossover experimental design. Two groups of four subjects were randomly assigned to two different diet treatment orders. Each diet regimen was fed for a 21-day period. The diets were similar in composition with respect to fatty acids and sterols. Wheat fiber content was maintained constant. The fat and protein content of both diets were similar and averaged 32% and 11% of total kilocalories, respectively. Caloric intake was adjusted to maintain subject weight. Diets were supplemented with vitamins and minerals as required to meet the recommended nutrient allowances. Fasting venous blood samples were collected at the beginning of the study and at 7-day intervals throughout the 42-day period. Serum was analyzed for total cholesterol and

triglyceride and plasma was analyzed for LDL and HDL cholesterol. Data was analyzed statistically using analysis of variance. There were not any significant ($P < 0.05$) differences between the PPD and the APD for serum total cholesterol or plasma LDL cholesterol. The overall mean serum cholesterol value for the PPD period was 154 ± 6^1 mg/dl compared to 156 ± 7^1 mg/dl for the APD period. The PPD period was associated with a mean LDL value of 82 ± 6^1 mg/dl which was the same as the APD period, 82 ± 11^1 mg/dl. However, significantly ($P < 0.05$) higher serum triglyceride values were associated with the PPD ($\bar{X} = 136 \pm 19^1$ mg/dl) compared to the APD ($\bar{X} = 84 \pm 12^1$ mg/dl) when the mean difference between the first week values for each diet treatment was analyzed. The results obtained for the two orders of diet presentation were not significantly ($P < 0.05$) different. At the end of three weeks, average serum triglyceride values were higher for the PPD ($\bar{X} = 118 \pm 18^1$ mg/dl) compared to the APD ($\bar{X} = 94 \pm 15^1$ mg/dl), but the result was not significant ($P < 0.05$). Mean plasma HDL cholesterol values for subjects fed the APD (48 ± 3^1 mg/dl) were significantly ($P < 0.05$) higher than those for the PPD (42 ± 2^1 mg/dl) at the end of three weeks, and

[1] Standard Error.

the mean differences for the two different orders of diet presentation were not significantly ($P < 0.05$) different. It is concluded that a diet which contains 55% of the total protein as animal protein does not have a hypercholesterolemic effect as measured by serum total cholesterol and plasma LDL cholesterol. An initial transitory rise in serum triglyceride levels was associated with the PPD which is consistent with the phenomenon of carbohydrate-induced hypertriglyceridemia. Whether the lower plasma HDL cholesterol levels associated with the PPD have a long-term etiological significance with regard to atherosclerosis remains to be elucidated.

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REVIEW OF LITERATURE

The Effect of Animal Protein and Plant Protein Diets on Blood Lipids

Diet, as an etiologic factor in the development of atherosclerosis, has been the subject of controversy for some time. In an effort to minimize the risk factors associated with this disease, several dietary constituents have been studied but the greatest emphasis has been on dietary fat and cholesterol. Nevertheless, evidence has accumulated which suggests that dietary protein can also have a significant influence on human blood cholesterol levels. More specifically, there is experimental evidence which shows that animal protein is more cholesterolemic and atherogenic than plant protein. Most of the data has been obtained from experiments in which the rabbit has been the experimental model for atherosclerosis. However, rabbit studies have failed to establish an overall relationship between dietary protein and atherogenesis because of the emphasis on the utilization of casein and soy protein diets which may not be representative of animal and plant protein diets in general. Furthermore, the limited number of studies

conducted with other animal species and man have focused on the hypocholesterolemic effect of soy protein and little attention has been directed to the effect of mixed plant or animal protein diets normally consumed by the human.

I. Rabbit Studies

A. Dietary Protein - A Historical Perspective

Protein quality was, in fact, the first aspect of diet to be implicated as a factor in the development of atherosclerosis. Ignatowski (1909) discovered that aortic lesions resembling those seen in human atherosclerosis could be produced in rabbits by feeding animal proteins in the form of meat, milk and eggs and concluded that the lesions of the arterial wall were attributed to the animal protein in the diets. This view was challenged by Stuckey (1912) who believed that the lipids associated with the animal products were responsible for the vascular injury. Later, Anitschkow and Chalatow (1913) showed that the feeding of cholesterol dissolved in vegetable oil to rabbits led to the development of typical atherosclerotic lesions. Since the substances in Ignatowski's atherogenic diet all contained cholesterol, it was concluded that this substance had been responsible for the

observed effects. Nevertheless, the impression that dietary protein is a factor in the production of experimental atherosclerosis persisted among some investigators. Newburgh and Squier (1920) observed atherosclerotic lesions in rabbits fed a high casein diet (30g daily). In a subsequent study, Newburgh and Clarkson (1923) fed a low cholesterol diet rich in powdered de-fatted beef (27% by weight) to rabbits and concluded that beef protein was responsible for the observed hypercholesterolemia and atherosclerosis after six months of feeding. The atherogenic effect was found to occur much earlier when the protein was fed at the 36% level. Meeker and Kesten (1941) subsequently confirmed that rabbits fed a high protein (38% by weight), low fat, cholesterol-free diet, with casein as the source of protein, became hypercholesterolemic and developed atherosclerosis. Conversely, a high protein diet (39% by weight) containing soybean flour diminished the incidence and degree of experimental atherosclerosis in rabbits. These studies which related to the dietary protein component of the diet, however, made no lasting impression and the discovery in the early 1950's that polyunsaturated fat diets resulted in a lowering of serum cholesterol levels in humans provided further impetus for future research on effects of dietary fat in atherosclerosis.

B. The Semisynthetic Diet Experimental Model

A renewed interest in the role of dietary protein in atherosclerosis came in the late 1950's with the observation that hypercholesterolemia and atherosclerosis could be produced in rabbits by feeding a semisynthetic diet (SSD) (Lambert, 1958; Wigand, 1959; Malmros and Wigand, 1959 and Gresham and Howard, 1962). This diet was typically low in fat and cholesterol and contained casein as the source of protein, a carbohydrate source, a fiber source, and salt and vitamin mixes. In contrast to the results obtained with the SSD, hypercholesterolemia and atherosclerosis did not develop in rabbits maintained on commercial laboratory chow diets which were mainly of a plant origin and commonly contained alfalfa and soybean meal, even when substantial amounts of saturated fats were added (Kritchevsky, 1964). Moreover, both a low and high fat SSD were associated with the atherogenic response and the higher levels of fat appeared to enhance the hypercholesterolemia (Carroll, 1971). This phenomenon served to indicate that a non-lipid component was responsible for the effects and the SSD became a popular diet model with proponents of the "protein theory" for the development of atherosclerosis. However, based on the observation that the hypercholesterolemic effect of the SSD was largely prevented

by the inclusion of substantial amounts of unsaturated fats, Malmros and Wigand (1959) suggested that the hypercholesterolemia associated with the SSD was due to a fatty acid deficiency. Contrary to this suggestion, Kritchevsky and Tepper (1968) demonstrated that addition of the extracted small amount of polyunsaturated fat normally present in laboratory chow to the SSD did not basically alter the results obtained by the two different types of diets. These results paved the way for further investigation into the non-lipid factor responsible for the differing cholesterolemic effects of semisynthetic and laboratory chow diets and the subsequent isolation of the protein component, namely casein.

C. Casein and Soy Protein Experimental Diets

Howard et al (1965) varied the composition of the basic SSD and found that only the isonitrogenous replacement of casein by whole soy flour or hexane - extracted soybean meal was effective in preventing the hypercholesterolemia and atherosclerosis in rabbits fed the SSD for periods of four months. The data suggested that the laboratory chow naturally consumed by rabbits contains an anti-atherogenic nutrient which is also present in extracted soybean meal. Similarly,

in a systematic variation of the composition of the SSD for a 28 day period, Hamilton and Carroll (1976) confirmed that the hypercholesterolemic response was due to the casein normally used as the protein source for these diets. When isolated soy protein or a crude preparation of soy replaced the casein in the SSD, the level of plasma cholesterol remained low, as in the rabbits fed the laboratory chow. Long-term feeding of the SSD containing casein has also shown that the hypercholesterolemia persists. Huff and Carroll (1977) noted significantly elevated levels of plasma cholesterol (approximately 200 mg/dl) in rabbits following ten months of feeding with a SSD. Aortic sudanophilia was clearly evident after the casein-containing SSD was consumed for ten months but it was not observed in rabbits fed the SSD containing soy protein isolate as the substituted protein source. The hypercholesterolemia was due primarily to an increase in the LDL fraction with some increase in the VLDL fraction. Carroll et al (1977) confirmed that this lipoprotein pattern persisted over a five month period and in addition, reported that phospholipid and protein components of the lipoprotein fractions were increased relative to those in lipoproteins from rabbits fed the soy protein SSD. The low and unchanged

plasma triglycerides in both dietary groups were hypothesized to be due to the low levels of dietary fat common to the SSD.

D. Animal and Plant Protein Experimental Diets

Protein sources other than soy and casein have been further investigated by Carroll and Hamilton (1975) who utilized the SSD model. They fed small groups of rabbits the SSD containing proteins from a variety of sources for 28 day periods and found that the animal protein diets were consistently more cholesterolemic than the plant protein diets in spite of considerable intravariation of response. However, some of the animal protein diets resulted in plasma cholesterol levels which were not significantly different from those obtained with plant protein diets. Animal protein diets which resulted in higher mean plasma cholesterol values were raw egg white, 105 $\pm$ 28 mg/dl; beef protein concentrate, 160 $\pm$ 60 mg/dl; casein, 200 $\pm$ 22 mg/dl; and skim milk, 230 $\pm$ 40 mg/dl. When the rabbits were fed plant protein diets, plasma cholesterol ranged from 25 $\pm$ 5 mg/dl for soy protein concentrate (60% protein) to 80 $\pm$ 21 and 80 $\pm$ 10 mg/dl for wheat gluten and peanut protein concentrate, respectively. Although some diets resulted in better growth than others,

there appeared to be no correlation between growth rate and the level of plasma cholesterol. In spite of the clear-cut evidence demonstrated for intact animal and plant protein sources by Carroll's group, the data must be interpreted with caution since similar data are not available for other animal species and man. Hence, conclusions regarding the distinctly different cholesterolemic responses obtained for the two classes of proteins in general are by no means justified.

E. The Influence of Other Dietary Constituents

Interpretation of results obtained for dietary proteins is further complicated by the existence among dietary variables of significant biological interactions which may influence the dietary response obtained. Consequently, the hypercholesterolemic action noted for casein must be assumed to hold only under the experimental dietary conditions specified. For example, Hermus (1975) reported that rabbits fed a SSD containing 20% casein resulted in mean serum cholesterol levels of 514 mg/dl whereas animals fed the same SSD containing a mixture of casein, gelatin and fish protein had mean values of 120 mg/dl. This suggested that animal proteins in mixed diets as normally consumed by man do

not always induce elevated cholesterol levels. Moreover, other researchers have demonstrated that other components of the diet can mediate the effect obtained with protein. Carroll and Hamilton (1975) reported that when the SSD contained casein or soy protein and included dextrose as a source of carbohydrate, casein was significantly more cholesterolemic, 220 mg/dl vs. 70 mg/dl. However, when the carbohydrate source was raw potato starch, serum cholesterol levels in both protein groups were normal for rabbits, 50 mg/dl. Rabbits maintained on the typical SSD, but high in fat (14% hydrogenated coconut oil), for ten months showed that casein was more cholesterolemic and atherogenic than soy protein only when the dietary fiber source was cellulose or wheat straw. The addition of alfalfa, an ingredient commonly used in laboratory chow for rabbits, rendered the two proteins equivalent in their cholesterolemic response and reduced the severity of atherosclerosis (Kritchevsky et al, 1977; Story et al, 1976). The results support the suggestion that dietary fiber may alter the hyperlipidemic effects of various diets (Trowell, 1973). Carroll (1971) further showed that saturated fats such as coconut oil, butter and beef tallow (15% by weight) tended

to enhance the hypercholesterolemic response observed for casein in the SSD, whereas replacement of the saturated fat by polyunsaturated fat rendered casein normocholesterolemic. Similarly, Howard et al (1965) found that corn oil at a level of 10% in a casein-containing SSD produced almost normal plasma cholesterol levels but did not prevent the atherosclerotic lesions. However, they concluded that the partially protective action of corn oil was not related to the content of essential fatty acids since the addition of other oils containing similar amounts of linoleic acid were without effect. The data emphasize the need to maximize control over other dietary variables when studying diet-induced atherosclerosis since the difference in atherogenic effects between casein and soy protein can be virtually eliminated by altering the non-protein component of the SSD.

F. Dietary Nature of the Response

Several investigators have attempted with limited success to isolate the specific dietary fraction responsible for the hypercholesterolemic effect observed for animal proteins. Most studies have compared casein and soy protein as representative of animal and plant proteins in general.

Since even the purest protein preparation tested contained at least 5 to 10% of non-protein material, it cannot be stated with absolute certainty that the differing effects of dietary proteins on plasma cholesterol in rabbits are necessarily due to the proteins *per se* (Carroll, 1978a). Contrary to other investigators, Howard *et al* (1965) found that only soy flour or hexane-extracted soy bean meal and not purified soy protein were effective in reducing serum cholesterol and the incidence of atherosclerotic lesions. These results supported the influence of a non-protein factor on serum cholesterol. However, further attempts to demonstrate the hypocholesterolemic property of soybean sterols were unsuccessful. Although these observations implicated non-protein effects, feeding trials were carried out with protein hydrolysates and mixtures of amino acids which simulated casein and soy protein, in order to further study the effects of protein. Huff *et al* (1977a) showed that an enzymatic hydrolysate of casein or a mixture of L-amino acids equivalent to casein resulted in elevated plasma cholesterol levels similar to those obtained with intact protein. Although plasma cholesterol levels remained low in rabbits fed an enzymatic digest of soy protein, a moderate, but not significant increase in plasma cholesterol was observed when a mixture of L-amino acids

equivalent to soy protein isolate was fed. The results suggested that the amino acid composition of the proteins may not be the only factor responsible for the observed effects. However, since the protein must be digested before the amino acids are available for absorption, feeding a mixture of amino acids is not biologically equivalent to feeding an intact protein containing amino acids in the same proportions in terms of availability of amino acids to the organism. This fact is supported by Hermus and Stasse-Wolthius (1977) who demonstrated that intact dietary proteins were less cholesterolemic than equivalent mixes of amino acids.

Further attempts to investigate this problem have yielded non-conclusive results. Supplementation of a soy protein diet with methionine, the first limiting amino acid, had relatively little effect on the plasma cholesterol level (Carroll and Hamilton, 1975; Hamilton and Carroll, 1976). Furthermore, attempts to reverse the observed effects of casein and soy protein isolate by adding amino acids to casein to simulate the overall composition of soy protein isolate, or by adding amino acids to soy protein isolate to give a mixture corresponding to casein have been unsuccessful (Huff et al, 1977b). Since the amino acid balance of the protein did not appear to have any effect on serum cholesterol, it

was concluded that the intact protein which made up about 50% of the total mixture in each case, exerted the predominant effect on plasma cholesterol. Examination of the amino acid spectra of casein and soy protein has revealed principle differences in levels of arginine, methionine, valine, aspartic acid and proline (Kritchevsky et al, 1977). Of particular interest has been the suggestion that the lysine/arginine ratio (L:A) in dietary proteins may be related to their atherogenicity (Kritchevsky, 1979). Since lysine has been shown to inhibit liver arginase activity (Cittadini et al, 1964), it has been hypothesized that in animals fed casein with a higher L:A, more arginine might be available to be incorporated into the arginine-rich apoprotein which is a constituent of an atherogenic lipoprotein in rabbits (Shore and Shore, 1974). Kritchevsky et al (1978) tested this hypothesis in rabbits by adding arginine to the casein diet to approximate the L:A of soy protein, 0.9, and lysine to the soy protein to make the L:A similar to casein at 2.0. The addition of lysine to soy protein consistently enhanced the atherogenicity of the diet containing the preparation and was further confirmed by Czarnecki and Kritchevsky (1980). However, more equivocal results were obtained following the

addition of arginine to casein. It has been further postulated that the L:A may be responsible for the differences in atherogenesis observed for wheat gluten, corn protein and lactalbumin for which the ratios are 0.43, 0.58 and 2.63, respectively. The possibility that the actual level of lysine or arginine is the determining factor rather than the ratio of the two has further been proposed. Obviously, more research is required before conclusions regarding the existence of a possible relationship between atherogenesis and specific amino acids can be defined. Nevertheless, it is safe to conclude that if dietary amino acids are responsible for the observed effect on plasma cholesterol in rabbits, it is more likely that the effects are due to an imbalance of amino acids, rather than a deficiency of any particular amino acid, since doubling the amount of soy protein isolate in the diet did not affect the serum cholesterol level (Huff et al, 1977a).

G. Mechanism of Action

Eventually, identification of the specific dietary component influencing cholesterol levels in rabbits fed cholesterol-free diets will undoubtedly help to elucidate

the biochemical mechanism of action which is as yet unknown. Nevertheless, various hypotheses of metabolism have been investigated in an effort to explain the biochemical nature of the effect of animal and plant protein on cholesterolemia in rabbits. Huff and Carroll (1977) investigated cholesterol oxidation, as measured by the conversion of (26 - ^{14}C) cholesterol to respiratory $^{14}CO_2$, and found that the rate of oxidation for rabbits fed diets containing soy protein isolate was twice that of the rabbits fed casein-containing diets. Further analysis of the plasma specific activity die-away curve following intravenous injection of (26 - ^{14}C) cholesterol indicated that the casein diet slowed plasma cholesterol turnover relative to the soy protein diet. Similarly, Suria (1970) found that rabbits fed a SSD which contained casein oxidized (26 - ^{14}C) cholesterol to $^{14}CO_2$ more slowly than rabbits fed commercial laboratory chow. Contrary to results reported by Huff and Carroll (1977), rates of oxidation were similar for the SSD which contained either casein or soy protein. Moreover, the plasma cholesterol levels did not correlate with the rates of oxidation, thereby suggesting another mechanism. Hermus (1975) was able to conclude from a cholesterol turnover study that rabbits fed

a SSD or commercial chow resulted in specific activity decay curves of serum cholesterol which conformed to a two-pool cholesterol model. Analysis revealed an increased size of plasma pool A and a decreased rate constant for removal of cholesterol from pool A for rabbits fed the casein-containing SSD compared with rabbits fed commercial chow. The results suggested that the casein-induced hypercholesterolemia in rabbits may be due to reduced plasma cholesterol turnover. Hermus and Stasse-Wolthius (1977) examined lipoprotein turnover by injecting labelled LDL and VLDL fractions from rabbits fed either casein or a mixture of casein, gelatin and fish protein into casein, mixed protein, and chow fed rabbits. The results indicated that lipoproteins from casein-donors were removed more slowly by all recipients and casein-fed recipients removed the lipoproteins more slowly than other animals. It was concluded that the type of dietary protein results in lipoproteins with characteristically different turnover rates and also affects the ability of an animal to remove lipoproteins from the blood.

A reduced level of degradation of endogenous cholesterol to bile acids has been reported as another mechanism responsible for the hypercholesterolemic effect of the SSD

by Kritchevsky et al (1975). Chow-fed rabbits excreted more labelled neutral steroid than did rabbits fed the SSD. The results are in agreement with Fumagalli et al (1978) who reported that replacement of casein by soybean meal in the SSD fed to rabbits resulted in an increase in fecal excretion of neutral steroids but not of bile acids. The ratio of cholesterol ingested to cholesterol and metabolites excreted fell from a mean of 1.6 to 0.6, suggesting a net loss of cholesterol.

Kritchevsky et al (1975) reported that more cholesterol was absorbed and more synthesized by rabbits who consumed the SSD. More radioactive cholesterol was also recovered from the aortic lesions. Contrary to these results, Carroll (1971) reported that the acetate (1 - ^{14}C) incorporation into cholesterol was decreased in liver slices for rabbits fed either a SSD or commercial chow with added casein. There was, however, some indication that the plasma cholesterol levels were higher under these dietary conditions. The contradictory findings were justified on the basis that the incorporation of labelled acetate into cholesterol is affected by other factors such as the size of the acetate pool in the liver and therefore, may not truly reflect the

absolute rate of biosynthesis. Moreover, liver cholesterol synthesis was found to be higher in rabbits fed the SSD relative to the corresponding commercial chow and no apparent relationship could be identified between liver cholesterol levels and the incorporation of acetate into cholesterol of liver slices.

As opposed to the absolute synthesis of cholesterol, it has been further suggested that the free to esterified cholesterol ratio is an important indicator of the severity of atherosclerosis; lower ratios are associated with greater severity (Kritchevsky and Tepper, 1965). Kritchevsky et al (1975) reported that the aortic ratio dropped from 20.0 to 1.78 for rabbits fed commercial chow compared to the SSD, respectively. However, the results are conflicting based on a report by Carroll (1971) that the ratios were much the same for the SSD and commercial chow in spite of the marked differences in plasma cholesterol.

The induction of hypercholesterolemia by the casein-containing SSD in rabbits has been explained experimentally by 1) increased absorption and biosynthesis of cholesterol, 2) decreased degradation and excretion of cholesterol to bile acids, and 3) decreased oxidation and excretion of

cholesterol and its neutral metabolites. These results have not been duplicated with other animal proteins or in other animal species and humans.

II. Other Animal Species Studies

In order to further investigate the effects of the type of dietary protein on cholesterolemia, animal species other than the rabbit have been fed the SSD. Malmros and Sternby (1968) reported that dogs who consumed a SSD which contained hydrogenated coconut oil developed a marked hypercholesterolemia and after one year, microscopic examination of the coronary arteries showed some atherosclerotic changes. Robertson et al (1972) confirmed this hypercholesterolemia in dogs with a similar diet but after four months of feeding, observed no intimal changes in the cardiovascular system. The results implicated the casein protein component of the SSD as responsible for the observed hypercholesterolemia since this diet is essentially cholesterol-free and low in fat content.

Malmros et al (1965) investigated the effect of feeding the SSD to cynomolgus monkeys when these diets contained either 17% hydrogenated coconut oil or corn oil. Over a period of 137 weeks, the mean serum cholesterol rose from 125 to 200

mg/dl with the coconut oil SSD and a less substantial increase from 100 to 157 mg/dl was reported for the corn oil SSD. Corey et al (1974) fed a SSD containing either 10% safflower oil or coconut oil with or without 0.1% cholesterol to four different species of monkeys; squirrel, cynomolgus, cebus and spider. Since most of the dietary changes related to the fat and cholesterol content of the diets and hypercholesterolemic responses were obtained with both coconut oil and cholesterol, only very tenuous hypotheses can be made regarding the cholesterolemic effect of the animal protein per se in the SSD.

Howard et al (1965a) reported that pigs maintained on a SSD which contained either 0% fat, 10% beef tallow, or 10% maize oil failed to develop a significant hypercholesterolemia as in the rabbit. In an earlier report, Gresham et al (1964) found that the incidence and severity of sudanophilic lesions was small in all groups. However, the inner medial arterial lesion was seen in all animals given the SSD. The results did not indicate that the casein protein in the SSD was associated with a hypercholesterolemic response. Contrary to these results, Kim et al (1978) compared diets which contained either 21% kilocalories from soy protein or casein.

The diets also contained 42% kilocalories as fat (38% butter, 4% corn oil), with one gram of cholesterol added. Each diet was fed to swine for a period of six weeks. Those swine who consumed the soy protein diet showed significantly lower mean serum cholesterol values, 114 mg/dl, than did those fed casein, 183 mg/dl. Conversely, Wiggers et al (1980) showed that there were no treatment differences in miniature swine for blood cholesterol concentration, blood lipoprotein cholesterol distribution, or aortic sudanophilia. The swine were fed for 12 week periods either (1) beef (ground round, 21% fat) and corn or (2) soy protein isolate, soybean oil, and corn. The results suggested that the hypercholesterolemic effect of animal protein may be specific to casein.

Chickens fed soybean protein in a low fat SSD (3% by weight corn oil) had depressed plasma cholesterol levels relative to similar diets which contained lactalbumin and egg white solids as the source of animal protein (Hevia and Visek, 1979). However, the differences were not statistically significant. It was suggested that alterations in plasma cholesterol caused by different dietary proteins are smaller in chickens than in rabbits perhaps as a result

of the higher rate of liver cholesterol synthesis in chickens which may confer a resistance to dietary manipulation. The results are in agreement with earlier reports by Kritchevsky et al (1959) who demonstrated that both germ-free and conventional chickens had higher serum cholesterol values in addition to higher liver lipids when fed low fat (5% by weight corn oil) casein diets as compared to soybean protein diets. Williams et al (1980) further showed that unlike cholesterol, the substitution of 40% of the protein with ground beef in basal diets which contained soybean meal as the sole protein source elevated the plasma apolipoprotein A-I constituent of the HDL fraction in tom turkeys.

Mice were used by Roy and Schneeman (1980) who tested the possibility that the lower digestibility and the trypsin inhibitor (TI) content of plant proteins could lead to alterations in bile acid metabolism and pancreatic function. Moreover, these metabolic alterations may explain the lower plasma cholesterol levels observed for different plant proteins compared to animal proteins. The mice were fed cholesterolemic diets containing casein, soy protein isolate or casein plus TI for four weeks. Lower plasma cholesterol levels were observed for the soy protein group and although soybean TI did not appear to affect lipid

metabolism, it greatly affected pancreatic secretion.

The cholesterol-free SSD has also been fed to baboons who develop arterial lesions resembling the fatty streaks of human atherosclerosis, show similar patterns of lipid metabolism, and have a phylogenetic proximity to man which may contribute to the understanding of the causes of human atherosclerosis (Strong and McGill, 1967). Kritchevsky et al (1974) showed that baboons maintained on a SSD containing casein will develop hyperlipemia and aortic sudanophilia compared to their normal diet consisting of bread, fruit and vegetables. Although proponents of the 'protein theory' have cited this data as evidence that animal protein is more cholesterolemic and atherogenic than plant protein, the authors concluded that the effects of the test diets were not due to any single dietary component, but to the interaction of carbohydrate, fat and fiber. In support of this conclusion, Lofland et al (1966) studied the interrelations of dietary protein and fat in pigeons and concluded that all the dietary variables in the study could influence one or more of the parameters related to atherosclerosis. Furthermore, they reported that any single dietary ingredient could be influenced by other components of the diet. For example, casein was

more atherogenic than wheat gluten when fed in combination with soybean oil (Crisco) but not when corn oil was the source of fat. The highly variable nature of the response observed for dietary protein agrees well with rabbit studies (Carroll and Hamilton, 1975) and is undoubtedly a general phenomenon for all species.

A number of the animal studies, mainly those using the rabbit, suggest that the cholesterol-free SSD which contains casein is more cholesterolemic and atherogenic than the normal diet for animal species which usually consists of mixed plant sources. However, interpretation of the results as meaning that animal protein is more cholesterolemic than plant protein is not justified in view of the fact that most studies have compared casein and soy protein with little consideration given to other animal and plant protein sources. Furthermore, often overlooked has been the existence among dietary variables of biological interactions which have served to alter the results obtained for casein relative to soy. Nevertheless, there appears to be little doubt that casein is hypercholesterolemic under certain dietary conditions and that soy protein is effective in maintaining low serum cholesterol levels in most animal species tested.

However, the magnitude of the difference between the two proteins varies from one species to the next and is further dependent on other dietary constituents present. Such factors have made quantitation of the cholesterolemic effect of casein and soy protein difficult. Moreover, it is yet to be determined whether the atherogenic nature of the casein-containing semisynthetic dietary model can be applied to man.

III. Human Studies

A. Epidemiologic Data

Studies related to the dietary factors affecting serum cholesterol in experimental animals have as their ultimate goal, the control of hypercholesterolemia and prevention of atherosclerosis in humans. In an attempt to elucidate the effect of dietary protein in humans, epidemiologic data have been examined. Such analysis of dietary intakes and disease among various population groups has led to the suggestion that dietary protein may have an effect on serum lipids and the development of atherosclerosis. As early as 1957, investigators called attention to the positive correlation between the incidence of coronary mortality and the amount of animal protein ingested in several countries

(Yudkin 1957; Yerushalmy and Hilleboe, 1957). This association was found to be even more pronounced than that noted between fat and heart disease mortality. Connor and Connor (1972) further examined the relationship of various nutrients in the diet to coronary mortality in men, aged 55 to 59 years, and found that animal protein had the highest positive correlation ($r = 0.782$), surpassing both cholesterol ($r = 0.762$) and total fat ($r = 0.676$), while vegetable protein showed a lower negative correlation ($r = 0.403$). A more recent analysis which reviewed data from 30 countries confirmed these results but found significant negative correlations between cereals and vegetables and heart disease mortality for men only (Armstrong et al, 1975). Similarly, associations for animal protein and coronary mortality have been made within individual countries such as Japan. As compared to other highly industrialized countries, Japan has low coronary mortality rates in addition to relatively low serum cholesterol values (Stamler, 1979). The traditional Japanese diet is vegetarian and low in animal products. However, the fact that the traditional diet is also low in total fat, saturated fat, dietary cholesterol, simple carbohydrate and total protein points to a major problem of interpretation associated with

epidemiologic correlations. Because of the lack of specificity, it is not possible to determine whether the association is unique to protein or merely reflects a diffuse relationship with other dietary factors. This problem is further illustrated by epidemiologic studies of individual population groups. For example, Connor et al (1978) studied the Tarahumara Indians of Mexico and found extremely low plasma cholesterol levels in all groups of the population; the adult group had a mean plasma cholesterol of 125 mg/dl. The Tarahumara Indians subsist mainly on corn and beans (90% of total kilocalories) and although the negligible intake of animal protein may have influenced the low plasma cholesterol levels observed, the low dietary intake of cholesterol (71 mg/day), fat (12% of total kilocalories) and saturated fat (2% of total kilocalories), and the high dietary intake of plant sterols (400 mg/day) and fiber (19 mg/day) can also be related to decreased plasma cholesterol. In contrast to this epidemiologic data, Dyerberg et al (1975) reported that Greenland Eskimos, who consumed a very large percentage of their total daily caloric intake as animal protein (26%), maintained below average serum cholesterol concentrations. The results suggested that

epidemiologic data lack quantitative information about specific dietary components and are not necessarily consistent from one population group to another.

1. Vegetarian Groups

Vegetarian groups afford a singular opportunity to study the relationship of dietary protein quality, serum lipids and atherosclerosis in a free-living population. In 1954, Hardinge and Stare reported that lacto-ovo-vegetarians (LOV) and pure vegetarians (PV) had consistently lower serum cholesterol levels at 16% and 29%, respectively, than did subjects ingesting the typical non-vegetarian diet (NV). There was a high degree of significance obtained only when the PV groups were compared with their respective LOV and NV groups. All NV groups ingested significantly higher total kilocalories, total fat, and total cholesterol than did the corresponding vegetarian groups. A group of 145 vegetarians (LOV) of the Seventh-day Adventist faith constituted the group studied by Walden et al (1964). They reported that not only were the median serum cholesterol levels of the LOV group lower than those of an age-matched NV group, but that the levels could further be lowered by

either eliminating the fat or by substituting unsaturated for saturated fat in the diet. West and Hayes (1968) confirmed that significantly lower mean levels of cholesterol were observed for Seventh-day Adventist vegetarians, mainly LOV, in a larger sampling of 233 carefully matched groups. The differences in serum cholesterol were significant only for subjects over 25 years and are in agreement with Hardinge and Stare (1954) who demonstrated that the adolescent age groups showed only slight differences in serum cholesterol. They suggested that with aging, there may occur a diminishing ability of the body to metabolize excess cholesterol, whether of endogenous or exogenous origin. Sacks et al (1975) further reported that for a group of 116 vegetarians who consumed macrobiotic diets (principally whole grains, beans and fresh vegetables as staples), LDL, VLDL and HDL cholesterol, total cholesterol and triglyceride values were significantly lower compared to a group of NV controls matched for age and sex. The large differences, ranging from 31% to 38%, could not be accounted for by the lower weights of the vegetarians. With the exception of one vegetarian, all vegetarians were classified as having normal lipoprotein patterns according to the Frederickson classification. A six-year prospective

study of 24,004 Seventh-day Adventists revealed progressively lower age-standardized coronary mortality rates for NV, LOV and PV groups than those found in the general population (Philips et al, 1978). The persistent difference between NV Adventists and the general population suggested that the reduced risk of coronary heart disease mortality among Seventh-day Adventists is partially due to some component of their lifestyle other than diet. Moreover, the fact that the risk of coronary mortality among NV males was three times greater than vegetarian males of comparable age ($p < 0.01$) suggested that diet plays a major role in the risk factors.

Although studies of vegetarians purport to demonstrate lower serum cholesterol values for this group, inferences regarding the specific effect of plant protein in ameliorating the cholesterolemic and atherogenic response are not possible in view of other concurrent dietary variables which may also alter the response. These variables include lower total and saturated fat, lower cholesterol, lower caloric and higher fiber intakes associated with the typical vegetarian diet. Moreover, some vegetarian groups such as the Seventh-day Adventists not only eat vegetarian diets but also

share a philosophy and lifestyle which further complicates interpretation. Multiple differences in the vegetarian lifestyle, including diet, smoking and drinking habits undoubtedly interact to produce the resultant superior prognosis with regard to atherosclerosis.

On the basis of the lack of consistency and specificity demonstrated by epidemiologic studies in both meat eating and vegetarian populations, it becomes clear that epidemiologic associations cannot be considered as being independently conclusive or constituting proof of a cause-effect relationship. They merely serve as guides for further research or as supporting evidence for a formulated hypothesis, namely, that dietary protein is an etiologic factor in arteriosclerotic and degenerative heart disease. Therefore, whether or not the statistically significant correlation presented for animal protein and coronary mortality is in fact etiologically significant must be evaluated in light of evidence derived from controlled laboratory studies with human subjects.

B. Metabolic Studies

1. Hypercholesterolemic Subjects

The most dramatic effects of dietary protein in

controlled human studies have occurred with hypercholesterolemic subjects in which soy protein has replaced the mixed dietary protein normally found in the average diet. The development of novel forms of commercially available isolated soy protein has greatly facilitated the use of soy protein in the study of the effect of plant protein on serum lipids in man. Hodges et al (1967) showed that the substitution of animal protein (15% of the total kilocalories) with soybean protein caused a marked fall in mean serum cholesterol levels of about 100 mg/dl in hypercholesterolemic subjects with average initial values of 297 mg/dl. Furthermore, the serum cholesterol levels remained low on the soybean protein diet regardless of the source of carbohydrate (sugar versus starch) or the level of fat (15% versus 45% of total kilocalories). However, Keys (1967) questioned whether the dramatic fall in serum cholesterol was due to the type of protein in the diet. On the basis of a critical examination of the data, it was revealed that almost all of the decrease in serum cholesterol could be explained by the changes in dietary fatty acids and cholesterol and the intrinsic characteristics of the particular men studied. Moreover, the experimental diets were not constant in

sitosterol and carbohydrate content which may have also influenced the results. In view of the uncontrolled dietary variables in this study, it is difficult to know whether or not there is real evidence for a special cholesterol-lowering effect of the soybean protein diet. However, Sirtori et al (1977) showed that the substitution of textured soybean protein for animal protein in the "prudent" diet used to treat type II hypercholesterolemic patients significantly enhanced the lipid-lowering properties of the diet. The usually prescribed low-lipid diet contained 58% of total kilocalories as carbohydrate, 21% fat (P/S, 2.2) and 21% protein (13% animal; 8% plant). The diet used by Sirtori contained less carbohydrate, 53%, more fat, 26% (P/S, 2.7), and similar amounts of protein (13% soybean; 6% other plant; 2% animal). The subjects showed a 14% decrease in plasma cholesterol after two weeks and 21% after three weeks on the soybean protein diet. This change was found to occur mainly in the LDL fraction. A subsequent experiment in which 500 mg of cholesterol was added to the soy protein diet showed that the hypocholesterolemic response was not influenced by the cholesterol content of the diet. Hermus et al (1977) suggested that the lack of effect of cholesterol could have been due to the very low

dietary fat and the high P/S ratio since there is some evidence of an interaction between these dietary variables. Moreover, Helms (1977) suggested that the hypolipidemic effects of textured soybean protein be ascribed to the carbohydrate fraction of the product and not the protein *per se*. Gatti and Sirtori (1977) responded to this suggestion by reporting that there was a difference of about eight grams of indigestible fiber content in the two diets in favour of the soybean protein diet which they concluded was unlikely to achieve any significant hypocholesterolemic effect. They also reported that the addition of methionine (1.39 grams/daily) to the soybean protein diet did not appear to alter the hypocholesterolemic effect as suggested by Helms (1977).

The effect of variation of the P/S fatty acid ratio fed to hypercholesterolemic subjects was investigated by Sirtori *et al* (1979). The subjects were fed either a soybean protein diet with a P/S ratio of 2.7 or a diet similar in all respects except that the P/S ratio was 0.1; each was fed for three weeks in a cross-over experimental design. The results showed that the soybean protein diet had a decreased effectiveness with the low P/S regimen but the lipid values

observed were still below baseline values. It is apparent that protein interacts with other components of the diet in man, as was also shown in animal studies, and that this interaction ultimately determines levels of serum lipids and lipoproteins.

These dietary studies provide evidence that the soy protein diet may be an effective regimen for inducing a significant serum cholesterol reduction in hypercholesterolemic patients refractory to standard low-lipid regimens. However, the suggestion by Sirtori et al (1977) that a mixed plant protein diet may be equally as effective is not justified until confirmed by further study.

2. Hypocholesterolemic Subjects

Hypocholesterolemic individuals have also been studied in an attempt to elucidate the effect of dietary protein on cholesterolemia and atherogenesis in man. It is a well documented fact that serum lipids are significantly reduced in protein deficiency and for this reason, kwashiorkor and atherosclerosis have been considered as reciprocal disorders. The fact that the hypocholesterolemia of kwaskiorkor can be readily restored to normal levels by skim milk or various fat-free amino acid or protein formulas

has been cited as evidence for the hypercholesterolemic effect of animal protein relative to plant protein. For example, Tripathy *et al* (1970) observed an increase in both serum cholesterol and phospholipid levels in malnourished adults when their dietary protein intakes which averaged from 15 to 30g daily were increased to 35 to 100g daily from mixed animal sources. However, Bagchi *et al* (1963) reported that the hypercholesterolemic response was more likely due to the amount of protein in the diet and not the animal protein *per se* since their study with malnourished children showed significant increases in serum cholesterol levels when the diets were supplemented with the same amount of plant or animal protein. Although there is evidence that the amount of dietary protein may also affect serum lipid levels in man, a number of reports in the literature have indicated that, within a wide range of normal intakes, a change in the protein content of the diet does not produce a significant change in the serum lipid levels (Anderson *et al*, 1971). Nevertheless, it should be apparent that unless purified protein sources are used in conjunction with a basal diet of constant protein composition, it is not possible to attribute with certainty blood lipid changes to the changes in the type of dietary protein.

3. Normolipidemic Subjects

There have been only a few direct comparisons of animal and plant dietary protein in normolipidemic human subjects. A marked decrease in serum cholesterol was reported by Olson *et al* (1958) when subjects were transferred from a diet containing 100g of animal protein to an isocaloric diet containing 25g of plant protein derived from cereal, rice and legumes. Each diet contained 50g of butterfat. This study has been classically cited by proponents of the 'protein theory' for atherosclerosis in spite of subsequent studies which demonstrated that the hypocholesterolemic response was more likely due to the lower absolute level of protein than the plant protein *per se* since diets consisting of 25g of a mixture of beef and milk protein still produced the hypocholesterolemic effect (Olson *et al*, 1961). Walker *et al* (1960) compared the effect on serum lipids of two protein diets, one derived the protein content from plant sources, with minimal soy protein, and the other from mixed animal sources. The protein content of both diets averaged 45 to 50g daily and the mixed fat content of the two diets was similar in amount (approximately 36% of total kilocalories)

and origin. Two different groups of six young women were subjects. Serum cholesterol levels decreased significantly ($p < 0.02$) in both groups but the decrease was greater for the group fed the plant protein diet. After six weeks, average serum cholesterol levels for the animal protein and plant protein diet treatment groups were 157 $\pm$ 9 mg/dl and 137 $\pm$ 8 mg/dl, respectively. However, the difference was statistically significant only at the end of two weeks ($p < 0.005$) and five weeks ($p < 0.025$) of the dietary regimen. The results must be interpreted with caution since there were different subjects for each dietary period.

Campbell et al (1965) tested the effect of the type of protein fed on serum lipids of men by comparing a diet containing wheat gluten as the chief source of nitrogen with a diet containing an isonitrogenous amount of casein-lactalbumin mixture equivalent to 30g of protein. They did not find significant differences in serum cholesterol levels for the different nitrogen sources regardless of whether 12% or 40% linoleic acid was fed. These results were confirmed by Anderson et al (1971) who substituted a lower proportion of total dietary protein but a higher absolute amount, 60g. The test protein represented half of the total daily intake (120g) as either wheat gluten or egg

white. In addition, no significant differences were found for triglyceride levels but phospholipid levels were significantly higher by 7 mg/dl ($p < 0.01$) when the men consumed the wheat gluten diet. They concluded that the type of protein in the diet is of no particular value in designing diets for the reduction of serum cholesterol in normocholesterolemic man.

Dietary protein has been shown to significantly influence the level of plasma cholesterol in rabbits and hypercholesterolemic human subjects. In response to these observations, Carroll et al (1978) investigated the effect of dietary protein in normolipidemic subjects. Utilizing simulated soy protein meat analogues, they showed a small, but significant decrease of 9 mg/dl ($p < 0.05$) in the plasma cholesterol levels of young adult women compared to feeding typical animal protein sources in a conventional diet. The mixed animal protein diet contained 70% of the total protein from beef, chicken, ham and milk. In the plant protein diet, the soy protein meat analogues were prepared to have a similar fat composition as the corresponding meats and together with soy milk, replaced the animal proteins in the diet. In addition to dietary fat, both diets were similar

with respect to sterol and carbohydrate composition. The relatively small response to changes in dietary protein noted in this study was judged by the authors as compatible with the larger changes reported by Sirtori et al (1977), since hypercholesterolemic individuals may be expected to show an exaggerated response to any measure that lowers serum cholesterol. The results are also in agreement with the earlier rabbit studies reported by Carroll and associates, although the magnitude of the response was considerably greater for rabbits. Koury and Hodges (1968) similarly studied the effect of feeding soy protein meat analogues on various biochemical parameters of hospitalized normolipidemic prisoners. The diets contained virtually no animal protein, five percent of total kilocalories as fat, with linoleic acid representing approximately half of the fat content. Hence, it is difficult to ascertain whether the reported decrease in serum cholesterol of approximately 100 mg/dl and the similar, roughly parallel, decrease in triglyceride values were largely due to the dramatically reduced fat intake or the soy protein present in the diet.

Clearly, there appears to be a hypocholesterolemic effect associated with soy protein fed to animal species and man. Whether a biological significance can be attached

to the small changes in serum cholesterol levels observed in normolipidemic individuals is questionable. Moreover, from a practical standpoint, it would not be possible to substitute all of the animal protein in the average Canadian diet with soy protein and whether a small level of substitution would have any direct effect on serum cholesterol is uncertain. It is more likely that the effect of substitution with soy protein would be indirect since such diets would have a much lower content of saturated fat.

Although there is evidence for a hypocholesterolemic effect associated with soy protein fed to man, the pursuit for a mechanism of action does not appear justified at this time based on the equivocal evidence available for other plant and animal proteins. Nevertheless, the amino acid constituents of the diet have been investigated in an attempt to provide a better understanding of the role of dietary protein in the control of serum cholesterol levels. Olson et al (1964) studied the effect of various pure L-amino acid mixtures to afford better control of the amino acid intake. Four male subjects were fed diets which contained 100g of protein, 36% of total kilocalories as fat, and all

of the essential nutrients for a four-week control dietary period. Following this period, a formula diet which contained either all of the L-amino acids in the same proportions as in the control protein mixture or a diet containing the eight essential amino acids in adequate amounts plus glutamate as a source of nonessential nitrogen was fed for three to four weeks. The caloric value and fat content of these diets were constant. No statistically significant change in serum lipids or β-lipoproteins from control values were found when all the amino acids were fed. However, when eight essential amino acids plus glutamate were fed, serum cholesterol decreased 37 mg/dl and phospholipids decreased 19 mg/dl, while triglycerides increased 49 mg/dl. The major alteration in lipoproteins, measured ultracentrifugally, was a decrease within the low-density cholesterol-rich fraction. It was concluded that the formation of β-lipoproteins by the liver may be a function of the nonessential as well as the essential amino acid nitrogen in the diet. A subsequent study by Olson et al (1970) confirmed the hypocholesterolemic effect when 137g of glutamate was fed as a nonessential source of nitrogen in conjunction with a mixture composed of the eight essential amino acids at three times Rose's

minimal daily requirement. Moreover, formula diets identical in all respects except for the equimolar replacement of glutamate by glycine plus ammonium acetate were not hypocholesterolemic. The hypocholesterolemic effect of the glutamate formula was found to be greater than that of a nitrogen-free diet identical in other respects and greater than the effect of feeding 25g of intact vegetable-cereal protein. The effect of glutamate, however, did not appear to be related to the absolute amount of glutamate present in the diet alone since the addition of glutamate to an otherwise normal diet was without effect (Bazzano and Olson, 1969). Anderson et al (1971) also showed that when diets which contained purified protein sources from either egg white or wheat gluten were fed, for which there was a difference of 21g glutamic acid in favour of the gluten diet, there were no significant changes in serum lipids. These results do not support the suggestion that the higher glutamic acid content per se characteristic of some plant protein diets relative to animal protein diets may be responsible for the observed hypocholesterolemic response.

Olson et al (1970) reported that since the hypolipidemic effects of glutamate and polyunsaturated fatty

acids were not additive, the two agents may be involved in a common pathway in the metabolism and distribution of serum lipids. In an attempt to explain the mode of action of glutamate in inducing hypocholesterolemia in man, Garlich et al (1970) investigated the changes in plasma free amino acid concentrations. They found that in spite of the altered plasma aminograms for subjects who received 137g of glutamic acid daily, no clear-cut difference in the proportions of amino acids could be identified. Furthermore, it was suggested that the mechanism of the glutamate effect was not related to altered availability of amino acids to the liver for apopeptide biosynthesis since the β-peptide is common to both LDL and VLDL fractions and only the LDL was preferentially depressed. The fall in plasma cholesterol and β-lipoproteins that occurs in human subjects fed amino acid formulas which contained glutamate may be due to a reduction in sterol biosynthesis as demonstrated in experiments which involved isotopic labelling of the plasma cholesterol pool and a sterol-balance study (Bazzano and Olson, 1969). In a later study, again using isotopic labelling, Olson et al (1970) suggested that the entry of cholesterol molecules into the plasma compartment from the

liver is retarded when the glutamate formula is fed, although the biochemical mechanism was not identified.

Studies using crystalline amino acids other than glutamate have consistently demonstrated no effect on serum cholesterol in man. Truswell (1964) tested the effect of supplementing the diets of normal adults with two and six grams of L-leucine daily based on the ability of L-leucine to be catabolized to β-hydroxy-β-methylglutaryl-CoA, a direct precursor of cholesterol. Serum cholesterol was not elevated by adding leucine to diets which already contained a high proportion of leucine. Later, in 1965, Truswell et al, tested the theory that the conjugation pattern of bile acids may influence the serum cholesterol concentration. On the basis that taurine conjugation of bile acids is generally associated with low serum cholesterol and glycine conjugation with high serum cholesterol levels, the diets of three adult men were supplemented with 1.5 grams taurine daily. The results showed that although the proportion of bile acids conjugated with taurine in human bile could be increased by supplementation, no effect on serum cholesterol concentration was produced over 10 to 15 days of study. It was concluded that the association of

increased taurine conjugation of bile acids with low serum cholesterol levels in man is an epiphenomenon and/or a coincidence. Gerber et al (1971) further showed that the long-term oral administration of L-histidine did not affect serum cholesterol concentration in spite of a significant ($p < 0.001$) increase of 0.31 mg/dl in the serum histidine concentration. These results are at variance with reports on rabbits and monkeys in which orally administered histidine produced significantly elevated plasma cholesterol concentrations (Geison and Waisman, 1970).

Clearly, the experimental evidence does not show a conclusive beneficial effect on serum lipids of mixed plant protein diets among man and animal species. Moreover, a conclusive detrimental effect on serum lipids associated with animal protein diets has not been revealed. The evidence merely purports to a hypocholesterolemic effect associated with soy protein fed to animal species and man. Similarly, casein fed as a purified protein source in a low fat cholesterol-free semisynthetic diet appears to be more cholesterolemic and atherogenic in a number of animal species. However, the exact dietary nature of the

cholesterolemic responses characteristic of these isolated proteins, together with their biochemical mechanisms of action, have not yet been elucidated. Until more experimental evidence becomes available which affirms that the cholesterolemic responses of soy and casein protein diets are in fact representative of plant and animal protein diets more typically consumed in the human diet, it is not scientifically justified to recommend the substitution of plant protein for animal protein in diets for human population groups with the goal of reducing the perceived risk to atherosclerosis.

INTRODUCTION

Investigations which have implicated various proteins in the diet as atherogenic have primarily utilized animal models, mainly the rabbit, and purified protein sources. Purified soy and casein proteins have been incorporated into semisynthetic diets which have not been standardized with respect to their non-protein composition. Such experimental diets cannot be considered to be representative of plant and animal proteins, nor do they simulate diets typical of human populations. Human studies have focused on the cholesterolemic effect of soy protein, fed mainly in the form of simulated soy protein meat analogues, for clinically defined hyperlipidemia. Subjects known to be hypercholesterolemic may show an exaggerated response to any measure that lowers blood lipids. Also, diets which include a major portion of protein as soy protein do not have a practical application for population groups. Moreover, diets fed to human subjects have not been consistently controlled with respect to all dietary constituents which have known effects on blood lipids, especially the composition of dietary lipid. Consequently,

it has not been possible to attribute the blood lipid changes to the changes in dietary protein *per se*. Hence, the effects of plant protein in diets which consist of a variety of plant sources typical to the Canadian diet need to be assessed with respect to serum lipid patterns in healthy free living individuals. The dietary variables known to affect serum lipids should be constant. Such an assessment is important before recommendations can be made for preventative dietary measures for atherosclerosis that will be beneficial to the Canadian population at large.

The primary objective of the research reported here was to compare the effects of two types of dietary protein on serum cholesterol, triglyceride, and plasma LDL and HDL levels of healthy, normolipidemic young men. One diet containeda variety of plant sources with minimal amounts of soy protein and the other diet was similar, but 55% of the plant protein was substituted by beef protein. The diets were formulated to consist of a variety of natural food sources typical to the Canadian diet, with the exception of commercial soy milk, and to approximate the mean daily nutrient intake for Canadian males.

According to the Nutrition Canada Survey[1] carried out between 1970 and 1972, males, aged 20 to 39 years, consumed on the average 15%, 42% and 43% of total kilocalories from protein, fat and carbohydrate, respectively. The percentage contribution by meat, poultry, fish and eggs to protein intake was 52% which compares closely to the amount of beef protein, 55%, in the animal protein diet fed in this study. The mean intakes of protein, fat and carbohydrate for the two diets in the present study were 11%, 32% and 57% of total kilocalories, respectively; a compromise between the difference in distribution of kilocalories characteristic of meat-eating and vegetarian population groups. The diets were adequate in energy and nutrients as judged by the 1975 Revised Canadian Dietary Standard for males, 19 to 35 years of age. In order to control other dietary variables, the types and amounts of fats and sterols in both diets were similar and the amounts of wheat fiber were constant for both diets. The data generated from this study should be useful in a further definition of the role of dietary protein in lipid metabolism in man.

1 Food Consumption Patterns Report, Nutrition Canada, Department of National Health and Welfare, 1977.

METHODS AND MATERIALS

I. Experimental Design and Statistical Analysis

The 42-day metabolic study was divided into two 21-day periods; the animal protein diet (APD) and the plant protein diet (PPD) periods. Each subject consumed the APD and the PPD in a crossover experimental design in order to minimize the effects of subject to subject variability and to overcome the effect of time periods. Four subjects were randomly assigned to start the study with the PPD treatment for a 21-day period and then cross over to the APD treatment for another 21-day period; another four subjects were randomly assigned to proceed in the reverse order of diet presentation. The crossover design used in this study is similar to the Replicated Latin Square Design described by Cochran and Cox (1957). Four replicates of the latin square were required to accommodate the eight subjects investigated in the study; 2 subjects in each latin square. The experimental design is illustrated in Figure 1.

Analysis of variance for diet treatment and diet order patterns with respect to serum cholesterol, triglyceride and plasma LDL and HDL were determined on the differences

Figure 1

Distribution of Subjects in Crossover Experimental Design

Subject	Diet Period I (21-Day)	Diet Period II (21-Day)
	Plant Protein	Animal Protein
AG		
JK		
WS		
MS		
	Animal Protein	Plant Protein
MH		
BY		
AS		
RP		

of the mean values for each subject between each diet treatment period and on the mean difference for each group of four subjects assigned a given order of diet presentation, respectively.[1] A paired-difference test (Mendenhall, 1979) was used to analyze the change in serum triglyceride response during the initial dietary period for both the APD and PPD groups.

II. Subjects

The subjects were eight healthy normolipidemic university students, aged 18 to 27 years ($\bar{x}$ = 21 years), selected from volunteers who responded to notices posted on the campus which advertised free meals plus a cash bonus for participation in a research project. Age, height and weights for each subject during the study are recorded in Table 1. Subjects were chosen on the basis of a physical examination by a university health officer and a personal interview in which adherence and co-operativeness were judged following a detailed explanation of the experimental protocol. None of the

[1] Personal communication. Dr. J. Brewster, Department of Statistics, University of Manitoba, Winnipeg, Manitoba.

Table 1

Age, Height and Weight of Subjects

Subject	Age yr	Height cm	Initial Weight kg	Animal Protein Diet Period		Plant Protein Diet Period	
				Mean Weight[1] kg	Mean Change in Weight[2] kg	Mean Weight kg	Mean change in Weight kg
MH	18	183	69.5	68.5 ± 0.6	-0.8	68.9 ± 0.8	-0.7
BY	23	175	61.8	61.7 ± 0.3	0.0	63.5 ± 0.5	+1.2
AS	23	168	56.4	57.2 ± 0.6	+0.9	58.5 ± 0.3	+0.4
RP	20	170	72.3	72.6 ± 0.4	+0.4	73.5 ± 0.4	-0.5
AG	18	168	60.9	61.2 ± 0.4	-1.3	61.7 ± 0.5	+0.9
JK	27	185	74.5	74.4 ± 0.3	-0.6	74.4 ± 0.3	+0.1
WS	19	173	66.8	67.1 ± 0.4	-1.1	67.6 ± 0.4	+1.0
MS	19	178	68.2	67.6 ± 0.4	-0.6	68.0 ± 0.4	+0.2

1 Mean ± SD for daily weighings.

2 Mean change between daily weighings.

subjects were obese, ingested drugs or lipid-altering medications, or had a personal history of cardiovascular disease. The initial mean serum cholesterol for the subjects was 184 ± 28[1] mg/dl; initial mean serum triglyceride, 114 ± 48[1] mg/dl; initial mean plasma LDL and HDL, 96 ± 33[1] mg/dl and 53 ± 11[1] mg/dl, respectively. The subjects resided in their own homes during the study and maintained their normal activities. All meals were served in the metabolic laboratory in the Home Economics Building. The subjects were typically sedentary students, with the exception of MH, who participated in a number of athletic and extra-curricular events. All subjects completed the study and maintained good health throughout its duration.

III. Diets

A. Description

Two experimental diets were formulated to differ only in the source of protein; in one the entire protein was obtained from mixed plant sources and in the other, beef was substituted for some of the plant sources. The diets

[1] ± Standard deviation.

were designated as the plant protein diet (PPD) and the animal protein diet (APD), respectively. The menus developed for each diet were designed to be comparable in both the proportion of nutrients and the types of food sources characteristic of the Canadian diet. A daily menu was repeated without alternation throughout the respective 21-day periods. The daily menus are shown in Appendix Table 1.

The plant sources of protein used included wheat and oatmeal products, vegetables, legumes, nuts, seeds and soy milk.[1] Soy milk provided 1.5g protein in the APD and 3.0g in the PPD. The single source of animal protein was lean ground beef. Both diets consisted of essentially the same foods except that in the APD, 55% of the plant protein (mainly in the form of legumes, nuts, seeds and whole wheat bread) was substituted by beef protein.

To ensure that the fat composition was similar between the two diets, a beef tallow margarine[2] was formulated for

[1] Prosobee, Mead Johnson and Company, Canada.

[2] Beef tallow supplied by Canada Packers Ltd., Winnipeg, Manitoba. Margarine produced by Dairy Science, University of Manitoba, under specification of 80% fat content.

the PPD in order to balance the tallow in the beef of the APD. A commercial sunflower oil margarine[1] was fed in the APD. The analyzed fat content of the ground beef was 12% by weight. An additional 6g beef tallow was added to the APD in order to make the ground beef equivalent to 15% total fat. Other major animal fat sources were the cookies and granola, both specially prepared with beef tallow. Peanut butter, sunflower seeds and commercial soy milk represented major plant sources of fat.

The wheat fiber was controlled in the two diets by the addition of 6.8g wheat bran (3.0g dietary fiber) to the APD; a level equivalent to the extra wheat fiber in the additional two slices of whole wheat bread of the PPD as calculated from McCance and Widdowson's Composition of Foods (Paul and Southgate, 1978).

The diets were supplemented with vitamin and mineral supplements in order to meet nutrient recommendations of the 1975 Revised Canadian Dietary Standard for males, 19 to 35 years of age. The calcium intake met the recommended

[1] Becel, Monarch Fine Foods Co. Ltd., Ontario.

allowances by utilizing a vitamin A/D preparation[1] which contained dicalcium phosphate; the PPD group received one capsule daily and the APD group, two capsules daily. Each subject was also given one vitamin B/C tablet[2] daily throughout the study in order to meet the thiamin and riboflavin recommended allowances.

All food supplies were purchased in bulk, with the exception of perishable fruits and vegetables, in order to minimize variability. Standardized recipes and procedures were utilized by a trained assistant. Granola, cookies and beef patties were prepared and frozen in advance of the study. Food portions were standardized by either weighing or measuring all foods and ingredients associated with the three meals plus between-meal snacks. Pre-portioned convenience foods were used to facilitate both accurate quantity control and meal preparation. Foods were prepared, heated and served in the same dishes to minimize losses. Participants ate all of the food provided in the daily menus. All food consumed by the subjects was supervised by the director of the study. Meals were eaten under supervision

[1] Natural Source, Adams Laboratories, Surrey, B.C., Canada.

[2] Neo-Bex, Neo Drug Co., Montreal, Canada.

in the metabolic unit, but between-meal snacks were portable. Condiments including ketchup, spices such as chili powder, and flavorings such as vanilla extract, were allowed in moderation to improve upon the palatability of certain foods. Clear tea and coffee were allowed ad libitum, in addition to limited amounts of tomato juice and calorie-free carbonated beverages.

Adjustments in kilocalories were made as required to maintain subject weight which was recorded at approximately the same time daily. The caloric intake from carbohydrate and fat in the diet was altered. The adjustments did not significantly effect the protein component of the diet and maintained the percentage of total kilocalories from fat constant for each individual.

B. Analysis

Aliquots representing one fifth of each diet were weighed using a Satorius top-loading balance (Model 2254)[1] and homogenized with 200 ml glass distilled water in a

[1] Satorius-Werke AG, Gottingen, Germany.

one-gallon Waring commercial blender (Model CB-5)[1]. Weighed aliquots of homogenate were lyophilized in a Virtis Freeze Dryer (Model 10-140 MR-BA)[2]. The lyophilized samples were pulverized to a fine homogeneous mixture and stored in Whirl-Pak plastic bags (#8992, 510-30g)[3] at -10 C. Diets were analyzed for total fat, sterols (cholesterol, β-sitosterol, campesterol, and stigmasterol), and protein as shown in Table 2. Energy was estimated from tables of food composition. Amino acid and fatty acid analysis for each diet are presented in Tables 3 and 4, respectively.

Total lipid was extracted from a known quantity of lyophilized food sample according to a method adapted from Bligh and Dyer (1959) utilizing a monophasic mixture of chloroform, methanol and water (1:2:0.8 v/v/v). The isolated chloroform layer was evaporated and dried thoroughly under nitrogen before lipid was determined gravimetrically. An aliquot of the lipid, dissolved in hexane, was transferred to a screw-top vial, flushed with

1 Waring Products Co., Winsted, Connecticut.

2 Virtis Co. Inc., Gardiner, New York 12525.

3 Canlab Laboratory Equipment, Winnipeg, Manitoba.

Table 2

Daily Energy, Protein, Fat and Sterol Composition of Basal Diets

Diet	Method	Energy Kj	Protein g	Fat g	Cholesterol mg	β-Sitosterol mg	Campesterol mg	Stigmasterol mg
Animal Protein	Calculation[1]	12357	94	114	162	ND[3]	ND	ND
	Analysis[2]	ND	94	117	184	194	42	22
Plant Protein	Calculation[1]	14602	84	115	27	ND	ND	ND
	Analysis[2]	ND	86	124	66	258	48	33

[1] Energy, protein and fat values calculated using USDA Handbook #8, Composition of Foods, (Watt and Merrill, 1963). Cholesterol values calculated from McCance and Widdowson's, The Composition of Foods, (Paul and Southgate, 1978).

[2] Mean value of triplicate analysis for protein; values within 1% of mean.
Mean value of quadriplicate analysis for fat and sterols; values averaged within 3% of mean.

[3] ND = No calculated or analytical data available, respectively.

Table 3

Amino Acid Composition of Experimental Diets[1]

Amino Acid	Plant Protein Diet[2] g/100g	Plant Protein Diet g/day	Animal Protein Diet[2] g/100g	Animal Protein Diet g/day	Essential Amino Acid Requirement[3] g/day
Histidine	2.31	2.04	2.84	2.69	
Isoleucine	3.74	3.30	4.18	3.95	0.70
Leucine	7.11	6.27	7.76	7.34	1.10
Lysine	4.02	3.55	6.16	5.82	0.80
Methionine	1.30	1.15	1.90	1.80	0.20[4]
Phenylalanine	4.92	4.34	4.12	3.90	0.30[5]
Threonine	3.46	3.05	3.74	3.54	0.50
Valine	4.52	3.99	4.80	4.54	0.80
Alanine	4.24	3.74	5.70	5.39	
Arginine	8.06	7.11	6.76	6.39	
Aspartic Acid	9.90	8.74	9.50	8.98	
Glutamic Acid	23.64	20.86	20.70	19.57	
Glycine	4.48	3.95	6.08	5.75	
Proline	7.22	6.37	7.02	6.64	
Serine	4.94	4.36	4.58	4.33	
Tyrosine	3.00	2.65	2.54	2.40	

1 Values based on duplicate analysis for each diet.
No analytical data available for tryptophan and cystine.
2 Values are expressed in grams of amino acid per 100 grams protein.
3 Food and Nutrition Board (1959).
4 In presence of adequate cystine.
5 In presence of adequate tyrosine.

Table 4

Fatty Acid Composition of Diets[1]

Fatty Acid	Plant Protein Diet	Animal Protein Diet
10:0[2]	tr[3]	tr
12:0	tr	1.01
14:0	1.21	1.94
14:1	tr	0.54
16:0	18.73	20.56
16:1	2.20	2.86
16:2	tr	tr
17:0	0.50	0.57
18:0	10.94	10.74
18:1	39.82	38.22
18:2	22.20	20.22
18:3	1.13	0.59
20:0	0.64	0.53
20:1	0.78	0.59
20:4	tr	tr
22:0	0.75	tr

1 Values expressed as percentage of total methyl esters. All values are mean of duplicate analysis.

2 Carbon number: number of double bonds.

3 Assigned to any value less than 0.5%.

nitrogen and stored at -10 C until required for fatty acid analysis.

For fatty acid analysis, the lipid extract was transesterified directly using the reagent (15% methanolic sodium methoxide, 60% petroleum ether, 25% diethyl ether) according to a method adapted from Shehata et al (1970). Fatty acid methyl esters were analyzed with a Varian Aerograph gas chromatograph (Model 1740-10)[1] equipped with dual flame ionization detectors and a Hewlett-Packard reporting integrator (Model 3380-S)[2]. The instrument was fitted with dual 6 feet long X 0.125" O.D. (medium wall thickness) stainless steel columns packed with 10% EGSS-Y on 100/120 mesh GAS CHROM Q[3]. Gas flow rates were 25, 30 and 250 ml/min for hydrogen, helium and air, respectively. The columns were operated isothermically at 190 C with injector and detector temperatures of 230 C and 250 C, respectively. Chromatographs were identified with respect to individual fatty acids by comparison of retention times to calibrated fatty acid

1 Varian Aerograph, Malton, Ontario.

2 Hewlett-Packard Ltd., Canada.

3 Applied Science Laboratories Inc., State College, Pennsylvania 16801.

reference standards.[1]

Sterol analysis of the lyophilized food composites was performed by the official method of the AOAC (1980). A Perkin-Elmer gas chromatograph (Model 3920B)[2] equipped with dual flame ionization detectors and a Hewlett-Packard reporting integrator (Model 3380-S)[3] was used. The trimethylsilyl sterols were resolved on a 6'x 4 mm id column packed with 1% SE-30 on 100/120 mesh GAS CHROM Q[4]. Gas flow rates were 20, 30 and 550 ml/min for hydrogen, helium and air, respectively. The column was operated at 260 C with injector and detector temperatures of 250 C and 260 C, respectively. Chromatographs were identified with respect to cholesterol and the three plant sterols, campesterol, stigmasterol and β-sitosterol by comparison of retention times to calibrated reference standards.[4] Sterol concentrations were calculated by comparison of the sterol peak areas with the peak area obtained for a known

1 Hormel Institute, Lipids Preparation Laboratory, Austin Minnesota.

2 Perkin-Elmer (Canada) Ltd., Downsview, Ontario.

3 Hewlett-Packard Ltd., Canada

4 Applied Science Laboratories Inc., State College, Pennsylvania 16801.

quantity of 5α-cholestane[1] used as an internal standard.

Total nitrogen content of the diets was determined using a boric acid modification of the Kjeldahl method (AACC, 1962). Titanium dioxide was used as the catalyst (Williams, 1973) instead of mercuric oxide and potassium sulphate recommended in the method. A factor of 6.25 was used to calculate the percentage protein present (AOAC, 1980).

Amino acid analyses of the diets were performed on a Beckman Automatic Amino Acid Analyzer (Model 121)[2] following the method of Spackman et al (1958). Protein hydrolysates were obtained by acid hydrolysis (6N hydrochloric acid) according to the method of Orth et al (1974). The percentage protein was calculated by applying a factor of 6.25 (AOAC, 1980).

IV. Blood Analysis

Fasting venous blood samples were taken by a medical technician on day 1 of the study and at 7 day intervals

1 Picker Nuclear Ltd., Winnipeg, Manitoba.

2 Beckman Instruments, Inc., Palo Alto, California.

throughout the 42-day study. Blood was drawn from each subject into two 15 ml BD vacutainer tubes (#4796)[1] at each collection period for serum analysis. This blood was allowed to clot for one hour at room temperature and then centrifuged (International Centrifuge, Model CS)[2] at 2000 rpm for approximately five minutes. Centrifuging was repeated if necessary to precipitate the clot. Sera was pipetted into two respective screw-top vials, flushed with nitrogen and stored at -10 C until required for analysis. On day 1, 22 and 43, an additional 5 ml of blood was drawn into a 7 ml BD vacutainer containing 10.5 mg powdered EDTA (#4735)[1] and transported on ice to the Metabolism and Endocrinology Laboratory, Health Science Centre, Winnipeg, for plasma low density lipoprotein (LDL) and plasma high density lipoprotein (HDL) cholesterol analysis. Plasma lipoproteins were fractionated and quantitated according to the method of Bronzert and Brewer (1977). Whole blood analysis was also performed at the Hematology Laboratory, Health Science Centre, Winnipeg, on the day 1, 22 and 43 blood samples

1 Canlab Laboratory Supplies, Winnipeg, Manitoba.

2 International Equipment Company, Boston, Massachusetts.

for all subjects.

Sera for each subject taken on day 1, 8, 15, 22, 29, 36 and 43 was analyzed for total cholesterol according to the method of Mann (1961). The Mann procedure incorporates the extraction and saponification procedure of Abell et al (1952) and the color reaction developed by Zlatkis et al (1953). Cholesterol was assayed colorimetrically by forming a color complex with iron-sulfuric acid in the presence of glacial acetic acid, followed by measurement of the optical density at 560 nm in a Coleman Junior Spectrophotometer (Model 6A)[1]. Comparison of the mean absorbance for quadriplicate analyses with a standard curve for cholesterol[2] derived the absolute amount of cholesterol in the serum.

Serum triglyceride analysis on day 1, 8, 22, 29 and 43 for each subject was performed using an enzymatic triglyceride kit[3]. The method involved specific lipase hydrolysis of

1 Instrument No. A-36715, Coleman Instruments, Inc., Maywood, Illinois, U.S.A.

2 Cholesterol reference standards from Fisher Scientific Co., Winnipeg, Manitoba.

3 Harleco TRI-ES 64983, Harleco, Gibbstown, N.J. 08027, U.S.A.

the triglycerides, followed by the oxidation of glycerol by NAD in the presence of glycerol dehydrogenase to form NADH and dihydroxyacetone. The NADH formed reacted with iodiphenylnitrophenyl tetrazolium chloride in the presence of diaphorase to form a colored complex for which the optical density was measured on a Pye Unicam Spectrophotometer (Model SP6-300)[1] at 505 nm. Serum triglyceride concentration of each sample was obtained by comparison of the mean absorbance for duplicate analyses to the absorbance of a known concentration of calibrator (standard).

1 Pye Unicam Ltd., Cambridge, England.

RESULTS

I. Serum Total Cholesterol

The type of protein in the diet did not have a significant ($P < 0.05$) effect on serum cholesterol levels as determined by analyses of variance. Individual cholesterol values for each subject at the beginning of the study and at weekly intervals throughout the 42-day metabolic trial, in addition to mean values for each subject for each diet treatment are reported in Table 5. Figure 2 further illustrates the serum cholesterol levels of the subjects throughout the study. When the data was compared for each diet treatment group, no significant ($P < 0.05$) differences in serum cholesterol between the plant and animal protein diet periods were found at the end of one, two or three weeks of each dietary treatment or for the mean serum cholesterol values for each dietary period (Appendix Tables 2, 3, 4 and 5, respectively). The overall mean serum cholesterol value for all subjects for the PPD period was 154 ± 6^{1} mg/dl compared to 156 ± 7^{1} mg/dl for the APD

[1] Standard Error.

Table 5

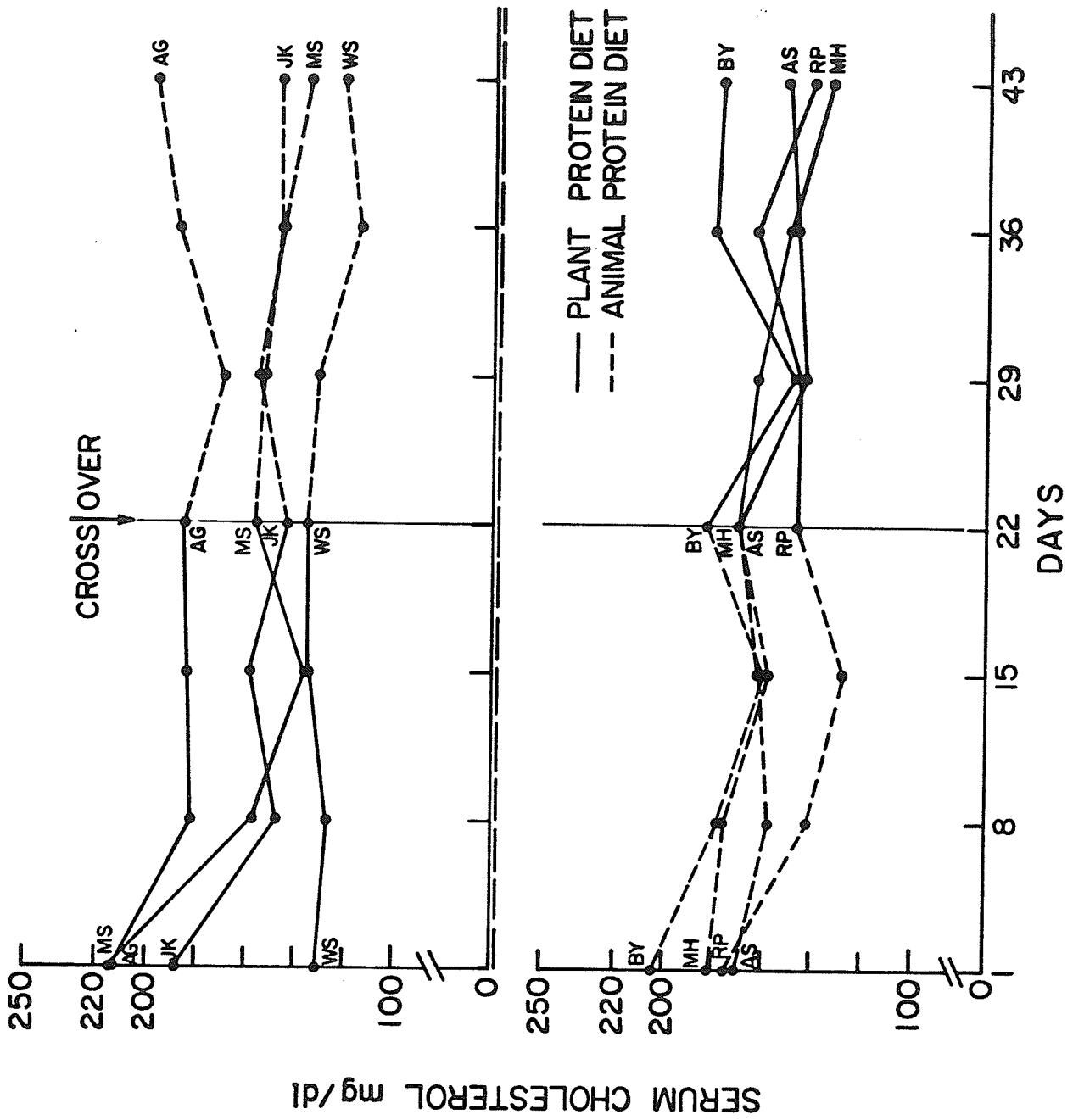
Serum Total Cholesterol of Subjects During Plant and Animal Protein Dietary Periods[1]

Subject	Initial	Plant Protein Diet				Animal Protein Diet			
		Day 8	Day 15	Day 22	Mean[2]	Day 8	Day 15	Day 22	Mean
AG	213	182	184	185	184 ± 1	170	188	198	185 ± 8
JK	178	148	159	144	150 ± 4	155	146	147	149 ± 3
WS	131	127	135	136	133 ± 3	131	115	121	122 ± 5
MS	214	158	136	156	150 ± 7	153	146	135	145 ± 5
MH	182	163	150	134	149 ± 8	176	158	171	168 ± 5
BY	205	148	181	178	169 ± 11	178	159	183	173 ± 7
AS	171	144	148	152	148 ± 2	158	162	170	163 ± 4
RP	174	145	164	141	150 ± 7	142	128	147	139 ± 6

[1] Subjects AG, JK, WS and MS were on the plant protein diet and subjects MH, BY, AS and RP were on the animal protein diet for the first 21-day period; the diets were reversed for the second 21-day period.
Values are the mean of quadriplicate analysis expressed in mg/dl serum.

[2] Mean cholesterol for period ± standard error.

FIG. 2. SERUM CHOLESTEROL LEVELS OF THE SUBJECTS THROUGHOUT THE STUDY



period. In addition, there was not a significant ($P < 0.05$) difference in serum cholesterol related to the two different orders of presentation of the diet treatments for all the statistical comparisons (Figure 3). However, a significant ($P < 0.05$) decrease was found in the mean serum cholesterol levels for both the PPD and the APD of the first 21-day period compared to initial values (Appendix Table 6). And, the two diet treatments did not significantly ($P < 0.05$) differ in their effects.

II. Plasma Low Density Lipoprotein Cholesterol

Consistent with the results for serum total cholesterol, the type of protein in the diet did not significantly ($P < 0.05$) affect the plasma low density lipoprotein (LDL) cholesterol as determined by analysis of variance (Appendix Table 7). Table 6 shows the LDL cholesterol values for each subject at the beginning of the study and at the end of three weeks (day 22) for both the PPD and the APD, together with the mean LDL cholesterol values for the eight subjects. It can be noted that the change in individual subject response from one dietary period to the next was inconsistent and variable. Hence, no significant ($P < 0.05$) overall

FIG. 3. MEAN SERUM CHOLESTEROL LEVELS OF THE SUBJECTS THROUGHOUT THE STUDY

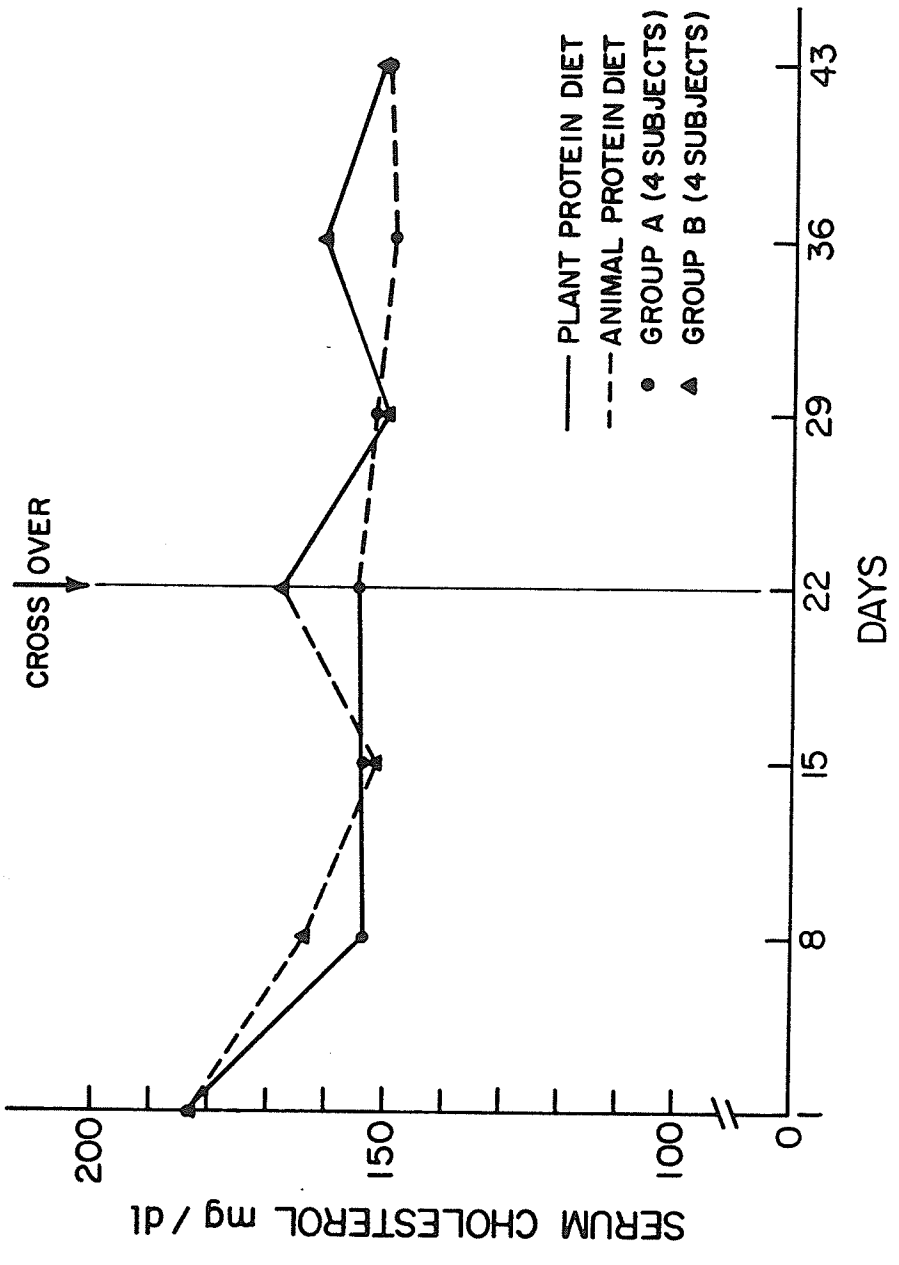


Table 6

Plasma Lipoprotein Cholesterol of Subjects at the End of Each Dietary Period[1]

Subject	LDL Cholesterol[2] Initial	22P[3]	22A[3]	HDL Cholesterol[2] Initial	22P	22A
AG	133	98	137	57	47	56
JK	71	82	73	62	43	50
WS	36	45	31	76	45	44
MS	103	83	65	41	44	35
MH	138	78	102	52	34	43
BY	107	105	90	47	44	52
AS	84	78	88	48	42	58
RP	100	87	70	44	36	43
Mean ± SE	96 ± 12	82 ± 6	82 ± 11	53 ± 4	42 ± 2	48 ± 3

1 Subjects AG, JK, WS and MS were on the plant protein diet and subjects MH, BY, AS and RP were on the animal protein diet for the first 21-day period; the diets were reversed for the second 21-day period. Values expressed in mg/dl plasma.

2 LDL = Low-density lipoprotein cholesterol. HDL = High-density lipoprotein cholesterol.

3 22 P/A = End of plant/animal dietary period (day 22 value).

difference in LDL cholesterol between the PPD and the APD periods was noted at the end of three weeks of each dietary treatment. The mean plasma LDL cholesterol value for all subjects fed the PPD was 82 ± 6[1] mg/dl compared to 82 ± 11[1] mg/dl for the APD. Moreover, the order of feeding did not affect the plasma LDL cholesterol ($P < 0.05$). Unlike the results obtained for serum total cholesterol, there was not a significant ($P < 0.05$) decrease in LDL cholesterol levels from initial values to day 22 when subjects AG, JK, WS and MS received the PPD and subjects MH, BY, AS and RP received the APD. Analysis of variance appears in Appendix Table 8. When the change in mean serum cholesterol between both diet treatments was compared to the change in plasma LDL cholesterol, the direction of change was similar for all subjects with the exception of BY, but the magnitude was highly variable. Thus, together with the small sample size ($n = 8$), linear regression analysis was not appropriate.[2]

1 Standard Error.

2 Personal communication. Dr. J. Brewster, Department of Statistics, University of Manitoba, Winnipeg, Manitoba.

III. Plasma High Density Lipoprotein Cholesterol

The type of protein in the diet had a significant ($P < 0.05$) effect on plasma high density lipoprotein (HDL) cholesterol as determined by analysis of variance (Appendix Table 9). Table 6 shows the HDL cholesterol values for each subject at the beginning of the study and at day 22 for both diets, together with the mean HDL cholesterol values for the eight subjects. The APD period was associated with significantly ($P < 0.05$) higher plasma HDL cholesterol than the PPD when the mean difference between diet treatments for all subjects was compared at the end of three weeks. All subjects, with the exception of WS and MS, showed higher levels of HDL cholesterol for the APD period. The mean plasma HDL cholesterol value for all subjects fed the APD was 48 ± 3^1 mg/dl compared to 42 ± 2^1 mg/dl for the PPD. In spite of the larger mean difference between diet treatments for those subjects fed the APD in the first period, the order of diet presentation did not have a significant ($P < 0.05$) effect. Moreover, because of the highly variable nature of the response, the mean difference in the change

[1] Standard Error.

of plasma HDL cholesterol during the first dietary period from initial values was not significant ($P < 0.05$) (Appendix Table 10). Also, there was not a significant ($P < 0.05$) difference between the APD and the PPD treatments in the response shown during this period relative to the baseline values.

IV. Serum Triglycerides

Individual and mean serum triglyceride values for each subject throughout the 42-day metabolic trial are reported in Table 7. The initial, day 8 and day 22 serum triglyceride values in each diet period are presented. The PPD treatment resulted in significantly ($P < 0.05$) higher mean serum triglycerides when the first week (day 8) values for each dietary period were compared (Appendix Table 11). The mean serum triglyceride value for the eight subjects at the end of the first week for the PPD treatment was 136 ± 19^{1} mg/dl compared to 84 ± 12^{1} mg/dl for the APD treatment. The mean differences between diet treatments for the two orders of presentation were not significantly ($P < 0.05$) different.

[1] Standard Error.

Table 7

Serum Triglycerides of Subjects During Plant and Animal Protein Dietary Periods[1]

Subject	Initial	Plant Protein Diet			Animal Protein Diet		
		Day 8	Day 22	Mean[2]	Day 8	Day 22	Mean[2]
AG	145	184	165	174 ± 10	75	137	106 ± 31
JK	64	68	68	68 ± 0	62	52	57 ± 5
WS	139	192	196	194 ± 2	127	124	126 ± 2
MS	199	156	142	149 ± 7	147	166	156 ± 10
MH	118	146	107	126 ± 20	72	91	82 ± 10
BY	116	186	134	160 ± 26	67	77	72 ± 5
AS	69	76	78	77 ± 1	60	55	58 ± 2
RP	63	76	54	65 ± 11	62	54	58 ± 4

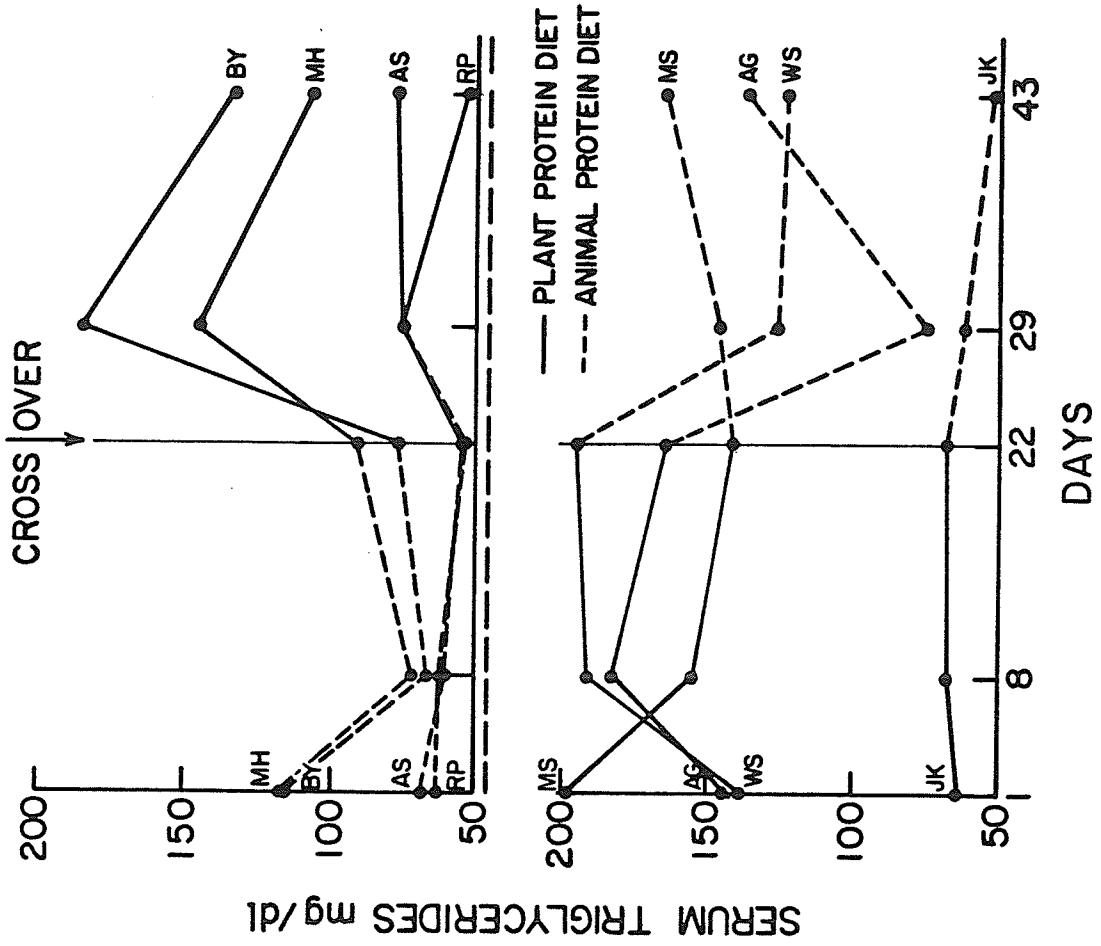
1 Subjects AG, JK, WS and MS were on the plant protein diet and subjects MH, BY, AS and RP were on the animal protein diet for the first 21-day period; the diets were reversed for the second 21-day period.
Values are the mean of quadriplicate analysis expressed in mg/dl serum.

2 Mean triglyceride for period ± standard error.

The PPD treatment was associated with higher levels of serum triglycerides regardless of whether it was fed in the first or second dietary period. Further comparison of the serum triglyceride values at the end of three weeks for the two diets showed that the PPD treatment was no longer associated with significantly ($P < 0.05$) higher serum triglyceride levels (Appendix Table 12). Again, the result was not significantly ($P < 0.05$) different for the two orders of presentation of the treatments. The mean serum triglyceride value for the eight subjects at day 22 for the PPD was only slightly higher (118 ± 18[1] mg/dl) than for the APD (95 ± 15[1] mg/dl). Figure 4 illustrates the individual subject response for serum triglycerides throughout the study. It can be noted that the response for all subjects, with the exception of MS, was similar. Subjects MH, BY, AS and RP demonstrated an initial fall in serum triglycerides on day 8 of the APD period compared to initial values, followed by relatively stable levels for the remainder of the period. The initial fall in serum triglycerides was not, however, statistically significant ($P < 0.05$) according

[1] Standard Error.

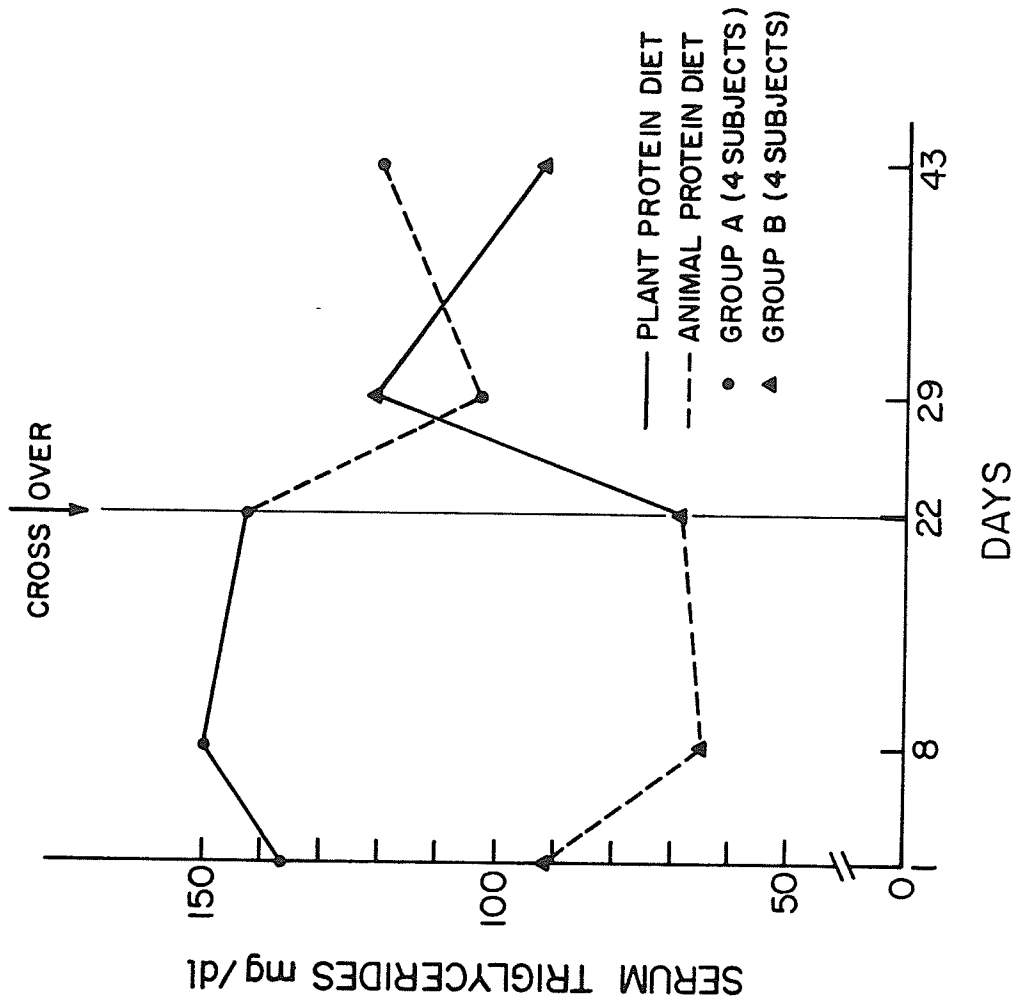
FIG. 4. SERUM TRIGLYCERIDE LEVELS OF THE SUBJECTS THROUGHOUT THE STUDY



to a paired-difference test. All four subjects proceeded to show an initial peak in the triglyceride response at the end of one week of the PPD period. However, triglyceride values returned to values approaching the initial values by the end of three weeks of the PPD for subjects BY, MH and RP. Subject AS appeared to reach a stable plateau by the end of the PPD period. Subjects AG, JK and WS were fed the diets in the opposite order and showed a similar response. That is, an initial rise in serum triglyerides occurred on day 8 of the PPD treatment compared to initial values, again followed by relatively stable levels for the remainder of the period. A paired-difference test revealed that the initial rise was not significant ($P < 0.05$) due to the variability in response, attributed largely by MS. For the APD period, subjects AG, JK and WS showed an initial fall in serum triglycerides which paralleled the peak response in the PPD period. And, at the end of the period, the triglyceride response of the subjects was more variable. Figure 5 illustrates the overall similar patterns of serum triglyceride response for the two groups of subjects, consistent with the different orders of diet presentation.

In an attempt to explain the individual variability in

FIG. 5. MEAN SERUM TRIGLYCERIDE LEVELS OF THE SUBJECTS THROUGHOUT THE STUDY



response, the initial triglyceride values prior to each given change in triglyceride response over the first week of each dietary period were compared to the magnitude of the specific change for each subject. Although linear regression analysis was not appropriate due to the limited number of subjects and the inconsistency of data, trends were apparent.[1] That is, subjects JK, AS and RP who had lower initial serum triglyceride levels of less than 100 mg/dl showed correspondingly smaller responses to diet treatment. However, the magnitude of the changes did not correlate directly with the magnitude of the corresponding initial values.

V. Subjects

All eight subjects completed the 42-day study and maintained good health. Body weights were relatively stable throughout the study. A mean increase of 0.3 kg and a decrease of 0.4 kg in body weight for all subjects occurred for the PPD and APD periods, respectively. For five of

[1] Personal communication. Dr. J. Brewster, Department of Statistics, University of Manitoba, Winnipeg, Manitoba.

the subjects, mean fluctuations in body weight were less than 1 kg in each dietary period (Table 1). However, subjects AG and WS showed a mean weight loss of 1.3 kg and 1.1 kg, respectively, during the APD. And, subject BY showed a mean weight gain of 1.2 kg during the PPD.

One subject (MH) required major increases in total energy intake during both dietary periods in order to maintain his weight. Appendix Table 13 illustrates the higher mean daily intake of energy, protein and fat for MH relative to the other subjects. The higher levels of nutrients were consistent with his active lifestyle.

D I S C U S S I O N

I. Serum Total Cholesterol

The hypocholesterolemic effect of plant protein diets relative to animal protein diets reported by Carroll et al (1978) and Walker et al (1960) for normolipidemic women and Sirtori et al (1979) and Hodges et al (1967) for hypercholesterolemic individuals was not confirmed in the present study. Carroll and co-workers reported that a small decrease of 5% (9 mg/dl) ($P < 0.05$) in plasma cholesterol for the plant protein diet was compatible with the larger changes reported by Sirtori and co-workers since hypercholesterolemic individuals may show a greater response to changes in dietary protein. The difference in results may be related to different components of the experimental design used by these investigators relative to the study reported here. That is, differences in diet, subjects and design of the dietary trials were observed between the studies and may explain why the present study does not show an effect of dietary protein on serum cholesterol.

Plant protein diets which utilize soy protein as the predominant source of plant protein (Carroll et al, 1978;

Hodges et al, 1967; Sirtori et al, 1979) may have a unique hypocholesterolemic effect which is not characteristic of other plant protein sources. The amount of soy protein used in the present study was minimal; 1.6% (1.5g) of the total protein for the APD and 3.6% (3.0g) for the PPD. Since isolated and textured soy proteins for human consumption have been exposed to a wide range of treatment conditions (Carroll et al, 1979) there is the potential for variability in the nutrient composition of processed soy products and in the biological availability of nutrients. Several investigators have attempted to explain the hypocholesterolemic effect of soy protein on the basis of amino acid composition and balance. Although soy is limiting in methionine, an essential amino acid for man, Gatti and Sirtori (1977) showed that the addition of methionine did not appear to reverse the hypocholesterolemic effect of soybean protein. A comparison of the PPD used in the present study to soy protein as illustrated in Table 8 does not show marked differences in the amino acid composition. The largest differences are the lower amounts of glutamic acid (-3.4g/100g) and proline (-2.0g/100g) and higher aspartic acid (+1.8g/100g) for soy protein relative

to the PPD. It is not known, however, whether these differences would have an effect on serum cholesterol. The low ratio of lysine to arginine (L:A) in soy protein was suggested by Kritchevsky et al (1978) to reduce serum cholesterol. A comparison of soy protein to the PPD used in this study (Table 8) showed that the PPD had a lower L:A ratio, 0.50 compared to 0.78 for soy protein. The APD had a ratio almost twice that of the PPD (0.90) and was associated with a similar cholesterolemic response in this study. Consequently, the L:A ratio for dietary proteins fed in this study cannot be considered to be the cholesterolemic factor. Factors in soy protein other than the balance of amino acids were thought to be related to the hypocholesterolemic effect of soy protein (Huff et al, 1977a). These investigators reported that an amino acid mixture which corresponded to soy protein induced higher levels of plasma cholesterol in rabbits than either intact soy protein or an enzymatic digest of soy protein. Helms (1977) suggested that the effect of textured soybean protein on blood lipids was ascribed to the specific carbohydrate fraction of the products rather than the plant protein per se. The sterol component of soybean has further been implicated

Table 8

Comparison of the Amino Acid Composition of the Plant Protein Diet and Soy Protein[1]

Amino Acid	Plant Protein Diet g/100g[2]	Soy Protein g/100g[2]
Alanine	4.2	3.9
Arginine	8.1	7.7
Aspartic Acid	9.9	11.7
Cystine	ND[3]	1.2
Glutamic Acid	23.6	20.2
Glycine	4.5	4.0
Histidine	2.3	2.5
Isoleucine	3.7	4.8
Leucine	7.1	7.6
Lysine	4.0	6.0
Methionine	1.3	1.1
Phenylalanine	4.9	5.3
Proline	7.2	5.2
Serine	4.9	5.4
Threonine	3.5	3.7
Tryptophan	ND	1.4
Tyrosine	3.0	3.7
Valine	4.5	4.7

1 Figures for soy protein are based on data for soy protein isolate reported by Huff et al (1977a).

2 Values are expressed in grams of amino acid per 100 grams protein.

3 ND = No analytical data available.

by Peterson (1951) who found that the simultaneous addition of soybean sterols and cholesterol to a diet fed to chicks did not produce the elevation in plasma and liver levels of cholesterol which was obtained when cholesterol was added alone or in vegetable oil. In summary, although the hypocholesterolemic factor of soy protein has not been specifically identified, the data from the present study suggest that this unknown factor is either not present in mixed plant proteins characteristic of the PPD used here or if so, it must also have been a constituent of the APD since similar cholesterolemic responses were noted for both diet treatments.

It is possible that the availability of amino acids in naturally constituted proteins may result in cholesterolemic responses which differ when compared one to another or to purified protein sources. Hence, the extrapolation of results obtained for purified proteins such as albumin and wheat gluten to plant and animal proteins in general as reported by Campbell et al (1965) and Anderson et al (1971) may not be warranted. Nevertheless, the results of the present study which has utilized diets composed of natural plant and animal protein sources are in concurrence with the data

reported by these investigators for purified protein sources. Anderson and co-workers reported that wheat gluten and albumin were not significantly ($P \ll 0.2$) different in their effects on serum cholesterol. Contrary to the results of the present study, Walker et al (1960) found that the serum cholesterol levels of normolipidemic subjects who received a mixed plant protein diet were significantly ($P \ll 0.05$) lower at the end of two weeks when compared to a mixed animal protein diet. Minimal amounts of soy protein were included in the diets. The difference in results could be related to the use of different combinations of animal protein sources by Walker et al. Dairy products, veal, turkey and cod were consumed as compared to beef protein used as the only animal protein source in the present study. Different types of animal proteins or plant proteins may be associated with a variety of cholesterolemic effects. Although this phenomenon has not been tested in man, a report by Hermus (1975) supports this theory. Rabbits fed a semisynthetic diet with 20% casein showed serum cholesterol levels of 514 mg/dl but

when a mixture of casein, gelatin and fish protein was substituted for the casein, serum cholesterol decreased to 120 mg/dl. Based on this observation in animals, it therefore follows that the hypocholesterolemic effects for plant protein observed by Carroll et al (1978) might be expected since the animal protein diet contained mixed sources of animal protein (beef, ham and chicken) compared to the present study in which beef was the sole source of animal protein. Perhaps the effects for protein on serum cholesterol are due to differences in amino acid composition or bio-availability of the amino acids which are not necessarily specific to the origin of the protein(s), but which may vary from one animal protein(s) to another. Likewise, the same explanation is possible for proteins of plant origin. Therefore, perhaps the more accurate description of the effect of dietary protein on serum cholesterol is in terms of individual proteins and their source as opposed to the origin of the proteins, namely plant or animal. The cholesterolemic response demonstrated for dietary protein may vary depending on whether the amino acids, the purified protein, or a combination of natural protein sources forms the dietary protein source. Moreover, when describing the

cholesterolemic effect of a given animal protein(s) by comparison to a given plant protein(s), it is essential that the effect be assumed to be related only to the plant protein(s) specified because quite a different effect might result for another plant protein(s) of different composition. For example, in the present study, the two diets showed similar amino acid compositions (Table 3) which may explain the similar cholesterolemic responses observed for the two diet treatments. However, for different combinations of proteins, variations in the composition may occur which induce different patterns of serum cholesterol response.

It is unlikely that the differences in the amount of protein in the diets designed by Walker et al (1960) and Carroll et al (1978) were a factor to be considered when results are compared with this study. Constant levels of protein for both experimental diets were maintained for all these investigations; 8%, 15.5% and 11% of total kilocalories, respectively. All three experimental regimens were adequate in protein according to recommended allowances. A number of reports in the literature have indicated that moderate changes in the protein content of the diet do not produce any significant change in the lipid

levels. Lutz et al (1959) reported that dietary protein levels of 8.5, 15 and 23% of total kilocalories maintained similar lipid levels in young male adults.

There is considerable documentation to show that the fatty acid composition of the diet has an effect on serum cholesterol (Reiser, 1973). The fatty acid composition of both diets in this study was similar (Table 4). The diets designed by Carroll et al (1978) contained similar amounts of fatty acids as fed to the subjects described here in spite of the fact that different fats were used. Carroll and co-workers used textured soy protein meat analogues specially prepared to contain the fats of the corresponding meats (beef, ham and chicken). Walker et al (1960) utilized similar fats for both experimental diets but the fatty acid composition was not reported. In both diets, vegetable fat in the form of hydrogenated soy oil shortening, hydrogenated soybean and cottonseed oil margarine and corn oil (30% of total fat) was the predominant source of fat. The type of fat in the semisynthetic diet fed to rabbits has been shown to mediate the cholesterolemic effect of casein (Carroll and Hamilton, 1975) and therefore, the fatty acid composition of the diets could have influenced serum cholesterol either as an additive effect with protein or as a dominant effect of the

fatty acids. Animal proteins contain fatty acids peculiar to that protein. In contrast to other reports, the study described here attempted to minimize the effects of fatty acids associated with animal protein on serum lipids by using only beef protein. The same amount of tallow was added to the PPD so that both diets were constant with respect to the animal fat associated with the beef.

The total amount of fat in the diet may also influence serum cholesterol. When compared to the diets used by Carroll et al (1978) and Walker et al (1960), subjects in this study received similar amounts of fat. The amount of fat in the experimental diets used by Carroll et al and Walker et al was 33.5% and 36% of the total kilocalories, respectively. Although the absolute amount of fat for both the APD and PPD in the present study were similar (Table 2), there was 4% less fat calories in the PPD. It is unlikely that this difference had a significant influence on serum cholesterol based on the report by Iacono et al (1975).

Although the amount of dietary cholesterol was higher for the APD than the PPD by 118mg according to analysis (Table 2), it is unlikely that this amount of cholesterol had an effect on the results obtained in this study. Based

on the prediction equation derived by Keys and his associates (1965) in which a change in serum cholesterol (mg/dl serum) = $1.5\ (Z_2 - Z_1)^1$, it is predicted that an increase in serum cholesterol of 5 mg/dl occurred for the APD. Thus, it follows that the mean serum cholesterol associated with the APD would have been reduced from 156 mg/dl to 151 mg/dl. This value is not significantly different from the mean cholesterol value of 154 mg/dl observed for the subjects fed the PPD. Moreover, the plant sterols (β-sitosterol, campesterol and stigmasterol) were very similar in total quantity for both the APD and PPD treatments and therefore, cannot be implicated as a confounding factor in the interpretation of the results for the two protein diets (Table 2). A specific hypocholesterolemic effect of plant sterols has been confirmed in rabbits and in man (Pollack, 1953; Shipley, 1955). It is possible that the plant sterols were involved in the overall depression of serum cholesterol in all subjects from pre-study values. Beveridge et al (1964) reported that a molar ratio of dietary sitosterol to cholesterol of at least 2:1 is required before a significant hypocholesterolemic effect can be detected.

[1] Z_1 and Z_2 represent the square root of the milligrams of cholesterol per 1,000 kilocalories of each of two diets.

The molar ratios calculated for the APD and the PPD were 1.0 and 3.9, respectively. The higher ratio for the PPD raises the question of whether the PPD would have been associated with a significantly greater cholesterolemic effect than the APD had the ratios been similar. Consistent with the sterol data for the present study, the diets investigated by Carroll et al (1978) were similar in dietary cholesterol and plant sterol composition. Also, the cholesterol content of the diets were low compared to the reported mean intake of 450 mg for the American population (Hegsted, 1979); 168 mg and 161 mg for the animal protein diet and the plant protein diet, respectively. In order to maintain the cholesterol contents constant for both diets in Carroll's study, a daily supplement of crystalline cholesterol (125 mg/day), dissolved in olive oil (6 ml), was added to the plant protein diet. The total plant sterol content for their diets was lower; a mean of 145 mg compared to 222 mg in the present study (Table 2). In summary, it is unlikely that dietary sterols played a significant role in influencing the effects on serum cholesterol for dietary protein in this study.

The specific composition of the carbohydrate fraction

of the diets in the present study is not known and consequently, an effect cannot be attributed to the different fiber constituents of the diets. Nevertheless, it may be speculated that the higher quantity of semi-digestible and non-digestible carbohydrate associated with the PPD may have served to lower the serum cholesterol levels relative to the APD. The PPD contained 142 grams (9.9% of total kilocalories) of carbohydrate in excess of the APD which was derived mainly from complex carbohydrate sources. There is evidence to show that the absolute level of carbohydrate *per se* has little effect on serum cholesterol (Grande, 1967; Hodges *et al*, 1967) compared to the effect of specific types of carbohydrate. Kay and Strasberg (1978) reviewed the specific lipidemic effects characteristic of different fiber components and reported that pectin generally reduces serum lipid levels, whereas a review by Truswell and Kay (1976) showed that wheat bran had little effect on plasma cholesterol levels. It was only possible to control the amounts of wheat bran for both diets in the present study. Grande *et al* (1965) reported an interesting hypocholesterolemic effect for mixed legumes in the human diet. At a level of 21% of total energy intake, a decrease in cholesterol of

18.5 mg/dl was noted. The legume content of the PPD in the present study accounted for 7% of the total kilocalories. Therefore, based on Grande's observation, it can be predicted that the PPD had the potential to reduce serum cholesterol by 6 mg/dl due to the legumes which were not contained in the APD. However, this decrease would not have significantly altered the results of the study. The total carbohydrate content of the APD (52% of total kilocalories) compared very well with the levels for both diets formulated by Carroll et al (1978), 51%, while the PPD contained 60%. It should be noted that these investigators used soy protein in the form of textured soy protein meat analogues as the predominant source of plant protein compared to mixed plant sources in the present study and consequently, the carbohydrate content for the two diets was constant. Plant protein derived from mixed food sources naturally occurs in association with carbohydrate and other nutrients, whereas protein isolates and animal proteins are more readily incorporated into the diet as protein sources per se. Carroll and co-workers did not report the actual composition of carbohydrate for the experimental diets which has been suggested to be the more important parameter

when measuring serum cholesterol status. A comparison of the specific effect of carbohydrate on the serum cholesterol observed for the subjects in this study is not in order.

It has been suggested that males and females may exhibit different blood cholesterol responses to dietary protein. Although the use of female subjects by Carroll et al (1978) and Walker et al (1960) may have accounted for some of the cholesterolemic effects found for animal versus plant proteins, it would seem more probable that some other element of the total experimental design influenced the results. However, until confirmed experimentally, it is not known whether sex differentiation is a significant factor for young adults.

The crossover experimental design used in the present study was also used by Carroll et al (1978) and consequently, the design per se cannot be used to explain the differences in results for the effect of protein on serum cholesterol. However, it may have accounted for the different results reported by Walker et al (1960) when compared to the present study for mixed protein diets. Walker and co-workers utilized a completely randomized design in which different groups of subjects (n=6) were fed the two experimental

diets. It is unlikely that the sample size was large enough to overcome the subject to subject variability inherent in this design. For the crossover design, the subject to subject variability is eliminated because each subject serves as his own control. Hence, greater reliability and validity are inherent in this type of design and subsequently in the results of the present research.

The fact that several investigators have reported that two to three weeks is adequate time for serum cholesterol levels to be reflective of a given change in diet lends further support to the validity of the results for the study reported here (National Diet Heart Study Group, 1968; Hegsted et al, 1965; Grande et al, 1965; Erickson et al, 1964; Keys, 1967). Also, previous investigations related to the effect of dietary protein on human serum cholesterol levels support the efficacy of a three week study period (Walker et al, 1960; Sirtori et al, 1977; Carroll et al, 1978). Most subjects showed a tendency for the serum cholesterol to plateau by the end of three weeks of either dietary regimen (Figure 2). However, subject AG showed a steady increase in serum cholesterol from 170 mg/dl to 198 mg/dl during the APD treatment and subject MH showed

the opposite trend (163 to 134 mg/dl) for the PPD. The data for these subjects appears to support the report by Mattson and co-workers (1972) that a slower rate of stabilization of up to four weeks for serum cholesterol concentration in response to a change in diet may occur for some individuals.

Several dietary factors may be implicated in the overall reduction ($P < 0.05$) of serum cholesterol found for both diet treatment groups during the first dietary period since the composition of the subjects' prior self-selected diets was not known. Although Carroll et al (1978) failed to show a significant decrease in plasma cholesterol during the first period, the results are in agreement with Walker et al (1960) who suggested that the reduction in serum cholesterol was due, in part, to the fact that a large percentage of fat in the experimental diets was of vegetable origin. In the present study, it is very likely that most subjects experienced a marked reduction in dietary cholsterol intake and saturated fatty acids. The daily cholesterol intakes for the APD and the PPD were 184 mg and 66 mg, respectively, compared to an average of 450 mg per day consumed by the American

population (Hegsted, 1979). However, whether or not the predicted lower cholesterol intake, reduced saturated fatty acid intake, or some other dietary factor(s) known to effect serum cholesterol levels were responsible for the observed effect cannot be ascertained. It can merely be suggested that both the APD and the PPD appeared to be associated with a more favorable response for serum cholesterol than the diets normally consumed by the subjects prior to the study; provided that the initial cholesterol values are truly reflective of the pre-study diets.

To conclude, the response of serum cholesterol is clearly dependent on several dietary variables. Although this study has attempted to isolate the effect of the dietary protein component on serum cholesterol, the utilization of mixed plant protein sources naturally altered the carbohydrate composition of the plant protein diet relative to the animal protein diet. Unfortunately, it was not possible to determine the specific effect of carbohydrate on the serum cholesterol results obtained in this study. Nevertheless, this study does not support a hypercholesterolemic effect for animal protein in normocholesterolemic subjects as has been previously reported. It appears that the effect of dietary protein on

serum cholesterol requires further investigation under more varied, but controlled experimental conditions in order to isolate the specific effect of different types of protein and to identify how other dietary components may interact to influence the effect obtained for dietary protein.

II. Plasma Low Density Lipoprotein Cholesterol

Results from biochemical and epidemiological studies have indicated that simple measurement of the total concentration of cholesterol in blood may not be an adequate indicator of the serum cholesterol risk factor for atherosclerosis. Nevertheless, previous investigations related to the role of dietary protein on blood lipid metabolism and atherosclerotic disease have chiefly monitored total serum cholesterol. Recently, it has been suggested that LDL are involved in both the development of atherosclerotic lesions and in the regulation of cholesterol metabolism. These lipoproteins transport cholesterol to the peripheral tissues of the body and may facilitate the accumulative uptake of cholesterol by atherosclerotic plaques (Marx and Kolata, 1978). Therefore, the failure of the present study to identify a significant ($P < 0.05$)

difference in the LDL cholesterol concentration associated with the PPD and the APD enhances the theory that the type of protein in the diet does not play an important role in the development of atherosclerotic disease. Unfortunately, the few studies in the literature which have monitored LDL cholesterol are not directly related to the present study and hence, a direct comparison of the results cannot be made. For example, Sirtori et al (1977) found that the total cholesterol and LDL cholesterol were significantly ($P < 0.001$) reduced in hypercholesterolemic patients fed a soy protein diet and, the pattern of response for both lipid fractions was similar. The present study does not facilitate a good comparison of the LDL and total cholesterol responses since only single third week (day 22) values for each of the PPD and APD periods were analyzed for plasma LDL cholesterol. Nevertheless, the highly variable nature of the responses observed upon comparison of any changes in mean serum total cholesterol and plasma LDL cholesterol values which occurred between dietary periods does not support a relationship between these two blood cholesterol parameters. The failure to establish such a relationship is of little consequence since both serum

total cholesterol and plasma LDL cholesterol were not significantly ($P < 0.05$) different for the different dietary protein periods.

It can be concluded that under the experimental conditions used in the present study, the data for plasma LDL cholesterol are in agreement with the data for serum total cholesterol in that the ingestion of two diets differing in the type of protein did not appear to significantly alter the levels of these two blood lipid parameters. It should be noted, however, that a more extensive analysis of plasma LDL cholesterol throughout each dietary period is necessary to document the response of this blood lipid parameter to changes in the type of dietary protein.

III. Plasma High Density Lipoprotein Cholesterol

The metabolic implication of the higher ($P < 0.05$) mean plasma HDL cholesterol found for the APD (48 ± 3^{1} mg/dl) compared to the PPD (42 ± 2^{1} mg/dl) is difficult to interpret based on the, as yet, undetermined role of this lipoprotein in the regulation of lipid metabolism and atherosclerosis.

[1] Standard Error.

Although this lipoprotein appears to be involved in the removal of cholesterol from peripheral tissues, it can merely be postulated that the HDL is somehow involved in diminishing the likelihood of cholesterol from accumulating in atherosclerotic plaques (Marx and Kolata, 1978). Consistent with this postulated physiological function are epidemiologic data which suggest that high concentrations of HDL decrease the risk of heart attack. Moreover, in addition to the precise role of HDL, little is known about the dietary factors which affect the level of this fraction in plasma and its relation to other lipoprotein classes (Truswell, 1978). With regard to the effect of dietary protein on the HDL in human subjects, there is a paucity of data available. Sirtori et al (1979) found no changes in the HDL cholesterol levels of hypercholesterolemic subjects when animal protein was substituted with textured soybean protein. An indirect study of the effect of dietary protein on HDL was reported by Sacks et al (1975) for a group of vegetarians who consumed a diet containing a minimal amount of animal protein. They showed significantly ($P < 0.001$) lower HDL levels (43 ± 11[1]) than a group of controls (49 ± 12[1]) who

[1] Mean $\pm$ SD.

consumed the typical American diet. However, the proportion of the total plasma cholesterol carried by the HDL fraction was greater in the vegetarian (33 percent) than in the controls (27 percent). The results are in partial support of the present study in which significantly ($P < 0.05$) lower HDL levels were associated with the PPD treatment compared to the APD treatment. Unlike the data presented by Sacks and co-workers, it was found that the percentage of total cholesterol carried by the HDL fraction remained consistently lower for the PPD group (27 percent) compared to the APD group (31 percent).

Although the differences in HDL cholesterol may have been related to the dietary protein, there is experimental evidence which suggests that the higher carbohydrate content characteristic of the PPD in the present study and the vegetarian (macrobiotic) diet studied by Sacks et al, may have been responsible for the lower HDL levels found for these diets. Blum et al (1977) showed that increasing the dietary carbohydrate content from 40% to 80% resulted in a lowering of HDL cholesterol and HDL protein concentration in normolipidemic subjects. The kinetic basis of the effect was shown to be a marked increase (mean 52.5%) in the rate

of catabolism of ^{125}I-labelled HDL from the intravascular compartment. Similarly, Schonfeld et al (1976) and Wilson and Lees (1972) found that carbohydrate intakes of 80% and 90% total kilocalories, respectively, led to a fall in HDL cholesterol concentration. The effect of lower carbohydrate intakes resembling those investigated in the present study have not been documented. Nevertheless, the increase in carbohydrate content from 52% of the total kilocalories in the APD to 61% in the PPD may have been a factor associated with the significantly ($P < 0.05$) lower plasma HDL values associated with the PPD. The fact that Sirtori et al (1979) did not report any changes in HDL cholesterol when the carbohydrate composition was constant in diets which varied in the type of protein, enhances the possibility of a carbohydrate-induced phenomenon as responsible for the HDL changes observed.

Other dietary factors, namely fat, have been implicated in the regulation of HDL cholesterol. Evidence has been reported for the effect of increased P:S ratios on HDL cholesterol levels. Shepherd et al (1978) reported a siginificant drop in HDL cholesterol and plasma HDL-apo A-I concentration in four normal subjects when the P:S dietary

fat ratio was increased from 0.25 to 4.0. However, it is unlikely that dietary fat played a role in altering the plasma HDL cholesterol response observed in the present study since both the APD and PPD were similar in fatty acid composition (Table 4).

An inverse correlation has been noted between triglyceride and HDL levels, and the familial hypertriglyceridemias are associated with particularly low plasma levels of HDL (Frederickson and Levy, 1972). Moreover, Furman et al (1964) reported a direct relationship between plasma triglyceride levels and the terminal rate of decay of intravenously injected ^{131} I-HDL. It is of interest that the present study supports this relationship, in that, at the end of the first week of the PPD, the mean serum triglyceride levels were significantly ($P < 0.05$) higher than the corresponding levels of the APD. And, the higher initial serum triglycerides associated with the PPD were inversely correlated to the lower plasma HDL levels found at the end of three weeks for the PPD. However, this relationship did not persist upon comparison of the third week serum triglyceride values for the PPD. Therefore, the data from the present study do not appear to support a

long term relationship whereby elevation of the flux or concentration of triglycerides may be linked to an increased rate of catabolism of HDL from plasma, unless the HDL metabolic pathway is slow to adapt.

Although the plasma HDL data appears to support the hypothesis that dietary protein may be involved in the regulation of this lipoprotein, interpretation of the data is complicated by the difference in the type and amount of carbohydrate in the two diets which may have also influenced the response. Further investigation of the HDL cholesterol response under similar experimental conditions, but with constant carbohydrate composition, and over a longer period would facilitate a better understanding of the effect of the type of dietary protein *per se* on plasma HDL concentration.

IV. Serum Triglycerides

The initial and temporary elevation of the serum triglyceride concentration associated with the PPD treatment in the present study is analogous to a phenomenon described by Ahrens *et al* (1961) as carbohydrate-induced hypertriglyceridemia. The amount of carbohydrate producing

these effects in experimental studies was reported to be from 65 to 90% of the total kilocalories. The calculated carbohydrate content of the PPD in this study was 530g or 61% of the total kilocalories, compared to a lower level of 388g or 52% of the total kilocalories for the APD. The additional carbohydrate associated with the PPD was derived mainly from complex sources including whole wheat bread, vegetables and legumes. It should be noted that there is a likelihood that part of the labile hypertriglyceridemic effect associated with the PPD may have resulted from complex semidigestible or nondigestible carbohydrates including pectins, gums and perhaps hemicellulose and cellulose (Hodges et al, 1967) since the two experimental diets were controlled only with regard to wheat fiber. It should be emphasized that the serum triglyceride elevations associated with the PPD were temporary. Longer term studies have indicated a return to base-line levels following feedings containing high amounts of complex carbohydrate (Antonis and Bershon, 1961). Perhaps the most dependable evidence for the transitory nature of carbohydrate-induced hypertriglyceridemia has been provided by epidemiologic studies where elevated

triglyceride levels were apparently rare in population groups such as the Tarahumara Indians of Mexico who were habituated to a high carbohydrate intake (Connor et al, 1978). Unlike serum cholesterol which responds rather rapidly to dietary change, and approaches a stabilized plateau in a few weeks, serum triglyceride is subject to slow adaptation, requiring as long as six months to reach a plateau for a given diet. The triglyceride data reported in this research clearly shows a short-term hypertriglyceridemic effect and a longer adaptation period. Only two subjects, AS and WS, displayed a tendency toward stabilization of serum triglyceride levels within the three week dietary treatment period. However, without more long-term data, it cannot be predicted with certainty whether these plateaus were, in fact, truly reflective of the diets fed. It can merely be suggested that based on well-documented evidence, it is expected that serum triglycerides would have stabilized for all subjects in response to a specific dietary change.

Consistent with the proposal of a carbohydrate-induced hypertriglyceridemia to explain the present results, Carroll et al (1978) reported that there was no effect of dietary protein on serum triglycerides when the

carbohydrate contents of the experimental diets were similar (51% of total kilocalories). The utilization of soy protein meat analogues as the predominant source of plant protein by these investigators permitted the diets to be isocaloric in carbohydrate. Moreover, Anderson et al (1971) reported that for isolated protein diets, egg white and wheat gluten with similar carbohydrate contents, the mean serum triglyceride levels were not significantly different. These results are in agreement with those reported by Campbell et al (1965) who found no significant differences in serum glycerides following the substitution of wheat gluten for a casein-lactalbumin mixture. Purified animal and plant proteins were purposely used by these investigators to avoid the difficulties inherent in using food as a protein source while attempting to maintain the carbohydrate content of the diets constant. Sirtori et al (1977) found that plasma triglycerides were slightly decreased by both a soybean protein diet and a typical animal protein diet during the first dietary period, and generally tended to stabilize during the second dietary period for hyperlipoproteinemic patients. The carbohydrate and fiber contents of the diets

were reported to be similar. The results of this study provide further evidence that plasma triglycerides are not altered by changes in dietary protein provided that carbohydrate and fat components of the diet are maintained constant.

There is evidence to show that the temporary rise in serum triglycerides induced by an excess of complex carbohydrates in the diet is particularly striking in hypertriglyceridemic individuals (Anderson, 1967; Glueck et al, 1969). In the present study, subject MS had the highest initial level of serum triglycerides, 199 mg/dl. However, serum triglycerides did not respond to the PPD relative to the high initial triglyceride value, in this case, nor was there the typical pattern of response demonstrated by all of the other subjects (Figure 4). It can be postulated that the atypical nature of this response was perhaps related to this subject's elevated serum triglycerides. Moreover, this data does not support a positive relationship between initial serum triglyceride concentration and a change in triglyceride response in normotriglyceridemic individuals. Anderson (1967) also failed to provide convincing evidence

for the tendency for correspondingly large triglyceride responses to occur with higher initial triglyceride levels among subjects without evidence of unusual lipid metabolism and with ordinary serum triglyceride concentration. Due to the large variability of response between individuals noted in both studies, it appears that more observations are needed for each dietary treatment to determine whether such a relationship exists.

In conclusion, the data from the present study appears to support the possibility that serum triglyceride is a function of carbohydrate and not the type of protein in the daily diet. Also, the data suggests that triglyceridemia induced in man by mixed carbohydrate is a temporary phenomenon and that serum triglyceride is subject to slow adaptation following a given dietary change.

V. Subjects

The weight change patterns observed in the present study can be partially explained by the difference in the mean daily energy intakes for the experimental diets (Appendix Table 13). The mean daily energy intake for all subjects fed the plant protein diet (PPD) was greater by 1775

kilojoules compared to the animal protein diet (APD). This extra energy was supplied largely by carbohydrate which resulted from the combination of foods to attain the desired level of plant protein in the PPD. In spite of the additional energy ingested during the PPD, the mean weight gain for all subjects was only 0.3 kg. This discrepancy may be explained in part by the reported mean difference between calculated energy intakes and actual available energy associated with high fiber diets. Mahalko and Johnson (1980) reported 6.5% less available energy for high fiber diets compared to calculated energy intakes. This value compares well with a report by Marshall et al (1975). Hence, the PPD may have actually contained lower available energy than was calculated from food composition tables due to the high fiber content of the diet. Moreover, several studies of overfeeding (Norgan and Durnin, 1980; Apfelbaum et al, 1971) have reported weight gains to be less than expected from the degree of overfeeding. The human body appears to have a homeostatic mechanism for maintenance of weight within certain limits. This phenomenon may serve to explain the relative constancy in weight noted in this study despite significantly different

caloric intakes. For five out of eight subjects in this study, weight maintenance was within 1 kg in each dietary period during the 42 days. Studies have generally shown a positive correlation between body weight and serum lipid levels (Galbraith et al, 1964; Nestel et al, 1969). However, it is unlikely that the small changes in body weight which occurred had major effects on the blood lipid levels of the subjects.

C O N C L U S I O N

It is concluded that the ingestion of two diets differing in the type of protein, either mixed plant or animal origin, could not be shown to have any effect on serum total cholesterol or plasma low density lipoprotein cholesterol with the number of subjects and the experimental conditions used in this study. However, in view of the reported investigations which have demonstrated a hypocholesterolemic effect for plant protein diets relative to animal protein diets when female subjects were fed other types of protein, it is clear that the present research requires further confirmation under more varied, but controlled experimental protocols in order to establish a more specific role for dietary protein in lipid metabolism. Because of the complexity of the mixed diet normally consumed by North Americans and the potential for significant biological interactions between all dietary components, the effect of dietary protein cannot be examined in isolation. The total composition of the diet studied must be considered as mediating whatever effect is isolated for dietary protein since there is evidence that non-protein constituents may alter the cholesterolemic

response obtained for protein. Hence, from a practical standpoint, further assessment of the effect of dietary protein under conditions of protein, fat and carbohydrate which are realistically achieved by the average population and which are consistent with the existing recommendations for a 'prudent' diet are in order. Only in this way can it be determined whether changing the type of protein in the diet has a practical application with regard to dietary recommendations aimed at ameliorating the risk for atherosclerosis.

Although this study suggests that the high density lipoprotein cholesterol is decreased by a plant protein diet, the data must be interpreted with caution since only individual values for each diet period were available. Thus, the specific effect of dietary protein on lipoprotein metabolism requires more thorough investigation. Moreover, the precise etiological significance of changes in this lipoprotein fraction in response to dietary manipulation must be ascertained before dietary recommendations are warranted.

The initial and transient hypertriglyceridemic effect for plant protein diet group is consistent with reports in the

literature of a carbohydrate-induced phenomenon as opposed to an effect by dietary protein. The data suggest that more than three weeks is required for stabilization of serum triglycerides in response to a given change in diet.

The present study does not support a beneficial cholesterolemic effect for the substitution of animal protein by mixed plant protein in the North American diet. Moreover, this study shows that adverse effects may result from such a substitution due to the effect of the naturally higher amounts of carbohydrate in the mixed plant protein diet on serum triglycerides and high density lipoprotein cholesterol. Therefore, until more conclusive evidence for the beneficial effect of plant protein on blood lipids is delineated, the advocation for changes in the type of dietary protein is not warranted.

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Appendix Table 1

Typical Daily Menus

Plant Protein Diet	g	Animal Protein Diet	g
	Breakfast		
Orange juice	240	Orange juice	240
Granola [1]	40	Granola	40
Soya milk (conc.)[2]	120	Soya milk (conc.)	60
Whole wheat bread	56	Whole wheat bread	28
Jam	30	Jam	30
	Morning Snack		
Raisins	100	Raisins	100
Sunflower seeds	25		
	Lunch		
Casserole		Beef pattie, lean	125
- Kidney beans	125	Lettuce	20
- Tomatoes (canned)	100	Tomatoes, fresh	100
Salad		Whole wheat bread	56
- Lettuce	20	Canned fruit	125
- Garbanzo beans	30		
Whole wheat bread	56		
Canned fruit	125		
Apple juice	284		
	Afternoon Snack		
Cookies[3]	50	Cookies	50
	Dinner		
Instant mashed potatoes	50	Beef pattie, lean	125
Cream-style corn	100	Instant mashed potatoes	25
Frozen peas	120	Frozen green beans	100
Whole wheat bread	56	Whole wheat bread	28
Canned fruit	125	Canned fruit	125
	Evening Snack		
Peanut butter	60	Peanut butter	20
Whole wheat bread	56	Whole wheat bread	56
Banana (A.P.)	190	Banana (A.P.)	190
Apple juice	284	Apple juice	284
	Fat		
Beef tallow margarine	45	Vegetable oil margarine	45

[1] Recipe for about 4 servings (40g) granola: (in grams) rolled oats, 80; bran, 10; beef tallow, 50; and brown sugar, 40.

[2] Prosobee Milk-Free Formula, Mead-Johnson, Canada.

[3] Recipe for 30 cookies: (in grams) flour, 215; salt, 3; rolled oats, 190; beef tallow, 150; brown sugar, 150; baking soda, 4.5; vanilla, 4; water, 50.

Appendix Table 2

Analysis of Variance: Serum Total Cholesterol[1]

Subject	Serum Total Cholesterol Difference (mg/dl)[2]
	Order (PPD to APD)
AG	+12
JK	- 7
MS	+ 5
WS	- 4
Mean	+ 1.5
	Order (APD to PPD)
MH	-13
RP	+ 3
AS	-14
BY	-30
Mean	-13.5
Overall Mean	- 6

Source of Variation	df	SS	MS	F ratio	P value
Mean (Difference)	1	288.00	288.00	2.24	n.s.
Order	1	450.00	450.00	3.51	n.s.
Error	6	770.00	128.33		
Total	8	1508.00			

1 Comparison of first week values (day 8) for each dietary period.

2 Plant protein diet (PPD) minus animal protein diet (APD).

Appendix Table 3

Analysis of Variance: Serum Total Cholesterol[1]

Subject	Serum Total Cholesterol Difference (mg/dl)[2]
	Order (PPD to APD)
AG	- 4
JK	+13
MS	-10
WS	+20
Mean	+ 5
	Order (APD to PPD)
MH	- 8
RP	+36
AS	-14
BY	+22
Mean	+ 9
Overall Mean	+ 7

Source of Variation	df	SS	MS	F ratio	P value
Mean (Difference)	1	378.12	378.12	1.03	n.s.
Order	1	36.12	36.12	0.10	n.s.
Error	6	2195.76	365.96		
Total	8	2610.00			

1 Comparison of second week values (day 15) for each dietary period.

2 Plant protein diet (PPD) minus animal protein diet (APD).

Appendix Table 4

Analysis of Variance: Serum Total Cholesterol[1]

Subject	Serum Total Cholesterol Difference (mg/dl)[2]
	Order (PPD to APD)
AG	-13
JK	- 3
MS	+21
WS	+15
Mean	+ 5
	Order (APD to PPD)
MH	-37
RP	- 6
AS	-18
BY	- 5
Mean	-16.5
Overall Mean	- 6

Source of Variation	df	SS	MS	F ratio	P value
Mean (Difference)	1	264.50	264.50	1.13	n.s.
Order	1	924.50	924.50	3.94	n.s.
Error	6	1409.00	234.83		
Total	8	2598.00			

1 Comparison of third week values (day 22) for each dietary period.

2 Plant protein diet (PPD) minus animal protein diet (APD).

Appendix Table 5

Analysis of Variance: Serum Total Cholesterol[1]

Subject	Serum Total Cholesterol Difference (mg/dl)[2]
	Order (PPD to APD)
AG	- 1
JK	+ 1
MS	+ 5
WS	+11
Mean	+ 4
	Order (APD to PPD)
MH	-19
RP	+11
AS	-15
BY	- 4
Mean	- 7
Overall Mean	- 1

Source of Variation	df	SS	MS	F ratio	P value
Mean (Difference)	1	15.12	15.12	0.15	n.s.
Order	1	233.28	233.28	2.25	n.s.
Error	6	622.60	103.77		
Total	8	871.00			

1 Comparison of mean total cholesterol values for each dietary period.

2 Plant protein diet (PPD) minus animal protein diet (APD).

Appendix Table 6

Analysis of Variance: Serum Total Cholesterol[1]

Subject	Serum Total Cholesterol Difference (mg/dl) Initial - PPD[2]
AG	+29
JK	+28
WS	- 2
MS	+64
Mean	+30
	Initial - APD[3]
MH	+14
BY	+32
AS	+ 8
RP	+35
Mean	+22
Overall Mean	+26

Source of Variation	df	SS	MS	F ratio	P value
Mean (Difference)	1	5408.00	5408.00	11.96	0.05
Order	1	112.50	112.50	0.25	n.s.
Error	6	2713.50	452.25		
Total	8	8234.00			

1 Comparison of initial total cholesterol values at the start of the study to mean total cholesterol values of the first dietary period.

2 PPD represents the mean total cholesterol value of the plant protein diet period.

3 APD represents the mean total cholesterol value of the animal protein diet period.

Appendix Table 7

Analysis of Variance: Plasma Low Density Lipoprotein Cholesterol[1]

Subject	Plasma LDL Cholesterol Difference (mg/dl)[2]
	Order (PPD to APD)
AG	-39
JK	+ 9
MS	+18
WS	+14
Mean	+ 0.5
	Order (APD to PPD)
MH	-24
RP	+17
AS	-10
BY	+15
Mean	- 0.5
Overall Mean	0

Source of Variation	df	SS	MS	F ratio	P value
Mean (Difference)	1	0.00	0.00	0.00	n.s.
Order	1	2.00	2.00	0.00	n.s.
Error	6	3310.00	551.67		
Total	8	3312.00			

1 Comparison of LDL values at the end of each dietary period (day 22).

2 Plant protein diet (PPD) minus animal protein diet (APD).

Appendix Table 8

Analysis of Variance: Plasma Low Density Lipoprotein Cholesterol[1]

Subject	Plasma LDL Cholesterol Difference (mg/dl) Initial - PPD[2]
AG	+35
JK	-11
MS	+20
WS	- 9
Mean	+ 9
	Initial - APD[3]
MH	+36
RP	+30
AS	- 4
BY	+17
Mean	+20
Overall Mean	+14

Source of Variation	df	SS	MS	F ratio	P value
Mean (Difference)	1	1624.50	1624.50	3.96	n.s.
Order	1	242.00	242.00	0.59	n.s.
Error	6	2461.50	410.25		
Total	8	4328.00			

1 Comparison of initial LDL values at the start of the study to LDL values of the first dietary period.

2 PPD represents the LDL value at the end of the plant protein diet period. (day 22).

3 APD represents the LDL value at the end of the animal protein diet period. (day 22).

Appendix Table 9

Analysis of Variance: Plasma High Density Lipoprotein Cholesterol[1]

Subject	Plasma HDL Cholesterol Difference (mg/dl)[2]
	Order (PPD to APD)
AG	+ 9
JK	+ 7
MS	- 9
WS	- 1
Mean	+ 1.5
	Order (APD to PPD)
MH	+ 9
RP	+ 7
AS	+16
BY	+ 8
Mean	+10
Overall Mean	+ 6

Source of Variation	df	SS	MS	F ratio	P value
Mean (Difference)	1	264.50	264.50	6.27	0.05
Order	1	144.50	144.50	3.43	n.s.
Error	6	253.00	42.17		
Total	8	662.00			

1 Comparison of HDL values at the end of each dietary period (day 22).

2 Animal protein diet (APD) minus plant protein diet (PPD).

Appendix Table 10

Analysis of Variance: Plasma High Density Lipoprotein Cholesterol[1]

Subject	Plasma HDL Cholesterol Difference (mg/dl) Initial - PPD[2]
AG	+10
JK	+19
MS	- 3
WS	+31
Mean	+14
	Initial - APD[3]
MH	+ 9
RP	+ 1
AS	-10
BY	- 5
Mean	- 1
Overall Mean	+ 6.5

Source of Variation	df	SS	MS	F ratio	P value
Mean (Difference)	1	338.00	338.00	2.47	n.s.
Order	1	480.50	480.50	3.52	n.s.
Error	6	819.50	136.58		
Total	8	1638.00			

1 Comparison of initial HDL values at the start of the study to HDL values of the first dietary period.

2 PPD represents the HDL value at the end of the plant protein diet period. (day 22).

3 APD represents the HDL value at the end of the animal protein diet period. (day 22).

Appendix Table 11

Analysis of Variance: Serum Triglycerides[1]

Subject	Serum Triglyceride Difference (mg/dl)[2]
	Order (PPD to APD)
AG	+109
JK	+ 6
MS	+ 9
WS	+ 65
Mean	+ 47
	Order (APD to PPD)
MH	+ 74
BY	+119
AS	+ 16
RP	+ 14
Mean	+ 56
Overall Mean	+ 51.5

Source of Variation	df	SS	MS	F ratio	P value
Mean (Difference)	1	21218.00	21218.00	8.52	0.05
Order	1	144.50	144.50	.06	n.s.
Error	6	14949.50	2491.58		
Total	8	36312.00			

1 Comparison of first week values (day 8) for each dietary period.

2 Plant protein diet (PPD) minus animal protein diet (APD).

Appendix Table 12

Analysis of Variance: Serum Triglycerides[1]

Subject	Serum Triglyceride Difference (mg/dl)[2]
	Order (PPD to APD)
AG	+28
JK	+16
WS	+72
MS	-24
Mean	+23
	Order (APD to PPD)
MH	+16
BY	+57
AS	+23
RP	0
Mean	+24
Overall Mean	+23.5

Source of Variation	df	SS	MS	F ratio	P value
Mean (Difference)	1	4418.00	4418.00	4.13	n.s.
Order	1	2.00	2.00	0.00	n.s.
Error	6	6414.00	1069.00		
Total	8	10834.00			

1 Comparison of third week values (day 22) for each dietary period.

2 Plant protein diet (PPD) minus animal protein diet (APD).

Appendix Table 13

Mean Daily Energy, Protein and Fat Intakes of Subjects[1]

Subject	Plant Protein Diet			Animal Protein Diet		
	Energy Kj	Protein g	Fat g	Energy Kj	Protein g	Fat g
MH	18698	115	164	16234	119	144
BY	14108	79	114	12355	94	114
AS	12945	76	107	12355	94	114
RP	13962	77	113	12836	94	114
AG	13485	77	113	11238	87	113
JK	14602	84	115	12355	94	114
WS	13878	79	114	12355	94	114
MS	14602	84	115	12355	94	114
Mean	14535	84	119	12760	96	118
± SE	626	5	6	521	4	4

[1] Calculated values using USDA Handbook #8, Composition of Foods (Watt and Merrill, 1963).