

THE UNIVERSITY OF MANITOBA

COMPARISON OF THE EFFECT OF BEEF TALLOW AND  
CORN OIL ON SERUM LIPID PATTERNS  
IN YOUNG MEN

by

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## ABSTRACT

The effects of dietary fat on serum lipids and lipoproteins were investigated in five healthy male subjects during two trials, each consisting of a six-day stabilization period, when a typical Canadian diet was fed, and a three-week experimental period, when either beef tallow or corn oil was fed in a mixed diet containing about 40 percent of the total calories as fat. Fasting sera samples were taken on Days 1, 6, 21 and 28 of each trial. Serum cholesterol levels decreased significantly ( $P < 0.005$ ) during the first two weeks on the test diets, with the decrease on the corn oil diet (48 mg. percent) being more than twice that on the beef tallow diet (20 mg. percent). Serum cholesterol levels tended to plateau during the third week on the test diets. The observed decrease in serum cholesterol on the beef tallow diet agreed well with the values that would be predicted on the basis of changes in dietary fatty acid composition if stearic acid was assumed to have no effect on serum cholesterol level. Lipid phosphorous followed a similar pattern to serum cholesterol although the magnitude of response was less and the changes were slower. Serum triglyceride levels did not change when the men were fed the beef tallow diet but decreased significantly ( $P < 0.005$ ) when they received the corn oil diet. The changes in serum beta-/pre-beta-lipoprotein ratio seemed to parallel those of serum cholesterol. The percent saturated and unsaturated fatty acids of the low density (LDL) and very low density (VLDL) lipoprotein fractions seemed to correspond to alterations in dietary fatty acids, with the response of the LDL fraction being more sensitive and congruous with changes in dietary fatty acids.

The results of the present study are consistent with the hypothesis that beef tallow per se is hypocholesterolemic. These findings suggest that restriction of beef to three 3-oz. portions weekly in serum lipid-reducing diets is a questionable practice.

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## INTRODUCTION

Atherosclerosis and related vascular diseases are the single greatest cause of death in men of the technologically developed countries. Coronary vessel disease has been correlated with a number of risk factors but the one that stands out more than any other, both in animal experimentation and in clinical observation, is the association of coronary disease with blood lipid abnormalities. Elevated serum cholesterol has received the most attention but virtually every blood lipid fraction has been incriminated (Kannel et al., 1971b). Almost every blood lipid fraction has been found to respond to diet manipulation especially changes in amount and source of dietary fat. Nutrition appears to be a key measure in any attempt to change the present high incidence of heart disease in the countries of the Western world. Diet is the only treatment we have today that may possibly slow down the infiltration of lipids into the blood vessels. It has long been recognized that dietary fat was in some way involved with serum cholesterol levels and that low-fat diets could depress serum cholesterol level and decrease the rate of incidence of coronary heart attacks (Keys, 1956). Theoretically, the risk of coronary heart disease drops three percent for every one percent reduction in serum cholesterol level (Cornfield, 1962).

With the demonstration that replacement of saturated with unsaturated fat had a similar effect on serum cholesterol (Ahrens et al., 1957), the emphasis was shifted to quality rather than quantity of fat.

In the last two decades, workers have found that while saturated fatty acids with fewer than 12 carbon atoms and more than 16 carbon atoms had little effect on serum cholesterol, these fatty acids exerted a hypertriglyceridemic effect. Since stearic acid (C18:0) per se has not always been found to have an effect on serum cholesterol and has been found to be hypertriglyceridemic (Grande et al., 1972), the question arises as to what are the effects of a natural fat, having a high stearic acid content, on blood lipid patterns.

A very important natural saturated fat containing high levels of stearic acid is beef tallow. About 84 lbs. of beef per capita were consumed in Canada during 1970. Since the general recommendation to Canadians is to decrease their intake of saturated fats and furthermore, as a severe restriction of beef intake (three 3-oz. portions weekly) is common in serum lipid-reducing diets, the effect of beef tallow on serum lipids needs to be better defined to justify these practices.

The present study investigated the effect of beef tallow as compared to the well-established effect of corn oil, on serum lipid levels in normal healthy young men.

## REVIEW OF LITERATURE

The leading causes of death in Canada and other developed societies are physiological manifestations of atherosclerosis, an entity characterized by the accumulation of cholesterol and other fatty substances in the walls of large blood vessels. Kannel (1971a) aptly described atherosclerosis as the "disease of living" because it is almost universally present in our population from adolescence onward. Persons with pronounced atheroma are excellent candidates for heart attacks, angina pectoris and strokes.

Canada ranks sixth among countries in the world in mortality from cardiovascular disease. About 50 percent of all reported deaths in Canada are due to various forms (angina pectoris, stroke, sudden death) of coronary heart disease (CHD). The increase in reported death rates has been especially significant in younger and middle-age males. The average, apparently healthy man has about one chance in five of experiencing a myocardial infarction before age 60 and one chance in fifteen of dying from a coronary attack.

In spite of the tremendous expenditure in human and dollar resources on this disease over the past 20 years, little progress has been made in solving or reducing the incidence of heart disease.

### Relationship of Coronary Heart Disease to Blood Lipids

A considerable body of evidence has accumulated concerning the role of lipids in atherogenesis, and, as a result, virtually every blood lipid fraction has been incriminated (Kannel et al. 1971).

Those lipid elements incriminated include cholesterol, triglyceride, phospholipid and non-esterified fatty acids. In addition, the lipoproteins to which the lipids are linked for transport have also been blamed. These include the cholesterol-rich beta-lipoproteins of the  $S_f$  0-20 class, the triglyceride-rich pre-beta-lipoproteins of the  $S_f$  20-400 class, the alpha-lipoproteins rich in phospholipid and the albumin fraction, which carries the fatty acids about. Whether one or several of these are fundamentally involved in atherogenesis is not clear. Furthermore, some serum lipid values can reach a high level through a number of mechanisms—diabetes, thyroid disease and a disordered or inborn error of lipid metabolism. However, the atherogenicity of each kind of disorder requires further clarification.

It must be realized also, that CHD is a disease of multiple causation—it is the result of an interplay of personal attributes (age and sex, genetic tendency to heart disease), metabolic disorders (hyperlipidemia, hypertension and diabetes) and personal habits (cigarette smoking, inactivity and overeating). Nevertheless, it is manifest that blood lipids can be used as a biochemical predictor of coronary events.

A disproportionate amount of CHD in the general population develops among individuals with serum cholesterol values in the upper quartile, i.e., cholesterol values between 250 to 350 mg. percent (Leren, 1966; Dawber et al., 1962; Gofman et al., 1966; Scrimshaw and Gunzman, 1968). As can be seen in Figure 1, there is a somewhat linear relationship between serum cholesterol concentration and incidence of CHD. This association is also buttressed by data from geographical studies showing a relationship between cholesterol level and morbidity and

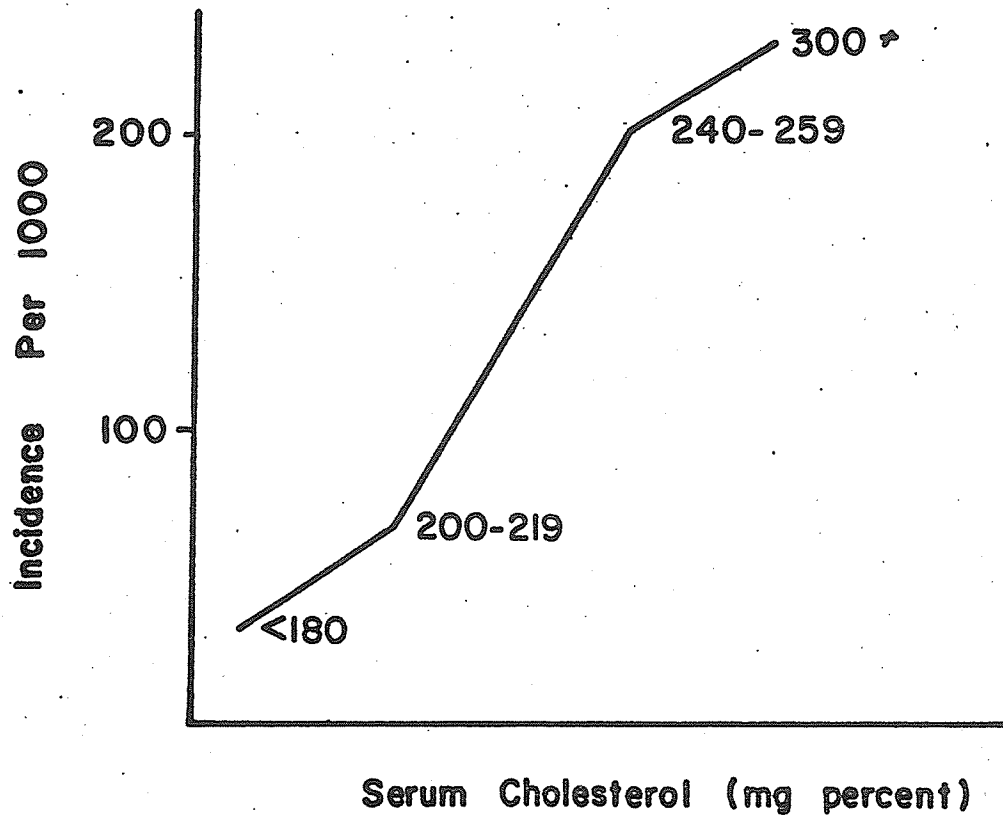


Figure 1. Incidence of coronary heart disease associated with serum cholesterol levels (Kannel, 1971a).

mortality from CHD all across the globe (Keys, 1956; Carlson and Bottiger, 1972).

Phospholipid is believed to be protective against a high cholesterol level so that the cholesterol:phospholipid ratio (C/P ratio) has been suggested as an index of potential risk to atherogenesis (Ladd et al., 1949). Yet, prospective risk to CHD can be shown to actually rise in proportion to the serum phospholipid value (Turpeinin et al., 1968). This is understandable since phospholipid values have been found to parallel those of serum cholesterol (Erickson et al., 1964). The C/P ratio, however, is unrelated to incidence of CHD (Kannel et al., 1971b). Since 1959, when Albrink and Man suggested that elevated serum triglyceride levels might be related to the pathogenesis of heart disease, several investigators (Brown, 1959; Albrink et al., 1961; Cramer et al., 1966; Allard and Goulet, 1967) have shown a firm relationship between elevated serum triglyceride concentrations and CHD.

Allard and Goulet (1967) reported results of a study carried out at the Montreal Heart Institute and suggested that a combination of both high cholesterol and high triglyceride levels would be more critical in producing morbidity than an increase in either lipid component alone. In addition, Carlson and Bottiger (1972), in a nine year follow-up of 3,168 men in Stockholm, reported that a combined elevation of plasma triglycerides and plasma cholesterol carried the highest risk for ischemic heart disease. These observations, together with the suggestion that serum phospholipid is a factor in CHD, suggest that serum lipoproteins, which are primarily involved in transport of cholesterol and triglyceride, would play a significant role in the development of atherosclerosis.



As early as 1949, Gofman et al. showed in humans that a spectrum of lipoproteins bearing cholesterol, phospholipid and triglyceride existed spontaneously or could be induced by a variety of experimental means.

In 1966, Gofman and his associates reported results from both the Framingham and Livermore studies which linked lipid and lipoprotein, especially the glyceride-rich  $S_f$  20-400, to the pathogenesis of atherosclerosis, especially during the early stages of the disease process or when only moderate involvement of the vessel has occurred. However, these parameters lose their predictive value for all de novo ischemic heart disease when individuals are above age 55.

The blood fat abnormalities defined by Drs. Frederickson, Levy and Lees (1967) provided an updated blueprint of the complicated chemical relationship between lipoproteins and heart disease. It has now become obvious that we cannot think about atherosclerosis in terms of one specific lipid, or consider high blood lipid levels the manifestation of one abnormality.

Important for an understanding of lipid transport is the concept that lipids (cholesterol, phospholipid and triglyceride) circulate bound to specific proteins and it is as lipid-protein complexes or lipoproteins that lipids enter and leave the plasma. In essence, lipid transport can be thought of in terms of two protein moieties and the triglycerides—the major lipid they are called upon to transport. The alpha- and beta-lipoproteins exist in plasma together with fixed amounts of phospholipid and cholesterol. The alpha-lipoprotein contains about 30 percent by weight phospholipid and 20 percent cholesterol. The beta-lipoprotein contains about 50 percent cholesterol by weight

and carries one-half to two-thirds of the total serum cholesterol in the fasting state. Kinetic studies demonstrate that the turnover of phospholipid and cholesterol is quite slow, both being less than one to two grams daily, while triglyceride has a turnover rate of 100 to 150 grams per day.

Hyperlipidemia may result from defective protein as well as defective lipid metabolism. The protein and lipid content of each lipoprotein determines its size, density and electrophoretic migration. With electrophoresis, using the difference in electrical charge on the different lipoprotein moieties, it is possible to separate serum lipoproteins into a non-migrating, a beta-migrating, a pre-beta-migrating and an alpha-migrating band. At present, at least five pathological patterns (Table 1) have been delineated. Type I is marked by tremendously increased chylomicrons while all other lipoproteins are low; Type II is characterized by an increased beta- or low density lipoprotein; Type III has elevated beta- as well as an increased pre-beta- or very low density lipoprotein; Type IV is characterized by a marked elevation in pre-beta-lipoprotein; while in Type V, both chylomicrons and pre-beta-lipoprotein are elevated.

More precise information is needed concerning the prevalence of the various types of hyperlipoproteinemia encountered in the general population and their contribution to clinical atherosclerotic disease.

In view of the fact that a number of lipids appear related to the incidence of CHD and that all are transported linked to lipoproteins, the question arises whether a battery of lipids, a lipid profile or the associated lipoprotein pattern would provide a better prediction of potential CHD risk than that given by a single lipid component.

Table 1. Types of hyperlipoproteinemia as defined by various indexes to serum lipid concentrations.

Type	Class Predominantly Elevated	Serum Triglyceride	Serum Cholesterol
I	Chylomicrons present; all other lipoproteins ↓	↑↑↑	+ -
II	Beta ↑↑↑ Pre-Beta ↑ ±	normal	↑↑↑
III	"Broad Beta" present; Pre-Beta ↑ ±	↑↑	↑↑
IV	Pre-Beta ↑↑↑	↑↑	normal or ↑
V	Chylomicrons present; Pre-Beta ↑↑	↑↑↑	↑

### Relationship of Diet to Blood Lipid Levels

None of the epidemiological studies of free-living affluent populations have satisfactorily demonstrated that either serum lipid values or atherosclerotic disease status relate to diet eaten by a particular population group (Scrimshaw and Gunzman, 1968; Keys, 1957; Carlson and Bottiger, 1972). Controlled studies with experimental animals and humans have shown that manipulation of a number of dietary factors produce almost predictable changes in serum lipid values (Keys et al., 1965 a - d). Nevertheless, Connor (1969) stated "to change the present high incidence of CHD in the countries of the Western world, nutrition is the key measure". Many others also believe that diet is the only treatment we have to offer today that may possibly slow down the atherosclerotic process. It is the principal treatment that can be safely offered to patients for reduction of blood lipids, although drugs may be of additional help in maintaining low blood lipid levels in certain types of hyperlipidemia. A very encouraging development is the reduced incidence of coronary artery disease in persons who have maintained a low-cholesterol level with fat-controlled diets for five years or more (Rinzler, 1968; Turpeinen et al., 1968, Christakis et al., 1966 and Kannel, 1971a). Theoretically, the risk of coronary heart disease drops by three percent for every one percent reduction in serum cholesterol level (Cornfield, 1962).

The dietary alterations involved include total fat, animal fats and vegetable oils, saturated and unsaturated fatty acids and cholesterol. Among non-fat dietary constituents are protein, starches, sugar, pectin and plant sterols. These latter components exert only

a minor effect on blood lipids as compared to effects of dietary fat and will not be discussed.

### Dietary Fat and Atherosclerosis

The incrimination of dietary fat as the cause of human atherosclerosis dates largely from World War I. During the war and the accompanying British blockade of Germany, it was noted extensively by German pathologists that the incidence of deaths and sickness from atherosclerosis fell sharply. Following the war, lifting of the food blockade and resumption of the customary fat intake in the diet, the incidence of deaths and morbidity from this disease again rose to pre-war levels in Germany (Hueper, 1944). During World War II, history repeated itself as regards the relationship of dietary fat and atherosclerosis. In this case the observations were the consequence of the German blockade of the Scandinavian countries (Morrison, 1952). Even more recently, additional information corroborating these findings was obtained from American military studies such as those of Enos et al. (1955). As a consequence, numerous investigations were begun into the role of dietary lipids in the production of human and animal atherosclerosis.

Before discussing these studies in more detail, however, it may be advantageous to briefly review the characteristics of dietary fat.

General properties of dietary fat. Dietary fat is composed of 98 percent triglyceride, a compound composed of a molecule of glycerol and three molecules of fatty acids. Since the glycerol moiety is the same in all triglycerides, fats differ from one another depending on the fatty acids making up these triglycerides. Each fatty acid is

made up of two parts—a carboxyl end and a hydrocarbon chain. It is the latter which distinguishes the various fatty acids.

Fatty acids are classified as being short-chain if they contain six or less carbons and an example of a fat which contains considerable quantities of short-chain fatty acids is butterfat. The medium-chain fatty acids are those with 8 to 12 carbon atoms and an example of a natural fat that is a rich source of medium-chain fatty acids is coconut oil. All other fatty acids are classed as long-chain fatty acids. The most common fatty acids in natural fats are those containing 18 carbons.

In addition to chain length, fatty acids may either be saturated or unsaturated. If they are unsaturated, they can be either monounsaturated—that is, contain only one double bond or they may be polyunsaturated—that is, contain two or more double bonds. Examples of these various classes of fatty acids are: stearic acid, an 18 carbon saturated fatty acid, a principal component of beef tallow; oleic acid, an 18 carbon monounsaturated fatty acid, the most common fatty acid in our diet; and linoleic acid, an 18 carbon fatty acid that contains two double bonds which are separated by a methylene group. Linoleic acid makes up 50 percent of the weight in corn, cottonseed and soybean oils and is the primary polyunsaturated fatty acid in today's diet.

Another characteristic of unsaturated fatty acids is their geometric configuration. The natural configuration of the fatty acids is the cis configuration wherein the carbon chain is doubled back on itself. The trans configuration, on the other hand, basically yields a straight chain configuration, except for the slight kink in the chain at the double bond. The importance of the cis and trans configuration is in the hydrogenation of oils in the manufacturing of margarine and

shortening where unnatural trans isomers of fatty acids are formed. Brown (1959) found no indication that trans fatty acids in amounts normally used in practical diets affect human beings adversely. The difficulty in using a hydrogenated product is the marked reduction of polyunsaturated fatty acids. Hydrogenation involves the addition of hydrogen, at high pressure and in the presence of a catalyst, to the double bond of a fatty acid, thereby converting an unsaturated fatty acid such as oleic to its saturated analog—stearic acid. In general the more unsaturated a fatty acid the more easily it is hydrogenated. Thus linolenic acid is much more readily hydrogenated than linoleic acid which is more easily hydrogenated than oleic acid. Thus in the hydrogenation of corn oil, the polyunsaturated fatty acid content can be markedly lowered while the saturated fatty acid content is only slightly increased.

Quantity of dietary fat. It was originally thought that the total amount of dietary fat was critical in a serum lipid-reducing diet because of the epidemiologic evidence (Keys et al., 1958; Joliffe, 1959) that populations eating low-fat diets (15 percent or less of total caloric intake) had lower blood cholesterol levels and much less cardiovascular disease than those eating high-fat diets (40 percent of total caloric intake). Keys (1957) ascribed the effect of low-fat diets to the decrease in animal fat, especially since some vegetable oils given in large amounts had been found to depress serum cholesterol. As a consequence the concept grew that it was the origin rather than the amount of dietary fat that affected serum lipid levels.

Origin of dietary fat. The original breakthrough on the relationship of serum lipids to dietary fat source was made by Kinsell et al. (1952) who showed that the ingestion of certain vegetable oils was followed by a pronounced drop in plasma cholesterol and phospholipid levels. This finding was soon confirmed by others (Ahrens et al., 1955; Beveridge et al., 1955; Malmros and Wigand, 1955). However, not all vegetable oils possessed this cholesterol-lowering effect. Bronte-Steward et al. (1956) clearly showed that certain marine and vegetable oils, which in their natural state lowered serum cholesterol levels in man, after hydrogenation acted to elevate serum cholesterol just as did certain naturally occurring highly saturated fats, e.g., those derived from coconuts and cow's milk. Thus, it became clear that the analysis of the effects of dietary fats on serum cholesterol should be made in terms of chemical composition rather than in terms of "animal" or "vegetable" origin. The highly saturated fats, such as butter and coconut oil, tended to elevate serum cholesterol levels while isocaloric amounts of highly unsaturated oils, such as safflower oils, corn oil and cottonseed oil depressed serum cholesterol.

It soon became evident, however, that serum lipid constituents other than cholesterol also responded to manipulations in dietary fat. Ahrens et al. (1957b) reported a decrease in the phospholipid levels of serum when a high polyunsaturated fat diet was fed; a decrease that paralleled the decrease in serum cholesterol. Shapiro et al. (1957) found that changes in serum phospholipid level were not proportional to the changes in serum cholesterol level and that the C/P ratio tended to be lower following the ingestion of unsaturated fatty acids.



Quality of dietary fat. Three main postulates regarding the relationship between dietary fats and serum cholesterol developed. The effectiveness of the polyunsaturated fatty acids in lowering serum cholesterol was ascribed by Ahrens et al. (1957b) to the total unsaturation of the fats as expressed by the iodine number (the number of double bonds per unit of carbon) and Gunning et al. (1964) to the square root of the iodine number. At the same time, Kinsell et al. (1958) and Sinclair (1956) postulated that the effect of polyunsaturates on cholesterol level was due to the content of essential fatty acids (EFA) in the dietary fat and that hypercholesterolemia in man might be due to a biochemical expression of EFA deficiency. This theory, however, was untenable in view of the fact that sardine, whale and pilchard oils, which are exceedingly poor in EFA but rich in other polyunsaturated fatty acids, caused a lowering of serum cholesterol, phospholipid and beta-lipoprotein (Malmros and Wigund, 1957). The third postulate was that of Keys et al. (1957) who, after six years of experimentation published their original prediction equation which related changes in serum cholesterol to changes in composition of dietary fat.

$$(1) \Delta C = 2.74 \Delta S - 1.31 \Delta P$$

Their equation suggested a relationship between the saturated and polyunsaturated fatty acid content of the dietary fat. From the variety of fats studied, saturated fatty acids, at least those of chain length longer than 10 carbon atoms, were about twice as effective in elevating serum cholesterol as the polyunsaturates were in depressing serum cholesterol. Further experimentation by the same group (Keys et al., 1958) showed the principal dietary monoene fatty acid, oleic acid, had absolutely no effect on serum cholesterol levels. This was

subsequently confirmed by Hegsted et al. (1965).

Effect of fatty acid chain length on serum lipids. At first, all saturated fatty acids were thought to affect serum cholesterol levels in the same way. Hashim et al. (1960) reported that addition of a mixture of glycerides of medium-chain length saturated fatty acids to the diet produced lower serum cholesterol levels than isocaloric amounts of butterfat. Furthermore, it was shown by Grande et al. (1961) and Hegsted et al. (1965) that saturated fatty acids containing 10 carbon atoms or less had essentially no hypercholesterolemic action. In fact, as early as 1957 Horlick and Craig had shown there was no discernable rise in blood cholesterol levels of humans when they were fed a low-fat diet to which supplements of corn oil, ethyl linoleate or ethyl stearate were added. In fact, with ethyl stearate there appeared to be a sustained fall in serum cholesterol. Fecal studies showed that 65 to 70 percent of the ethyl stearate was absorbed when it constituted 25 percent of the total daily calories and so the cholesterol-lowering effect was not thought to be an effect of poor absorption. These findings by Horlick and associates were subsequently confirmed by Malmros et al. (1957), Keys et al. (1965d) and Hegsted et al. (1965). In fact, Keys et al. (1965d) postulated that since stearic acid is not involved, palmitic acid (C16:0) must be the saturated acid of primary importance. However, there is no complete agreement on this point. Hegsted et al. (1965) proposed that proportions of myristic (C14:0), palmitic and polyunsaturated fatty acids in dietary fats seem as important and perhaps more important than the percent of calories contributed by these acids. This conclusion, however, is limited to

the range of fat intakes studied, 22 to 40 percent of the total calories. Nevertheless, this range of fat intakes include the limits of practical acceptable diets in the United States.

Grande et al. 1961 reported that saturated fatty acids with 12 to 14 carbon atoms in the chain have a slightly greater serum cholesterol-raising effect in man than equal weights of the saturated fatty acids with 16 to 18 carbon atoms. Considering the results of the Minnesota group, it is possible to explain why Ahrens et al. (1957<sub>a</sub>) reported lower serum cholesterol values with cocoa butter in the diet than when the fat was isocalorically replaced by butterfat. These two fats have almost identical iodine values and composition in terms of fatty acids classified as saturated, monounsaturated and polyunsaturated. The most obvious difference between these two fats is that about one-third of the saturated fatty acids in butterfat have 14 or fewer carbon atoms in the chain, whereas in cocoa butter almost all of the saturated fatty acids are C16 and C18 in length. Most of the experimental findings so far available on man are consistent with the theory that as far as saturated fatty acids are concerned, any increase in chain length beyond C16 and any decrease below C12 result in marked decreases in serum cholesterol level.

Hegsted et al. (1965) investigated the quantitative effects of several dietary fats on serum cholesterol in man and found the percent myristic acid to correlate highly,  $R = 0.817$ , with increases in serum cholesterol level. Palmitic acid was correlated to a much lesser degree,  $R = 0.538$ . Nevertheless, the combination of myristic and palmitic acids provides nearly as good a fit as the combination

of myristic and polyunsaturated fatty acids. It is surprising to find that myristic acid appears to be such an important dietary variable in influencing serum cholesterol levels. It appears to be a constituent of all animal fats although the amount may vary from one to 12 percent. Most common vegetable oils other than coconut oil contain very little myristic acid. However, Hegsted cautions that the regression equations are primarily descriptive of the information from which the equations are derived and it is therefore hazardous to attach as much functional significance to the regression equations as has been done by Keys et al. (1965d).

In 1970, Grande et al. specifically tested the effects of stearic and palmitic acids on serum cholesterol concentration. They concluded that stearic acid had no effect on serum cholesterol in man and that palmitic acid had a definite cholesterol-raising effect. On the other hand, McGandy et al. (1970) found that lauric acid and stearic acid were hypercholesterolemic under the conditions of their experiment, although they were less so than myristic and palmitic acids. McGandy's group fed a semisynthetic mixture of triglycerides wherein the fatty acids of natural fats were transesterified and therefore, equally distributed in all positions on the glyceride moiety. The hypercholesterolemic effect of stearic acid in the semisynthetic mixtures contrasts its lack of effect in tallow and cocoa butter. Thus, it appears that in addition to the known effects of chain length and saturation, the position of a fatty acid on the glyceride molecule also influences its metabolism.

Because the cholesterol-raising effect of saturated fatty acids with 12 to 16 carbon atoms is twice as great as the cholesterol-

depressing effect of polyunsaturated fatty acids (Keys et al., 1965d), fats containing two parts of polyunsaturated fatty acid to one part saturated fatty acid should have no effect on serum cholesterol concentration. Grande et al. (1972) tested this hypothesis using two such fat mixtures—olive oil-safflower (OS) at 73 and 27 percent by weight, respectively, and safflower oil-palm oil (SP) at 61 and 39 percent by weight, respectively. These were fed at different levels—OS at 23 and 68 grams, OS-23 and OS-68, respectively, and SP at 63 grams, SP-63. In addition to the fat supplements, the effects of an isocaloric substitution of carbohydrate (CHO) for fat were also studied. There was no difference in the serum cholesterol levels among the fat supplements and CHO diets.

The comparison between diets SP-68 and OS-68 amounted to the replacement of 9.8 percent of calories provided by monoene fatty acid glycerides by a mixture of saturated (C12 to C16 [S']) and polyunsaturated [P] fatty acid glycerides. The S' and P fatty acids provided 3.2 and 6.6 percent, respectively of the energy content of the diet. This study demonstrated that at this calorie level, any mixture of saturated and polyunsaturated glycerides with the value  $2S' - P$  equal zero is equivalent to monoene fatty acid glycerides with regard to its effect on serum cholesterol. The fact that the three diets SP-68, OS-68 and OS-23 produced serum cholesterol levels that were not significantly different from that observed on the carbohydrate diet demonstrated that the fat mixtures contained in these diets could be isocalorically replaced by carbohydrates as well as monoene fatty acids without affecting the serum cholesterol concentration. These results are in agreement with previous work—Keys et al. (1965d), Hegsted et al. (1965),

Fetcher et al. (1967), McGandy et al. (1970). Also in agreement with general experience, serum phospholipids closely parallel serum cholesterol.

Grande et al. (1972) have also reported saturated fatty acids from 12 to 16 carbon atoms seem to have little effect on serum triglycerides. They showed that the plasma triglyceride level, under conditions of their experiment, increased when carbohydrate was substituted for dietary fat containing primarily C12 to C16 fatty acids, which agrees with previous observations by Anderson (1967). The significance of these observations to CHD, however, remains to be answered. Carlson and Bottiger (1972) reported that both serum triglyceride and cholesterol have a direct causal relationship with CHD. These latter findings suggest that one must consider dietary effects on both serum lipids simultaneously.

In addition, Grande et al. (1972) included a butter supplement in the preliminary and final periods of their experiment to provide an initial and final reference point that was identical for all men. Serum cholesterol and phospholipid levels were higher with the butter than with the other test diets. Changing from the test fat diets to the butter-supplemented diet resulted in an average increase in serum triglyceride levels of 25 mg. per 100 ml. whereas changing from the test-fat diets to the carbohydrate-supplemented diet resulted in a 75 mg. per 100 ml. increase in serum triglyceride levels. The authors postulated that the relative hypertriglyceridemic effect of their butter diet may be, at least in part, related to its content of stearic acid, which in this particular experiment was about six percent of the total calories. Anderson (1967) and Grande et al. (1970) had previously reported higher serum triglyceride levels when diets containing beef

tallow were substituted for diets containing coconut oil. There is a higher proportion of long-chain saturated fatty acids (namely, palmitic and stearic) in beef tallow as compared to coconut oil. It would appear that the long-chain saturated fatty acids in the diet resulted in an increase of serum triglyceride levels. In fact, the Minnesota group (Grande et al., 1972) have concluded that saturated fatty acids with fewer than 12 carbon atoms and stearic acid, which do not affect serum cholesterol, produce elevations of serum triglycerides. On the other hand, saturated fatty acids from 12 to 16 carbon atoms, which elevate serum cholesterol concentration, seem to have little effect on serum triglyceride levels.

However, the final word has not been written on the role of dietary fat and blood lipids in relation to atherogenesis.

#### Relationship of Dietary Cholesterol to Blood Lipids

The dietary intake of cholesterol in human adults varies between 200 and 800 mg. per day and is highest among individuals with high intakes of saturated fat. The body synthesizes approximately 2,000 mg. daily — 80 percent of this is synthesized in the liver and another 11 to 12 percent is formed in the intestine. In general, increased cholesterol intake results in a suppression of cholesterol synthesis in the liver. On the other hand, dietary cholesterol apparently has no direct effect on intestinal synthesis. Keys et al. (1965b) reported that a 50 percent decrease in dietary cholesterol will produce an average decrease in serum cholesterol of only about 7 mg. per 100 ml. Thus, in spite of the effect on cholesterol synthesis, level of dietary cholesterol has a <sup>a minor</sup> effect on serum cholesterol concentrations.

The true significance of cholesterol in fat-controlled diets

appears when considered in relationship to the amount and proportion of fatty acids and total fat in the diet. The triangle diagram of Hegsted et al. (1965) illustrated how a change in one fatty acid component—saturates, monounsaturates or polyunsaturates—alters the proportion among all three. Furthermore, Hegsted et al. (1965) found the proportion of fatty acids to be of greater importance in predicting changes in serum cholesterol level than was the percentage of calories from each of these as proposed by Keys et al. (1965d). Ahrens (1957) states that in order to obtain an adequate reduction in serum cholesterol, the polyunsaturated fatty acid requirement increases as the dietary cholesterol rises. For a reduction of 18 to 22 percent in serum cholesterol level, a diet with 200 mg. of cholesterol requires 15 percent of calories as polyunsaturated fatty acids and one with 500 mg. cholesterol requires 23 percent of total calories as polyunsaturated fatty acids. The relationship of polyunsaturated fatty acids to dietary cholesterol is not linear—because intakes above 750 mg. of dietary cholesterol daily have been found to have little effect on serum cholesterol levels.

#### Effect of Total Calories on Blood Lipids

In addition to dietary fat and cholesterol, total calorie intake has been associated with the level of blood lipids in man. Anderson et al. (1955) showed that there were significant decreases in total cholesterol levels in young volunteers on a low-calorie diet even when the dietary glycerides and cholesterol were constant.

In contrast to calorie restriction, Kartin et al. (1944) reported a significant increase in serum cholesterol, large rises in phospholipids and critical changes in triglycerides in man during total starvation.



These changes were reversed by ingestion of carbohydrate. Although these studies are not directly comparable, they demonstrate the critical balance between calories and blood lipids.

Like calorie deficit, the effect of excess calories on serum lipid levels has not been well defined. However, Anderson et al. (1957) reported that each one percent increase in the amount of calories consumed above the eucaloric intake will lead to an increase of about 2 mg. per 100 ml. in the serum cholesterol concentration. A gain in weight is most frequently associated with an excessive intake of calories. Anderson et al. (1957) also reported that a continued excessive intake of calories for 10 weeks resulted in a constant increase in serum cholesterol level until both body weight and cholesterol levels reached plateaus. Both increases were readily reversible by a reduction in the consumption of calories. Galbraith et al. (1964) have reported that when body weight is rapidly reduced, the fall in serum cholesterol concentration exceeds that which can be attributed solely to changes in the composition of the food.

An additional factor apparently contributing to the excessive mortality from CHD seems to be overweight and obesity—both associated with dietary calorie excess. The relationship between incidence of CHD and changes in body weight, in the 12-year follow-up to the Framingham study (Kannel, 1971a), have shown that men whose weight increased more than 20 percent over this time ran a much higher risk of developing a coronary than men who maintained their weight within 90 to 120 percent of their original weight. These observations may not be the result of increased body weight per se because the concomitant increase in serum cholesterol level may strongly contribute to CHD

risk.

It is interesting to note that the method of weight reduction, be it through diet modification or increased energy expenditure, i.e., physical exercise, does not play a significant role on the effect of serum lipid concentrations.

#### Trends in Fat Disappearance

Fat disappearance in Canada, 1960 - 1970. In view of the facts quoted concerning dietary fat and its relation to blood lipids and atherosclerosis, it is of interest to study the changes in available food supplies in this country over the last decade with particular emphasis on fat supplies. Data in Table 2 give the apparent per capita domestic disappearance of food in the last decade. There are limitations in applying food disappearance data to the calculation of food consumption patterns. No allowance is made for waste between retail and ingestion; no distinction is made between individuals, households, restaurants or institutions and some items may be omitted because of lack of data. Further details on the methodology involved in collecting such data and limitations of the interpretation of information thus gathered have been presented by Call and Sánchez (1967).

Per capita consumption of fats and oils has increased 4.4 percent since 1965 and 3.2 percent since 1960. The increase is primarily due to margarine, shortening and shortening oils. There was a slight increase in butter consumption. By contrast, lard consumption has decreased markedly. In general then, the increased consumption of fat from the "fats and oils" category is in the polyunsaturated fatty acids.

Table 2. Apparent pound per capita domestic disappearance of food in Canada, 1960, 1965 and 1970<sup>1</sup>

	Pounds per capita				
			1970	1970	
	<u>1970</u>	<u>1965</u>	<u>1960</u>	<u>1965</u>	<u>1960</u>
<b>Oils and Fats</b>					
Margarine	9.3 <sup>2</sup>	7.0	7.5	+2.3	+1.8
Lard	N.A. <sup>2</sup>	7.2	9.4	- -	- -
Shortening and shortening oils	15.2	9.9	9.4	+5.3	+5.8
Other oils and fats	5.7	4.7	4.1	+1.0	+1.6
Butter	15.7	15.1	13.7	+0.6	+2.0
<b>Total oils and fats</b>	<b>45.9</b>	<b>43.9</b>	<b>44.1</b>	<b>+2.0</b>	<b>+1.8</b>
<b>Meat (carcass weight)</b>					
Pork	55.3	49.2	55.2	+6.1	+0.1
Beef	84.0	78.7	69.2	+5.3	+14.8
Veal	4.5	8.0	7.6	-3.5	-3.1
Mutton and lamb	3.5	2.8	3.2	+0.7	+0.3
Offal	3.4	3.4	4.9	0.0	-1.5
Canned meat	4.7	4.2	7.5	+0.5	-2.8
<b>Total meat</b>	<b>155.4</b>	<b>146.3</b>	<b>147.6</b>	<b>+9.1</b>	<b>+7.8</b>
<b>Poultry</b>					
Chicken	30.5				
Fowl	3.8	18.7	15.1	+25.6	+29.2
Turkey	10.0				
Duck	0.3	8.0	5.5	-7.5	-5.0
Goose	0.2				
<b>Total poultry</b>	<b>44.8</b>	<b>26.7</b>	<b>20.6</b>	<b>+18.1</b>	<b>+24.2</b>
<b>Fish</b>					
Total	12.9	15.5	12.7	-2.6	+0.2
<b>Milk and Cheese</b>					
	58.0	60.6	65.4	-2.6	-7.1
<b>Eggs</b>					
	32.7	32.1	36.7	+0.6	+4.0

<sup>1</sup> Dominion Bureau of Statistics, (Statistics Canada) Ottawa, Ont., Catalogue No.32 - 226 Annual (1960, 1965, 1970).

<sup>2</sup> Not available at release date.

Another significant source of dietary fat is meat. This is of particular interest because much of the fat is saturated. Total meat (carcass weight) disappearance has increased 5.9 percent since 1965 and 5.1 percent since 1960. Pork disappearance was the same in 1970 as 1960 although there was an 11 percent decrease from 1960 to 1965 and a corresponding increase from 1965 to 1970. Beef disappearance has steadily increased—up 6.3 percent since 1965 and up 17.5 percent since 1960. Veal consumption has steadily declined.

Total poultry intake has almost doubled since 1965 (40.4 percent) and has more than doubled since 1960 (59.5 percent). Egg disappearance has changed little since 1965 (1.8 percent) but has decreased considerably since 1960 (12.2 percent). Milk and cheese disappearance has fallen 4.5 percent since 1965 and 12.8 percent since 1960.

It appears from Canadian market disappearance trends that Canadians are purchasing more oils and fats, more red meats, considerably more poultry, less cheese, milk and eggs today than in 1960 or 1965. The Canadian diet today appears to contain more polyunsaturated fatty acids and less saturated fatty acids and cholesterol.

Although not identical, food disappearance data from the United States has been available since 1909 and may give more information on long term trends in fat disappearance. In addition, the American data provide information on the changes that have occurred in the consumption of the fatty acids of food fat.

Fat disappearance trends in United States, 1909 - 1961. Data reported by Antar et al. (1964) on changes in the United States retail market supplies from 1909 - 1961 show interesting changes in total

retail supplies of dairy products, lards and fat pork. As a consequence of these changes, fat from these foods reached a peak of availability in the late twenties which was maintained until 1950 and then began to decrease. Supplies of margarine, shortenings and other vegetable oils increased during the same period. Egg supplies reached a peak around 1950, then began to decrease.

Total fat available per person in the U.S. has increased only 12 percent over the period 1909 - 1961 and this increase is mainly in the market supply of oils which provide an increased percentage of unsaturated fatty acids. There was a considerable decrease in supplies of the saturated fatty acids furnished from dairy products, lard, fat pork and eggs from 1954 to 1961. This was compensated for by the increased supply from meats and poultry. The increase in the saturated fatty acids from 1909 to 1961 was relatively small (7 percent) in comparison to the increase in the polyunsaturated fatty acids which amounts to 37 percent. Consequently, the ratio of polyunsaturated to saturated fatty acids has increased from 0.24 to 0.31 (about 29 percent) over the 53-year period, 1909 to 1961.

In this period it seems that approximately one-third of the saturated fatty acids came from dairy products, another third was furnished by the fat of meat, poultry, fish and eggs and the rest is provided by all other fats and oils. Another intriguing finding is that the change in supplies of dietary cholesterol in the same time period has been relatively small (7.5 percent). A peak was reached in 1950, after which cholesterol available in market supplies began to decrease primarily as a result of a decrease in egg supplies.

### Present Dietary Recommendations For Serum Lipid-Reducing Diets

In spite of the trends which have occurred in fat consumption (on basis of food disappearance data) it has been strongly suggested that fat intake continues to be too high and that dietary fat contains too much saturated fatty acids. The American Heart Association has recently issued a statement on "Diet and Heart Disease"<sup>1</sup> which strongly supports the theory that diet is of value in reducing the frequency of heart disease in general and myocardial infarcts in particular. It recommends a decrease in the intake of saturated fats and an increase in the intake of polyunsaturated fats. An intake of less than 40 percent of total calories from fat is considered desirable. Of this total, polyunsaturated fats should probably equal twice the quantity of saturated fats. Alfin-Slater (1969) has suggested one way to follow these recommendations is to substitute chicken or fish for beef. Frederickson et al. (1970) in The Dietary Management of Hyperlipoproteinemia. A Handbook for Physicians also recommend the substitution of fish or poultry for beef and in most cases restrict beef intake to three 3-ounce lean portions weekly. They further suggest that hyperlipidemics avoid prime grades of beef because these cuts are most heavily marbled. These restrictions may be very difficult to follow for middle-age men who enjoy prime quality roasts, steaks, etc. Perhaps, the restrictions imposed on beef intake may be too severe in view of the fact that oleic and stearic acid seem to have little effect on blood lipids, particularly cholesterol. The fat in beef is primarily tallow which has a fatty

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<sup>1</sup>Presented at the 51st Annual Meeting of The American Dietetic Association in San Francisco on October 18, 1968.

acid content as presented in Table 3. Oleic and stearic acid account for 63 percent while palmitic acid accounts for about 25 percent of the total fatty acids. Even though palmitic acid accounts for one-quarter of the total fatty acids in beef tallow, if a polyunsaturated oil is consumed in the same meal as the beef it could negate the hypercholesterolemic effect of palmitic acid.

Another point to emphasize is that many of the studies quoted were carried out on hyperlipidemic or mentally defective subjects or prisoners. The various fatty acids may exert different effects on normal, healthy, free-living subjects having a mixed diet containing a typical distribution of calories from protein, carbohydrate and fats. In fact, there is a paucity of data for what might be considered normal, free-living individuals.

Table 3. Fatty acid content of edible beef tallow.

Fatty Acid		% of Total Fatty Acids
Myristic	C12:0	2.7
Palmitic	C16:0	24.5
Stearic	C18:0	23.5
Oleic	C18:1	39.5
Linoleic	C18:2	3.6



## OBJECTIVES OF STUDY

The object of the present research was to; (1) investigate the effect of beef tallow on serum lipid patterns in healthy young men when beef tallow served as the prime source of dietary fat and provided about 40 percent of the total calories in a mixed diet; (2) compare the serum lipid response on the beef tallow diet to that observed on the same diet having an isocaloric substitution of corn oil; and (3) confirm that a high level of stearic acid in the diet is not hypercholesterolemic.

## METHODS

The purpose of the present investigation was to compare the effect of beef tallow with that of corn oil on blood lipid patterns in healthy young males. Men were maintained on a test diet having, as the sole source of dietary fat, an isocaloric substitution of beef tallow for corn oil.

### Experimental Design

The study included two 28-day metabolic trials, designated as Trial A and Trial B, conducted during September, October and November, 1971. The trials were separated by a 15-day non-experimental period. The first six days of each trial served as a stabilization period during which a standardized diet typical of that eaten by Canadians was fed. The purpose of this period was to determine individual caloric requirement, to allow the blood lipid patterns to stabilize and to provide time for the introduction of the men to the routine of the study.

The following 22 days of each trial served as the experimental period when subjects received diets containing approximately 40 percent of their total daily caloric intake as fat. In Trial A, 95 percent of the fat was provided by beef tallow whereas in Trial B, corn oil provided 95 percent of the fat.

Meals generally were served at the customary hours although attempts were made to accommodate individuals' lecture time-tables. In addition to three meals daily, the subjects received three snack items daily. Fixed recipes (Ravensdale, 1972) were followed. All food servings were standardized and were weighed or measured for each

individual.

Experimental fats were purchased in single lots and stored in sealed containers. Beef tallow, tallow margarine and corn oil margarine were stored at 7°C in a home-style electric refrigerator while the corn oil was kept at room temperature. Other staples were similarly bought in single lots and stored at appropriate temperatures for the form of the product. Fresh milk, bread and produce were purchased from a single local source. All entrees and snack items were prepared in advance and stored at -10°C until needed.

Subjects weighed-in daily before breakfast and individual caloric intake was periodically adjusted to maintain constant body weight.

Fasting venous blood samples were obtained before breakfast on Days 1, 6, 21 and 28 of each trial. Sera were removed and stored until used for chemical analysis.

Two menus were alternated daily in both the stabilization period and the experimental period. During the stabilization period, the menus were designated SM1 and SM2 whereas during the experimental period the menus were designated as FM1 and FM2. SM1 and SM2 were identical for both stabilization periods. Similarly, FM1 and FM2 were the same in each trial except for the fat source; beef tallow in Trial A and corn oil in Trial B.

The symmetrical block design of the study facilitated obtaining representative samples of all meals from each menu for each individual during both trials. The procedure was to weigh out a duplicate meal to the one consumed by the subject whose meals were being collected for that day. Daily composites were made of all meals and snacks and the composites were stored at -10°C until analyzed.

During the pre-experimental period, composites were made on two separate days for three of the subjects in Trial A and for the other three in Trial B. Single daily composites also were collected for each subject during two 5-day intervals—Days 9 - 13 and Days 24 - 28 inclusive—in both Trial A and Trial B. Thus composite menus were collected on two separate days for each of the subjects during both Trial A and Trial B.

### Subjects

Six healthy college males, aged 22 - 34 (average 25.6 years) were chosen from volunteers who responded to notices advertising the study. The subjects were selected on the basis of physical examinations, blood lipid analysis, expressed cooperativeness and availability. They were of average height and weight (Table 4) with no diagnosed metabolic disorders or recent history of poor health. The men maintained their normal activities and resided in their own homes. Any unusual activities were recorded. All were fed in the Home Economics Building, University of Manitoba. Particular emphasis was placed on the fact that no other foods were to be consumed. General instructions given to the participants are found in Appendix Table 1.

Five of the six subjects served successfully. One subject was replaced for Trial B. It was believed that the subject (M.T.), who was replaced, had not adhered to the regime and subsequent analysis confirmed this suspicion. The subjects were friendly and cooperative and ate well at all times.

### Test Fats

The test fats used in the experiment were beef tallow—bleached,

Table 4. Physical data of subjects.

Subject	Age (yr.)	Height (cm.)	Weight (Kg)		
			Initial	Trial A (Beef Tallow Diet)	Trial B (Corn Oil Diet)
C.W.	22	177	75.6	76.0 ± 0.3 <sup>1</sup>	76.4 ± 0.4
L.R.	23	183	82.5	86.0 ± 0.7	86.8 ± 0.6
P.B.	34	175	82.8	82.6 ± 0.4	83.5 ± 0.4
C.B.	27	149	63.5	64.0 ± 0.3	63.8 ± 0.6
V.M.	22	171	95.0	94.7 ± 0.4	94.1 ± 0.3
M.T. <sup>2</sup>	25	180	79.6	80.3 ± 0.8	- -
R.H. <sup>3</sup>	32	182	76.9	- -	76.8 ± 0.3

<sup>1</sup> Mean ± S.D. for 28 daily weighings.

<sup>2</sup> Participated in Trial A only.

<sup>3</sup> Participated in Trial B only.

clarified, deodorized,<sup>1</sup> a specially prepared tallow margarine,<sup>2</sup> corn oil,<sup>3</sup> and soft corn oil margarine (Fleischmann's).<sup>4</sup> Fatty acid composition of the various products was analyzed by gas-liquid chromatography.

#### Dietary Composition

The fat-controlled test diet was designed to closely resemble a typical Canadian diet but with the fat portion derived solely from one source; beef tallow or corn oil. A two-day menu rotation, FM1 and FM2 (Appendix Table 2), which provided about 40 percent of the total calories from fat, was formulated using textured soy protein (TVP)<sup>5</sup> skim milk and egg albumin as the primary protein sources. Three different physical forms of TVP was used to prepare beef stew, meatballs, pork casserole and meatloaf. Two of these entrees per day provided approximately 54 percent of the total fat intake. Addition of tallow or corn oil to cooked cereals, scrambled egg albumin, cookies and muffins provided 35 percent of the fat. The remainder of the fat was supplied by the corn oil or tallow margarine. Variety was provided by random variation of the fruit and vegetables served. Bread was included at all meals to utilize the margarine and also to permit subjects to wipe up any visible fat remaining on the serving dishes. Pickles, soy

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<sup>1</sup>Canada Packers Ltd., Winnipeg, Man.

<sup>2</sup>Canada Packers Ltd., Toronto, Ont.

<sup>3</sup>Best Foods Division of the Canada Starch Co. Ltd., Montreal, P.Q.

<sup>4</sup>Standard Brands Ltd., Winnipeg, Man.

<sup>5</sup>Archer Daniels Midland Co., 4666 Faries Parkway, Decatur, Ill. 62526.

sauce and ketchup were available.

Addition or removal of such items as sucrose, bread, potato or fruit permitted alteration in the caloric intake if subjects demonstrated weight loss or gain. A corresponding alteration in margarine intake maintained the percentage of fat calories at approximately 40 percent.

Each daily menu was designed to include all food groups and provide variety in texture and flavour. All recommended nutrient allowances were met according to the Canadian Dietary Standard.

The stabilization diet was similar to the experimental diet except that ordinary meat, whole milk and eggs, as well as the usual fats and oils (butter, mayonnaise, lard and vegetable oil shortening) were used (Appendix Table 3). As in the experimental diets, fat provided approximately 40 percent of the total daily caloric intake.

#### Meal Analysis

Daily meal composites for each individual were thawed at room temperature, weighed and homogenized with 200 - 300 ml. of distilled water in a one-gal. Waring commercial blender<sup>6</sup> (Model 50119). A 70-gm. portion of the homogenate was lyophilized in a Model 10-140BA Virtis Freeze Dryer.<sup>7</sup> Analysis of meals was completed by Ravensdale, (1972).

#### Blood Investigation

Sera collection. Fasting blood samples were drawn from the

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<sup>6</sup>Rotor Electric Co., Toronto, Ont.

<sup>7</sup>Virtis Co., Inc., Gardner, N.Y., 12525.

antecubital vein (a tourniquet being applied briefly) prior to break-fast on Days 1, 6, 21 and 28. Subjects had been standing for at least 10 minutes prior to sampling. Approximately 35 ml. of whole blood was drawn from each person into 10-ml. Vacutainer tubes (BD No. 470).<sup>8</sup> The blood was allowed to clot in a slanted position at room temperature for one hour. The clot was separated from the walls of the tube with the tip of a Pasteur pipet. The Vacutainers were then centrifuged<sup>9</sup> at 1,400 x g for 10 minutes. Five 2 - 3 ml. portions of the sera were pipetted into 10-ml. screw-top glass vials and stored at -10°C for later analysis.

Prior to analysis, sera were thawed at room temperature for a half hour. Sera from each subject were randomly analyzed in duplicate (two readings taken for each duplicate) for total cholesterol, free fatty acids, lipid phosphorous and triglyceride. A standard (Moni-trol I, Lot No. Ltd. 112 A, B)<sup>10</sup> was run for all determinations. In addition, lipoprotein patterns were determined by electrophoresis and the fatty acid composition of the lipoprotein fractions, obtained by ultracentrifugation, was determined.

#### Chemical Analysis

Total cholesterol. Total cholesterol was determined by the method described by Pearson et al. (1952). This procedure utilizes a modified Leibermann-Burchard reaction, which consists of treating the

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<sup>8</sup>Canlab Laboratory Equipment, Winnipeg, Man.

<sup>9</sup>Model HN-2368P-2, Centrifuge, International Equipment Co., Needham Hts., Mass.

<sup>10</sup>Dade Division, American Hospital Supply Corp., Miami, Fla. 33152.



serum with acetic acid, p-toluenesulfonic acid, acetic anhydride and sulfuric acid.

Free fatty acids. The method of quantitation used was Mosinger's (1965) photometric adaptation of Dole and Meinertz's (1960) micro-determination of free fatty acids. The method requires only 0.1 ml. of serum.

Lipid phosphorous. Determination of lipid phosphorous was carried out according to the procedure of Fiske and Subbarow (1925) with the following modifications. The samples were evaporated to dryness in 30-ml. micro-Kjeldahl flasks on a steam bath. They were then charred with 1.0 ml. sulfuric acid on the Kjeldahl digestion apparatus. Peroxide was added directly to the hot acid mixture to give quicker oxidation.

Serum triglyceride. Serum triglycerides were determined by the method of Van Handel and Zilversmit (1957).

Serum lipoproteins. (i) Electrophoresis. Electrophoresis of the sera was carried out according to the method of Beckering and Ellifson (1970). The cellulose acetate strips were scanned at a resolution of five on a Model 552 Densicord<sup>11</sup> densitometer fitted with filter No. 5265. The areas of the peaks were determined by means of a planimeter and the ratio of the beta- to pre-beta-lipoproteins was calculated.

(ii) Ultracentrifugation. The different lipoprotein fractions were separated by centrifugation with a Model L350 preparative centri-

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<sup>11</sup>Photovolt Corp., N.Y., N.Y.

fuge<sup>12</sup> equipped with an F30.2 rotor according to a modification of the method described by Havel et al. (1955). Two ml. of sera were pipetted into cellulose nitrate tubes to which 7.0 ml. of saline solution (final solvent density-- $P \frac{20^{\circ}}{40} - 1.063 \pm 0.005$  gm. per ml.) were added. The very low density lipoprotein fractions (VLDL) were separated by centrifuging at 27,000 rpm for 20 hours at 15°C and the top 1.0 ml. portion was removed using a Pasteur pipet and collected in a 1.0 ml. volumetric tube. This portion was then transferred to a screw-top glass vial. The low density lipoprotein fractions (LDL) were then separated by adding 1.0 ml. saline solution (final solvent density-- $P \frac{20^{\circ}}{40} - 1.019 \pm 0.005$  gm. per ml.). The LDL fraction, concentrated in the top 2.0 ml. portion, also was transferred to screw-cap vials as described for VLDL. All fractions were stored at -10°C until analyzed.

Lipid extraction from lipoprotein fractions. The saline portions containing the lipoprotein fractions separated by centrifugation were extracted using chloroform:methanol (2:1 V/V) according to Folch et al. (1957).

Fatty acid analyses. Methyl esters of the fatty acids were prepared for chromatographic analysis using the method of Barnes and Holloday (1972). The chloroform extract was evaporated to dryness under nitrogen and 1.0 ml. of 0.5 N methanolic NaOH was added. The tightly stoppered vials were heated four minutes in an 80°C waterbath. The vials were cooled; 1.0 ml. portion of  $BF_3 - CH_3OH$  was added directly to each vial and the vials reheated at 80°C for two minutes. One ml.

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<sup>12</sup>Beckman Instruments Inc., Standford Industrial Park, Palo Alto, Cal.

of saturated NaCl and 1.0 ml. of n-pentane were added to each vial and the methyl esters extracted by shaking. The pentane layer was removed, concentrated under nitrogen and injected directly into the gas chromatograph.

The fatty acid methyl esters were resolved on 2.7 m. by 2 mm. i.d. stainless steel columns packed with 10 percent EGSS-Y on 100 - 120 mesh Gas CHROM Q<sup>13</sup> using a Varian Aerograph Model 1740-1<sup>14</sup> gas chromatograph fitted with dual columns, hydrogen flame detectors, a Varian Aerograph Model 20<sup>14</sup> single pen recorder and a Varian Aerograph Model 477<sup>14</sup> digital integrator. The flow rates were 30 ml./min. for helium,<sup>15</sup> 25 ml./min. for hydrogen and 250 ml./min. for air.<sup>15</sup> The columns were operated isothermally at 200°C with injector and detector temperatures maintained at 250°C and 230°C, respectively. Identification of the fatty acid methyl esters was made by comparison with known standards.<sup>16</sup>

#### Statistical Analysis

Data from various analyses was subjected to the analysis of variance technique for a complete randomized block design as described by Snedecor and Cochran (1967). Only the values obtained for the subjects who completed the entire study were used. The Treatments sum of squares

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<sup>13</sup>Applied Science Lab. Inc., P.O. Box 440, State College, Pa. 16801.

<sup>14</sup>Varian Aerograph, 6358 Viscount Rd., Malton, Ont.

<sup>15</sup>Welder's Suppliers, 25 McPhillips St., Winnipeg 3, Man.

<sup>16</sup>Hormel Institute, Lipids Preparation Lab., 801 - 16th Ave. N.E., Austin, Minn. 55912.

was partitioned to make appropriate orthogonal comparisons to establish which of the treatments were significantly different from one another.

## RESULTS AND DISCUSSION

### Subjects

The subjects remained in good health throughout both experimental periods. There were no digestive upsets or symptomology of illness that might be attributed to the diets. Body weight remained essentially constant during the entire experiment. As a consequence, the changes in serum lipid patterns were attributed to dietary modifications and not to changes in energy balance. One subject did not serve successfully and was replaced by a seventh subject for Trial B. Their data are not included in the text and are found in Appendix Table 11.

### Dietary Fats

Data concerning the dietary fat sources used in this investigation are summarized in Tables 5, 6 and 7. Fatty acid composition of the diets (Table 5) represents the proportion of the total daily fat that was contributed by each of the fatty acids. Several criteria have been used to explain the effect of the change in dietary fats on serum lipid patterns, e.g., total dietary fat and the ratio of polyunsaturated to saturated fatty acids in the diet. On the other hand, Keys et al. (1965d) have suggested a relationship between the polyunsaturated and saturated dietary fatty acids in terms of the calories they provide. Key's group showed that, in general, long-chain saturated fatty acids are about twice as effective in increasing serum cholesterol as polyunsaturated fatty acids are in decreasing it. However, stearic acid, like the monoenoic fatty acids, was found to have no effect on serum cholesterol (Keys et al. 1958 and Grande et al. 1970). Thus fats like

Table 5. Percent fatty acid composition of diets.

Fatty Acid	Fatty Acid Composition, %			
	Diet/	Stabilization	Beef Tallow	Corn Oil
Lauric, C12:0		3.2	tr	0.8
Myristic, C14:0		6.7	2.7	0.7
Palmitic, C16:0		24.9	24.5	12.2
Palmitoleic, C16:1		3.7	3.5	tr
Stearic, C18:0		13.3	23.3	3.1
Oleic, C18:1		33.4	39.2	30.6
Linoleic, C18:2		7.3	3.9	50.5
Linolenic, C18:3		tr	tr	0.6

Table 6. Percent total daily calories contributed by glycerides of saturated, saturated minus stearic, monounsaturated and polyunsaturated fatty acids for each diet.

Diet	% Total Daily Calories from Fatty Acids				P/S	% Total Calories From Fat
	S <sup>1</sup>	S' <sup>2</sup>	M <sup>3</sup>	P <sup>4</sup>		
Stabilization	18.7	13.4	14.8	2.9	0.2	36.4
Beef Tallow	20.3	10.9	15.8	1.5	0.1	37.6
Corn Oil	6.1	5.1	12.2	20.7	3.4	39.0

<sup>1</sup> S - Total saturated fatty acids.

<sup>2</sup> S' - Total saturated fatty acids minus stearic acid.

<sup>3</sup> M - Monounsaturated fatty acids, e.g. C18:1.

<sup>4</sup> P - Total polyunsaturated fatty acids.

Table 7. Comparison of the differences among diets in the daily calories derived from glycerides of saturated, saturated minus stearic, monounsaturated and polyunsaturated fatty acids.

Diet Comparison	Differences Among Diets in Calories From Fat (% of total daily calories)				Total Fat
	S <sup>1</sup>	S'	M	P	
Beef Tallow vs Stabilization	+1.6	-2.5	+1.0	-1.4	+1.2
Corn Oil vs Stabilization	-12.6	-8.3	-2.6	+17.8	+2.6
Corn Oil vs Beef Tallow	-14.2	-5.8	-3.6	+19.2	+1.4

<sup>1</sup> See footnotes bottom Table 6.



beef tallow are not hypercholesterolemic, whereas corn oil, because of its high proportion of linoleic acid (a polyunsaturated fatty acid) relative to its saturated fatty acid content, is hypocholesterolemic.

Calculation of the percentages of the total calories contributed by the glycerides of saturated (S), saturated minus stearic acid (S'), monounsaturated (M) and polyunsaturated (P) fatty acids are presented for the stabilization and experimental diets in Table 6. The major differences in percentages of total daily calories contributed by various classes of fatty acids in the three diets are shown in Table 7. This information will be considered further in the discussions of the observed changes in serum cholesterol levels in response to the experimental diets.

The fatty acid composition of the diets differed considerably. There was about a 50 percent decrease in the levels of myristic and linoleic acids and a 55 percent increase in stearic acid associated with the change from the stabilization diet to the one containing beef tallow. The change from the stabilization to the corn oil diet resulted in 90, 76 and 50 percent decreases in myristic, stearic and palmitic acids, respectively, and an 85 percent increase in linoleic acid. Thus the tallow diet contained about twice as much palmitic acid, eight times as much stearic acid, but only one-thirteenth as much linoleic acid as the corn oil diet. Since stearic and oleic acid have been reported to have little effect on serum cholesterol levels, the main effect on serum lipid levels of the beef tallow diet as compared to the corn oil diet, would appear to be due to myristic, palmitic and linoleic acids which comprise 31.1 and 63.4 percent of the total fatty acids and provide 12.3 and 25.4 percent of the average total daily calories for beef tallow and

corn oil diets, respectively.

#### Total Serum Cholesterol

Mean serum cholesterol levels at specific times throughout the two dietary trials are presented in Table 8. Statistical analysis of the data (Appendix Tables 4 and 5) showed significant ( $P < 0.005$ ) decreases in serum cholesterol on both experimental diets, with the decreases being greater for the corn oil diet. Individual serum cholesterol levels differed as indicated by the significant F-value for People in the analysis of variance table (Appendix Table 4). In general, the relative differences among subjects remained throughout the experiment. These data agree with the observations of Keys et al. (1965c) that the average inter-individual standard deviation in serum cholesterol level is at least 20 mg. percent for free-living men fed a rigidly controlled diet. Consequently, Keys et al. (1965d) suggested it is advantageous to consider group mean values in any comparison of the effect of diet on serum cholesterol for a particular group of individuals, as grossly discrepant responses among individuals would be common for all treatments.

There was little change in serum cholesterol level in response to the stabilization diet (Day 1 vs Day 6) during either trial. Mean serum cholesterol levels rose slightly during the stabilization period in Trial A and fell slightly during Trial B, resulting in similar mean values after only six days on the stabilization diets. During the first two weeks of each experimental diet (Day 6 to Day 21 for Trial A and Trial B) mean serum cholesterol concentration decreased from about 190 mg. per 100 ml. of serum to 171 and to 142 mg. on the beef tallow

Table 8. Total serum cholesterol (mg. per 100 ml. serum) for subjects fed beef tallow and corn oil diets.

Subject	Period						Tallow vs Corn Oil	
	Stabilization		Experimental Diet		Δ Cholesterol			
	Day/	1	6	21	28	Day 28 vs Day 6		Day 28
		<u>Trial A - Beef Tallow</u>						
C.W.	127 <sup>2</sup>	135	130	118		-17		
L.R.	180	187	169	177		-18		
P.B.	208	225	207	197		-28		
C.B.	208	190	170	171		-19		
V.M.	219	216	178	189		-27		
Mean	188	191	171	171		-20		
		<u>Trial B - Corn Oil</u>						
C.W.	147	150	101	113		-37	+5	
L.R.	188	208	154	164		-44	+13	
P.B.	220	223	155	157		-66	+40	
C.B.	183	168	140	140		-28	+31	
V.M.	222	200	154	137		-63	+52	
Mean	192	190	141	142		-48	+29	

<sup>1</sup> Mean square error (MSE) = 434.97

<sup>2</sup> Mean of duplicate readings for only Day 1 Trial A  
All other values are means of two duplicate analyses.

and corn oil diets, respectively (Figure 2). There was a much greater decrease on the corn oil diet, 48 mg. percent than on the beef tallow diet, 20 mg. percent. The difference in the decrease of serum cholesterol on the two diets was significant ( $P < 0.005$ ) as indicated by the Diet x Day interaction in the analysis of variance table (Appendix Table 4). The data presented in Figure 2 illustrate how rapidly serum cholesterol levels responded to a change in diet. A shift from the stabilization diet to the experimental diets (Day 6 vs Day 21) was accompanied by a marked change in serum cholesterol levels. Similarly, when the subjects returned to a free-choice diet for the 15 days between trials, cholesterol level rose from a value of 170 mg. percent at the end of Trial A (tallow diet) to 192 mg. percent at the start of Trial B.

However, serum cholesterol values appeared to reach a stable plateau by the fourteenth day (Day 21) irrespective of the experimental diets, as there was little change from Day 21 to Day 28. These results coincide with those of Keys *et al.* (1957) who concluded that the major changes in serum cholesterol level, following a change in dietary fat, takes place within the first week. By the end of the second week, a plateau is reached and no further significant changes are observed within the next one to two months. Unfortunately, serum cholesterol values were not measured at the end of the first week on the experimental diets in the present study so the pattern of response during the first 14 days on the two diets is not available.

It is interesting to note that subjects who had higher initial serum cholesterol levels showed a greater magnitude of change in serum cholesterol response to the diets than those who had lower initial

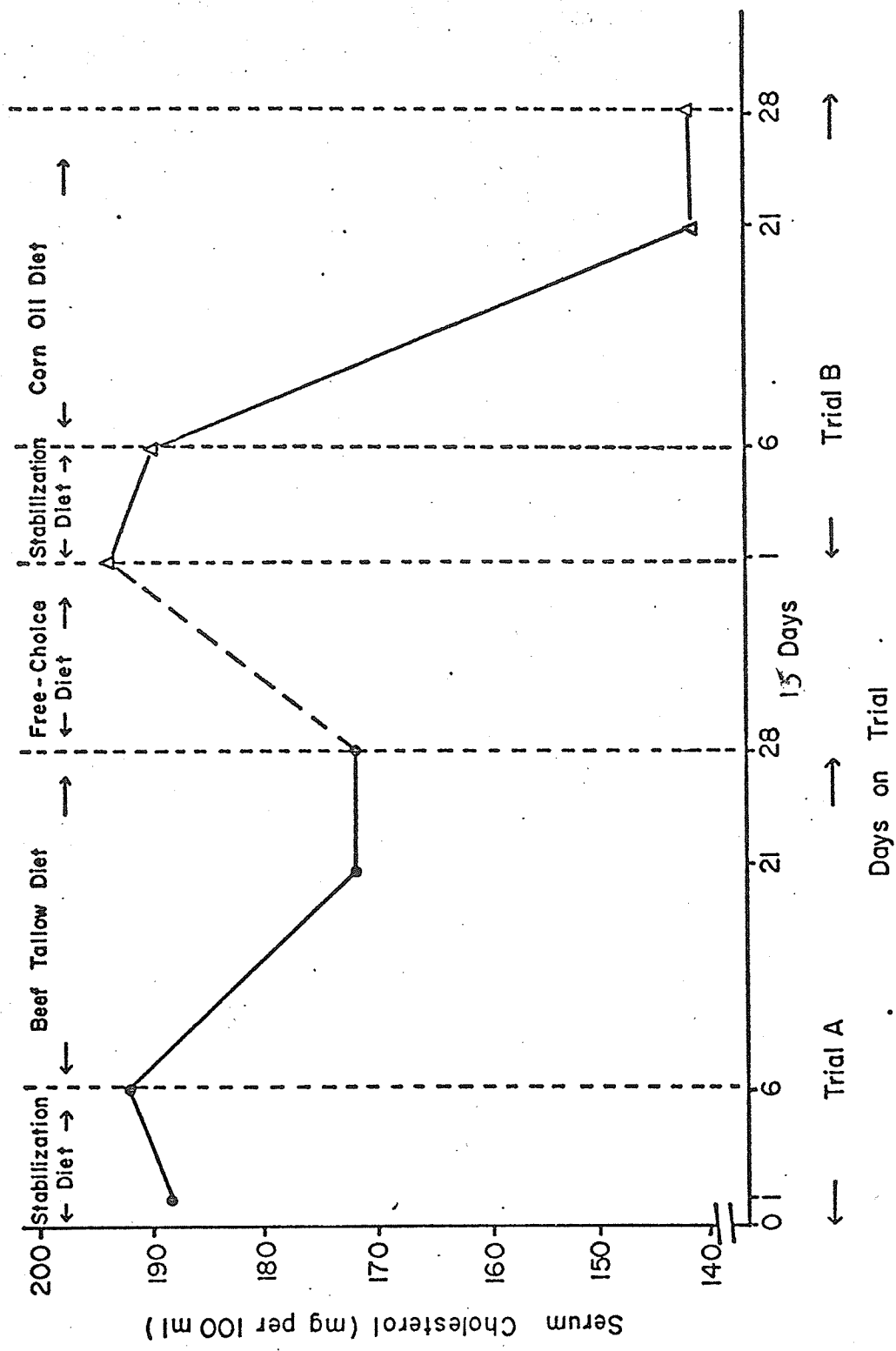


Figure 2. Average serum cholesterol levels of subjects receiving stabilization and experimental diets.

levels (Table 8).

The change from the stabilization diet to the experimental diets involved an alteration in dietary fat source as well as a change from an animal protein to a primarily vegetable protein diet. The transfer from the stabilization diet to the tallow diet also involved a shift from a diet containing normal cholesterol levels to one low in cholesterol. The change from the stabilization to the corn oil diet involved a similar marked decrease in dietary cholesterol but the corn oil diet contained a relatively high level of plant sterols compared to the beef tallow diet.

Anderson et al. (1971) found that the source (animal vs vegetable) of dietary protein did not significantly alter serum cholesterol levels. On the other hand, Olson et al. (1964, 1970) have found that glutamate, the major component of most vegetable proteins, can cause lower serum cholesterol levels although Bazzano and Olson (1969) have disputed the effect of glutamate on serum cholesterol levels.

Keys et al. (1965d) suggested the primary response of serum cholesterol to changes in dietary fat is related to the percent of dietary calories contributed by the different groups of fatty acids. They have proposed a simplified form of a more elaborate multiple regression equation for predicting the change in serum cholesterol in response to a specific change in dietary fatty acids. This simplified equation

$$(1) \Delta \text{ serum cholesterol, mg. percent} = 1.2 (2\Delta S' - \Delta P)$$

suggests that the change in serum cholesterol level is dependent on the changes in the dietary levels of C12 to C16 saturated fatty acids, relative to the changes in the polyunsaturated fatty acids, both expressed as percent of the total calorie intake. It should be noted that stearic acid, a major component of beef tallow is not included

among the saturated fatty acids in this equation (Eq. 1).

The observed changes in serum cholesterol levels in response to the two experimental diets are compared with the predicted changes on the basis of Keys et al. (1965d) formula in Table 9. The observed change in serum cholesterol in response to the beef tallow diet was 20 mg. percent whereas on the basis of equation (1) the predicted value was only 4 mg. percent. With the corn oil diet, the observed change was 48 mg. percent whereas the predicted change was 41 mg. percent. In fact, the slightly higher observed value as compared to the predicted value for corn oil has been observed by Grande et al. (1958). However, the predicted values do not coincide with the observed values for beef tallow. Thus other dietary modifications such as level of cholesterol, source of protein, etc. might be of importance in accounting for discrepancies between the observed and predicted responses of serum cholesterol for beef tallow.

Mattson et al. (1972) have recently suggested that dietary cholesterol is an important determinant of serum cholesterol level. The analyzed cholesterol content of the stabilization beef tallow and corn oil diets were 320, 98 and 0 mg. per 1000 Kcal, respectively. The expected change in serum cholesterol levels as a result of changes in dietary cholesterol levels also was calculated using equations proposed by both Keys et al. (1965b) and Mattson et al. (1972) (Table 9). On the basis of Keys' et al. (1965b) equation, one would expect a change in serum cholesterol level of 12 mg. percent accompanying the change from the stabilization to the beef tallow diet and a change of 27 mg. percent in the change to the corn oil diet. Calculations based on the equation derived by Mattson et al. (1972) resulted in considerably higher

Table 9. Observed and predicted changes in serum cholesterol levels (mg. per 100 ml.) of subjects fed tallow and corn oil diets.

Diet Comparison	Observed Decrease	Predicted Decreases in Serum Cholesterol		
		E (1) <sup>1</sup>	E (2) <sup>2</sup>	E (3) <sup>3</sup> (E 1 + Eg 2)
Beef Tallow vs Stabilization	20	4	12	28
Corn Oil vs Stabilization	48	41	27	39
Expected Difference Corn Oil vs Beef Tallow	29	37	15	13
				52

<sup>1</sup>  $\Delta$  serum cholesterol =  $1.2 (2\Delta S' - 4P)$ ; Keys et al. (1965d) on the basis of changes in dietary fatty acids (Table 5)

<sup>2</sup>  $\Delta$  serum cholesterol =  $1.5 (Z_2^{0.5} - Z_1^{0.5})$ ; Keys et al. (1965b) where Z is level of dietary cholesterol

<sup>3</sup>  $\Delta$  serum cholesterol =  $1.6 + 0.118 (Z_2 - Z_1)$ ; Mattson et al. (1972) where Z is the level of dietary cholesterol



predicted decreases for both experimental diets than those obtained by using the equation of Keys et al. (1965b). However, the expected difference between the two diets was the same with both equations.

Perhaps worthy of note at this point is the fact that Mattson et al. (1972), in formulating their equation (Eq. 3) (Table 9), misinterpreted Keys' et al. (1965b) equation in that they presented equation 2 as being

$$\Delta \text{ serum cholesterol} = 1.5(Z_2 - Z_1)^{0.5}$$

instead of

$$\Delta \text{ serum cholesterol} = 1.5(Z_2^{0.5} - Z_1^{0.5})$$

where Z is the level of dietary cholesterol in mg. per 1000 Kcal.

Since Mattson's et al. (1972) equation was derived by recording the increase in serum cholesterol levels when subjects were changed from an essentially cholesterol-free diet to one containing graded levels of cholesterol, the question arises as to whether similar decreases would occur if cholesterol was removed from the diets of subjects receiving normal dietary cholesterol levels. As discussed in the review of literature, a regression equation is really descriptive of only a particular set of data and may not be applicable to all circumstances.

The experimental design employed by Keys and associates (1965b) in deriving their equation (Eq. 2) was similar to that of the present study which may explain why the predicted changes in serum cholesterol level on the basis of Eq. 2 seem more applicable to the data under consideration.

If the change in serum cholesterol in response to alterations in dietary fatty acids and cholesterol are directly additive, and if there is no interaction, one would expect a change of 16 and 68 mg.

percent for beef tallow and corn oil diets, respectively, on the basis of a combination of equations (Eq.1 and Eq.2). Although the predicted values for the change from stabilization to the beef tallow diet agree well with the observed value, it will be recalled that the observed value and predicted value on the basis of only equation (1), agreed reasonably well for the corn oil diet. The predicted value for the change in serum cholesterol on the beef tallow diet agrees with Hegsted's et al. (1965) suggestion that changes in serum cholesterol as a consequence of changes in dietary cholesterol are independent of the fatty acid composition of the diets. The question arises as to whether the prediction equations, such as those used in the above calculations, are valid or whether there is something peculiar associated with the change in serum cholesterol level in response to the corn oil diet.

Nevertheless, the results of the present investigation agree with Grande et al. (1972), McGandy et al. (1970) and Hegsted et al. (1965) that there is no hypercholesterolemic effect of beef tallow when fed at 40 percent of the total calorie intake. In fact, beef tallow appears to have a hypercholesterolemic effect. Whether this effect is due to the spatial configuration of stearic acid on the triglyceride molecule as suggested by McGandy et al. (1970) or due to stearic acid per se as suggested by Grande et al. (1970) remains to be resolved.

Moreover, the results of the present experiment cast considerable question on the popular recommendation that hypercholesterolemics restrict their beef intake to three 3-oz. servings weekly.

#### Serum Triglycerides

Serum triglyceride levels (Table 10), which were observed at the same time as serum cholesterol, were unaffected by the dietary regimen

Table 10. Serum triglyceride (mg.per 100 ml.) response of subjects fed beef tallow and corn oil diets.

Subject	Period						Day 28 vs Day 6	Day 28	Tallow vs Corn Oil
	Stabilization		Experimental Diet		Triglyceride				
	1	6	21	28	Day 28 vs Day 6	Day 28			
	<u>Trial A - Beef Tallow</u>								
C.W.	73 <sup>2</sup>	72	63	71		- 1			
L.R.		44	40	33	33	- 7			
P.B.		163	91	107	114	+23			
C.B.		51	42	39	37	- 5			
V.M.		124	119	112	108	-11			
Mean		91	73	71	73	0			
	<u>Trial B - Corn Oil</u>								
C.W.	94	65	47	42		-23		+29	
L.R.	95	55	47	50		- 5		-17	
P.B.	109	90	75	73		-17		+41	
C.B.	75	72	69	51		-21		-14	
V.M.	124	93	78	72		-21		+36	
Mean	99	75	63	55		-20		+18	

<sup>1</sup> MSE = 1,169.02

<sup>2</sup> Mean of two duplicate sets of analyses.

except for the significant ( $P < 0.005$ ) decrease during the stabilization period in both trials (Appendix Tables 6 and 7). As for serum cholesterol levels, considerable variation in serum triglyceride levels was found among subjects. In fact, the variation in serum triglyceride levels among subjects was significant ( $P < 0.005$ ) and in general, this variability was greater than that observed for serum cholesterol. Hegsted et al. (1965) and Turpeinen et al. (1968) also noted that serum triglyceride levels were characterized by a greater coefficient of variation, about 47 percent, than the 21 percent coefficient of variation observed for serum cholesterol levels.

A considerable portion of the variation among subjects was attributable to one subject, P.B., who had elevated serum triglyceride levels at the beginning of the first trial. Serum triglyceride levels for this individual decreased markedly during the stabilization period of Trial A and then increased during the feeding of the beef tallow diet, whereas all other subjects showed no change or a slight decrease during Trial A. Part of the variation associated with subject P.B. may be due to age, in that P.B. was 34 years old as compared to a mean age of 23.6 years for the other subjects (Table 4). Shilling et al. (1969) reported that serum triglyceride levels reach a maximum level at about 34 years of age. The pattern of response for subject P.B. in Trial A, a decrease in serum triglyceride from 163 to 91 mg. percent during the stabilization period followed by a gradual increase from 91 to 114 mg. percent on the beef tallow diet, suggests that there may have been an "overshoot" in the response of the serum triglyceride level to the stabilization diet. This possibility of an "overshoot" is sustained by the fact that his serum triglyceride response in Trial B followed

similar trends to those of the other subjects.

The apparent age-diet interreaction for serum triglyceride levels may explain why many investigators find serum triglyceride response so variable and difficult to interpret. In spite of the variation among individuals, there was a very appreciable decrease in serum triglyceride levels during the stabilization period (Day 1 to Day 6) for both Trials A and B (Table 10 and Figure 3). In fact, the only significant change in serum triglyceride levels in response to the dietary treatments was the decrease during the stabilization period as evidenced by the significant F-value for orthogonal comparisons of Day 1 vs other days (Appendix Tables 6 and 7). As in the case of serum cholesterol, serum triglyceride levels tended to stabilize at this decreased level on the beef tallow diet. However, there was a trend for the serum triglyceride values to decrease on the corn oil diet (Figure 3). Also of interest, is the fact that mean serum cholesterol and triglyceride levels reached almost identical values at the end of the stabilization period in both trials. The trend toward decreased serum triglyceride levels on the corn oil diet suggests that saturated fatty acids, which do not affect serum cholesterol levels, affect serum triglyceride levels. The results of the corn oil diet agree with the suggestion of Grande *et al.* (1972), that saturated fatty acids with fewer than 12 carbon atoms and stearic acid, which do not affect serum cholesterol concentrations, produce elevations of serum triglycerides.

The primary difference between the stabilization and the corn oil diet was that the corn oil contained 10 percent units less stearic acid, as well as six and 12 percent units less of myristic and palmitic acids, respectively. In fact, these differences are greater than those that can be calculated from the analyzed data herein reported because 46 percent

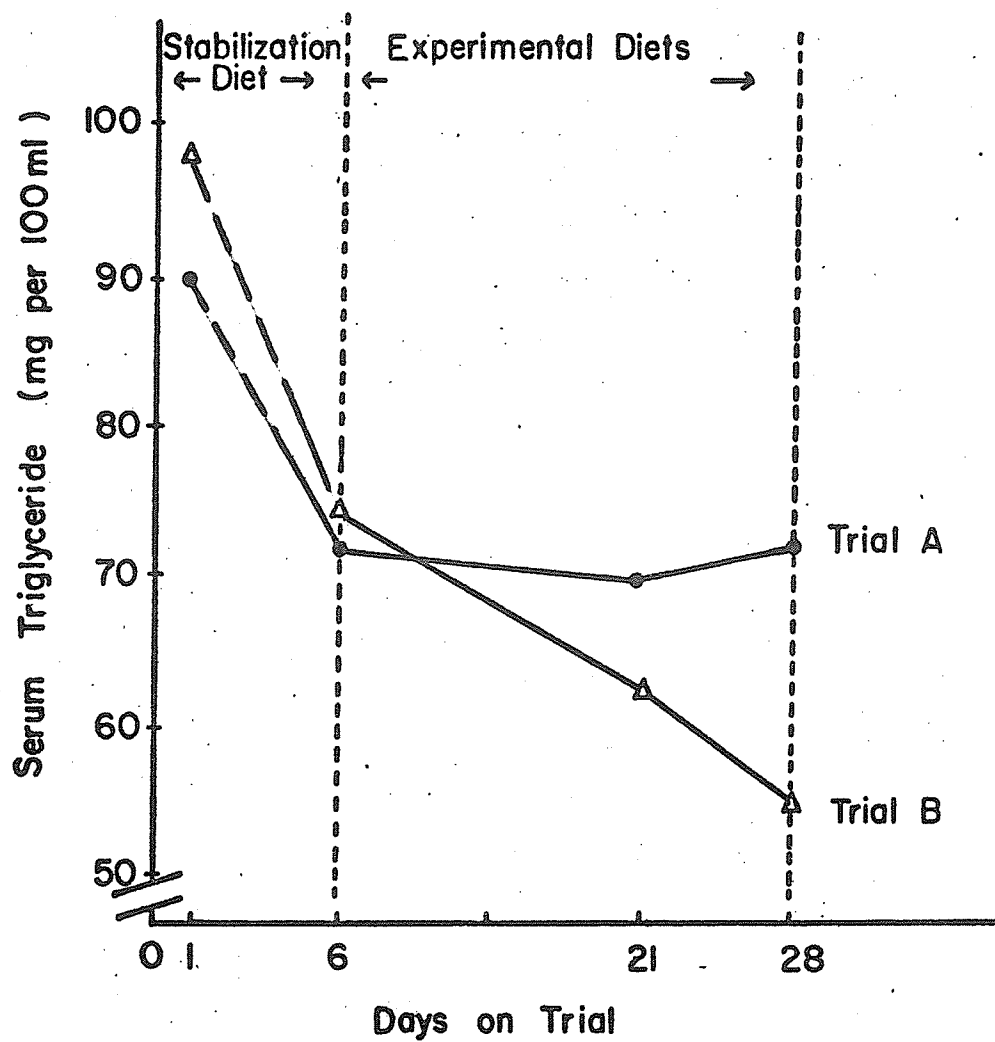


Figure 3. Average serum triglyceride level of subjects receiving stabilization and test diets; ●—● Trial A (beef tallow) and ▲—▲ Trial B (corn oil).

of the fat calories of the stabilization diet were supplied by butter fat and the analysis procedure used did not resolve the short- and medium-chain triglycerides. The failure to observe a response on the beef tallow diet is probably due to the slight difference in the change from the stabilization to the beef tallow diet in the level of short- and medium-chain triglycerides and stearic acid.

However, no linear relationship has been established between levels of dietary stearic acid and serum triglyceride concentration. If there is a relationship, it is not unlikely that it may be confounded by other variables such as age, weight and other constituents in the diet. In fact, Anderson et al. (1971) found greater serum triglyceride levels in men having animal protein as compared to vegetable protein in their diets, though this difference was not significant. There is no evidence however, of a relationship between serum triglyceride levels and dietary sterols.

Although an increase in serum triglyceride levels appears to be associated with coronary heart disease, Carlson et al. (1972) found that this association is independent of and additive to the risk associated with elevated serum cholesterol levels. Furthermore, Kannel et al. (1971) suggest that an elevation of endogenous triglyceride in the serum is associated with an increased risk of coronary disease only if accompanied by an increased cholesterol value. Moreover, dietary fat has not been found to be of great importance in treating hypertriglyceridemia of the disease types described by Frederickson et al. (1970) except for the rare Type I, where there is very poor clearance of exogenous triglyceride resulting in an enormous increase in the fasting concentration of chylomicrons.

On the basis of the results of the present investigation, there is no suggestion that beef tallow, when fed at 40 percent of the total daily calories, is associated with increased serum triglyceride levels.

#### Lipid Phosphorous

Table 11 presents the responses in serum lipid phosphorous to the beef tallow and corn oil diets. There was a significant variation ( $P < 0.005$ ) among individuals which is analagous to that observed for serum cholesterol. These observations agree with Turpeinen et al. (1968) who reported coefficients of variation for lipid phosphorous and cholesterol of 22 and 21 percent, respectively. Furthermore, statistical analysis of the data (Appendix Tables 8 and 9) showed lipid phosphorous response to be similar to that of serum cholesterol.

Figure 4 illustrates graphically the serum lipid phosphorous response to the diets. Both initial mean serum lipid phosphorous levels, 8.13 and 7.62 mg. percent for Trial A and Trial B, respectively decreased about 0.41 mg. percent during the 6-day stabilization periods. This contrasts to both serum cholesterol and triglyceride, in that, initial Day 1 values, despite differences in magnitude would each come to identical values for Day 6.

During the first 14 days on the experimental diets, there was a significant decrease ( $P < 0.005$ ) of 0.81 mg. percent in both the beef tallow and corn oil diets. This response seemed to parallel to that of serum cholesterol except that the magnitude was much less. Similar findings were reported by Hegsted et al. (1965) and Grande et al. (1972). However, during the third week on the experimental diets, the serum lipid phosphorous reached a plateau at about 6.98 to 6.99 mg. percent on the



Table 11. Serum lipid phosphorous (mg.per 100 ml.) levels of subjects fed tallow and corn oil diets.

Subject	Period						Δ Phospholipid	
	Stabilization		Experimental Diet		Tallow vs Corn Oil		Day 28 vs Day 6	Day 28
	Day/ 1	6	21	28	Day 28 vs Day 6	Day 28		
	<u>Trial A - Beef Tallow</u>							
C.W.	6.46 <sup>2</sup>	5.25	5.15	4.97			-0.26	
L.R.	7.15	8.75	6.75	6.73			-2.02	
P.B.	10.73	9.80	8.67	9.02			-0.78	
C.B.	8.57	6.64	6.72	6.45			-0.19	
V.M.	7.75	8.12	7.63	7.76			-0.36	
Mean	8.13	7.71	6.98	6.99			-0.72	
	<u>Trial B - Corn Oil</u>							
C.W.	6.63	5.41	5.31	5.32			-0.09	-0.35
L.R.	7.47	7.78	6.88	6.63			-1.15	+0.10
P.B.	9.90	8.78	7.08	7.16			-1.62	+1.86
C.B.	6.69	7.13	6.43	5.03			-2.10	+1.42
V.M.	7.43	6.91	6.24	6.03			-0.88	+1.73
Mean	7.62	7.20	6.39	6.03			-1.17	+0.96

<sup>1</sup> MSE = 1.56

<sup>2</sup> Mean of two duplicate sets of analyses.

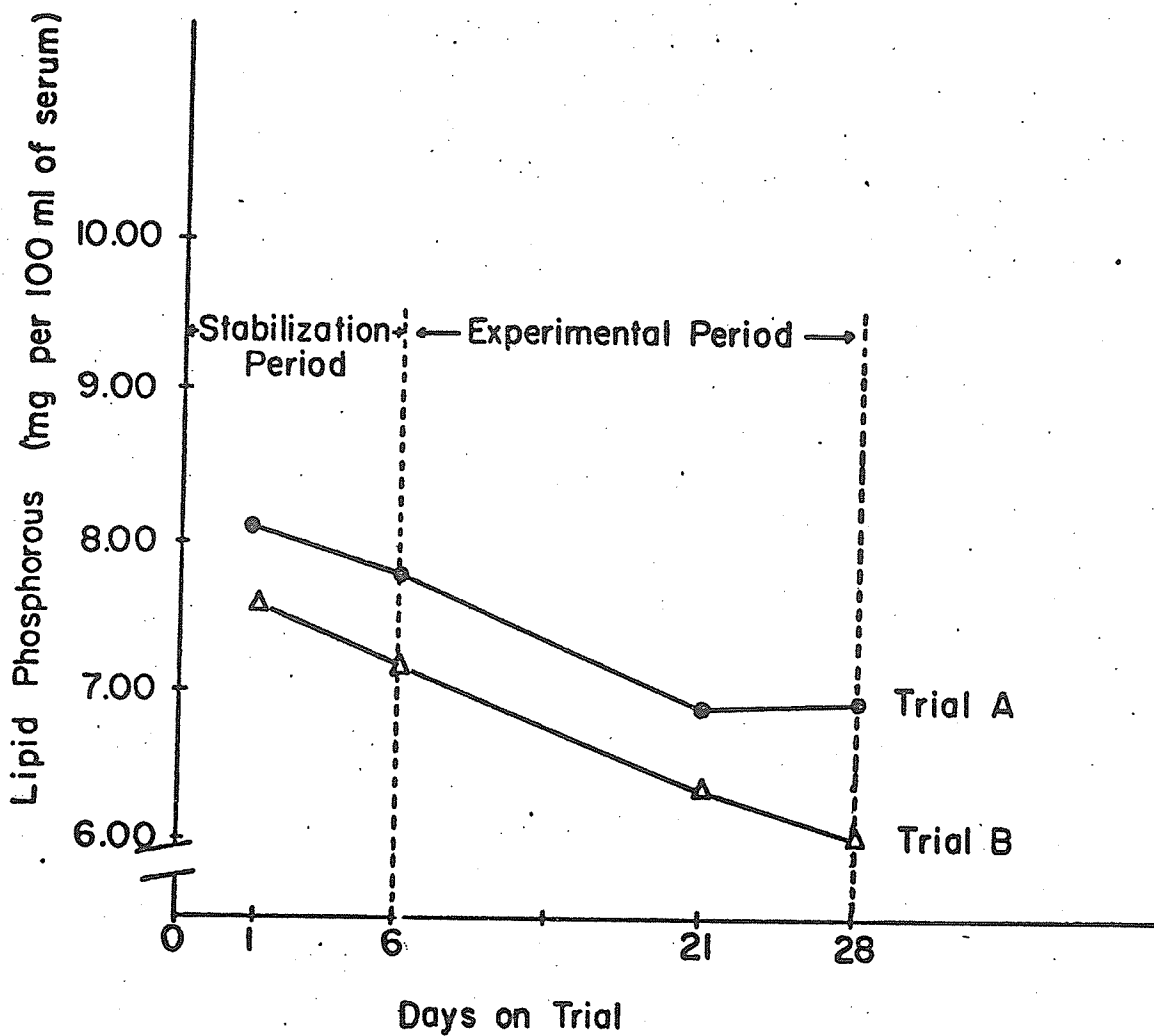


Figure 4. Average lipid phosphorous level of subjects receiving stabilization and test diets;  $\bullet$ — $\bullet$  Trial A (beef tallow) and  $\Delta$ — $\Delta$  Trial B (corn oil).

beef tallow diet, whereas the level continued to decrease from 6.39 to 6.03 mg. percent on the corn oil diet. From examination of the orthogonal comparisons (Appendix Table 9), it appears that the primary difference between the beef tallow and corn oil diets is the decrease from Day 21 to Day 28 on the corn oil diet.

Serum lipid phosphorous response to dietary fat alteration seems to follow that observed for serum cholesterol but is of a lesser magnitude and somewhat slower. This is evidenced by the fact that the lipid phosphorous value for Day 1<sup>-</sup> of Trial B had not returned to the original level of Trial A during the 15 days between trials and that lipid phosphorous continued to decrease during the entire corn oil diet.

Nevertheless, there was a decrease in lipid phosphorous as with serum cholesterol but whether this is of any importance in lipidemic disorders or diseases associated with blood lipid patterns is questionable. Levy (1969) states that his group has never felt that measurement of serum phospholipids has contributed any additional information on the effect of dietary change on blood lipids. Connor (1969) agrees with Levy (1969) but feels measurement of phospholipid is useful because it reinforces the analysis of the data for serum cholesterol since lipid phosphorous responds in a similar manner.

Results of the present experiment agree with Connor (1969), Erickson et al. (1964), McGandy et al. (1970) that the lipid phosphorous response to changes in dietary components is similar to that of serum cholesterol. Results of this study suggest the pattern of response may not be identical to serum cholesterol as it seems slower and lesser in magnitude.

### Free Fatty Acids

Neither the stabilization nor the experimental diets had any effect on serum free fatty acid concentrations (Table 12 and Appendix Table 10.) Dole (1956) similarly found no change in fasting plasma free fatty acids when corn oil was fed in amounts sufficient to cause gross lipemia. In fact, Gordon and Cherkes (1956) have indicated that lipemia per se cannot be correlated with free fatty acid levels. The concentration of free fatty acid rises only in relation to the need for fat as an energy-yielding substrate for oxidative catabolism.

As with other blood lipid parameters, there was considerable variation among subjects (F-value for People significant,  $P < 0.005$ ; Appendix Table 10). Furthermore, there was also considerable variability among duplicate analyses using this method. However, there was no suggestion from the present study that dietary fat source has any apparent effect on serum free fatty acids when subjects are fed isocaloric diets where fat provides approximately 40 percent of the calories.

### Serum Lipoproteins

If we assume that the primary defect in coronary heart disease is the lipoprotein transport vehicle rather than the lipids it carries, the risk factor should be more closely related to the lipoprotein fractions than to the lipid content of the serum. It has been shown (Frederickson *et al.*, 1967) that lipoprotein patterns in certain genetic hyperlipidemic disorders respond to dietary treatment. However, little is known about the response of lipoproteins to dietary manipulation in the normal individual. Since the change in lipoprotein patterns should be similar to the change in serum cholesterol and triglyceride levels, it was thought

Table 12. Serum free fatty acid levels (mg. per 100 ml.)<sup>1</sup> of subjects fed beef tallow and corn oil diets.

Subject	Period						Free Fatty Acids	
	Stabilization		Experimental Diet		Tallow vs Corn Oil		Day 28 vs Day 6	Day 28
	1	6	21	28	Day 28 vs Day 6	Day 28		
	Trial A - Beef Tallow							
C.W.	22.8 <sup>2</sup>	23.6	22.1	22.1			-1.5	
I.R.	25.9	23.9	25.1	25.5			+1.6	
P.B.	24.6	24.1	23.4	24.9			+0.8	
C.B.	24.6	23.4	25.8	26.9			+3.5	
V.M.	26.7	25.9	25.1	25.7			-0.2	
Mean	24.9	24.2	24.3	25.0			+0.8	
	Trial B - Corn Oil							
C.W.	23.6	24.2	24.0	23.3			-0.9	-1.2
L.R.	22.0	21.3	23.1	23.6			+2.3	+1.9
P.B.	23.7	24.8	24.1	27.4			+2.6	-2.5
C.B.	24.4	24.2	26.0	26.5			+2.3	+0.4
V.M.	26.2	26.2	26.4	25.8			-0.4	-0.1
Mean	24.0	24.2	24.7	25.3			+1.1	-0.3

<sup>1</sup> MSE = 5.49<sup>2</sup> Mean of two duplicate sets of analyses.

of interest to study the response of the lipid transport vehicles in the present investigation.

(i) Electrophoresis of serum lipoproteins. Serum lipoproteins were separated on cellulose acetate strips and the relative amounts of each fraction determined from densitometer scans of the electrophoretic strips rather than by visual inspection (which is the usual method in diagnosing familial hyperlipidemic disorders). Only the beta-/ pre-beta-lipoprotein ratios have been reported because these moieties are associated with cholesterol and triglyceride transport in the blood. The results obtained by this procedure, however, must be interpreted with some reservation because satisfactory methods have not been developed for the quantitation of serum lipoproteins. Problems in quantitation stem from the lack of uniformity in the uptake of stain by the lipoproteins and the lack of satisfactory lipoprotein standards against which electrophoregrams of unknown samples can be compared. In addition, sera used in the present study had been frozen and stored. Freezing causes considerable tailing of the electrophoretic bands, which in turn alters the resolution among the various fractions, particularly the beta-/pre-beta-lipoprotein moieties. A representative cellulose acetate strip and the densitometer scan of this electrophoregram showing tailing of the beta-band is presented in Figure 5.

As observed for serum cholesterol, the beta-/pre-beta-lipoprotein ratio varies appreciably among subjects (Table 13). Changes in the ratios for the individual subjects were highly variable during the stabilization period of Trial A whereas a consistent decrease was observed during the stabilization period in Trial B. During the first two weeks on both experimental diets, the ratio decreased with the decrease on the

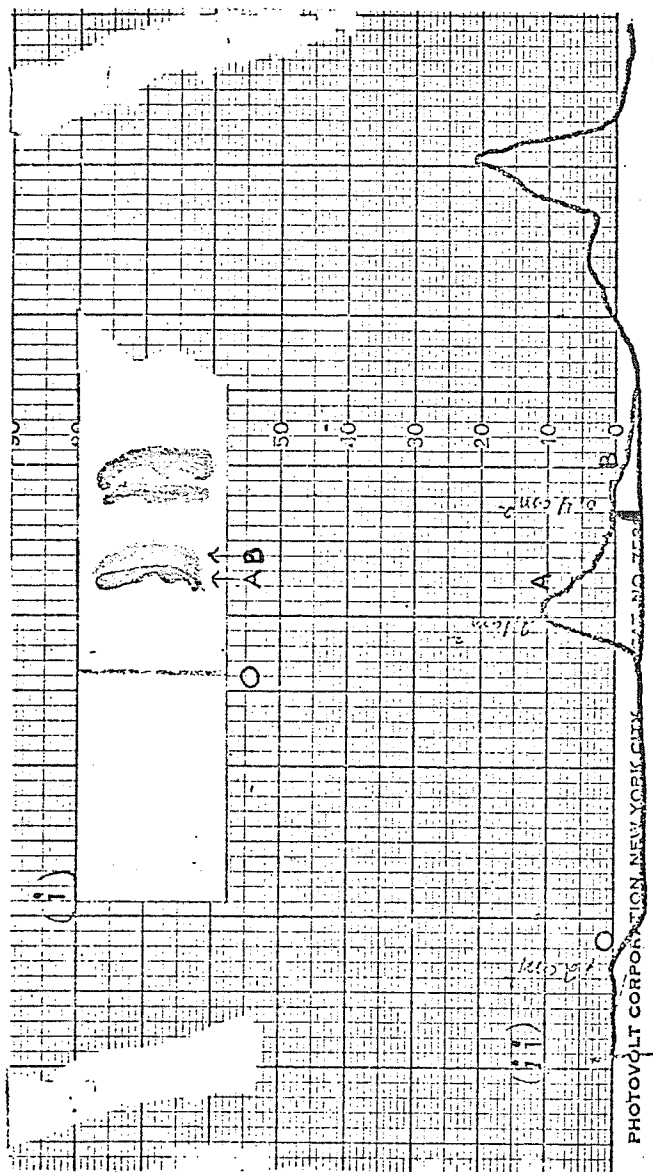


Figure 5. (i) Electrophoretic pattern of serum lipoproteins for subject C.W., Trial A (tallow), Day 28. Where: O is the origin; A is the beta-lipoprotein fraction and B is the pre-beta-lipoprotein fraction.  
(ii) Densitometer scan of (i).

Table 13. Beta- pre-beta-lipoprotein ratios of subjects fed beef tallow and corn oil diets.

Subject	Period				Δ Beta- / Pre-Beta Lipoprotein Ratio	
	Stabilization		Experimental Diet		Day 28 vs Day 6	Tallow vs Corn Oil Day 28
	Day/ 1	6	21	28		
	<u>Trial A - Beef Tallow</u>					
C.W.	11.0 <sup>1</sup>	17.0	15.0	13.0	-4.0	
L.R.	10.6	6.0	4.6	6.5	+0.5	
P.B.	6.7	2.7	2.0	1.8	-0.9	
C.B.	2.2	4.0	2.6	5.3	+1.3	
V.M.	2.8	2.0	3.0	1.6	-0.4	
Mean	5.9	6.3	5.4	5.6	-0.9	
	<u>Trial B - Corn Oil</u>					
C.W.	15.0	12.0	3.3	4.5	-7.5	+8.5
L.R.	6.5	5.0	4.0	4.0	-1.0	+2.5
P.B.	3.5	2.8	2.3	1.7	-1.1	+0.1
C.B.	4.4	3.0	3.0	3.3	+0.3	+2.0
V.M.	7.0	2.6	1.8	m <sup>2</sup>	-	-
Mean	7.3	5.1	2.9	2.7	-2.4	+2.9

<sup>1</sup> Ratio of areas for beta- and pre-beta-lipoprotein determined from densitometer scans of electrophoretic strips.

<sup>2</sup> m = missing value.



corn oil diet (2.2) being more than twice that observed for the beef tallow diet (0.9). These observations tend to parallel the response in serum cholesterol; serum cholesterol dropped 48 mg. percent on the corn oil compared to a decrease of 20 mg. percent on the beef tallow diet. Furthermore, there was little change in the serum triglyceride levels on the beef tallow diet, whereas triglyceride levels decreased on the corn oil diet (12 mg. percent). The beta-/pre-beta-lipoprotein ratios appeared to reach a fairly stable plateau during the third week on each test diet. Again, as with serum cholesterol, the ratios seemed to respond quickly to changes in dietary fat and then stabilize at the new level.

Since the beta-/pre-beta-lipoprotein ratios varied for subjects within trials as well as between trials, the question arises as to whether there was a change in the fatty acid composition of the triglycerides of the serum. Changes in the ratios are a consequence of an elevation in the very low density lipoprotein fraction (pre-beta-lipoprotein) or a decrease in the low density lipoprotein (beta-lipoprotein) or both. Thus, the percent changes in saturated and unsaturated fatty acids in response to the dietary treatments were investigated.

(ii) Ultracentrifugation of serum lipoproteins. Table 14 represents a summary of the main changes observed in the levels of saturated and unsaturated fatty acids in both the very low density (VLDL) and low density lipoprotein (LDL) fractions. No data are available for subject V.M. and there were missing observations for several other subjects. Lack of sufficient fraction, i.e., VLDL and LDL made it impossible to carry out satisfactory fatty acid analysis on these samples. Because of the limitations associated with sample size and

Table 14. Mean response of fatty acids of the very low and low density lipoprotein fractions in subjects fed beef tallow and corn oil diets.

Lipoprotein Fraction	Fatty Acid Class, %	Period						Fatty Acids, % Day 28 vs Day 6	Tallow vs Corp Oil Day 28
		Stabilization		Experimental Diet		Fatty Acids, %			
		Day/ 1	6	21	28	Day 28 vs Day 6	Day 28 vs Day 6	Day 28	
Trial A - Beef Tallow									
Very Low Density	Saturated <sup>1</sup>	43.0 <sup>3</sup>	38.1	53.4	46.2		+ 8.1		
	Unsaturated <sup>2</sup>	57.0	61.4	46.5	53.8		- 7.6		
Low Density	Saturated	40.1	46.7	50.3	59.2		+12.5		
	Unsaturated	59.9	53.3	49.7	40.8		-12.5		
Trial B - Corn Oil									
Very Low Density	Saturated	42.1	42.8	45.8	39.6		- 3.2	+ 6.6	
	Unsaturated	57.4	57.1	54.2	60.4		+ 3.3	- 6.6	
Low Density	Saturated	26.9	56.2	58.8	45.9		-10.3	+13.3	
	Unsaturated	73.1	43.8	41.2	54.1		+10.3	-13.3	

<sup>1</sup> Total percent saturated fatty acids: C16:0 and C18:0

<sup>2</sup> Total percent unsaturated fatty acids: C16:1, C18:1 and C18:2

<sup>3</sup> Mean for two subjects for Day 1 only. Mean for four subjects on all other days.

methodology, caution must be exercised in discussing the observations in relation to alterations in dietary fatty acids.

The fatty acid composition of both the VLDL and the LDL appeared to be responsive to changes in dietary fatty acids. In general, the percent saturated fatty acids for both the VLDL and the LDL tended to increase, while the unsaturated fatty acids tended to decrease on the beef tallow diet. However, the reverse pattern prevailed on the corn oil diet. The trend seemed to be more consistent and more pronounced for the LDL. This may be due to a slower turnover rate of the fatty acids of the LDL fraction.

Thus, the results of this study suggest that a saturated fat, such as beef tallow, when fed at approximately 40 percent of the total calories, produces an increase in the percent saturated fatty acids of both the VLDL and LDL fractions.

#### General Discussion

The present study indicates that substitution of a standardized Canadian diet by a mixed diet in which beef tallow and corn oil served as the *prime* sources of dietary fat resulted in a significant ( $P < 0.005$ ) decrease in serum cholesterol levels of healthy young men. The decrease in serum cholesterol level on the beef tallow diet was only one-half that observed on the corn oil diet; approximately 190 mg. percent on the stabilization diet to 170 and 140 mg. percent after three weeks on the beef tallow and corn oil diets, respectively. The decrease occurred within the first two weeks on both test diets with no further decrease in the third week. Similarly, there was a significant ( $P < 0.005$ ) decrease in lipid phosphorous levels, with the pattern of response being the same as serum cholesterol levels on the beef tallow diet but with a

continued decrease into the third week on the corn oil diet. For both experimental diets, the lipid phosphorous response was slower and of a lesser magnitude than that of serum cholesterol. Keys et al. (1965d) and Hegsted et al. (1965) have reported similar findings in serum cholesterol and lipid phosphorous levels in response to diets containing beef tallow. However, the observed decrease in serum cholesterol levels was greater for the beef tallow diet than that which would have been expected on the basis of Keys' et al. (1965b, d) equations for predicting changes in serum cholesterol. On the other hand, the decrease on the corn oil diet, although slightly greater than predicted (Eq. 1), coincided with the observation reported by the Minnesota group (Keys et al., 1965d) who suggested discrepancies between observed and predicted changes in serum cholesterol levels with corn oil are associated with the relatively high plant sterol content of corn oil.

Serum triglyceride levels, on the other hand, showed no change on the beef tallow diet whereas a significant ( $P < 0.10$ ) decrease was observed on the corn oil diet; from approximately 75 to 55 mg. percent after three weeks. Grande et al. (1972) found significant increases in serum triglyceride levels using diets high in stearic acid. They also found short- and medium-chain fatty acids were hypertriglyceridemic. Part of the reason for the failure to observe a hypertriglyceridemic effect in the present study may be due to the fact that the main difference between the stabilization and the beef tallow diet was the replacement of the short- and medium-chain fatty acids by stearic acid. However, the results of the present study agreed with the suggestion by Grande et al. (1972) that short- and medium-chain fatty acids as well as stearic acid are hypertriglyceridemic.

There was substantially more variation among individuals in the

response of serum triglycerides to diet than observed for either serum cholesterol or serum lipid phosphorous, which coincides with Keys et al. (1965d) and Turpeinen et al. (1968). Factors such as age, body weight, smoking and physical activity which are known to affect blood lipid patterns, were not standardized for the different subjects in this present experiment. This may partially explain the substantial variation in serum triglycerides among subjects.

Since the change in serum lipoprotein patterns should reflect changes in serum cholesterol and triglyceride levels, serum lipoprotein patterns were determined. The decrease in the beta-/pre-beta-lipoprotein ratio observed on both diets was similar to that of serum cholesterol.

Determination of percent fatty acid composition of the very low density (VLDL) and low density (LDL) lipoprotein fractions suggested that there was an increase in percent saturated fatty acids in these fractions on the beef tallow diet, whereas on the corn oil diet there was an increase in the percent unsaturated fatty acids. These results reflect the change in dietary fatty acids but the results were not quantitatively related to the fatty acid composition of the various diets. The changes in the LDL fraction seem more closely related to diet than those of the VLDL fraction.

Beef tallow, under the present experimental design, seems to have a hypolipidemic effect similar to that of corn oil but of a lesser magnitude. The results question the particular recommendation of the restriction of the level of beef in diets of hyperlipidemics to three 3-oz. portions weekly. Also the recommendation for the general population to decrease their intake of saturated fats without specifying the fat source is to be questioned.

## SUMMARY AND CONCLUSIONS

The present experiment reports the response of serum lipid patterns in healthy young men when diets, in which beef tallow or corn oil provide the sole source of fat at approximately 40 percent of the total daily calories, are fed. The experiment consisted of two 28-day trials. The first six days of each trial served as a stabilization period (a typical Canadian diet was fed) while the following 21 days served as the experimental period when diets, using "TVP" and the test fats, were fed to the subjects. There was about a 50 percent increase in the levels of myristic and oleic acids and a 55 percent decrease in stearic acid associated with the change from the stabilization diet to the one containing beef tallow. The change from the stabilization to the corn oil diet resulted in 90, 76 and 50 percent decreases in myristic, stearic and palmitic acids, respectively and an 85 percent increase in linoleic acid. Thus, the tallow diet contained about twice as much palmitic acid, eight times as much stearic acid, but only one-thirteenth as much linoleic acid as the corn oil diet. The main difference between diets was the dietary fatty acids but the level of dietary cholesterol was also included in interpretation of the data.

Serum cholesterol, lipid phosphorous, triglyceride and the lipoprotein fractions were measured in fasting serum samples drawn on Days 1, 6, 21 and 28 of each trial. The results obtained with the beef tallow diet were compared to those with the corn oil diet. Results of the present study agree with previous observations that there is con-

siderable variation in lipid patterns among subjects in the response to a given dietary change.

Serum cholesterol and phospholipid levels were lowered significantly ( $P < 0.005$ ) by both test diets; the response on the corn oil diet was nearly twice as great as that on the beef tallow diet. There was a marked decrease during the first two weeks on the test diets and no further change occurred during the trials. The response was greater on both diets than that predicted by the change in fatty acids alone. When both the changes in dietary fatty acids and cholesterol were considered, the predicted change in serum cholesterol agreed well with that observed on the beef tallow diet, whereas the decrease on the corn oil diet was lower than expected. Changes in serum phospholipid in response to the test diets tended to follow a similar pattern to that of serum cholesterol, although the decreases appeared to be slower and of a lower magnitude. There were no changes in serum triglyceride levels on the beef tallow diet as it contained more palmitic acid that has been shown to be less hypertriglyceridemic than the short- and medium-chain fatty acids of the stabilization diet. A marked decrease in serum triglycerides was observed on the corn oil diet.

Since the lipoproteins are the transport vehicle for serum cholesterol and triglyceride, the beta-/pre-beta-lipoprotein ratio as well as the fatty acid content of the VLDL and the LDL fractions were studied. Similar to serum cholesterol, the ratio decreased on both test diets during the first two weeks and then seemed to plateau. The percent saturated fatty acid content of the lipoprotein fractions increased on the beef tallow diet and the reverse was observed on the corn oil diet. These results seem to parallel the changes in dietary saturated

and unsaturated fatty acids.

The results of this study emphasize the important influence of dietary fat and cholesterol upon serum lipid patterns. On the basis of the data presented, it is concluded that the high level of stearic acid in beef tallow is not hypercholesterolemic. In fact, beef tallow appears to be hypocholesterolemic when fed as the prime source of dietary fat at approximately 40 percent of the total calories. Beef tallow per se appears to have no effect on serum triglycerides. The response of serum lipid patterns to beef tallow is similar to that of corn oil but of a lesser magnitude. These results suggest that the general recommendation for the population to reduce their saturated fat intake should consider the kind of fat. Also, the severe restriction of beef in serum lipid-reducing diets is questionable.



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Appendix Table 1. General instructions - human metabolic study.

1. Meals will be served in Room 402, Home Economics Building, 7 days a week at the following times:

Breakfast 8:00 - 8:30 A.M.

Lunch 12:00 - 12:30 P.M.

Dinner 5:00 - 5:30 P.M.

Please be prompt for all meals. Remember to inform the persons preparing meals if you expect to be early or late for lunch or dinner.

2. All of the food must be eaten since it will have been carefully weighed or measured.
3. Only food or drink served or specified by the director of the project will be permitted. Nothing else is to be eaten during the period you are on the study.
4. Weigh yourself daily before breakfast. A scale and form for recording weight will be available in Room 402, Home Ec. Building. An effort will be made to maintain a constant weight for you by adjusting the intake of certain food items.
5. The Home Ec. Building is locked on weekends. Please obtain an entrance key, to be signed out at the secretary's office. It should be returned at the conclusion of the study.

BLOOD SAMPLES

1. Three fasting blood samples will be taken during each diet trial. Dates and times will be announced beforehand.
2. Do not consume any food for 8 hours before blood sample is to be taken (clear coffee, tea or water are permitted). DO NOT drink coffee or smoke a cigarette for 1 hour before the time blood sample is to be taken.

COLLECTION OF FECES

1. Two 5-day feces collection periods will be conducted during each diet trial.
2. Collect all feces in the containers provided. The containers may be obtained (in Room 402) and should be signed out in the record book (in Room 402).
3. Label all containers carefully and clearly. Complete the label with your name, the day and the time the collection was made.
4. Use a separate container for each fecal collection. In other words, this is not to be a composite sample for a 24 hour period.
5. Place containers in the freezer in Room 400. If not within reach of the Home Economics Department, keep in as cool a place as possible. The success of this metabolic study depends largely on you . . . on your cooperation in eating all the food that you are given AND NOTHING ELSE and in making careful collections of feces. We will greatly appreciate such cooperation on your part in carrying out this study. If you have any questions, contact some of the laboratory personnel.



Appendix Table 2. Experimental diet menus.

Breakfast	FM1	FM2
	Juice	Juice
	Rolled Oats <sup>1</sup>	Cream of Wheat <sup>1</sup>
	Scrambled Egg Albumin <sup>2</sup>	Scrambled Egg Albumin <sup>2</sup>
	Toast	Toast
	Jelly	Jelly
	Skim Milk	Skim Milk
	Sugar	Sugar
	Margarine	Margarine
<u>Lunch</u>	Tossed Salad	Tossed Salad
	Sweet and Sour Pork	Meatballs/tomato sauce
	Rice	Spaghetti
	Fruit <sup>3</sup>	Fruit <sup>3</sup>
	Skim Milk	Skim Milk
	Bread	Bread
	Margarine	Margarine
<u>Dinner</u>	Meat loaf/gravy	Beef Stew
	Mashed Potato	Tossed Salad
	Vegetable <sup>4</sup>	
	Fruit <sup>3</sup>	Fruit <sup>3</sup>
	Skim Milk	Skim Milk
	Bread	Bread
	Margarine	Margarine
<u>Snacks</u>	Ginger Muffin	Ginger Muffin
	Fruit Square	Fruit Square
	Oatmeal Cookies	Oatmeal Cookies

<sup>1</sup> Subjects could choose rolled oats or cream of wheat.

<sup>2</sup> Omelette or French toast could be selected.

<sup>3</sup> Rotation of canned apricots, fruit cocktail, peaches, pears and pineapple.

<sup>4</sup> Rotation of frozen carrots, green beans and peas.

Appendix Table 3. Stabilization diet menus.

Breakfast	SM1	SM2
	Juice <sup>1</sup>	Juice <sup>2</sup>
	Scrambled Eggs/butter	Dry Cereal <sup>3</sup>
	Bacon	Boiled Egg
	Toast	Toast
	Jelly	Jelly
	Whole Milk	Whole Milk
<u>Lunch</u>		
	Tossed Salad/ French Dressing	Cream of Tomato Soup
	Hamburger Pattie/ cheese slice	Ham & Cheese Sandwich
	Hamburger Bun/relishes	Carrot & Celery Sticks
	Fruit <sup>4</sup>	Fruit <sup>4</sup>
	Whole Milk	Whole Milk
<u>Dinner</u>		
	Pork Chops	Roast Turkey
	Mashed Potato	Mashed Potato
	Vegetable <sup>5</sup>	Vegetable <sup>5</sup>
	Fruit <sup>4</sup>	Fruit <sup>4</sup>
	Whole Milk	Whole Milk
	Bread	Bread
	Butter	Butter
<u>Snacks</u>		
	Gingersnaps	Gingersnaps
	Oatmeal cookies	Oatmeal cookies
	Oreos	Oreos

<sup>1</sup> Subjects had choice of apple or pineapple juice.

<sup>2</sup> Subjects had choice of orange or grapefruit juice.

<sup>3</sup> Subjects could choose either corn flakes or bran flakes.

<sup>4</sup> Rotation of canned apricots, fruit cocktail, pears, peaches and pineapple.

<sup>5</sup> Rotation of frozen carrots, green beans and peas.

Appendix Table 4. Analysis of variance: total serum cholesterol.

Source	df	TSS	MS	F-value	p <sup>1</sup>
Total	149	166,709.0720			
Treatments	7	60,719.1582	8,674.1654	19.9420	0.005
Diet	1	5,309.0736	5,309.0736	12.2057	0.005
Days	3	45,254.8886	15,084.9628	0.0346	ns
Diet x Days	3	10,155.1960	3,385.0653	7.7823	0.005
People	4	84,857.6130	21,214.4032	48.7726	0.005
People x Treatment	28	12,179.0359	434.9655		
Duplicates	35	3,213.6111	91.8174	1.1997	ns
Readings	75	5,739.6538	76.5287		

<sup>1</sup> p = probability of chance occurrence.

Appendix Table 5. Orthogonal comparisons: total serum cholesterol.

No.	Comparisons	$(\sum c_i T_i)^2$	$\sum c_i^2$	F-value <sup>a,b</sup>	P <sup>c</sup>
1	Day 6 vs Days 21, 28	7,673,675.62	240	73.51	0.005
2	Day 21 vs Day 28	5,320.24	80	0.15	ns
3	Tallow vs Corn Oil	1,270,174.08	120	24.34	0.005
4	Tallow Days 6,21,28 vs Corn Oil Days 6,21,28	1,148,026.53	240	11.00	0.005
5	Tallow Days 21,28 vs Corn Oil Days 21,28	511.66	80	0.02	ns
6	Trial A Day 1 vs Trial B Day 1	3,814,052.76	30	292.29	0.005
7	Day I vs Days 6,21,28	401,320,286.68	390	2,365.77	0.005

<sup>a</sup> MSE = 434.97

<sup>b</sup> F-values are not clear-cut due to bias of unbalanced nature. There is no effect on significance.

<sup>c</sup> P = probability of chance occurrence.

Appendix Table 6. Analysis of variance: serum triglyceride.

Source	df	SS	MS	F-value	P <sup>1</sup>
Total	159	176,746.1743			
Treatments	7	32,773.7720	4,681.9674	4.0050	0.005
Diets	1	1,157.4532	1,157.4532	0.9901	ns
Days	3	27,822.4666	9,274.1555	7.9333	0.025
Diet x Days	3	3,793.8522	1,264.6174	1.0817	ns
People	4	2,032.7200	23,008.1800	19.6816	0.005
People x Treatment	28	32,732.4508	1,169.0161		
Duplicates	40	8,467.7237	134.4938	0.6368	ns
Readings	80	10,759.5074	211.1930		

<sup>1</sup> P = probability of chance occurrence.

Appendix Table 7. Orthogonal comparisons: serum triglyceride.

No.	Comparisons	$(\sum c_{1r})^2$	$\sum c_{1r}^2$	F-value <sup>a</sup>	p <sup>b</sup>
1	Day 6 vs Days 21,28	135,799.62	240	0.48	ns
2	Day 21 vs Day 28	204.78	80	0.00	ns
3	Tallow vs Corn Oil	80.10	120	0.00	ns
4	Tallow Days 6,21,28 vs Corn Oil Days 6,21,28	39,636.83	240	0.14	ns
5	Tallow Days 21,28 vs Corn Oil Days 21,28	2,170.63	80	0.02	ns
6	Trial A Day 1 vs Trial B Day 1	190,348.96	40	4.07	0.100
7	Day 1 vs Days 6,21,28	13,081,386.91	480	23.31	0.005

<sup>a</sup> MSE = 1,169.02

<sup>b</sup> p = probability of chance occurrence.

Appendix Table 8. Analysis of variance: lipid phosphorous

Source	df	SS	MS	F-value	P <sup>1</sup>
Total	159	419.6224			
Treatments	7	62.4974	8.9282	5.7191	0.005
Diet	1	9.8857	9.8857	6.3325	0.025
Days	3	51.7873	17.2624	11.0578	0.005
Diet x Days	3	0.8244	0.2748	0.1760	ns
People	4	190.3460	47.5865	30.4826	0.005
People x Treatment	28	43.7110	1.5611		
Duplicates	40	120.6527	3.0163	0.0099	ns
Readings	80	2.4153	0.0301		

<sup>1</sup> P = probability of chance occurrence.

Appendix Table 9. Orthogonal comparisons: lipid phosphorous.

No.	Comparisons	$(\sum c_i T_i)^2$	$\sum c_i^2 r$	F-value <sup>a</sup>	P <sup>b</sup>
1	Day 6 vs Days 21,28	4,883.35	240	13.04	0.005
2	Day 21 vs Day 28	48.99	80	0.39	ns
3	Tallow vs Corn Oil	1,076.46	120	5.75	0.025
4	Tallow Days 6,21,28 vs Corn Oil Days 6,21,28	130.18	240	0.35	ns
5	Tallow Days 21,28 vs Corn Oil Days 21,28	52.85	80	0.42	ns
6	Trial A Day 1 vs Trial B Day 1	48.46	40	0.78	ns
7	Day 1 vs Days 6,21,28	14,473.29	480	19.31	0.005

<sup>a</sup> MSE = 1.56

<sup>b</sup> P = probability of chance occurrence.



Appendix Table 10. Analysis of variance: free fatty acids.

Source	df	SS	MS	F-value	P <sup>1</sup>
Total	159	427.3433			
Treatments	7	32.5558	4.6508	0.8477	ns
Diet	1	0.1601	0.1601	0.0291	ns
Days	3	21.7050	7.2350	1.3187	ns
Diet x Days	3	10.6907	3.5635	0.6495	ns
People	4	154.0756	38.5189	7.0210	0.005
People x Treatments	28	153.6147	5.4862		
Duplicates	40	43.0117	1.0752	1.9513	0.250
Readings	80	44.0855	0.5510		

<sup>1</sup> P = probability of chance occurrence.

Appendix Table 11. Data for subject M.T. on Trial A replaced by subject R.H. for Trial B.

Lipid Parameter	Period				Day 28 vs Day 6
	Stabilization		Experimental Diet		
	1	6	21	28	
	M.T. Trial A - Beef Tallow				
Beta- / Pre-Beta- Lipoprotein Ratio	15.5	9.0	1.6	2.5	+13.9
Total Cholesterol <sup>1</sup>	195	203	169	165	-38
Free Fatty Acids <sup>1</sup>	24.2	23.0	26.8	27.3	+ 4.3
Lipid Phosphorous <sup>1</sup>	7.78	8.70	6.98	6.99	- 0.72
Triglyceride <sup>1</sup>	86	71	121	77	+ 6
	R.H. Trial B - Corn Oil				
Beta- / Pre-Beta- Lipoprotein Ratio	9.5	6.8	m <sup>2</sup>	2.8	+ 1.7
Total Cholesterol <sup>1</sup>	192	233	162	142	-81
Free Fatty Acids <sup>1</sup>	25.7	25.4	25.7	25.7	0.0
Lipid Phosphorous <sup>1</sup>	7.62	7.20	6.39	6.03	- 1.17
Triglyceride <sup>1</sup>	90	71	65	59	-12

<sup>1</sup> mg per 100 ml serum.<sup>2</sup> m = missing observation