CHANGES IN SERUM LIPID PATTERNS OF HEALTHY YOUNG MEN FED DIETS RICH IN LARD AND SUNFLOWER OIL

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by

Susan Karen Cobden

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A dissertation submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

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ABSTRACT

The effects of sunflower oil and lard (40 percent of calories) on serum lipid patterns and cholesterol turnover was investigated in eight healthy young men. The 39-day metabolic trial consisted of 1) a 10-day stabilization period when a fat mix with a fatty acid composition representative of the average Canadian consumption was fed; 2) a 21-day experimental period when either the lard or sunflower oil diet was fed; and 3) a 7-day post-experimental period when the mixed fat diet was refed. Fasting venous blood samples were taken on days 4, 11, 18, 25, 32 and 39. Serum total cholesterol decreased ($P < 0.001$) 56 mg/100 ml on the sunflower oil diet and increased ($P < 0.001$) 26 mg/100 ml on the lard diet. Serum free and esterified cholesterol followed the pattern of total cholesterol as illustrated by the fact that the proportion of free and esterified cholesterol remained fairly constant within each group throughout the experiment. Although there were differences among the groups, serum cholesterol, serum lipid phosphorus and serum triglycerides followed similar patterns. There was little change in the fatty acid patterns of the serum phospholipids in response to dietary fat source. Little is known of the effects of dietary fat on the turnover of plasma cholesterol in normal, healthy men. Thirty-two days prior to the start of the study, each subject was infused with 50 microcuries of tritium labelled cholesterol and the decline in radioactivity in the plasma

was monitored during the study. The decrease of ^{3}H-cholesterol in the blood was twice as great on the sunflower oil diet as on the lard diet. However, there was no change in the slopes of the specific activity-time curve on the two diets which suggests that the rate of turnover of cholesterol differed on the two diets but synthesis and absorption of cholesterol remained constant. During the final seven days of the experiment when the mixed fat diet was fed, the effects of protein source on serum lipids was investigated. Two subjects from each of the lard and sunflower oil treatments consumed the same identical mixed fat diet of days 1 to 10, whereas the remaining four subjects consumed the same diet except that the soy protein was replaced by lean beef. Protein source was found to have no effect on any of the serum lipid parameters measured. The appreciable effects of sunflower oil and lard on serum lipid patterns is consistent with the hypolipidemic effect of polyunsaturated fatty acids and hyperlipidemic effect of saturated fatty acids although the recommendation of substituting animal fats with vegetable fats without considering the fat source is to be questioned.

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INTRODUCTION

The relation of angina pectoris to coronary atherosclerosis was recognized over two centuries ago. Myocardial infarction was first recognized clinically over a century ago. Today, syndromes characterized by cardiac pain are precisely diagnosed and treated (Altschule, 1974). Generally, when a disease is easily and satisfactorily treated, the pressure on scientists to elucidate the etiology and define preventive measures is minimal. But, when a disease is increasing in frequency - as is the case with coronary atherosclerosis - and when treatment of some of its manifestations is not satisfactory, the pressure for a solution is persistent and heavy.

Atherosclerosis, the leading cause of death in North America, is an entity characterized by the accumulation of cholesterol and other fatty substances in the walls of the large blood vessels (Gresham, 1972). From adolescence onwards, the disease is almost universally present in our population; persons with extensive disease become candidates for heart attacks and angina pectoris when the coronary arteries are involved, and for strokes when the cerebral arteries are involved. In North America, a male under 60 has one chance in five of developing atherosclerotic ischemic heart disease. Almost one-third of the initial heart attacks are fatal within a few hours and more than 60 percent before the victim receives medical attention. For the survivors the outcome is often gloomy (Brusis, 1971).

Clinical and epidemiological studies have indicated that the causes of atherosclerosis and the clinical events leading up to a heart attack are multifactorial (Epstein, 1972). These causes include genetic, cultural and environmental factors. Some of the cultural and environmental factors which are known to increase the risk of coronary heart disease (CHD) are under the control of the individual. These include dietary patterns (high intake of cholesterol, saturated fats and calories), sedentary living habits (Paffenbarger and Hale, 1975) and cigarette smoking. Other factors, which have been implicated but which are less well documented, are large coffee intakes (Paul, 1968), hardness of the drinking water, and emotional stress and tension (Schroeder, 1974). Some of the predisposing factors are not responsive to preventive intervention; men, for example, are more prone to CHD than pre-menopausal women. Diabetes mellitus, obesity and a positive family history of vascular disease also increase the risk of CHD (Kannel et al, 1967; Epstein, 1972).

The degree to which each of these factors has contributed to atherosclerosis has been confounded by many problems. Some of the factors are interrelated; obesity can be due to genetic factors or to an imbalance of caloric intake and energy expenditure (Katz et al, 1958); both can cause hypercholesterolemia. The complexity of the tissue and haemodynamical factors influencing the location and evolution of the atherosclerotic lesion (Altshule, 1974a), the difficulty at present to assess the presence and extent of

atherosclerosis in the living person and the lack of a satisfactory experimental animal model for atherosclerosis (Gresham, 1971), although work with primates appears promising (Jones et al, 1975), have added to the problem in elucidating and separating all the factors implicated in atherosclerosis.

All serum lipids have been implicated in atherosclerosis. Elevated serum cholesterol values appear to be a factor present in all forms of CHD, hence interest has been focused on serum cholesterol. Examination of atherosclerotic fatty streaks in human thoracic aorta have shown average cholesterol values five times those of normal aorta, and phospholipid values 1.5 times those in adjacent normal intimae (Insull and Bartsch, 1966). High fasting concentrations of plasma triglycerides have been shown to be a significant risk factor in atherosclerotic disease (Carlson and Boltiger, 1972) and it has been suggested that hypertriglyceridemia may have a risk independent of associated hypercholesterolemia (Albrink, 1973).

There have been many studies on man in which the diet has been closely regulated. These studies have shown that changes in the fat composition of the diet can bring about a change in serum lipid values. Diets high in saturated fats, containing high proportions of C12:0, C14:0 and C16:0 fatty acids, have been shown to elevate serum cholesterol values (Grande et al, 1972). On the other hand, saturated fatty acids of fewer than 12 carbon atoms (Keyes et al, 1965c) and stearic acid (Keyes et al, 1965c; Grande

et al, 1970; and Losier, 1972) have been found to have little effect on serum cholesterol values. Epidemiological studies have shown that populations who consume diets low in fat, such as those which characterize the Bantu, have little ahterosclerotic heart disease. But, these diets tend to be unpalatable and too extreme for North American society. Substitution of vegetable oils, rich in polyunsaturated fatty acids and low in saturated fatty acids and cholesterol, in place of animal fats can essentially achieve the same effect on serum lipids. This approach has offered a way to maintain a palatable high fat diet. Several controlled clinical trials have established the quantitative effects of saturated and polyunsaturated fat, as well as of dietary cholesterol on serum cholesterol in man (Hegsted et al, 1965; Keyes et al, 1965a; Keyes et al, 1965b; and Keyes et al, 1965c).

It has been suggested that to counteract the present incidence and mortality from CHD in North America, the diet must be modified to minimize elevations of serum lipids. However, many of the earlier studies implicating various fats were conducted with subjects who were fed forumula diets and who were not free-living individuals consuming mixed diets. Saturated fats are generally regarded as hypercholesterolemic although Losier (1972) found beef tallow, a fat high in stearic acid, to have a hypocholesterolemic effect. Thus, more precise information on the effects of specific fats and fatty acids is needed. However, if it is accepted, in general, that polyunsaturated fatty acids decrease serum cholesterol and that saturated fatty acids increase serum

cholesterol, the question arises as to how they bring about these effects; whether it is by increasing turnover of cholesterol or by a mechanism of redistribution of cholesterol between the various body tissues. The present study was undertaken to investigate the effects of lard, a saturated fat high in palmitic acid, and sunflower oil, a polyunsaturated fat high in linoleic acid, on cholesterol turnover and on serum lipid levels in healthy, free-living individuals consuming a mixed food diet.

REVIEW OF LITERATURE

The main features of cholesterol metabolism were elucidated in 1933 by Schoenheimer & Breusch who concluded that synthesis, absorption and destruction of cholesterol in mammals were controlled by a complex system of interrelated mechanisms in which cholesterol itself was a principal mediator. However, even today the means by which the regulatory mechanisms are integrated is poorly understood largely because adequate methodology for accurately quantitating sterol synthesis, absorption, excretion and degradation in the intact animal has been developed only recently. In addition, the miscibility of cholesterol among tissues and organs makes the dissection of control mechanisms in the various organs difficult. This review will attempt to summarize the current concepts of the measurement and regulation of cholesterol turnover.

Turnover, in the case of cholesterol, reflects the balance between input of cholesterol from endogenous tissue synthesis and from dietary sources, and the loss of cholesterol which occurs in the feces in the form of bile acids and neutral sterols. There have been several studies of cholesterol turnover. In addition to studies on cholesterol turnover, other studies have provided estimates of the flow of cholesterol among the body pools. Many of the studies have focused on assessing serum cholesterol levels because of the association between atherosclerosis and high serum cholesterol values. In general, the changes in plasma

cholesterol are thought to reflect the changes in other body tissues.

Studies in cholesterol turnover require considerable time because turnover is slow in some tissues. The measurement of cholesterol turnover demands steady state conditions in which there is a constant sterol intake and stable body weight. Cholesterol is lost almost exclusively in the feces. Fecal collections are problematic; total feces must be collected and corrections must be made for losses of neutral sterols due to bacterial degradation. Methods which measure the turnover of radioactive cholesterol in the plasma are more simple than chemical methods but do not yield the amount of information that direct quantitation of fecal sterols or a combination of both methods can give.

A. METHODS OF MEASURING CHOLESTEROL TURNOVER

Nestel (1970) and Grundy & Ahrens (1969a) have reviewed the four major techniques for measuring cholesterol turnover in man. These include the chemical balance technique, isotopic balance methods, estimations based on analysis of radioactive cholesterol die-away curves in serum[1] and a combination of these methods.

Two types of isotopic balance methods have been used to distinguish between fecal products of endogenous and exogenous origin. The simplest method involves a single

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1. A plot which takes into account radioactive decay and biological turnover of the labelled compound.

intravenous injection of labelled cholesterol and the subsequent measurement of radioactivity in the plasma and fecal steroid fractions. The labelled cholesterol is administered at least 30 days prior to sampling to allow complete equilibration of the isotope among the various body tissues. The second approach, the steady state isotopic balance method, has been suggested as a means for calculating the amount of cholesterol absorbed. A constant amount of labelled cholesterol is ingested daily until a steady state is reached. Once a steady state has been reached, cholesterol turnover can be calculated.

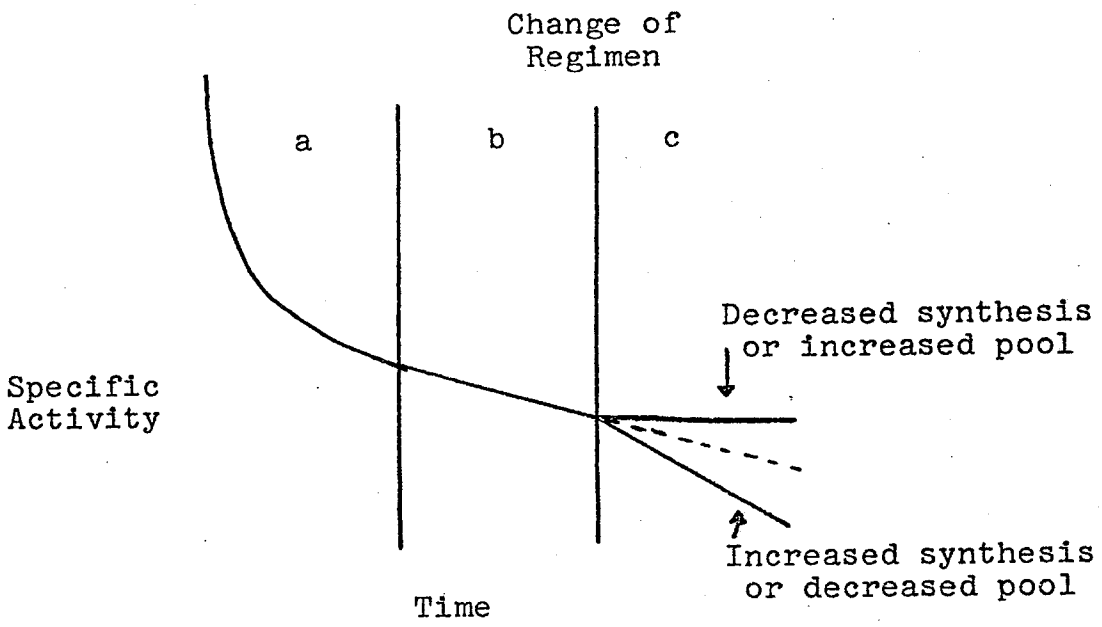
There have been several approaches for the measurement of cholesterol turnover by analysis of specific activity die-away time curves. One of the simplest techniques is the single intravenous injection of labelled cholesterol followed by monitoring of plasma radioactivity (Goodman and Noble, 1968). The limitation of this method is that no direct measurement of cholesterol synthesis and absorption is made.

Chobanian et al (1962) proposed a method for calculating the rate of total turnover of cholesterol on the basis of the die-away time curve of radioactive cholesterol. His approach reflected the turnover of a single pool of readily-miscible cholesterol excluding that in nerve tissue. This model did not account for the fact that the change in specific activity in serum cholesterol is not linear until 30 days following the injection of labelled cholesterol (Figure 1).

It is well documented that plasma cholesterol specific activity declines more slowly after the first few

FIGURE 1

SCHEMATIC LOG SPECIFIC ACTIVITY-TIME CURVE FOR SERUM CHOLESTEROL AFTER ADMINISTRATION OF A SINGLE DOSE OF RADIOACTIVE CHOLESTEROL



a = Period of Rapid Decay (non-linear fall off).

b = Period of Isotopic Equilibrium Between Readily Miscible Pools (linear fall-off).

c = After Change of Regimen.
(Pool is conceived of as all the body cholesterol except that in nervous tissue. Its size can be increased by decreased excretion, increased synthesis, or increased absorption of endogenous cholesterol. After a change in regimen, a decreased slope of the specific activity-time curve is due to decreased endogenous synthesis or increased pool size; increased slope is due to increased synthesis or decreased pool size.)

Grundy & Ahrens (1966).

days with the rate of disappearance becoming essentially linear after the fourth week (Figure 1). In 1968, Goodman and Nobel proposed that cholesterol exchanged within a system of two pools rather than within a single pool. The linear decrease of specific activity reflected the rate of cholesterol turnover in the slower of the two pools and not the whole body. Under this scheme, Pool A reflects a pool of rapidly miscible cholesterol comprised of the liver, bile, plasma, erythrocytes and possibly the intestine. In Pool B, turnover is much slower and is comprised of cholesterol in the viscera (other than in Pool A), skeletal muscle and adipose tissue. It should be noted that these two pools only represent mathematical models and have no true physical boundaries.

Analysis by chemical balance was developed by Grundy et al (1965) and Miettinen et al (1965). This method affords direct measurement of quantitative and qualitative excretion patterns of fecal neutral and acidic sterols. In addition, the chemical balance method allows for the quantitative analysis of plant sterols and their conversion products independent of cholesterol. However, a major limitation is that it does not distinguish between metabolites of endogenous and exogenous origin.

When isotopic balance techniques and chemical analyses are combined, a fairly accurate estimation of cholesterol absorption can be made. This method affords results which are not possible by either method alone. Fecal steroids of endogenous origin can be estimated by the

isotope method while chemical analyses give an estimation of steroids of endogenous and exogenous (dietary) origin. The difference between the two represents that portion of cholesterol which has not been absorbed during its passage through the intestine.

B. TURNOVER OF CHOLESTEROL

The development of adequate methodology for accurately quantitating cholesterol and its metabolites has made it possible to measure cholesterol turnover. Plasma is one of the more accessible tissues and turnover of plasma cholesterol has been extensively studied in a number of species. The most common method involves administration of isotopically labelled cholesterol or a biosynthetic precursor of cholesterol and determining the radioactivity of plasma cholesterol in the ensuing weeks. It has been consistently shown (Nestel et al, 1969; Nestel, 1970; and Quardordt & Greenfield, 1973) that, during the first few weeks after isotopic administration, the semilogarithmic plot of cholesterol radioactivity versus time is curvilinear (Figure 1). The changing slope of the curve during the first few weeks is due to the slow rates of equilibration between the plasma and other body tissues. As the changes in plasma cholesterol are believed to reflect what is happening in the rest of the body, Goodman and Nobel (1968) suggested that turnover conformed to a two-exchangeable pool model; Pool A - the rapidly equilibrating pool - and Pool B - one which exchanged more slowly. Dietschy and Wilson (1970a) and

Goodman et al (1973) have presented evidence for a three-pool model. In this model, cholesterol was viewed as a rapidly-exchanging pool (A), a more slowly-exchanging pool (B) and a pool with a neglible exchange rate (C). Entry and exit from the system occurs primarily through Pool A. However, no matter how solidly based these models are, it must be remembered that the body actually is comprised of multiple tissues, each with its own turnover and exchange rate, and that some of these tissue pools may be too small or too slowly exchanging to contribute to the overall dimensions of cholesterol turnover. Nevertheless, they may be critical in the localized accretion of cholesterol in pathological states.

1) Synthesis of Cholesterol: Every mammalian tissue has the capacity for de nova synthesis of cholesterol from acetate although there is great variation in synthetic activity among organs (Dietschy and Wilson, (1968). The highest rates of sterol synthesis are in the liver and ileum, with the synthetic ability of the gastrointestinal tract varying according to site. Synthesis is relatively low in the proximal jejunum and increases to a maximum at the terminal small bowel. The abdominal and thoracic viscera and the skin have lower rates of synthesis than the liver and gastrointestinal tract, while the rate of synthesis in muscle and mature nerve tissue is very low.

The difference in potential for sterol synthesis between the liver and gastrointestinal tract and other

tissues is striking when organ weight is taken into account. Approximately 98 percent of all detectable sterol synthesis takes place in the liver, gastrointestinal tract and skin; all other tissues account for only two percent. Muscle, which accounts for nearly half of the body weight, contributes only 0.5 percent of cholesterol synthesis in the squirrel monkey; 98 percent occurs in the liver and gastrointestinal tract (Dietschy and Wilson, 1970a).

2) Absorption of Cholesterol: A major source of cholesterol entering the body is exogenous or dietary cholesterol. The dietary cholesterol can be free or esterified with fatty acids. It becomes mixed with endogenous cholesterol from the bile and desquamated mucosal cells (Dietschy and Wilson, 1970b), and the two become indistinguishable. Only free cholesterol is absorbed.

Pancreatic cholesterol esterase hydrolyses the cholesterol esters. If sufficient bile, monoglycerides and fatty acids are present, free cholesterol is solubized in mixed micelles and brought into contact with the brush boarder where it is moved across the membrane into the intracellular pool of cholesterol. Here esterification with long chain fatty acids takes place. These esters are incorporated into chylomicrons which are then released into the lymph.

There are several potential sites where the rate of absorption can be controlled. These include the

permeability characteristics of the mucosal membrane, the relative activity of cholesterol esterase and the rate of chylomicron formation. Exogenous cholesterol is more readily absorbed when it is given in conjunction with a high fat diet (Wilson, 1962), although the exact mechanism by which fat enhances absorption has not been elucidated.

The bile acid pool also affects the rate of absorption. If bile is prevented from entering the gastrointestinal tract, cholesterol is not absorbed (Siperstein et al, 1952) whereas, if the amount of bile acid is increased, there is an increased rate of absorption (Dietschy and Wilson, 1970b). Sylven and Borgstrom (1968) have suggested that the rate-limiting step is the release of cholesterol to the lymph, although no mechanism has been proposed. Other factors affecting absorption include the amount of cholesterol and fat in the diet.

The understanding of cholesterol absorption has been confounded by many factors. Cholesterol absorption can only be reliably assessed when dietary intake remains constant. This is because cholesterol can be taken up into the intestinal cell and, subsequently, discharged into the lumen of the intestine again without even reaching the circulatory system. In addition, there is enterohepatic recycling of cholesterol. Three different approaches have been proposed to overcome these confounding factors, namely, the isotopic balance technique (Wilson and Lindsay, 1965), the chemical

balance technique (Miettinen et al, 1965) and combinations of these two. Only net absorption is measured by these methods; there is no means for assessing enterohepatic circulation of cholesterol. By these methods it is estimated that, of the 0.5 to 2.0 gm of cholesterol ingested daily by persons eating a typical North American diet, only 20 percent is absorbed (Wilson and Lindsey, 1965).

3) Excretion of Cholesterol: Elimination of cholesterol and its metabolites occurs via the gastrointestinal tract. The excretory products of cholesterol appear in the neutral sterol and bile acid fraction of the feces. McIntyre and Isselbacher (1973) have reviewed the role of the intestine in cholesterol turnover. They estimate that the pool of bile salts amounts to between 2.5 and 5.0 gm. Approximately 200 to 600 mg of bile acids are lost in the feces each day. Factors determining the magnitude of losses of bile acids and neutral sterols is not clear (Dowling, 1972). The conjugated bile acids - taurocholic acid, glycocholic acid, taurochenodeoxycholic acid and glycochenodeoxycholic acid - are the primary end products of hepatic catabolism of cholesterol. Deconjugation may occur, resulting in the formation of cholic and chenodeoxycholic acid which, in turn, may be dehydroxylated to form deoxycholic acid and lithocholic acid respectively. Bile acids can be absorbed from the intestine. The deconjugated bile acids are again conjugated

with glycine or taurine and re-secreted into the bile.

The excretion of cholesterol has been extensively studied. When there is a high rate of absorption of cholesterol in the rat or dog, increased conversion of cholesterol into bile acids appears to be an important excretory mechanism (Dietschy & Wilson, 1970c). These authors have suggested that increased hepatic excretion of fecal bile acids may account for the cholesterol lowering effect when polyunsaturated fats are fed.

Other studies, using radioisotopes, have shown that bile acid losses can be increased by cholestyramine. This resin binds bile acids in the lumen, thereby increasing fecal losses. This, in turn, causes plasma cholesterol levels to fall (Moore et al, 1968). However, this fall in plasma cholesterol may not simply be the result of an increased excretion of bile acids, but could also be due to a decreased absorption of cholesterol because bile acids are required for the absorption of cholesterol.

The fecal neutral sterol fraction in man includes plant and animal sterols of dietary and endogenous origin (Danielsson, 1963). Endogenous sterols consist of cholesterol and its precursors - lanosterol and 7-dehydrocholesterol (McIntyre & Isselbacher, 1973). Many of the bacteria present in the large intestine are capable of degrading cholesterol, thus accounting for

the many other sterols present in the feces. Coprostanol is one of the major bacterial degradation products.

Endogenous cholesterol can enter the small intestine via the bile and shed epithelial cells. Cholesterol which reaches the distal intestine is unavailable for resorption and may contribute significantly to fecal neutral sterols (Nestel, 1970). The loss of endogenous sterols in man can be determined by the loss of radioactivity in the fecal neutral sterols following infusion of labelled cholesterol. Labelled cholesterol has to be equilibrated with the cholesterol of the bile and the intestinal mucosal cells for the loss of endogenous sterols to be accurately assessed (Grundy and Ahrens, 1969a). In addition, neutral sterols are extensively degraded. These losses are postulated to be the result of bacterial degradation and can only be determined if an internal standard, such as β-sitosterol, is fed. β-sitosterol is poorly absorbed but can be degraded by the intestinal flora.

Substitution of polyunsaturated fats for saturated fats in the diet cause serum cholesterol values to fall. Ahrens et al (1957) proposed that this decrease in serum cholesterol values may be because of increased excretion of fecal neutral sterols of endogenous origin. Work by Conner et al (1969) supports this hypothesis, namely, that the excretion of neutral sterols and bile acids is

increased on a diet rich in polyunsaturated fatty acids, but Spritz (1965) failed to demonstrate increased excretion of fecal acidic and neutral sterols in response to polyunsaturated fats.

C. REGULATION OF CHOLESTEROL METABOLISM

Total body cholesterol is regulated by the interaction of absorption, synthesis and excretion.

Three physiologic variables influence cholesterol biosynthesis, namely, the amount of cholesterol in the diet, the caloric intake of the animal and the enterohepatic circulation of bile acids.

Grundy et al (1969b) demonstrated that the total synthesis of cholesterol was related to the amount of cholesterol absorbed from the lumen. Interruption of enterohepatic recycling of bile acids inhibited cholesterol absorption, thereby causing reduced plasma cholesterol values. However, the reduction in plasma cholesterol was limited by a compensatory increase in cholesterol synthesis (Dietschy & Wilson, 1970c). This work clearly demonstrated that cholesterol synthesis in man is under feedback regulation by cholesterol itself. Further work by Quintão et al (1971) on the interaction of cholesterol absorption, synthesis and excretion, using the sterol balance technique, indicated that absorption of cholesterol increased with an increase in dietary cholesterol. As much as 1 gm of cholesterol was absorbed by patients who were fed 3 gm of

cholesterol per day. The compensatory mechanisms evoked were increased excretion of cholesterol (but not bile acids) and decreased total synthesis of cholesterol.

Although total body synthesis of cholesterol is reduced by cholesterol feeding, only hepatic cholesterogenesis is promptly suppressed by dietary cholesterol (Gould, 1951). Hence, the gastrointestinal tract (especially the ileum) becomes the major site of endogenous sterol synthesis when liver synthesis is suppressed.

It has been demonstrated that fasting, as well as the feeding of cholesterol, causes a reduction in hepatic cholesterogenesis. This was demonstrated in 1952 by Tomkins and Chaikoff and, subsequently, confirmed by Dietschy and Wilson (1970c). Dietschy and Wilson (1968) found that there was little reduction in the synthetic rate of other tissues in the monkey when cholesterol was fed.

A model for the control of hepatic cholesterogenesis was proposed by Weiss and Dietschy in 1969. In this model, it was assumed that cholesterol absorbed from the lumen controlled hepatic synthesis of cholesterol. The size of the bile acid pool was assumed to have an indirect effect on hepatic cholesterol synthesis (at one time it was thought to have a direct effect) because bile acids are required for the absorption and transport of intestinal cholesterol to the lymph. Thus, an increase in the size of the bile acid pool allows for increased absorption of cholesterol which, in turn, reduces cholesterol synthesis.

D. DIETARY FACTORS AFFECTING CHOLESTEROL TURNOVER

There are many factors affecting cholesterol turnover in man, and a great deal of attention has focused on the elucidation of the control mechanisms involved. Elevated serum cholesterol levels are associated with atherosclerosis. Since diet has been implicated with elevated serum cholesterol levels, an enormous volume of literature has been devoted to the effects of various dietary constituents on serum cholesterol.

In brief, all aspects of diet have been incriminated. For many years the focal point was concentrated on dietary fat. However, several other dietary factors recently have been implicated. A lack of dietary ascorbate has been cited as a predisposing factor in elevating serum cholesterol values (Krumdieck and Butterworth, 1974), a lack or excess of various macro and trace minerals has been cited as elevating serum cholesterol levels (Schroeder, 1974), and a lack of dietary fiber has been incriminated (Kritchevsky et al, 1975). That the level of dietary protein may have an effect on serum lipid levels has been indicated by Elson et al (1971). Recently Carroll and Hamilton (1975) reported that protein source, as well as amount of protein, is important in promoting hypercholesterolemia in the rabbit.

For many years dietary fat, both the source and the amount, was the primary point of focus, and many experiments were undertaken to determine its effects on serum lipids. The effects of dietary fats on serum lipids have been reviewed

by Losier (1972), and the topic will be reviewed only briefly in this thesis.

That polyunsaturated fats lower serum cholesterol values has been accepted for some years (Keyes et al, 1974; and Pikaar & Fernandes, 1966), but studies on plasma cholesterol levels tell little of the overall turnover of cholesterol. Gordon and Danielsson (cited by Wood et al, 1966) found increased excretion of cholesterol and its metabolites following a change to a polyunsaturated fat diet, and concluded that this accounted for the decrease in plasma cholesterol. However, Nestel (1970) has suggested that the analytical methods used by Gordon and Danielsson to measure the excretion rates may have been unsatisfactory. Nevertheless, Connor et al (1969), Grundy & Ahrens (1966), Moore et al (1968) and Sodhi et al (1967) have shown increased excretion of bile acids and neutral sterols on diets rich in polyunsaturated fatty acids. As yet there is little agreement on which fecal sterol fraction was increased. Grundy & Ahrens (1966) found that there was an increase in the neutral fraction but not the acidic fraction. On the other hand, Sodhi et al (1967) and Moore et al (1968) found an increase in both the acidic and neutral fractions. Other researchers have given different explanations for the serum cholesterol lowering effects of polyunsaturated fats. Spritz et al (1965) and Grundy & Ahrens (1970) found no consistent change in excretion rates when polyunsaturated fats were substituted for saturated fats. Grundy & Ahrens (1970) proposed that

redistribution of cholesterol among the various body pools was a possible mechanism to account for the hypocholesterolemic effect of polyunsaturated fatty acids.

The administration of phytosterols has been found to increase the excretion of cholesterol in the stool. Sterols have been shown to reduce the reabsorption of endogenous neutral sterols derived from the liver and intestine as well as dietary cholesterol (Grundy et al, 1969a. The effect of plant sterols on serum cholesterol has been reviewed by Keyes et al (1974) who state that ingestion of 6 to 10 gm daily of phytosterols is necessary in order to produce a discernable decrease in serum cholesterol. This amount is far in excess of that contained in most diets. Corn oil, which has a relatively high phytosterol content, contains only 0.58 to 1.00 gm per 100 gm of oil (Lange, 1950).

There is controversy in the literature concerning the effects of saturated fats on serum cholesterol levels (Reiser, 1974; Keyes et al, 1974; and Altschule, 1974b). It is generally accepted that saturated fats increase serum cholesterol levels, but only C12:0, C14:0 and C16:0 fatty acids have a hypercholesterolemic effect (Grande et al, 1972). Stearic acid (C18:0) and saturated fatty acids with fewer than 12 carbon atoms have been reported to have little effect on serum cholesterol levels (Keyes et al, 1965c; and Grande et al, 1970). A recent study by Losier (1972) at the University of Manitoba demonstrated a modest decrease in

serum cholesterol values when 40 percent of total calories in a mixed food diet were derived from beef tallow. In the same study, corn oil was found to cause a marked decrease in serum cholesterol levels. However, these studies gave no indication of turnover of cholesterol.

There is still much to be learned about the effects of dietary constituents, including dietary fat sources, on serum lipid levels in man. Furthermore, there is very little known about the effects of various dietary constituents on cholesterol turnover and serum lipid patterns of healthy individuals as much of the work on the effects that various dietary constituents have on cholesterol turnover and serum lipid patterns was undertaken on hospitalized people.

OBJECTIVES OF STUDY

The primary objective of the study was to investigate the effects of lard and sunflower oil as the major sources of dietary fat on the serum lipid patterns and cholesterol turnover in healthy young men when these fats provided 40% of total calories in a mixed diet.

In addition, the response in serum lipid patterns of subjects fed a diet containing soy protein was compared with that observed in subjects fed the same diet but having an equivalent protein substitution of meat for soy protein.

EXPERIMENTAL METHODS

A. EXPERIMENTAL DESIGN

The study, a 39-day metabolic trial, was divided into three periods. The first 10 days of the study served as a stabilization period during which time a mixed fat diet was fed. The fat was formulated to simulate the amount and composition of fat in the average Canadian diet. The purpose of this period was to provide time for introducing the subjects to the routine of the study, to establish individual requirements for calories and to allow serum lipid patterns to stabilize. The next 21 days constituted the experimental period during which time either lard or sunflower oil supplied nearly all of the fat in the diet. The test diets were followed by a further seven days on the mixed fat diet. During the final seven-day period, two subjects from each of the experimental fat groups were fed diets in which the soy protein in the diet was substituted, on a protein equivalent basis, by lean ground beef.

Fasting venous blood samples were obtained from each subject before breakfast on days 4, 11, 18, 25, 32 and 39. Sera was removed and stored at $-10^{o}C$ until used for chemical analysis.

The experimental plan is diagramed in Figure 2.

B. SUBJECTS

The initial group of subjects were nine healthy young men age 20-31 years ($\bar{x}$ = 25 years), selected from a group

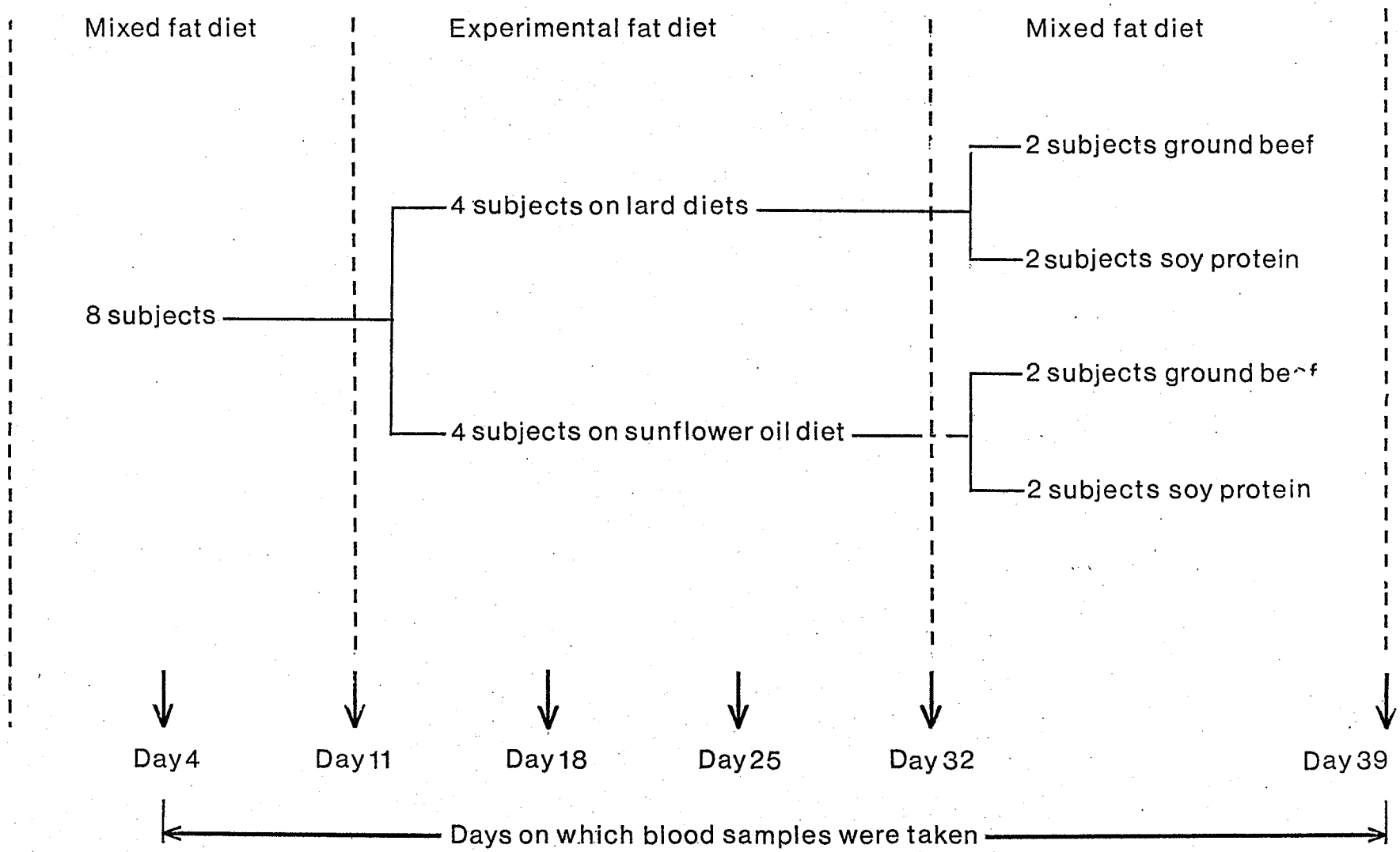


Figure 2: Design of Experiment

who responded to posted notices advertising the study. The subjects were chosen on the basis of an interview with the project directors, a physical examination and expressed co-operativeness. They were of average height and weight (Table 1) with no diagnosed metabolic disorders or recent history of poor health. All but one subject, (R.R.), were full-time students at the University of Manitoba; R.R. was employed by the University and worked on campus. Eight of the nine subjects successfully served for the entire study. One person who had been recruited resigned at breakfast on Day 1 and so his physical data were not included in Table 1. The eight subjects were friendly and co-operative at all times. They remained in good health although R.L. was diagnosed as having abnormal white blood cells due to mono-nucleosis on the basis of blood taken on Day 4. This complaint continued for the duration of the study, but he was able to pursue his normal routine without feeling any ill effects. H.P. also was diagnosed with an abnormal leucocyte count in blood taken on Day 3. He was thought to have been affected by poison oak. On Days 5 through 13 he took two antihistamine tablets three times a day (4 mg Chlor-Tripolon tablets). His blood haematology was normal again on Day 10.

C. INFUSION OF THE ISOTOPE

On Monday, August 26, 1974, 35 days prior to commencing the feeding trial, the subjects were infused with

TABLE 1

PHYSICAL DATA OF SUBJECTS

Subject	Age (Years)	Height (cms)	Weight (kgm) Initial	Weight (kgm) Average During Study
J.B.	25	175.3	75.0	74.3 ± 0.3[1]
H.P.	20	175.3	94.8	93.2 ± 1.3
R.R.	31	185.4	89.1	87.2 ± 1.2
R.L.	27	168.9	59.1	57.7 ± 0.7
J.G.	22	176.5	68.4	67.2 ± 0.8
R.M.	29	167.6	60.9	63.0 ± 0.6
T.B.	26	180.3	83.2	81.6 ± 0.7
B.M.	22	177.8	83.2	82.4 ± 0.3

1. Mean ± S.D. for 39 daily weighings.

50 microcuries of cholesterol-1,2-^{3}H(N)[1] (0.36 ug cholesterol in 0.5 ml sterile ethanol). The infusions were carried out under the supervision of Ms. Helen Bowan in consultation with Dr. John Moorhouse at the Health Sciences Centre, Winnipeg, Manitoba. A Harvard Parallel/Reciprocal Pump, Model Series 940[2] was used for the infusions. The ^{3}H-cholesterol in 0.5 ml of sterile ethanol was taken into a 3 cc plastic disposable syringe[3]. The vial which had contained the isotope was rinsed with 1.5 cc of sterile ethanol which also was taken into the syringe. Air was carefully removed from the syringe, and the syringe was fitted to the Harvard Pump. A similar 3 cc disposable syringe (auxillary syringe) containing 3 cc of saline also was fitted to the pump. Disposable plastic tubing, calibrated to contain exactly 2 cc of saline, was used to deliver the infusate from the pump to the subjects' arm veins. The arrangement of the syringes on the pump is diagramed in Figure 3.

The delivery tube was filled with 2 cc of physiological saline[4]. The ^{3}H-cholesterol was delivered into the tubing at a rate of 0.786 ml/min (setting 3). The saline,

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1. Lot No. 787-164, obtained July 1974 from New England Nuclear, 575 Albany Street, Boston, Massachusetts 02118.

2. Purchased June 1970 from Harvard Apparatus Co., Inc., 150 Dover Road, Millis, Massachusetts 02054.

3. Plastipak Disposable Syringe, Beckor Dickinson & Co. Canada Ltd., 2464 South Sherridan Way, Mississauga, Ontario.

4. 0.9% Sodium Chloride Injection, USP (Normal Saline), Baxter Laboratories of Canada Ltd., Malton, Ontario.

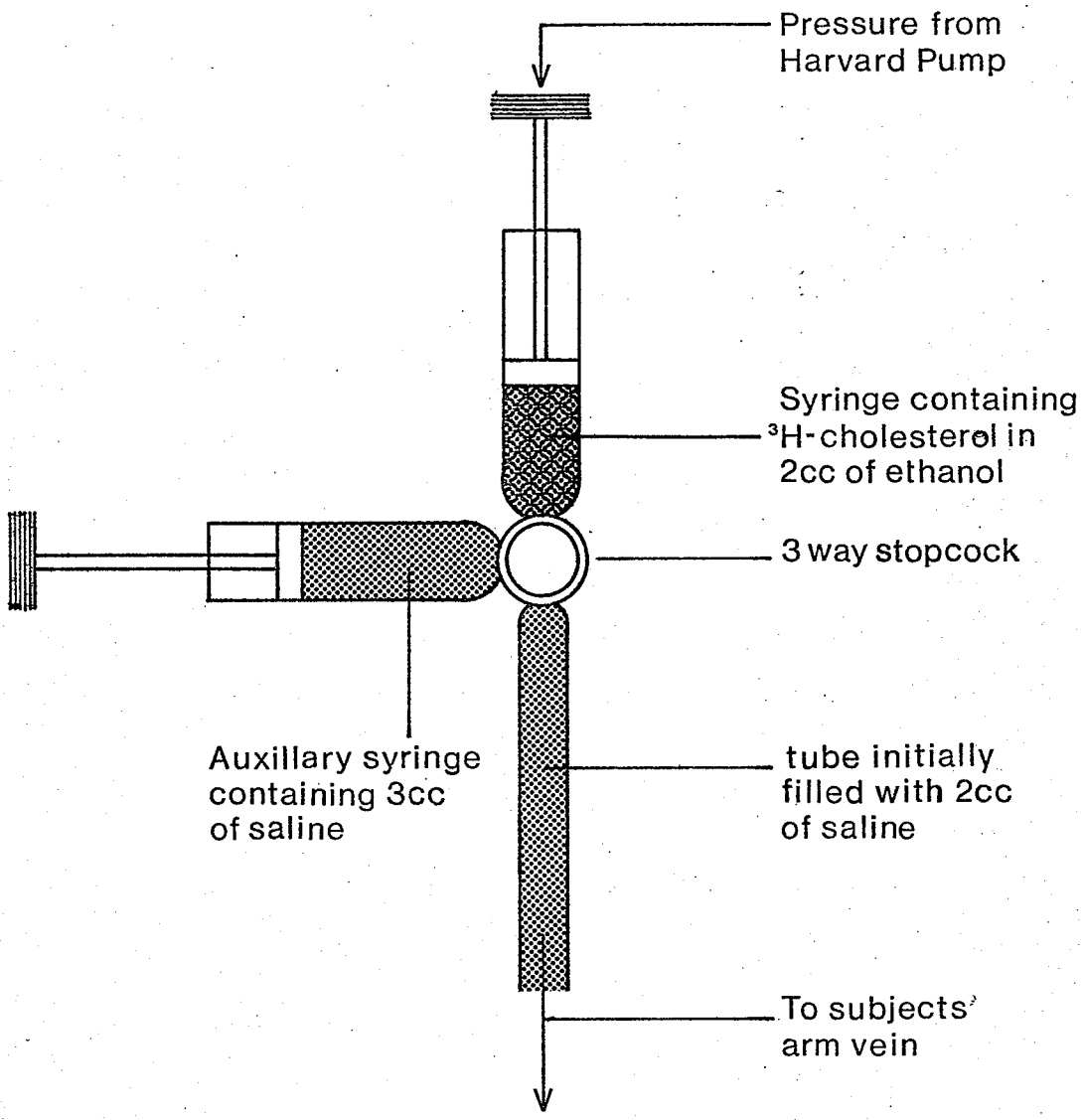

Figure 3:

The arrangement on the Harvard Pump of the syringes used to deliver the infusate.

in turn, was infused into the subject. When the contents of the first syringe had been delivered, the auxillary syringe was moved into position and its contents delivered into the tubing at a rate of 0.079 ml/min (setting 6), thus infusing the ^{3}H-cholesterol into the subject. After the 2 cc of ^{3}H-cholesterol and 0.4 cc of saline had been infused at this setting, another 0.60 cc of saline was infused at a rate of 0.786 ml/min (setting 3).

D. DIET COMPOSITION

Two menus (Table 2) were alternated daily on both the mixed fat and experimental fat regimens. Each daily menu, which was designed to provide approximately 40% of the total calories from fat and to contain complementary textures and flavours, included all food groups. The experimental diets were similar to the mixed fat diet except that lard and lard margarine or sunflower oil and corn oil margarine were substituted for the fats in the mixed fat diet.

All food servings were standardized and were weighed or measured for each individual. In addition to the three regular meals, between-meal and post-dinner snacks were provided for each subject. Standardized recipes (Appendix Tables 1-9) were followed in the preparation of all food items.

Breakfast was essentially the same for both menus; variety was provided through different cereals and juices. Lunch menus included scalloped potatoes with cooked peas or spaghetti with tomato sauce and a lettuce and tomato salad.

TABLE 2

COMPOSITION OF DIETS

Menu I	Menu II
Breakfast[1,2]	
120 gm Apple Juice	120 gm Orange Juice
30 gm Rolled Oats[3] + 25 gm fat	30 gm Cream of Wheat[3] + 16 gm fat
100 gm albumin mix[3] + 5.16 gm fat	100 gm albumin[3] + 5.16 gm fat
1 slice Bread	1 slice Bread
Jam or Jelly (1 serving)	Jam or Jelly (1 serving)
16.3 gm Sugar (brown or white)	16.3 gm Sugar (brown or white)
Lunch[1,2]	
Scalloped Potatoes[3] + 10 gm fat	Spaghetti[3]
50 gm Peas	50 gm Lettuce
1 slice Bread	50 gm Tomato
1 fresh Pear	1 slice Bread
	1 fresh Apple
Dinner[1,2]	
Chili[3]	Beef Stew[3]
100 gm Rice + 6.5 gm fat	Mashed Potatoes + 15 gm fat
50 gm Cabbage	75 gm Creamed Corn
15 gm Green Pepper	50 gm Peas
2 slices Bread	50 gm Carrots
120 gm canned Peaches	2 slices Bread
	120 gm Fruit Cocktail
Spread: 25 gm[4]	Spread: 25 gm[4]
Oil: 13.3 gm[5]	Oil: 13.3 gm[5]
Milk: 450 gm/day (150 gm/meal)	Milk: 450 gm/day (150 gm/meal)
Snacks[1,2]	
1 x 10 oz can 7-Up of subst.	1 x 10 oz can 7-Up or subst.
2 cookies	2 cookies
1 carrot and pineapple square	1 carrot and pineapple square

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1. Coffee and tea allowed ad lib. Alcohol and other beverages prohibited.
2. For quantities of each item, see diet calculations, Appendix Tables
3. See recipes, Appendix Tables 1-9.
4. 25 gm butter as spread per day during mixed fat diet, 25 gm corn oil margarine or hard margine during experimental diets.
5. Provided on mixed fat and sunflower oil diets.
6. Substitute for one 7-Up: 5½ oz apple juice + 20 gm hard candy.

A choice of a fresh apple or pear provided variety. Chili with rice and coleslaw salad or stew and mashed potatoes were the entrees at dinner. Either canned fruit cocktail or canned peaches were provided as dessert. On the mixed fat diet and sunflower oil diet 13.3 gm of oil was served with the salad. Vinegar was allowed so that an oil and vinegar dressing could be made by the subject if desired. On the lard diet, where no oil was served, the additional 13.3 gm of lard was incorporated into the entrees (Appendix Tables 10 and 11). Bread was included at each meal to utilize the spread and to permit the subjects to wipe up any visible fat remaining on the serving dishes. Worchestershire sauce, tabasco sauce, vinegar and ketchup were available at all meals. The subjects were asked to use the same amounts of these condiments daily throughout the study.

Three entrees - stew, chili and spaghetti with tomato sauce - were prepared in advance in individual foil containers[5], frozen and stored at -10°C for up to four months. No detectable changes were observed as a result of freezing and storing. Entrees for the various meals were taken directly from the freezer, heated and served in the foil container. The fourth entree - the scalloped potatoes - was not prepared in advance but ingredients were weighed out in advance for preparation on the day of serving.

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5. Sizes 685LL and 705-35, EKCO Foil Containers with Lids, Price Wilson Ltd., 830 King Edward Street, Winnipeg, Manitoba.

The mixed fat diet, formulated to simulate the amount and composition of fat in the average Canadian diet, was similar to that used previously in the Department of Foods & Nutrition (King, 1974; Lake, 1975). Since a considerable decrease in serum cholesterol had been observed on this diet in previous studies (Le Blanc, 1973; King, 1974; Lake, 1975), the formulation of the fat mix (Table 3) was changed to coincide with fat disappearance figures for Canada. The figures used were provided by Dr. Paul Sims of the Food Research Institute, Ottawa (personal communication). The relative proportions of the various fat sources used in the mixed fat diet are given in Table 4. Composition of the fat mix incorporated into the snacks and entrees is shown in Table 5. During the stabilization period, butter was used as the spread and the other fats were incorporated into the menu items.

Textured soybean protein, fluid skim milk and spray dried egg albumin[6] were utilized as the primary protein sources. The soy products used were TVP[7] and Bontrae.[8] Both are essentially fat free. The effects of dietary protein source on serum lipids were studied by substituting

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6. Chan Foods Ltd., Jarvis Street, Winnipeg, Manitoba.

7. Trade Name for Textured Vegetable Protein, Archer Daniels Midland Co., 733 Marquette Avenue, Minneapolis, Minnesota 55440.

8. Registered Trade Name of General Mills, Inc., 5000 Plymouth Avenue North, Minneapolis, Minnesota 55427.

TABLE 3

RELATIVE PERCENTAGE OF FATTY ACIDS IN MIXED FAT DIET

	Saturated	Monoun-saturated	Polyun-saturated
Literature values[1]	37.6	49.5	13.4
Calculated values[2]	37.6	49.5	13.1
Analysed values	38.6	40.6	19.9

1. Paul Sims, Food Research Institute, Ottawa.

2. Fat Acid Content of Food Fats. Compiled by Department of Foods & Nutrition, University of Manitoba, 1970-71.

TABLE 4

COMPOSITION OF THE FAT MIXTURE[1]

Ingredient	Per cent
Hydrogenated Soy	30
Lard	20
Tallow	25
Butter Oil	15
Corn Oil	10

1. See Section F), Test Fats, for details.

TABLE 5

FATTY ACID COMPOSITION OF THE FAT MIXTURE[1]

Fatty Acid	% of Total Fatty Acids
Myristic, C14:0[2]	3.0
Myristoleic, C14:1	1.7
Palmitic, C16:0	20.1
Palmitoleic, C16:1	2.4
Heptadecanoic, C17:0	0.8
Heptadecenoic, C17:1	0.7
Stearic, C18:0	14.5
Oleic, C18:1	38.3
Linoleic, C18:2	13.4
Linolenic, C18:3	0.9

1. Composition determined by gas-liquid chromatography.

2. Carbon Number: number of double bonds.

lean ground sirloin tip[9,10] for the TVP[7] and Bontrae[8] when the mixed fat diet was resumed on Day 32. The beef tallow in the fat mix was decreased proportional to the amount of fat supplied by the sirloin so that the fatty acid composition of the fat mix in the diet remained relatively unchanged.

Since lard contains cholesterol, the lard was analysed for cholesterol content[11] and an amount of pure, crystalline cholesterol, equivalent to that supplied by 133 gm of lard, was added to the 25 gm of corn oil margarine spread for each subject. Details of the analysis are explained in Section H under Ingredient Analysis.

Each menu was designed for young men and was calculated to provide 3,000 calories daily. Calories and nutrient composition of the menus are shown in Table 6, and the calculated nutrient composition of the menus is presented in Appendix Tables 12-19. Although Menu I did not meet the recommended daily intake for Vitamin A specified in the 1974 Canadian Dietary Standard, nor did Menu I or Menu II meet the 1974 recommended allowances for niacin, these menus were adequate in meeting the recommended daily intakes specified in the 1968 Dietary Standard for Canada.

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9. Analysed by Kjeldahl Method.

10. Analysed for total lipid by the Bligh and Dyer Method (1959).

11. Cholesterol content of the lard was analysed at 65.1 mg of cholesterol per 100 gm lard by the method of Tu et al (1967).

TABLE 6

CALORIE & NUTRIENT COMPOSITION OF DIETS[1]

Composition	Diet		Recommended[2]
	Menu I	Menu II	
Calories	3050.0	2977.0	3000.0
Protein (gm)	73.5	73.0	56.0
Fat (gm)	137.6	136.0	------
Carbohydrate (gm)	384.6	375.7	------
Calcium (mg)	1538.0	836.0	800.0
Phosphorus (mg)	1209.0	973.0	800.0
Iron (mg)	16.2	24.3	10.0
Vitamin A (ug RE)	623.6	1010.0	1000.0
Thiamine (mg)	1.5	1.5	1.5
Riboflavin	1.9	2.2	1.8
Niacin (mg)	15.9	14.0	20.0
Vitamin C (mg)	176.2	166.2	30.0

1. Calculated values using USDA Handbook #8, Composition of Foods (Watt & Merril, 1963).

2. Based on Revised Dietary Standard for Canada (1974). Values given for males, 75 kgm, Activity Level A.

E. STUDY ROUTINE

The subjects maintained their normal activities and resided in their own homes throughout the study. Meals were served in the Home Management Apartments in the Home Economics Building on the University of Manitoba campus. All meals were served at customary hours, although attempts were made to accommodate individual lecture time-tables. Particular emphasis was placed on the fact that no other foods were to be eaten. The general instructions given to subjects are presented in Appendix Table 20.

Each subject weighed himself daily before breakfast. Individual calorie intake was adjusted when necessary by altering the carbohydrate and fat intakes in an effort to maintain body weight constant.

F. TEST FATS

The fat sources used for the mixed fat diet included butter[12], corn oil[13], beef tallow[14], lard[15] and hydrogenated soybean oil.[16] Lard[15], specially prepared lard

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12. Modern Dairies Brand, Winnipeg, Manitoba.

13. Mazola, Best Foods Division, Canada Starch Co. Ltd., Montreal, Quebec.

14. Bleached, clarified, deodourized, Canada Packers Ltd., Winnipeg, Manitoba.

15. Kindly supplied by Mr. B.F. Teasdale, Canada Packers Ltd., Toronto, Ontario.

16. Crisco, Proctor & Gamble, Toronto, Ontario.

margarine[15] (from the same batch), sunflower oil[17] and corn oil margarine[18] were the fats used for the experimental diets.

G. STORAGE AND HANDLING OF DIETARY FATS AND OTHER FOOD STAPLES

Fats for the mixed fat diet and experimental diets were purchased as a single lot and refrigerated at 7°C in a home-type refrigerator until required. All fats and oils were stored in sealed containers.

Other staples were bought as single lots and stored under conditions considered appropriate for each food item.

Lean sirloin tip was purchased as a single lot, trimmed of all visible fat, ground and stored frozen at -10°C until required.

Fresh skim milk and bread were purchased biweekly from a single local source. The bread was frozen until required.

All entrees and snack items prepared in advance were stored at -10°C until needed.

H. INGREDIENT ANALYSIS

Samples of TVP, Bontrae protein crumbles and ground

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17. Co-op Vegetable Oils, Altona, Manitoba.

18. Fleishmans 100% Soft Corn Oil Margarine, Standard Brands Ltd., Winnipeg, Manitoba.

sirloin tip were analysed for total lipid and nitrogen content. Total lipid was extracted by the method of Bligh & Dyer (1959). Percent nitrogen was determined by the boric acid modification (AACC, 1962) of the AOAC (1960) Kjeldahl procedure for total nitrogen except that the mercuric oxide and potassium sulphate were replaced by 2 gm of a pre-mixed catalyst[19]. The analysed values for lipid and protein in the three samples are given in Table 7. On the basis of Kjeldahl nitrogen, 59.3 gm of meat was required to replace the TVP in the stew and 45.6 gm to replace the Bontrae in the chili. Since sirloin tip contains 5.2% and the soy products 1% fat, the amount of beef tallow in the stew and chili was reduced by 2.9 gm and 2.2 gm respectively when made with sirloin tip.

Total cholesterol of lard was determined by the Tu et al (1967) modification of the method by Mann (1961). The micrograms of cholesterol were obtained by comparing the sample to a reference analysed in the same manner. The optical density of all readings was taken in a Coleman Junior Spectrophotometer[20] standardized with a reagent blank. The lard was found to contain 65.1 mg of cholesterol per 100 gm and thus 133 gm of lard, the daily amount in the lard diet, provided 86.8 mg of cholesterol daily. This amount of cholesterol was added to the daily allotment of 25 gm of

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19. No. 4 Kel-pak, Canlab Supplies Limited.

20. Model No. 6A-36715, Coleman Instrument Inc., Maywood, Illinois.

TABLE 7

ANALYSIS OF TVP, BONTRAE & GROUND SIRLOIN TIP

	TVP	Bontrae	Sirloin Tip
% Nitrogen analysed[1]	48.4	49.8	20.4
% Fat analysed[2]	1.0	1.0	5.2
Protein (gm) per serving	12.1	9.3	----

1. Kjeldahl Method, AOAC (1960).
2. Bligh & Dyer (1959).

corn oil margarine in the sunflower oil diet.

Total phytosterol content of the sunflower oil was estimated by the method of Tu et al (1967). Although this method was developed for the analysis of cholesterol in meat products, all sterols with a hydroxyl group at carbon 3 in the equatorial position and the cyclohexane rings in the chair configuration will undergo precipitation with digitonin and colour reactions with ferric ions in H_2SO_4 (Lange, 1950). Total sterols were calculated as 374 mg/100 gm of sunflower oil (Table 8). This is in close agreement with the values reported by Itoh et al (1973) who list the major phytosterols in sunflower oil as campesterol (8%), stigmasterol (8%), β-sitosterol (60%), Δ^5-avenasterol (4%), Δ^7-stigmasterol (15%) and Δ^7-avenasterol (4%).

I MEAL ANALYSIS

Composites were made of each daily menu of the mixed fat and experimental fat diets. The individual food items were weighed to the nearest gram using a Satorius top-loading balance (Model 2254)[21]. Individual food items were thawed and composites of the meals were homogenized with approximately 200 ml of distilled water in a one-gallon Waring commercial blender (Model CB-5)[22]. The homogenate was weighed and a 145 to 190 grams aliquot was lyophilized

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21. Satorius-Werke AG, Gottingen, Germany.

22. Waring Products Co., Winsted, Connecticut.

TABLE 8

STEROL ANALYSIS OF SUNFLOWER OIL

	mg/100 gm Oil
Analysed Values	374
Literature Values[1]	392

1. Itoh et al (1973).

in a Model 10-140 MR-BA Virtis Freeze Dryer[23]. The dried sample was reduced to a fine particle size by pounding in Whirl-Pak plastic bags (#8992, 510-30 gm)[24] and stored at -10°C for later analysis.

Total lipid was extracted from the lyophilized food samples by the method of Bligh & Dyer (1959). An aliquot of the lipid was dissolved in petroleum ether and transferred to a 20 ml screw top vial. The vials were flushed with nitrogen and stored at -10°C until required for gas-liquid chromatography (GLC). At the time of analysis, the petroleum ether was evaporated under a stream of nitrogen and methyl esters of the fatty acids were prepared according to the method of Metcalfe et al (1966). Analyses were carried out with a Varian Aerograph gas chromatograph (Model 1740-1)[25] equipped with dual columns, flame ionization detectors, a Varian Aerograph single pen recorder (Model 20)[25] and a Varian Aerograph digital integrator (Model 477)[25].

Samples were resolved on 2.7m x 3.2mm steel columns packed with 10% EGSS-Y on 100/120 mesh GAS CHROM Q[26]. The flow rates were 30 ml/min for helium[27], 25 ml/min for

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

24. Canlab Laboratory Equipment, Winnipeg, Manitoba.

25. Varian Aerograph, 6358 Viscount Road, Malton, Ontario.

26. Applied Science Laboratories Inc., P.O. Box 440, State College, Pennsylvania 16801.

27. Welder's Supplies, 25 McPhillips Street, Winnipeg, Manitoba.

hydrogen[27] and 250 ml/min for air[27]. The columns were operated isothermically at 195°C with the injector and detector temperatures at 230°C and 250°C respectively. The individual fatty acids were identified by comparing them with linear-log plots of retention time versus carbon number of fatty acid reference standards[28].

Nitrogen content of the meals was determined by the Kjeldahl method as described previously. The factor of 6.25 was used to calculate the percent protein present.

Energy content of the diets was measured using a Parr Adiabatic Calorimeter (Model U30M) equipped with a Parr #1241 oxygen bomb calorimeter and a Parr #1541 water heater[29]. Calculated and analysed daily intakes of fat, energy and protein are given in Table 9.

Total sterol content of Menu I and Menu II for each experimental diet was determined by the method of Mann (1961) as modified by Tu et al (1967). The micrograms of sterols were obtained by comparing the samples to a reference analysed in the same manner in a colorimeter standardized with a reagent blank. Losses (= 5.3%) were corrected for on the recovery of a known amount of cholesterol run with each determination. Table 10 gives the analysed values for total sterol content of the sunflower oil and lard diets.

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28. Hormel Institute, Lipids Preparation Laboratory, 801 - 16th Avenue N.E., Austin, Minnesota 55912.

29. Parr Instrument Co., 211 Fifty-third Street, Moline, Illinois 61625.

TABLE 9

TOTAL DAILY FAT, ENERGY & PROTEIN INTAKES

	Mixed Fat		Lard		Sunflower	
	Menu I	Menu II	Menu I	Menu II	Menu I	Menu II
Fat (gm)						
Calculated	138	136	138	136	138	136
Analysed[1]	126	121	129	128	125	130
Energy (Kcal)						
Calculated[2]	3051	2977	3051	2977	3051	2977
Analysed[3]	3223	3180	3130	3163	3329	3229
Protein (gm)						
Calculated[4]	74	73	74	73	74	73
Analysed[5]	78	81	74	78	78	82

1. Analysed using the procedure of Bligh & Dyer (1959).
2. Calculated values using USDA Handbook #8, Composition of Foods (Watt & Merril, 1963).
3. Results obtained by bomb calorimetry.
4. Calculated values using USDA Handbook #8, Composition of Foods (Watt & Merril, 1963).
5. Results obtained by the Kjeldahl Method.

TABLE 10

STEROL ANALYSIS OF THE DIET (mg/100 gm)

	Lard Diet	Sunflower Oil Diet
Menu I	256	657
Menu II	242	651

J. BLOOD ANALYSIS

Blood samples were taken between 7:30 a.m. and 8:00 a.m., following a 10-hour overnight fast, on Days 4, 11, 18, 25, 32 and 39. Blood from each person was drawn into three 15 ml BD vacutainer tubes (#4796)[30]. A 15 ml sample (BD vacutainer tube #4759 containing EDTA)[30] also was drawn for whole blood analysis. The blood was allowed to clot at room temperature for one hour. The 15 ml clotted samples were centrifuged[31] at 1400 x g for five minutes to remove any contaminating red cells. The clear sera was pipetted into clear vials, and the vials were flushed with nitrogen and stored at -10°C until used for analysis.

Sera from each subject was analysed in duplicate for radioactivity, total and free cholesterol, triglyceride content and lipid phosphorus. Serum phospholipids were precipitated from acetone and the fatty acid composition determined by GLC. Haemoglobin, haematocrit, leucocyte counts and platelet counts were determined on the whole blood at the Haematological Laboratories, Health Sciences Centre, Winnipeg, Manitoba.

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30. Canlab Laboratory Equipment, Winnipeg, Manitoba.

31. Model HN-2368P-2, Centrifuge, International Equipment Co., Needham Heights, Massachusetts.

K. CHEMICAL ANALYSIS OF SERUM

1) Radioactivity: Two ml of serum were extracted for total lipid by the method of Folch et al (1957). Part of the solvent (chloroform) was evaporated under a stream of nitrogen. The dissolved lipid was transferred to a 25 ml screw top scintillation vial and the solvent removed under a stream of nitrogen. Ten ml of scintillation fluid (Appendix Table 21) was added. Samples were counted for 20 minutes or 4,000 counts in a liquid scintillation spectrometer[32] using the following settings: Data H.V. and Gate H.V. - 9.0; dial A, level 3 - 0.5 and level 5 - 9.9; and dial B, levels 3 and 4 - 0.5 and 2.3 respectively. The data attenuator was set at zero.

2) Cholesterol: Total and free cholesterol were determined by the method of Zak et al (1954). Esterified cholesterol was determined as the difference between total and free cholesterol. Samples were compared to standards of known cholesterol content in a Coleman Junior Spectrophotometer[20].

3) Lipid Phosphorus: Phospholipids were determined by the method of Chen et al (1956), except that ashing was carried out at 250°C in a heating block (Model #120C)[33] for one hour. Samples were compared to standards of

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32. Model 725, Nuclear-Chicago Corporation, 333 East Howard Avenue, Des Plaines, Illinois.

33. Hallikainen Instrument, Slaco Division, Richmond, California.

known phosphorus content in a Unicam SP600 Series 2 Spectrophotometer[34].

4) Serum Triglycerides: Serum triglycerides were extracted by the method of Ryan & Rasho (1967), except that 0.2 ml of serum was used for each determination. The saponification and colour reaction were by the method of Van Handel & Zilversmit (1967), except that sodium bisulphite was used rather than sodium arsenite as suggested by Jagannathan (1964). Samples were compared to standards of known triglyceride content. The optical density was measured at 570 mu in a Coleman Junior Spectrophotometer[20].

5) Phospholipid Fatty Acid Patterns: Total lipid was extracted from 2 ml of serum by the procedure of Folch et al (1957), and the phospholipids precipitated from acetone according to the method of Beare - Rogers (1969). Methyl esters of the fatty acids were prepared for analysis by the method of Barnes & Halloday (1972) with the following modifications. Saponification was carried out in screw top vials using 0.50 ml of 0.5N methanolic NaOH. The vials were heated in a water bath at 80°C for five minutes. The vials were cooled and 0.25 ml of BF_3/CH_3OH solution was added directly to the saponified mixture and the contents reheated at 80°C for three

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34. Model #46511, Pye Unicam Ltd., York Street, Cambridge, CB12 PX, England.

minutes. Once the vials had cooled, 1.5 ml of saturated NaCl solution was added and the methyl esters extracted by shaking with 2 ml of petroleum ether. The petroleum ether layer was removed, concentrated under nitrogen and injected directly into the gas-liquid chromatograph. The fatty acid methyl esters were resolved as previously described under Section I for Meal Analysis.

L. STATISTICAL ANALYSIS

The data was subjected to analysis of variance (Larmond, 1970) to determine whether the dietary treatments had any effect on serum lipid patterns. The Students T-test was used to determine whether the pattern of response to a particular dietary regimen varied with time (Mendenhall, 1971). The activity of labelled cholesterol in the serum on days 18, 25, 32 and 39 was expressed as a ratio of the activity on day 11 prior to subjection to analysis of variance.

RESULTS AND DISCUSSION

A. SUBJECTS

All the subjects successfully served for the duration of the study. They remained in good health with the exception of R.L. who was diagnosed as having mononucleosis when he was found to have an abnormal leucocyte count on the basis of blood taken on Day 4. This complaint did not affect his participation in the trial. The weight of three subjects remained essentially constant throughout the study. Subject R.M. gained 2.1 kgm, while subjects R.L., J.G., R.R. and H.P. lost 2.1 kgm, 2.5 kgm, 3.5 kgm and 3.6 kgm, respectively. These losses were moderate but may have had some bearing on the changes seen in serum lipid values. Nestel et al (1969) have reported decreases in serum cholesterol in response to a decrease in body weight.

B. EFFECT OF DIET ON SERUM CHOLESTEROL

1) Serum Total Cholesterol: The pattern of response of total serum cholesterol to dietary fat differed appreciably depending upon the fat source (Figure 4). Serum cholesterol decreased during the stabilization period when the subjects were fed a diet in which the fat was supplied by a mixture formulated to simulate the average disappearance of fat in Canada. When the diet was changed from mixed fat to the experimental fat, the pattern of response for the two test fats differed appreciably. Serum cholesterol continued to

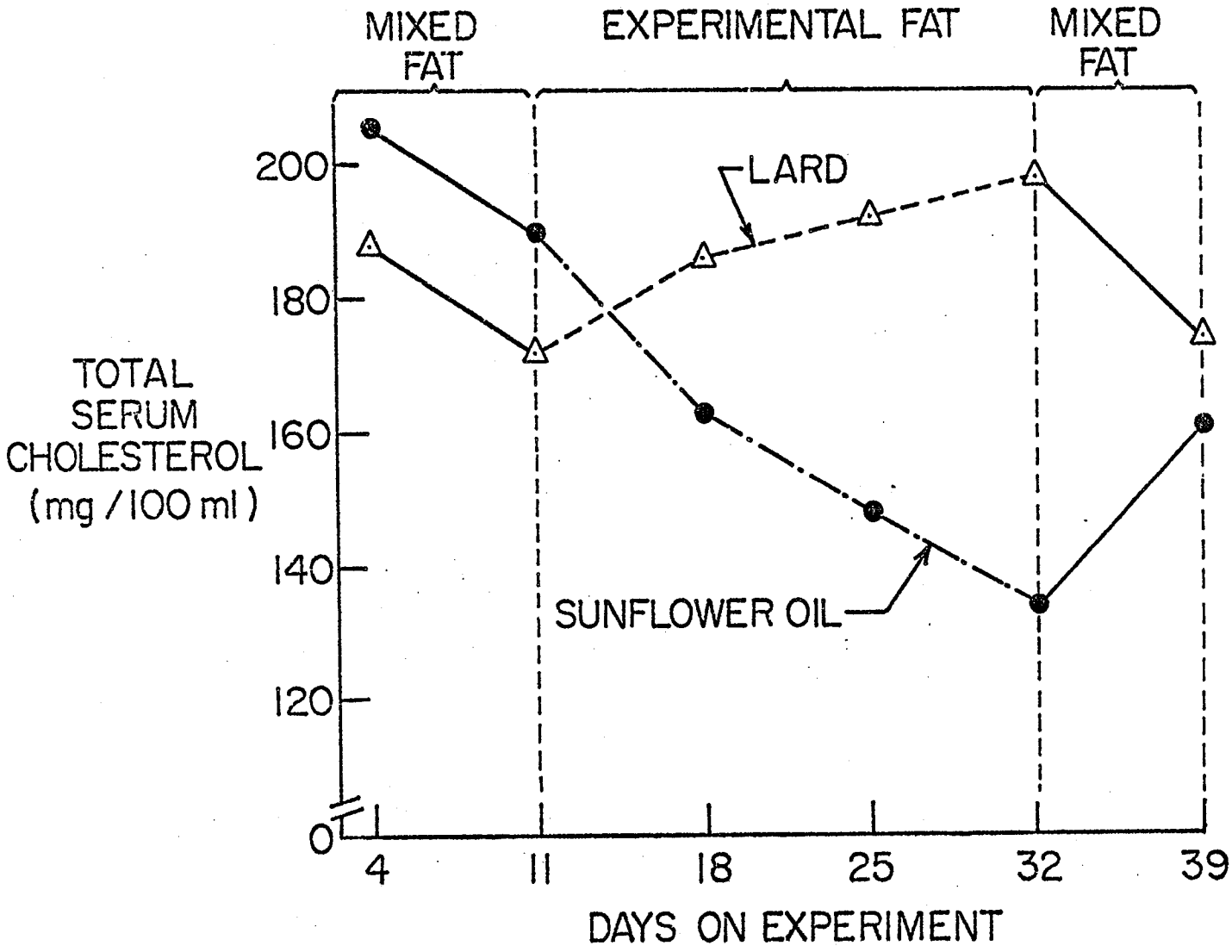

Figure 4: Mean serum cholesterol levels of subjects in response to dietary fat.

decrease for subjects fed the sunflower oil diet but increased for those on the lard diet. The patterns of response on the test fats were reversed when the mixed fat diet was again fed during the final week of the experiment. Serum total cholesterol decreased for the group who had been fed the lard diet and increased for those fed the sunflower oil diet.

The overall decrease in serum total cholesterol during the first seven days of the stabilization period was approximately 15 mg for both groups (Tables 11 and 12). Although there were no differences between the groups on Day 4 and Day 11, there was a significant decrease in serum cholesterol with time ($P < 0.001$, Appendix Table 22). This decrease in serum total cholesterol in response to a mixed fat diet has been observed previously with diets similar to that used in the present study (King, 1974; Lake, 1975). The decrease during the stabilization period, when the mixed fat diet was fed, may be attributed to a number of factors. One possibility may be that the composition of the fat ingested by the subjects prior to the start of the experiment was very different from the mixed fat diet. The decrease may also be attributed to the fact that the mixed fat diet was a low cholesterol diet. Mattson et al (1972) found that serum cholesterol was approximately 25 percent lower in men fed a cholesterol free diet than when they were fed a typical North American diet. Another factor which might have played a role is

TABLE 11

TOTAL SERUM CHOLESTEROL OF SUBJECTS IN RESPONSE TO DIETARY FAT[1]

Subject	Day of Experiment 4	11[2]	18	25	32[2]	39
	mg Cholesterol/100 ml Serum					
J.B.[3]	194.2	173.2	158.1	144.2	137.7	172.9
H.P.[3]	204.9	186.5	165.6	148.0	120.6	150.8
R.R.[3]	200.1	185.4	151.9	139.1	122.9	142.5
R.L.[3]	224.3	213.9	176.5	159.8	153.4	176.0
Group Mean	205.9	189.7	163.0	147.8	133.7	160.6
J.G.[4]	170.6	154.0	177.6	180.8	197.6	156.8
R.M.[4]	216.9	201.2	211.4	212.0	206.5	187.4
T.B.[4]	174.2	164.3	173.2	180.3	187.9	165.3
B.M.[4]	181.0	168.1	181.4	200.0	201.7	188.6
Group Mean	185.7	171.9	186.0	193.3	198.4	174.5

1. Mean of Duplicate Analyses.

2. Days on which Dietary Regimen was changed. Diets included:
   a) mixed fat diet, Days 1-10 inclusive
   b) lard or sunflower oil diet, Days 11-31 inclusive, and
   c) mixed fat diet, Days 32-39 inclusive.

3. Subject on Sunflower Oil Diet.

4. Subject on Lard Diet.

TABLE 12

CHANGES IN SERUM TOTAL CHOLESTEROL IN RESPONSE TO DIETARY FAT

	Experimental Period				
	Mixed Fat	Lard or Sunflower Oil Diet			Mixed Fat
Subject	Day 4 vs 11	Day 11 vs 18	Day 18 vs 25	Day 25 vs 32	Day 32 vs 39
	mg Cholesterol/100 ml Serum				
J.B.[1]	-21.0	-15.1	-13.9	- 6.5	+35.2
H.P.[1]	-18.4	-20.9	-17.6	-27.4	+30.2
R.R.[1]	-14.7	-33.5	-12.8	-16.2	+19.6
R.L.[1]	-10.4	-37.4	-16.7	- 6.4	+22.6
Group Mean	-16.1	-26.7	-15.2	-14.1	+26.9
J.G.[2]	-16.6	+23.6	+ 3.2	+16.8	-40.8
R.M.[2]	-15.7	+10.2	+ 0.6	- 5.5	-19.1
T.B.[2]	- 9.9	+ 8.9	+ 7.1	+ 7.6	-22.6
B.M.[2]	-12.9	+13.8	+18.1	+ 1.7	-13.1
Group Mean	-13.8	+14.1	+ 7.2	+ 5.2	-23.9

1. Subject on Sunflower Oil Diet.

2. Subject on Lard Diet.

the fact that the daily caloric intake was maintained constant and the distribution of calories among the meals was stabilized as Fábry et al (1964) observed higher serum cholesterol levels in men eating less than three meals a day.

Serum total cholesterol decreased on the sunflower oil diet ($P < 0.005$). The decrease during the first week on the diet averaged 26.7 mg/100 ml of serum (Tables 11 and 12). This downward trend continued with decreases of 15.2 and 14.1 mg during the second and third weeks. By contrast, serum total cholesterol increased ($P < 0.005$) for subjects consuming the lard diet. Average increases of 14.1 mg/100 ml of serum were observed during the first week on this regimen, with further increases of 7.2 and 5.2 mg respectively in the following two weekly periods.

Keyes and associates (1957) found that the major change in serum cholesterol, following a change of diet, occurred during the first week. The present study appears to support this observation. On the lard regimen, the changes were of a lower magnitude during the second week than during the first week and little change occurred in the third week, except for B.M. whose serum cholesterol increased 18.1 mg during the second week and J.G. whose serum total cholesterol increased 16.8 mg during the third week. Although serum total cholesterol continued to decrease over the second and third weeks on the sunflower oil diet, the decrease

observed during the third week is largely attributable to H.P. and R.R. whose values decreased 27.4 and 16.2 mg, respectively. H.P. and R.R. lost weight during the course of the study; Nestel et al (1969) have reported decreases in serum cholesterol in response to a decrease in body weight. However, the decrease cannot be attributed solely to weight loss as the serum total cholesterol of H.P. and R.R. was found to increase 30.2 and 19.6 mg respectively during the post-experimental mixed fat period while these subjects were still losing weight.

It is generally accepted that the fatty acid composition of the dietary fat plays an important role in serum cholesterol levels. Saturated fatty acids are regarded as being hypercholesterolemic and polyunsaturated fatty acids as hypocholesterolemic. Keyes et al (1965c) have suggested that the response in serum cholesterol to a change in dietary fat can be predicted when the change in fatty acid composition of the diet is expressed in terms of total calories. These authors have derived a simple equation, by multiple regression analysis, to express this relationship. In their prediction equation:

$$\Delta \text{ serum cholesterol} = 1.2(2\Delta S^1 - \Delta P)$$

where: Δ serum cholesterol is the change in serum cholesterol in mg/100 ml of serum,

ΔS^1 is the change in the percent of calories contributed by C12:0, C14:0 and C16:0 fatty acids, and

ΔP is the change in the percent of calories contributed by polyunsaturated fatty acids.

According to this equation, C12:0, C14:0 and C16:0 fatty acids were twice as effective in elevating serum cholesterol as polyunsaturated fatty acids were in decreasing it. Also worthy of note is the fact that stearic acid, short chain saturated fatty acids and the monoencic fatty acids are not included in this equation because changes in the levels of these fatty acids in the diet were not found to bring about a change in blood cholesterol (Keyes et al, 1958; Grande et al, 1970).

The fatty acid composition of the diets used in the present study is given in Table 13. Lauric, myristic and palmitic acids, the major fatty acids regarded as having a hypercholesterolemic effect (Keyes et al, 1965c), together contributed 24.2 percent of the total fatty acids in the mixed fat diet, 27.4 percent in the lard diet and 8.5 percent in the sunflower oil diet. The linoleic acid content of the mixed fat diet (18.3 percent) was higher than that of the lard diet (11.9 percent), while linoleic acid accounted for 65.4 percent of the total fatty acids in the sunflower oil diet.

Fat contributed 34 to 37 percent of the total calories in these diets. In terms of the percentage of total calories contributed by the various classes of fatty acids, saturated fatty acids other than stearic acid contributed 1.7 percent more calories in the lard

TABLE 13

PERCENT FATTY ACID COMPOSITION OF DIETS

	Fatty Acid Composition		
Fatty Acid	Diet/ Mixed Fat	Lard	Sunflower Oil
Lauric, C12:0[1]	0.6	0.8	tr
Myristic, C14:0	2.9	1.8	tr
Palmitic, C16:0	20.7	24.8	8.5
Palmitoleic, C16:1	2.0	2.5	tr
Stearic, C18:0	13.4	15.9	4.8
Oleic, C18:1	38.0	40.4	20.4
Linoleic, C18:2	18.3	11.9	65.4
Linolenic, C18:3	1.8	0.8	0.8

1. Carbon number: number of double bonds.

diet than in the mixed fat diet while the polyunsaturated fatty acids contributed 2.3 percent less calories (Table 14). On the other hand, the sunflower oil diet contained 5.4 percent less calories from lauric, myristic and palmitic acids than the mixed fat diet and 16.2 percent more calories from polyunsaturated fatty acids (Table 14).

The observed changes in serum total cholesterol levels in the present study were greater on the experimental fats than those predicted by the Keyes et al equation. There was a decrease of 56 mg on the sunflower oil diet (Tables 11 and 15), whereas the predicted change on the basis of Keyes equation was 32.4 mg/100 ml of serum (Table 15). Similarly, when the lard diet was fed, total serum cholesterol was found to increase 26.5 mg/100 ml of serum, whereas the predicted change was 6.8 mg/100 ml of serum. Le Blanc (1973) also observed slightly lower values than those predicted by the Keyes equation when a diet rich in low erucic acid rapeseed oil was fed, and Losier (1972) found the decrease in serum cholesterol was considerably greater than that predicted by the Keyes equation when a diet rich in corn oil was fed. Keyes et al (1965d) also found that the observed decrease exceeded the predicted decrease when a diet rich in corn oil was fed and have suggested that this apparent discrepancy with corn oil is associated with the relatively high plant sterol content which is estimated at

TABLE 14

PERCENT TOTAL DAILY CALORIES CONTRIBUTED BY GLYCERIDES OF SATURATED MINUS STEARIC AND POLYUNSATURATED FATTY ACIDS FOR EACH DIET

Diet	% Total Daily Calories From Fatty Acids		% Total Calories From Fat
	S[1]	P[2]	
Mixed Fat	8.4	7.0	34.4
Sunflower Oil	3.0	23.2	34.9
Lard	10.1	4.7	36.7

1. Total saturated fatty acids minus stearic.

2. Total polyunsaturated fatty acids.

TABLE 15

OBSERVED AND PREDICTED CHANGES IN SERUM CHOLESTEROL LEVELS (mg per 100 ml) OF SUBJECTS FED SUNFLOWER OIL AND LARD DIETS

Diet Comparison	Observed Change	Predicted Change[1]
Sunflower vs Stabilization	-56.0	-32.4
Lard vs Stabilization	+26.5	+ 6.8

1. According to Keyes et al, 1965c. $\Delta C = 1.2\ (2\Delta S^{1} - \Delta P)$

580-1000 mg/100 gm of oil (Lange, 1950). Plant sterols have been found to inhibit the absorption of cholesterol, but approximately 6 to 10 gm daily is the minimum amount needed to produce a discernible reduction in serum cholesterol (Keyes et al, 1974). This does not explain the present results or those of Le Blanc. The sunflower oil used in this present study was analysed and found to contain 374 mg of phytosterols/100 gm of oil.

It may not be justified to attach too much functional significance to a regression equation such as that derived by Keyes et al (1965c). Hegsted et al (1965) has cautioned that regression equations are primarily descriptive of the information from which they are derived.

When the subjects were returned to the mixed fat diet on Day 32, the patterns which had been observed on the experimental fats were reversed (Figure 4). Serum total cholesterol increased 26.9 mg/100 ml of serum ($P < 0.001$) for those who had consumed the sunflower oil diet and decreased 23.9 mg for those who had consumed the lard diet. In spite of this reversal in pattern of response on the mixed fat diet, the value for the group that had been fed the sunflower oil was significantly lower ($P < 0.005$) on Day 39 than on Day 11. There was no difference ($P > 0.05$), however, in mean serum cholesterol values on Day 39 and Day 11 for the group that had been fed the lard diet. There also was no difference ($P > 0.05$) between the two groups on Day 39.

The data from the present study confirms the hypothesis that sunflower oil, a rich source of linoleic acid, is hypocholesterolemic whereas lard, a fat with a higher proportion of palmitic acid, is hypercholesterolemic.

2) Serum Free and Esterified Cholesterol: Free and esterified cholesterol followed the same overall pattern of response to diet as total serum cholesterol, although the relationship appeared more consistent with free cholesterol than with esterified cholesterol. Serum free and esterified cholesterol decreased on the sunflower oil diet and increased on the lard diet.

As in the case of serum total cholesterol, there was a significant decrease ($P < 0.001$, Appendix Tables 23 and 24) in both free and esterified cholesterol between Days 4 and 11 when the mixed fat diet was fed. However, there was no difference between the groups on Day 4 or Day 11. The pattern of response differed for the two test fats. There was a 24.7 mg decrease in serum free cholesterol on the sunflower oil diet (Tables 16 and 17) and an increase of 15.9 mg on the lard diet. This upward trend on the lard diet was observed in all subjects with the exception of B.M. whose serum free cholesterol decreased 4.0 mg during the third week. In spite of the marked difference in pattern of response to the test fats, the levels of free cholesterol did not differ significantly between the

TABLE 16

SERUM FREE CHOLESTEROL OF SUBJECTS IN RESPONSE TO DIETARY FAT[1]

Subject	Day of Experiment 4	11[2]	18	25	32[2]	39
	mg Free Cholesterol/100 ml Serum					
J.B.[3]	75.2	66.6	59.8	53.0	46.4	70.6
H.P.[3]	72.1	59.0	51.0	41.5	35.1	44.6
R.R.[3]	76.4	68.6	68.3	60.9	55.4	61.5
R.L.[3]	107.2	103.2	82.3	72.6	62.0	83.7
Group Mean	82.7	74.4	65.4	57.0	49.7	65.1
J.G.[4]	62.2	51.5	57.9	60.2	66.7	54.7
R.M.[4]	78.6	66.6	75.9	80.1	79.6	66.4
T.B.[4]	64.0	53.6	59.5	69.2	78.5	66.1
B.M.[4]	68.0	55.5	61.5	69.8	65.8	58.9
Group Mean	68.2	56.8	63.7	69.8	72.7	61.5

1. Mean of Duplicate Analyses.
2. Days on which Dietary Regimen was changed.
3. Subject on Sunflower Oil Diet.
4. Subject on Lard Diet.

TABLE 17

CHANGES IN SERUM FREE CHOLESTEROL IN RESPONSE TO DIETARY FAT

	Experimental Period				
	Mixed Fat	Lard or Sunflower Oil Diet			Mixed Fat
Subject	Day 4 vs 11	Day 11 vs 18	Day 18 vs 25	Day 25 vs 32	Day 32 vs 39
	mg Free Cholesterol/100 ml Serum				
J.B.[1]	- 8.6	- 6.8	- 6.8	- 6.6	+24.2
H.P.[1]	-13.1	- 8.0	- 9.5	-10.4	+ 9.5
R.R.[1]	- 7.8	- 0.6	- 7.4	- 5.4	+ 6.1
R.L.[1]	- 4.0	-20.9	- 9.7	-10.6	+21.7
Group Mean	- 8.4	- 9.1	- 8.4	- 8.2	+15.4
J.G.[2]	-10.7	+ 6.4	+ 2.3	+ 6.5	-12.0
R.M.[2]	-12.0	+ 9.3	+ 4.2	+ 0.5	+13.2
T.B.[2]	-10.4	+ 5.9	+ 9.7	+ 9.3	-12.4
B.M.[2]	-12.5	+ 6.0	+ 8.3	- 4.0	- 6.9
Group Mean	-11.4	+ 6.9	+ 6.1	+ 3.1	-11.1

1. Subject on Sunflower Oil Diet.

2. Subject on Lard Diet.

subjects fed lard and those fed sunflower oil. However, there were significant changes in free cholesterol with time ($P < 0.001$). Failure to find a difference between the groups may be attributed to the fact that the mean serum free cholesterol values for the group fed the lard diet were lower on Day 11 and higher on Day 39 than for those fed the sunflower oil diet.

Serum esterified cholesterol levels decreased 31.3 mg/100 ml of serum (Tables 18 and 19) for subjects fed the sunflower oil diet. Conversely, serum esterified cholesterol increased 10.7 mg/100 ml of serum for subjects fed the lard diet. However, as mentioned previously, the pattern of change in esterified cholesterol was less consistent than for free cholesterol. For example, values for R.M. and T.B. decreased 8.2 and 4.3 mg respectively during the last two weeks on the lard diet, whereas there was no change in serum esterified cholesterol for J.B. in the final week on the sunflower oil diet.

The response in serum free and esterified cholesterol to the mixed fat diet during the final seven days of the study was opposite to that observed on the experimental fats. Serum free cholesterol increased 15.4 mg/100 ml for those who had consumed the sunflower oil diet, whereas serum free cholesterol levels fell 11.1 mg/100 ml for those who had consumed the lard diet. Esterified cholesterol levels followed a similar pattern to free cholesterol. There was an increase of

TABLE 18

ESTERIFIED CHOLESTEROL OF SUBJECTS IN RESPONSE TO DIETARY FAT[1]

Subject	Day of Experiment 4	11[2]	18	25	32[2]	39
	mg Esterified Cholesterol/100 ml Serum					
J.B.[3]	119.0	106.6	98.3	91.2	91.3	102.3
H.P.[3]	132.8	127.5	114.6	107.5	85.5	106.2
R.R.[3]	123.6	116.8	83.6	78.2	67.5	81.0
R.L.[3]	113.1	110.7	94.2	87.2	91.4	92.3
Group Mean	122.1	115.4	97.7	91.0	83.9	95.5
J.G.[4]	118.4	102.5	119.7	120.6	130.9	102.1
R.M.[4]	168.3	134.6	135.5	131.9	126.9	121.0
T.B.[4]	110.2	110.7	113.9	111.1	109.4	99.2
B.M.[4]	113.0	112.6	120.4	130.2	135.9	129.7
Group Mean	120.0	115.1	122.3	123.5	125.8	113.0

1. Mean of Duplicate Analyses.
2. Days on which Dietary Regimen was changed.
3. Subject on Sunflower Oil Diet.
4. Subject on Lard Diet.

TABLE 19

CHANGES IN SERUM ESTERIFIED CHOLESTEROL IN RESPONSE TO DIETARY FAT

	Experimental Period				
	Mixed Fat	Lard or Sunflower Oil Diet			Mixed Fat
Subject	Day 4 vs 11	Day 11 vs 18	Day 18 vs 25	Day 25 vs 32	Day 32 vs 39
	mg Cholesterol/100 ml Serum				
J.B.[1]	-12.4	- 8.3	- 7.1	+ 0.1	+11.0
H.P.[1]	- 5.3	-12.9	- 8.1	-12.0	+20.7
R.R.[1]	- 6.8	-33.2	- 5.4	-10.7	+13.5
R.L.[1]	- 2.4	-16.5	- 7.0	- 4.2	+ 0.9
Group Mean	- 6.7	-17.7	- 6.9	- 6.7	+11.5
J.G.[2]	-15.9	+17.2	+ 0.9	+10.3	-28.8
R.M.[2]	- 3.7	+ 0.9	- 3.2	- 5.0	- 5.9
T.B.[2]	- 0.5	+ 3.0	- 2.6	- 1.7	-10.2
B.M.[2]	- 0.4	+ 7.8	+ 9.8	+ 5.7	- 6.2
Group Mean	- 5.1	+ 7.2	+ 1.2	+ 2.3	-12.8

1. Subject on Sunflower Oil Diet.

2. Subject on Lard Diet.

11.5 mg/100 ml for those who had consumed the sunflower oil diet and a 12.8 mg/100 ml decrease for those who had consumed the lard diet. Although there was a trend for the values to return to the levels that prevailed on Day 11, the increase in serum esterified cholesterol from Day 32 to Day 39 was not sufficient to offset the decrease which had occurred on the sunflower oil diet. However, the mean serum esterified cholesterol value on Day 39 of the subjects who had been fed the lard diet did not differ statistically from that on Day 11. Similarly, the mean serum free cholesterol levels on Day 39 approached those of Day 11 for both groups, but the values on Day 39 for those who had been fed lard or sunflower oil were statistically different from Day 11 ($P < 0.005$). However, there was no significant difference between the two groups on Day 39 in either free or esterified cholesterol.

Serum free and esterified cholesterol closely followed the pattern of total serum cholesterol as illustrated by the fact that the proportion of free and esterified cholesterol remained fairly constant within each group throughout the experiment (Tables 20 and 21) although there was considerable variation among individuals. Free and esterified cholesterol (Tables 20 and 21) comprised 40 and 60 percent of the total cholesterol, respectively, for the sunflower oil group, and 35 and 65 percent respectively for the lard group.

TABLE 20

PERCENT FREE CHOLESTEROL
OF TOTAL SERUM CHOLESTEROL

Subject	Day of Experiment 4	11	18	25	32	39
H.P.	35.2	31.6	30.8	29.9	30.0	30.0
J.B.	38.7	38.5	37.8	36.7	33.7	40.8
R.R.	38.1	37.0	44.9	43.8	45.1	43.2
R.L.	47.8	48.2	46.6	45.7	40.4	47.5
Group Mean[1]	40.0	38.8	40.0	39.0	37.3	40.4
J.G.	34.4	33.4	32.6	33.2	35.7	34.9
R.M.	36.2	33.1	35.9	37.8	38.6	35.4
T.B.	36.7	32.6	34.3	38.4	41.7	39.9
B.M.	37.6	33.0	33.8	34.9	32.6	31.2
Group Mean[2]	36.2	33.0	34.2	36.1	37.2	35.4

1. Group on Sunflower Oil Diet.

2. Group on Lard Diet.

TABLE 21

PERCENT ESTERIFIED CHOLESTEROL
OF TOTAL SERUM CHOLESTEROL

Subject	Day of Experiment 4	11	18	25	32	39
H.P.	64.8	69.4	69.2	70.1	70.0	70.0
J.B.	62.3	62.5	62.2	63.3	66.3	59.2
R.R.	61.9	63.0	55.1	56.2	54.9	56.5
R.L.	52.2	51.8	53.4	54.3	59.6	52.5
Group Mean[1]	60.3	61.7	60.0	61.0	62.7	59.6
J.G.	65.6	66.6	67.4	66.8	64.3	65.1
R.M.	63.8	66.9	64.1	62.2	61.4	64.6
T.B.	63.3	67.4	65.7	61.6	58.3	60.1
B.M.	62.4	67.0	66.2	65.1	67.4	68.8
Group Mean[2]	63.8	67.0	65.9	63.9	62.9	64.7

1. Group on Sunflower Oil Diet.

2. Group on Lard Diet.

Examination of the proportions which free and esterified cholesterol comprised of the total serum cholesterol for each of the subjects indicates that esterified cholesterol comprised between 60 and 70 percent of the total cholesterol except for R.L. where esterified cholesterol made up 54 percent of the total. Goodman (1965) has reported that cholesterol esters account for between 60 and 80 percent of the total serum cholesterol.

The data of the present study support the reports (Goodman and Nobel, 1968; Nestel, 1970) that there is a rapid equilibration between free and esterified cholesterol. Nestel et al (1969) found that equilibration between plasma free and esterified cholesterol was complete within four days following a single intravenous injection of cholesterol-4-^{14}C complexed with plasma lipoprotein. Nestel (1970) has estimated that the turnover of esterified cholesterol in the plasma is about 100 mg per hour with the plasma being the major site of cholesterol ester formation.

3) Turnover of Plasma Cholesterol: The turnover of plasma cholesterol in response to changes in diet was monitored by measuring the rate of disappearance of tritium-labelled cholesterol from the plasma. The mean decrease in ^{3}H-cholesterol on the mixed fat diet (Days 4-10) was similar for the four subjects who

eventually consumed the sunflower oil diet and for those who eventually consumed the lard diet (Figure 5, Table 22). However, there was an appreciable difference in the rate of decrease of ^{3}H-cholesterol ($P < 0.001$, Appendix Table 25) for the two groups when fed the lard and sunflower oil diets. The different responses for the two diets was evident within a week (Figure 5, Table 22). Loss of labelled cholesterol was twice as great on the sunflower oil diet as on the lard diet. The marked decrease in labelled cholesterol observed on the sunflower oil diet coincided with the appreciable decrease in serum cholesterol on this diet. Similarly, the much lower decrease in cholesterol turnover on the lard diet relative to that on the sunflower oil diet coincided with the increase in serum cholesterol on this diet. The level of labelled cholesterol (dpm/ml serum) was significantly lower on Day 32 for the group fed the sunflower oil diet than the group fed the lard diet. However, there was no difference between the two groups on Day 39 after they had been fed the mixed fat diet for seven days.

Specific activity of cholesterol in the serum (Table 23, Figure 6 and Appendix Table 26) was similar for both groups. The fact that there was no obvious change in the slope of the specific activity-time response curve to changes in dietary fat source in the present study, whereas plasma cholesterol levels and

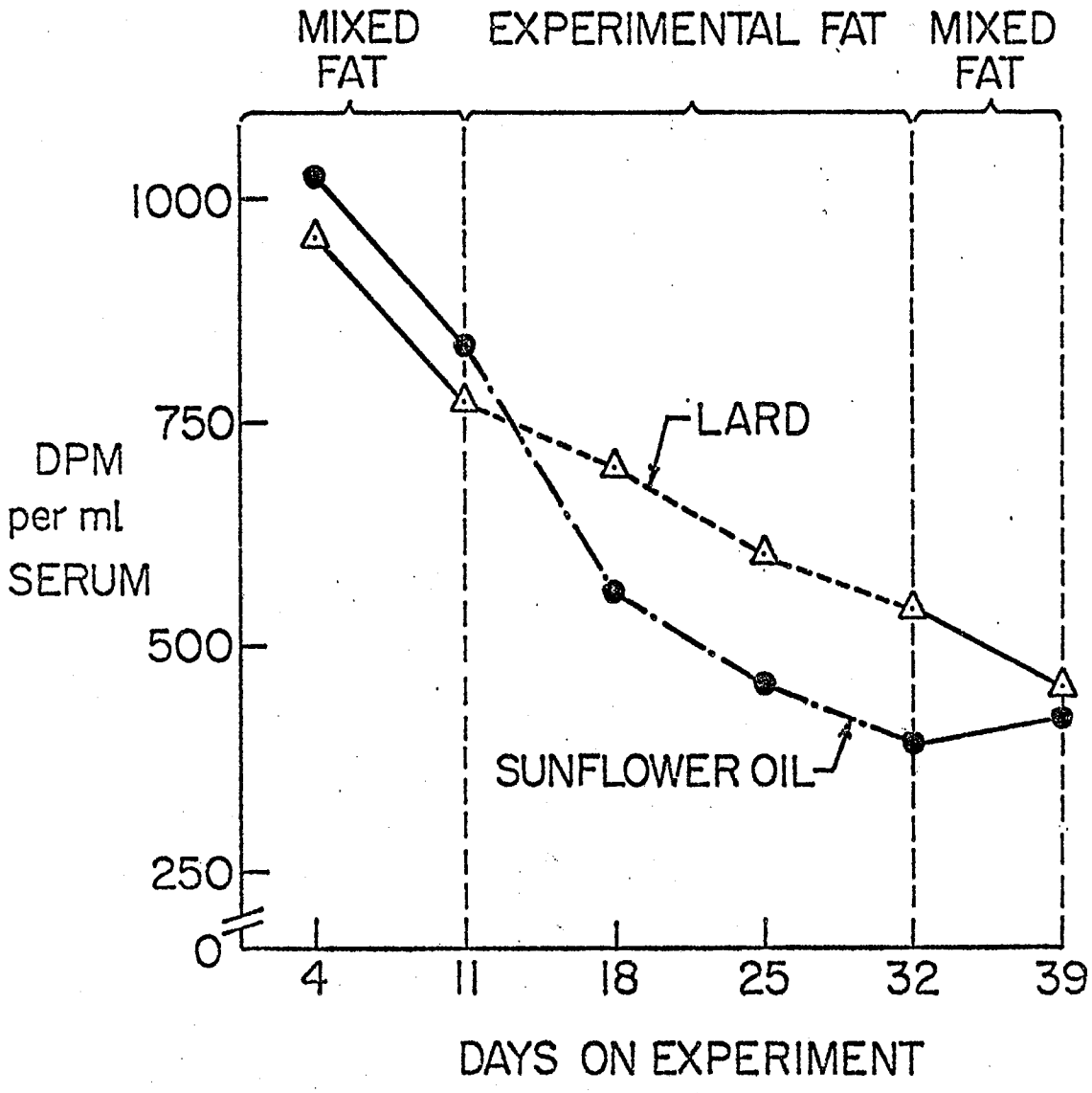

Figure 5: Mean DPM of serum cholesterol of subjects following infusion with 1,2 - ^{3}H cholesterol in response to dietary fat.

TABLE 22

DPM IN 1 ml OF SERUM OF SUBJECTS IN RESPONSE TO DIETARY FAT[1]

	Day of Experiment					
Subject	4	11[2]	18	25	32[2]	39
J.B.[3]	1276	1084	749	599	540	578
H.P.[3]	488	462	299	269	234	251
R.R.[3]	1059	799	567	440	393	413
R.L.[3]	1296	1028	653	534	430	440
Group Mean	1030	843	567	461	399	421
J.G.[4]	847	662	607	520	508	415
R.M.[4]	1028	817	776	670	589	503
T.B.[4]	591	440	415	370	339	270
B.M.[4]	1397	1170	1020	849	776	634
Group Mean	966	772	705	602	553	456

1. Mean of Duplicate Analyses.
2. Days on which diet was changed.
3. Subject on Sunflower Oil Diet.
4. Subject on Lard Diet.

TABLE 23

SERUM SPECIFIC ACTIVITY OF
SUBJECTS IN RESPONSE TO DIETARY FAT

	Day of Experiment					
Subject	4	11[1]	18	25	32[1]	39
J.B.[2]	657	624	471	415	390	640
H.P.[2]	238	248	181	181	194	166
R.R.[2]	530	431	373	316	320	304
R.L.[2]	578	478	370	334	277	250
Group Mean	501	445	349	312	295	340
J.G.[3]	469	430	341	290	257	270
R.M.[3]	472	406	367	316	285	268
T.B.[3]	339	268	240	206	181	167
B.M.[3]	772	696	561	430	385	337
Group Mean	513	450	377	311	277	261

1. Days on which diet was changed.
2. Subject on Sunflower Oil Diet.
3. Subject on Lard Diet.

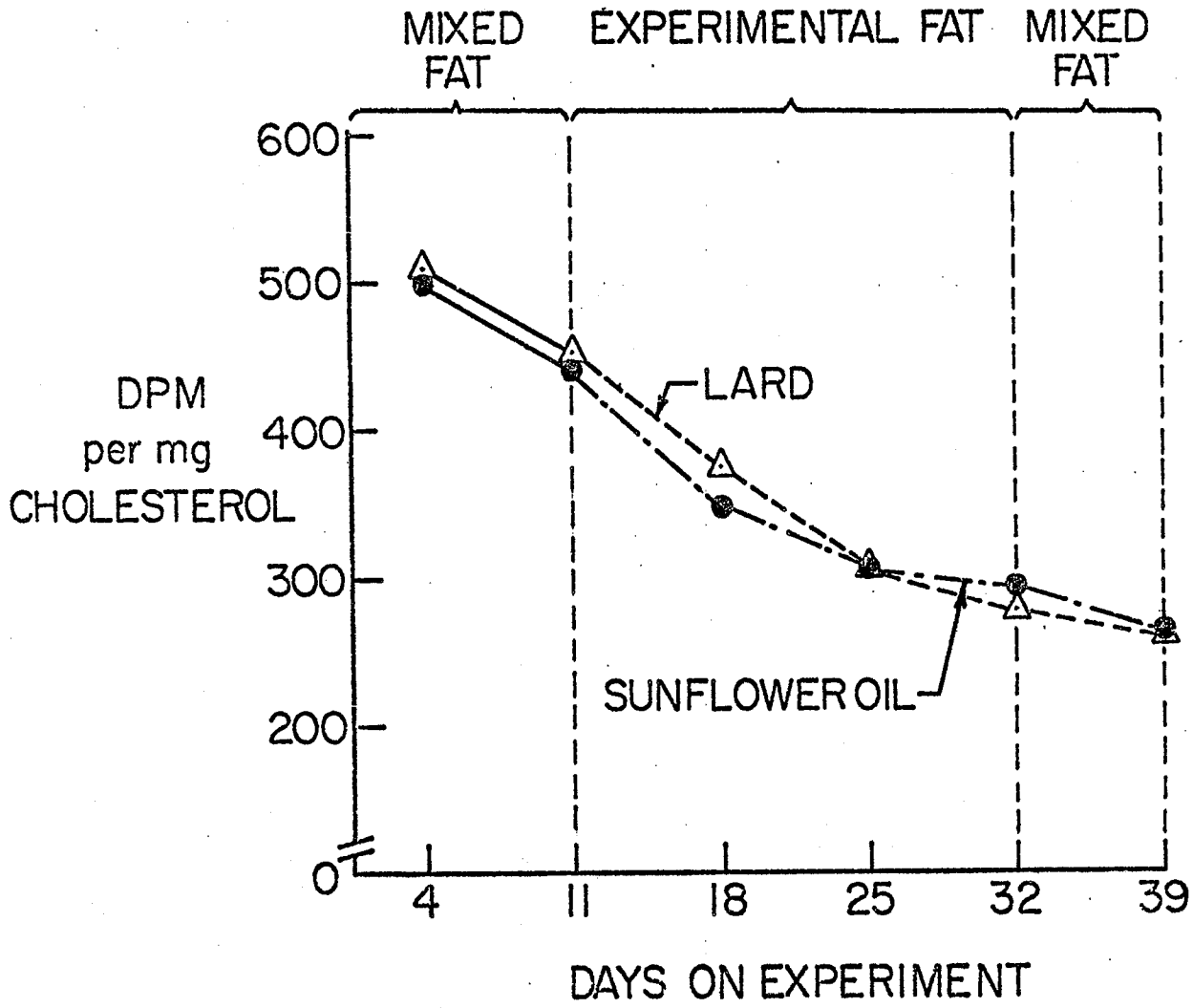

Figure 6: Mean serum specific activity of cholesterol of subjects in response to dietary fat.

the level of labelled cholesterol differed appreciably between the dietary treatments when the lard and sunflower oil diets were fed, indicates that there was a difference in the rate of turnover of plasma cholesterol on the two test diets. Thus, lard and sunflower oil appear to differ appreciably in their effects on the rate of excretion of cholesterol or on the distribution of cholesterol between the plasma and other tissue pools. Furthermore, the results of the present study suggest that dietary fat source had no effect on the rate of synthesis of cholesterol because the slopes of the specific activity-time curves were essentially the same (Figure 6). A downward deflection in the slope of the specific activity-time curve (Figure 1) in response to diet would reflect an increase in total body cholesterol as a result of increased exogenous cholesterol synthesis or increased absorption of exogenous cholesterol. An upward deflection in the slope of the specific activity-time curve would suggest a decrease in the rate of endogenous synthesis or a decrease in the absorption of exogenous cholesterol. The literature is inconclusive on the effect of dietary fat on cholesterol synthesis. Grundy and Ahrens (1966) found a transient upward deflection in the slope of the specific activity-time curve for plasma cholesterol when a butter oil diet was followed by a diet rich in corn oil. However, the same authors (Grundy and Ahrens,

1970) also have reported no change in the slope of the curve when a diet rich in butter oil was followed by a diet rich in corn oil.

Body cholesterol is generally regarded as conforming to a model involving two pools; a pool of rapidly equilibrating cholesterol (Pool A) and one which exchanges more slowly (Pool B). Entry into and exit from the system occurs primarily through Pool A. On the basis of the two pool system, it is possible to postulate various mechanisms by which sunflower oil and lard could induce the effects observed in the present study.

Pool A was found to decrease on the sunflower oil diet as indicated by the decrease in the concentration of serum cholesterol. There are several means by which sunflower oil may effect this change. The size of Pool A may be decreased because of increased excretion of cholesterol (Figure 7.1). Since the exchange between Pool A and Pool B is relatively slow, equilibrium between the two may take up to three to four weeks. Alternatively, sunflower oil may bring about a decrease in the size of Pool A through an increased transfer of cholesterol from Pool A into Pool B (Figure 7.2a), or a decrease in the rate of transfer of cholesterol from Pool B into Pool A (Figure 7.2b). Pool A also may be decreased in size due to a decrease in the rate of cholesterol synthesis or a decrease in the rate of absorption of cholesterol. However, these latter two

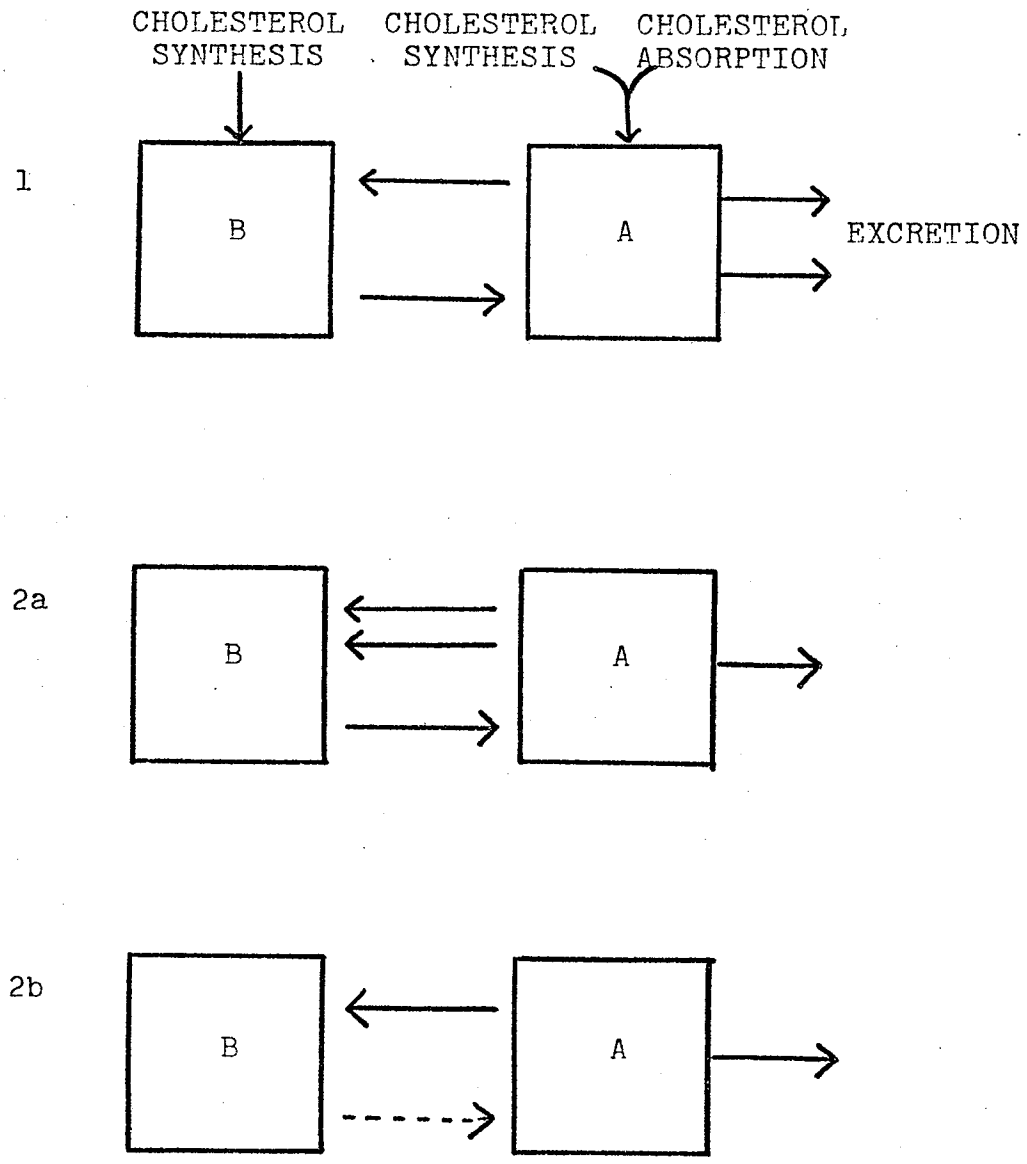

Figure 7: Possible mechanisms by which sunflower oil may effect changes in the amount of cholesterol in pool A.

possibilities would bring about a change in the specific activity-time curve. As there was no change in the slope of the specific activity-time curve in the present study, it can be concluded that the rate of synthesis and absorption were not affected appreciably by dietary fat source.

Lard was found to bring about an increase in the size of Pool A in the present study, as evidenced by the increase in plasma cholesterol concentration. This hypercholesterolemic effect may be brought about because the excretion of cholesterol from Pool A was reduced (Figure 8.1). Alternatively, lard may disrupt the equilibrium between Pool A and Pool B (Figure 8.2a) in a manner similar but opposite to that produced by sunflower oil. A third possibility, as in the case of sunflower oil, is that absorption of, and synthesis of, cholesterol may be changed. However, as mentioned previously, this possibility is ruled out by the fact that there was no change in the slope of the specific activity-time curves in response to changes in dietary fat source.

At the present time, it is only possible to speculate on which of these mechanisms was operating. Analysis of the fecal lipids[1] should help to resolve the mechanisms. However, if the changes in serum cholesterol levels in response to diets in the present study are found to be due

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1. Quantitation of the fecal lipids is the responsibility of Mr. Gary Sloan, Masters' candidate in the Department of Foods & Nutrition, University of Manitoba, Winnipeg, Manitoba.

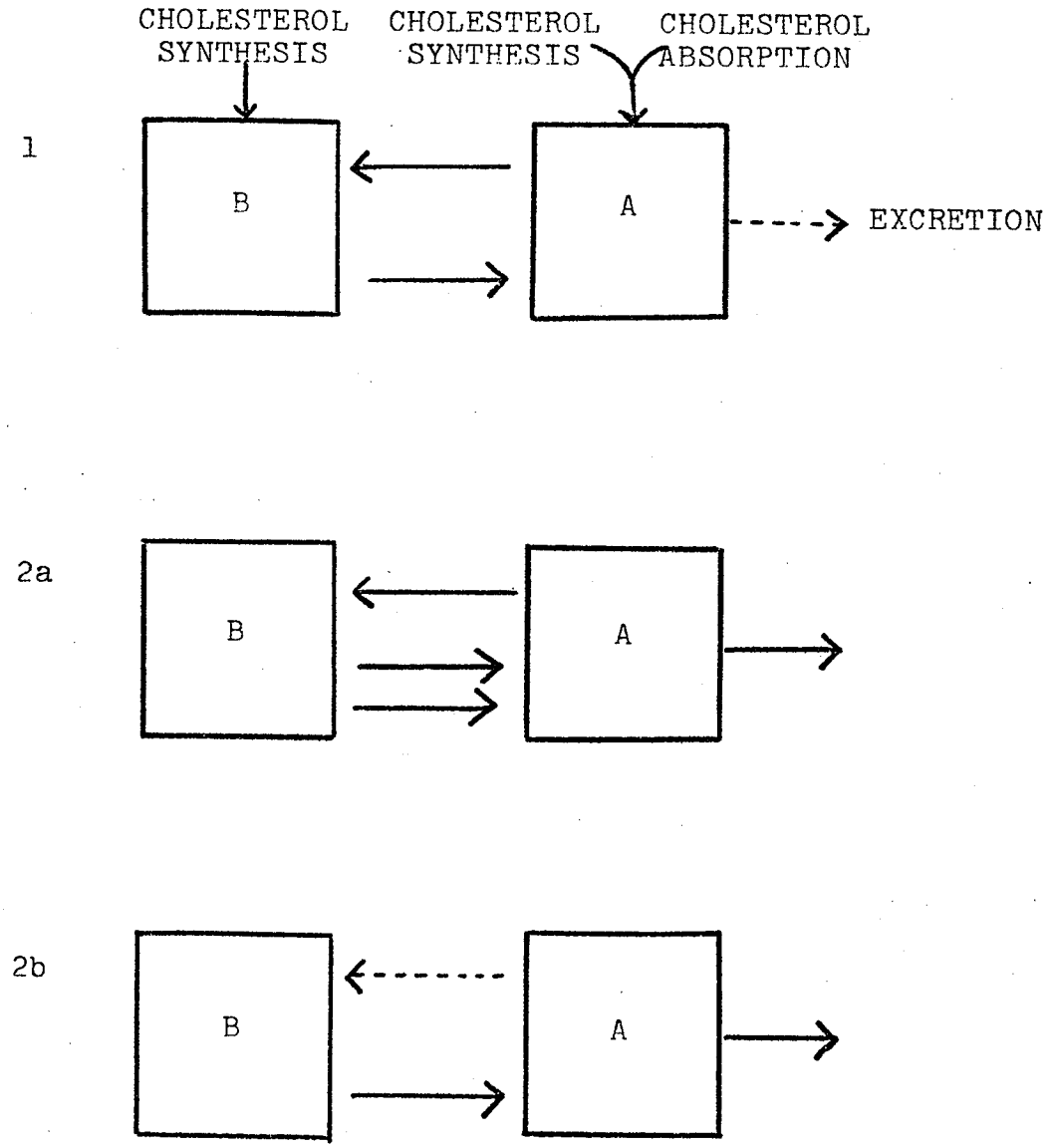

Figure 8: Possible mechanisms by which lard may effect changes in the amount of cholesterol in pool A.

to redistribution between Pool A and Pool B, it will not be possible from the data gathered in this study to determine the mechanism by which the new equilibrium between Pool A and Pool B is established. Even if the rate of excretion of cholesterol and its metabolites is found to change in response to a change in dietary fat source, there is also the possibility that a change in the rate of exchange of cholesterol between Pool A and Pool B also had occurred.

C. EFFECT OF DIET ON SERUM TRIGLYCERIDES

Serum triglycerides decreased significantly ($P < 0.001$, Appendix Table 27) during the stabilization period (Days 4-10) when the mixed fat diet was fed. A decrease of 14.2 mg/100 ml was observed for the group subsequently fed the lard diet and a decrease of 8.2 mg for those who went onto the sunflower oil diet (Figure 9, Table 24). Although the decrease in serum triglycerides was statistically significant, there was no difference among the two groups on either Day 4 or Day 11. Serum triglycerides continued to decrease for the subjects fed the sunflower oil diet. A decline of 19.4 mg percent was observed during the first week (Table 25) with decreases of 7.9 and 9.0 mg respectively in the next two weeks. A substantial portion of the decrease between Days 11 and 18 was due to the 53.8 mg decrease of serum triglycerides for H.P. When the study was commenced, this subject had higher serum triglycerides (151.3 mg percent) than the other subjects. He also was overweight on the basis of weight for

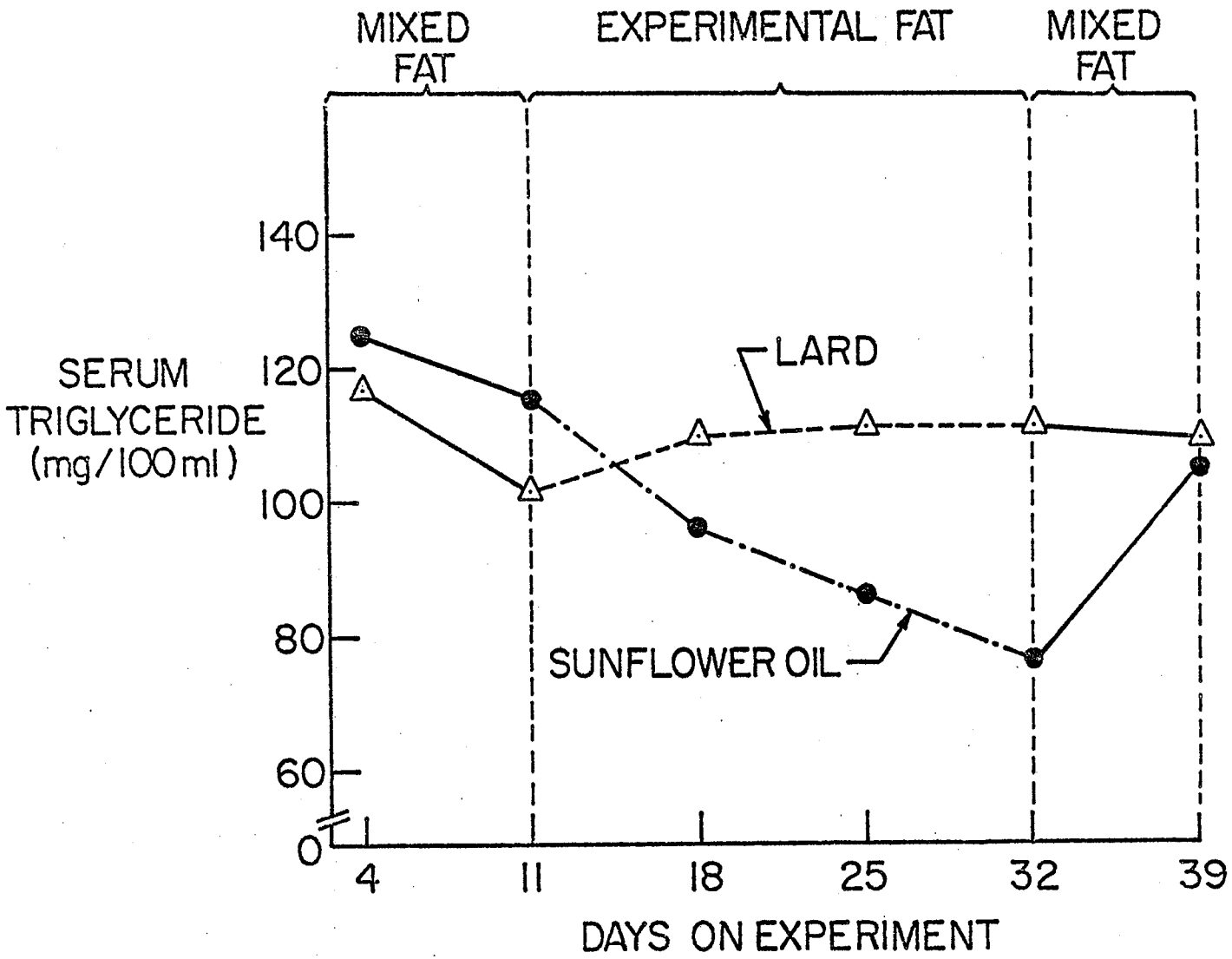

Figure 9: Mean serum triglyceride levels of subjects in response to dietary fat.

TABLE 24

SERUM TRIGLYCERIDES OF SUBJECTS IN RESPONSE TO DIETARY FAT[1]

Subject	Day of Experiment 4	11[2]	18	25	32[2]	39
	mg Triglyceride/100 ml Serum					
J.B.[3]	127.7	120.2	108.3	93.9	84.2	122.0
H.P.[3]	151.3	145.4	91.6	89.5	87.3	113.3
R.R.[3]	118.2	106.8	98.4	92.2	80.0	101.0
R.L.[3]	101.4	93.1	89.4	80.5	68.2	87.6
Group Mean	124.7	116.4	96.9	86.5	77.4	
J.G.[4]	94.1	90.0	97.0	98.5	99.7	97.0
R.M.[4]	141.2	125.7	135.6	136.8	135.3	131.6
T.B.[4]	115.4	102.7	106.4	110.9	116.2	112.8
B.M.[4]	115.9	90.0	101.7	100.0	97.8	96.6
Group Mean	116.6	102.1	110.2	111.6	112.3	109.5

1. Mean of Duplicate Analyses.

2. Days on which Dietary Regimen was changed.

3. Subject on Sunflower Oil Diet.

4. Subject on Lard Diet.

TABLE 25

CHANGES IN SERUM TRIGLYCERIDES
IN RESPONSE TO DIETARY FAT

Subject	Experimental Period: Mixed Fat Day 4 vs 11	Sunflower Oil or Lard Diet: Day 11 vs 18	Day 18 vs 25	Day 25 vs 32	Mixed Fat Day 32 vs 39
	mg Triglyceride/100 ml Serum				
J.B.[1]	- 7.5	-11.9	-14.4	- 9.7	+37.8
H.P.[1]	- 5.9	-53.8	- 2.1	- 2.2	+26.0
R.R.[1]	-11.4	- 8.4	- 6.2	-12.0	+21.0
R.L.[1]	- 8.3	- 3.7	- 8.9	-12.3	+19.4
Group Mean	- 8.2	-19.4	- 7.9	- 9.0	+26.0
J.G.[2]	- 4.1	+ 7.0	+ 1.5	+ 1.2	- 2.7
R.M.[2]	-15.5	+ 9.9	+ 1.2	+ 1.5	- 3.7
T.B.[2]	-12.7	+ 3.7	+ 4.5	+ 5.3	- 3.4
B.M.[2]	-25.9	+11.7	- 0.3	- 2.2	- 1.2
Group Mean	-14.5	+ 8.1	+ 1.7	+ 1.5	- 2.7

1. Subject on Sunflower Oil Diet.

2. Subject on Lard Diet.

height (Guthrie, 1971). Although H.P. lost 3.6 kgm, and Albrink (1973) has stated that weight loss can result in a reduction in elevated serum triglyceride levels due to obesity, the observed decrease cannot be attributed only to weight loss. Weight loss occurred throughout the experiment whereas the major loss in serum triglycerides occurred during the first week on the sunflower oil diet. Furthermore, H.P. reacted in the same manner as the other subjects during the post-experimental period when the mixed fat diet was fed (Days 32 to 39). The mean increase in serum triglycerides for the sunflower oil group was 26 mg/100 ml, and the increase in serum triglycerides for H.P. also was 26 mg.

By contrast, serum triglycerides increased 8.4 mg/100 ml of serum during the first week on the lard diet, and had a tendency to plateau at this level (Tables 24 and 25). When the mixed fat diet was refed serum triglycerides decreased slightly (2.7 mg/100 ml). This difference in the pattern of response, the slight increase in serum triglycerides on the lard diet and the decrease on the sunflower oil diet, was real as indicated by the significant diet x day interaction (Appendix Table 27).

Grande et al (1972) have suggested that saturated fats of fewer than 12 carbon atoms and stearic acid, which Keyes et al (1965c) found had little effect on serum cholesterol, elevate serum triglycerides. The sunflower oil diet contained 8.8 percent less stearic acid than the mixed fat diet. In addition, the mixed fat diet included 20 gm of butter oil which would contribute a small proportion of short

and medium chain fatty acids but these were not resolved by the analysis procedure used in this study. Hence a decrease of serum triglycerides would be expected on the sunflower oil diet. However, the lard diet contained only 2.5 percent more stearic acid than the mixed fat diet and, hence, the change in fatty acid composition does not explain the 11.3 mg increase observed on the lard diet.

On the basis of the present investigation, sunflower oil brought about a significant ($P < 0.001$) decrease in serum triglycerides, whereas lard was associated with a slight but significant ($P < 0.001$) increase in serum triglycerides when these fats are fed at 40 percent of total calories.

D. EFFECT OF DIET ON SERUM LIPID PHOSPHORUS

The response of serum lipid phosphorus to diet (Figure 10) tended to parallel changes in serum total cholesterol (Figure 4). Serum lipid phosphorus decreased 0.5 mg from Day 4 to Day 10 of the stabilization period when the mixed fat diet was fed ($P < 0.025$, Appendix Table 28). This downward trend continued for the subjects fed the sunflower oil diet (Table 26), with decreases of 0.7, 0.6 and 0.3 mg/100 ml of serum. For those on the lard diet, serum lipid phosphorus values increased during the first week and then tended to plateau (Figure 10, Table 26). During the final week of the study when the mixed fat diet was again fed, serum lipid phosphorus values increased 0.9 mg/100 ml of serum for the subjects who had been fed the sunflower oil diet and decreased 1.0 mg/100 ml of serum for the subjects

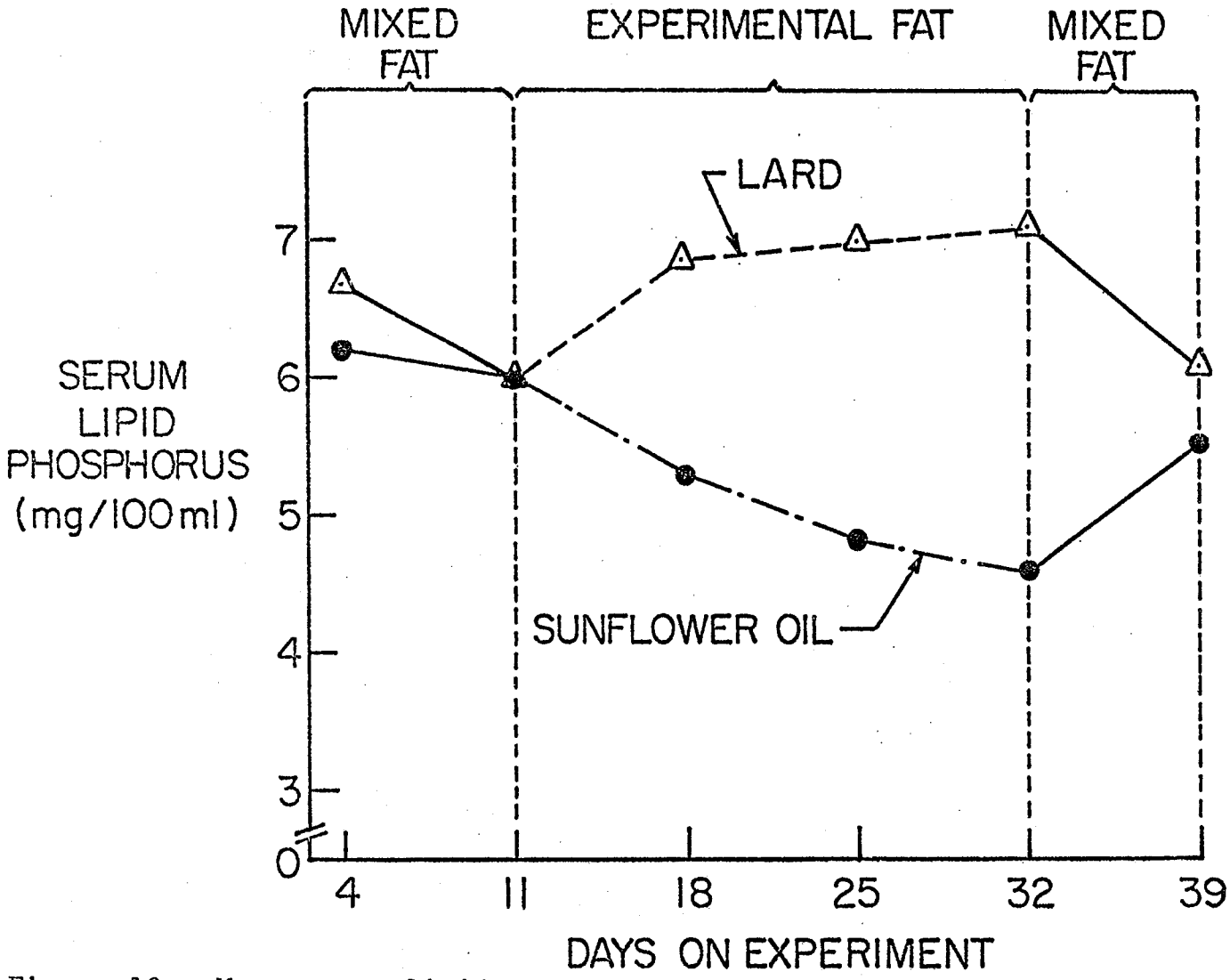

Figure 10: Mean serum lipid phosphorus levels of subjects in response to dietary fat.

TABLE 26

SERUM LIPID PHOSPHORUS OF SUBJECTS IN RESPONSE TO DIETARY FAT[1]

Subject	Day of Experiment 4	11[2]	18	25	32[2]	39
	mg Lipid Phosphorus/100 ml Serum					
J.B.[3]	6.7	6.4	6.3	5.4	4.6	5.2
H.P.[3]	5.6	5.5	4.7	4.6	4.4	6.5
R.R.[3]	6.2	6.1	5.3	4.6	4.9	5.0
R.L.[3]	6.2	6.1	5.1	4.5	4.5	5.3
Group Mean	6.2	6.0	5.3	4.8	4.6	5.1
Δ Lipid Phosphorus[5]		-0.2	-0.7	-0.5	-0.2	+0.8
J.G.[4]	5.7	5.5	5.9	6.2	6.2	5.5
R.M.[4]	7.2	6.1	7.8	7.6	7.4	6.0
T.B.[4]	6.3	5.9	6.1	6.4	7.2	5.8
B.M.[4]	7.4	6.4	7.6	7.7	7.6	7.1
Group Mean	6.7	6.0	6.8	7.0	7.1	6.1
Δ Lipid Phosphorus[5]		-0.7	+0.8	+0.1	+0.1	-1.0

1. Mean of Duplicate Analyses.
2. Days on which diet was changed.
3. Subject on Sunflower Oil Diet.
4. Subject on Lard Diet.
5. Expressed as difference of preceding day.

who had been fed the lard diet. Although the values obtained on Day 39 approached those of Day 11 with both groups, serum lipid phosphorus levels were different on Day 11 and Day 39 ($P < 0.005$). However, there was no statistical difference between the two groups on Day 39.

The results of the present study agree with those of Connor et al (1969) and McGandy et al (1970) who found that changes in lipid phosphorus in response to dietary fat were similar to those of serum cholesterol. Losier (1972) observed the same pattern, although she found that the response in serum lipid phosphorus lagged behind that of cholesterol.

E. EFFECT OF DIET ON PHOSPHOLIPID FATTY ACID PATTERNS

The fatty acid patterns for the serum phospholipid fraction precipitated from acetone are given in Table 27 for the group fed the lard diet and in Table 28 for those on the sunflower oil diet. There was very little change in the fatty acid patterns of the phospholipid in response to the lard diet. Even the changes in the phospholipid fatty acid patterns observed on the sunflower oil diet were small considering that this diet provided 47 percent more linoleic acid than the mixed fat diet (Table 13). The linoleic acid of the phospholipids increased slightly on the sunflower oil diet. The change was rapid as it occurred during the first week on this diet with little change in the ensuing two weeks. During the post-experimental mixed fat period (Days 32 to 39), there was a tendency for linoleic acid to return to the values

TABLE 27

PERCENT FATTY ACID COMPOSITION OF SERUM PHOSPHOLIPIDS[1] OF GROUP OF SUBJECTS FED LARD DIET

	Day of Experiment					
Fatty Acid	4	11[2]	18	25	32[2]	39
Myristic, C14:0[3]	2.0	2.1	3.0	0.9	1.3	1.2
Pentadecanoic, C15:0	1.2	1.1	0.9	0.3	0.9	1.0
Palmitic, C16:0	30.1	28.5	31.6	29.6	31.7	26.8
Palmitoleic, C16:1	1.7	2.0	2.7	1.2	1.0	1.5
Heptadeconoic, C17:0	1.1	1.2	0.7	0.5	0.5	0.9
Stearic, C18:0	17.2	16.6	17.2	18.8	17.6	16.7
Oleic, C18:1	13.4	13.9	14.6	12.8	13.5	13.1
Linoleic, C18:2	24.5	25.2	22.5	26.7	23.1	25.8
Eicosatrienoic, C20:3	1.6	1.9	1.0	2.0	1.9	2.1
Arachidonic, C20:4	6.6	6.3	5.1	6.7	6.2	7.6

1. Mean of four subjects.
2. Days on which Dietary Regimen was changed.
3. Carbon number: number of double bonds.

TABLE 28

PERCENT FATTY ACID COMPOSITION OF SERUM PHOSPHOLIPIDS[1] OF GROUP OF SUBJECTS FED SUNFLOWER OIL DIET

	Day of Experiment					
Fatty Acid	4	11[2]	18	25	32[2]	39
Myristic, C14:0[3]	1.9	2.1	2.1	1.5	0.8	1.0
Pentadeconoic, C15:0	1.1	1.2	0.8	0.8	0.5	0.7
Palmitic, C16:0	29.2	28.8	27.0	27.3	26.3	29.8
Palmitoleic, C16:1	1.4	1.4	0.8	1.1	0.7	1.2
Heptadeconoic, C17:0	0.9	1.5	0.6	0.3	0.7	0.5
Stearic, C18:0	17.2	16.2	17.9	18.3	18.2	16.1
Oleic, C18:1	14.7	13.9	10.3	9.8	9.7	13.6
Linoleic, C18:2	23.6	25.5	33.1	32.1	33.6	28.5
Eicosatrienoic, C20:3	1.7	1.7	0.4	1.3	1.1	1.3
Arachidonic, C20:4	7.3	6.4	6.4	7.0	7.9	6.8

1. Mean of four subjects.

2. Days on which Dietary Regimen was changed.

3. Carbon number: number of double bonds.

observed on Day 11.

The small changes exhibited in the phospholipid fatty acid patterns appear to parallel the changes in fatty acid composition of the diet. The data also suggest that the turnover of fatty acids in the phospholipid fraction of the serum is rapid. Ahrens et al (1957) found only small changes in the fatty acid patterns of the phospholipids when the customary diet of men was replaced by corn oil or menhaden oil. These authors concluded that serum phospholipids had the most stable fatty acid patterns of any of the serum lipid components.

F. EFFECT OF DIETARY PROTEIN SOURCE ON SERUM LIPIDS

The effects of dietary protein source on serum lipid levels in man is poorly understood. In the present study, the effects of substituting beef protein on a nitrogen equivalence basis for soy protein was studied during the post-experimental period (Days 32 to 39) when the mixed fat diet was again fed. Four subjects, two of whom had been fed each of the experimental fats, were assigned to the beef diet while the other four received the regular mixed fat-soy protein diet fed during the stabilization period.

There was no evidence in the present study that protein source had any appreciable effect on the blood parameters measured (Appendix Tables 22, 23, 24, 27 and 28). Mean serum cholesterol levels were slightly higher on the soy diet than on the beef diet (Table 29), but the difference between the two groups was not statistically significant ($P > 0.05$). A

TABLE 29

MEAN RESPONSE IN SERUM CHOLESTEROL TO DIETARY PROTEIN SOURCE

	Serum Cholesterol (mg/100 ml)		
	Lard	Sunflower	$\bar{X}_4$
Beef	- 41 - 20	+ 20 + 23	-5
Soy	- 23 - 13	+ 35 + 30	+7
$\bar{X}_4$	- 24	+ 27	

similar trend was observed in serum triglycerides. Serum triglycerides were somewhat higher on the soy diet than on the beef protein diet (Table 30) but, again, the difference was not significant. Serum lipid phosphorus levels, on the other hand, were slightly higher on the beef protein diet than on the soy diet (Table 31) but, as with serum cholesterol and serum triglycerides, the groups did not differ statistically ($P > 0.05$).

The results of the present study suggest that protein source had no effect on serum cholesterol levels. However, the amount of beef and soy consumed was small. Of the total protein supplied by the diet, beef and soy comprised 14.1 percent (9.3 gm of protein daily) on Menu I and 16.9 percent (12.1 gm of protein daily) on Menu II. The diet also contained protein from egg albumin (15.8 percent) and skim milk (18.8 percent). Thus, on the beef diet, animal protein provided nearly 50 percent of the total protein intake, whereas, on the soy diet, animal protein supplied 34.6 percent of the total. It is possible that the 15.4 percent extra animal protein may have been insufficient to effect changes in serum lipids. Furthermore, the effect of protein source was studied over just a seven-day period in the present experiment. This time may have been too short for any effects of protein source on serum lipids to be observed. Rabbits have to be fed a particular protein for three weeks before any effect on serum cholesterol is observed (Huff, 1975); serum cholesterol was found to be lower in the rabbit when the protein source was of plant origin (Carroll and Hamilton, 1975). However,

TABLE 30

MEAN RESPONSE OF SERUM TRIGLYCERIDES TO DIETARY PROTEIN SOURCE

	Serum Triglycerides (mg/100 ml)		
	Lard	Sunflower	$\bar{X}_4$
Beef	- 3 - 4	+ 21 + 19	+ 9
Soy	- 4 - 1	+ 39 + 26	+15
$\bar{X}_4$	- 3	+ 26	

TABLE 31

MEAN RESPONSE IN SERUM LIPID PHOSPHORUS TO DIETARY PROTEIN SOURCE

	Serum Lipid Phosphorus (mg/100 ml)		
	Lard	Sunflower	$\bar{X}_4$
Beef	-0.7 -1.4	+0.1 +0.8	-0.3
Soy	-1.4 -0.5	+0.6 +2.1	-0.8
$\bar{X}_4$	-1.0	+0.9	

Anderson et al (1971) conducted a 28-day study on the effects of dietary protein source on serum lipid levels in man and found no significant differences in serum cholesterol when vegetable proteins were substituted for animal proteins. Anderson et al (1971) also investigated the effects of dietary protein on serum triglyceride and phospholipid patterns. They found that serum triglycerides were somewhat higher in men fed animal protein than those fed vegetable protein although the differences were not statistically significant. These authors also found a small but significant increase in serum lipid phosphorus levels when men were fed a vegetable protein diet rather than animal protein.

The evidence on hand in the present study indicates that the level of beef or soy provided in the diet has no effect on serum lipid levels.

G. GENERAL DISCUSSION

The present study has shown that source of dietary fat has an appreciable effect on serum lipid levels. Substitution of a mixture of dietary fats, representative of the average Canadian consumption, by sunflower oil resulted in a significant decrease ($P < 0.001$) in serum cholesterol, whereas replacement of the mixed fat by lard resulted in an increase ($P < 0.001$) in serum cholesterol values.

Serum cholesterol decreased 15 mg/100 ml during the stabilization period when the mixed fat diet was fed (Days 4 to 10). Similar observations have been reported by Lake (1975), King (1974) and Le Blanc (1973), when analagous fat

mixtures were fed. However, the magnitude of change varied. King (1974) reported a decrease of 13 mg/100 ml during a ten-day period while Le Blanc (1973) and Lake (1975) reported decreases of 29 and 32 mg/100 ml, respectively. The decrease in serum cholesterol observed on the mixed fat diet continued when sunflower oil was the sole source of added dietary fat. There was a decrease of 56 mg/100 ml of serum when sunflower oil was fed during the 21-day experimental period. Conversely, serum cholesterol increased 26 mg/100 ml for the subjects fed the lard diet. The patterns observed on the sunflower oil and lard diets were reversed when the mixed fat diet was again fed (Days 32 to 39).

Ahrens et al (1957a), Keyes et al (1957a) and Koranyi et al (1961) also reported increases in serum cholesterol when diets rich in lard were fed. Similarly, Suzuki et al (1963) found that serum cholesterol levels were increased by 18 percent when 60 gm of lard was fed to girls for seven days, which was slightly higher than the increase of 13 percent observed in the present study. The fatty acids responsible for the hypercholesterolemic effect of dietary fat are thought to be lauric, myristic and palmitic acids (Keyes et al 1965c), and, hence, the higher amount of palmitic acid in the lard diet, when compared to the mixed fat diet, may be regarded as the hypercholesterolemic factor. Stearic acid (Keyes et al, 1965c), monoenoic fatty acids (Keyes et al, 1958) and medium chain saturated fatty acids (Gjone et al, 1972) are not thought to affect serum cholesterol levels.

There is abundant evidence in the literature that

polyunsaturated fatty acids lower serum cholesterol. Losier (1972), for example, found that serum cholesterol decreased 25 percent when corn oil provided 40 percent of the dietary calories for 21 days. This was slightly less than the 29 percent decrease observed on the sunflower oil diet in the present study. Suzuki et al (1971) also reported a decrease of 26 percent when sunflower oil provided 40 percent of calories in the diets of young girls, and Moore et al (1968) found that safflower oil reduced serum cholesterol by 28 percent.

In general, saturated fats have been found to increase serum cholesterol and polyunsaturated fats to decrease serum cholesterol, although the magnitude of response does not appear to be directly related to the fatty acid composition of the fat. Keyes et al (1957a) found that there was a more marked decrease in serum cholesterol when corn oil was fed than when either sunflower oil or safflower oil were fed, even though the latter provided much higher levels of polyunsaturated fatty acids than corn oil. Hegsted et al (1965) found that the decrease in serum cholesterol was greater when safflower oil was fed than when corn oil was fed. Cottonseed oil, which contains more linoleic acid than corn oil but less than sunflower oil, was found to decrease serum cholesterol more than either corn oil or sunflower oil (Keyes et al, 1957a). King (1974) and Lake (1975) found that serum cholesterol was not significantly decreased by either high or low erucic acid rapeseed oil or by soybean oil, although Gjone et al (1972) found that serum cholesterol decreased when soybean oil

provided 40 percent of the daily calories. On the other hand, saturated fats are regarded as being hypercholesterolemic, although Losier (1972) found that beef tallow, a fat high in stearic acid, caused a modest reduction in serum cholesterol levels. Hence, it would appear that more attention should be focused on the source of fat in the diet because fatty acid composition alone does not appear to account for all the changes observed under experimental conditions.

The major changes in serum cholesterol occurred during the first week on each of the experimental fat diets, with changes of a lesser magnitude in the second and third weeks. Similar observations also have been noted by Keyes et al (1957a) and Le Blanc (1973). Serum free and esterified cholesterol were found to follow a similar pattern to serum total cholesterol.

There is a paucity of information on the effects of dietary fat source on the turnover of plasma cholesterol in the normal free-living individual. Turnover of plasma cholesterol was monitored in the present study by measuring the decline in radioactivity in the plasma of subjects who had been infused with 50 microcuries of ^{3}H-cholesterol 32 days prior to commencing the study. The decline in radioactivity per millilitre of serum was approximately twice as great on the sunflower oil diet as on the lard diet. Wood et al (1966) also have reported a more rapid decline in labelled-cholesterol in the plasma when a diet high in polyunsaturated fats replaced a diet high in saturated fats. However, there was no difference among the fat sources in the rate of decline

of specific activity in the present study. The fact that the slope of labelled plasma cholesterol declined more rapidly on the sunflower oil diet than on the lard diet, whereas the slopes of the specific activity-time curves among the fats did not differ, suggests that there was no dilution of the label on the experimental diets. This implies that synthesis and absorption of cholesterol remained constant and that the turnover of plasma cholesterol differed on the two diets. Avigan & Steinberg (1965) and Moore et al (1968) also observed no differences in the specific activity-time curve when diets rich in polyunsaturated fatty acids were compared to those rich in saturated fatty acids. However, Wood et al (1966) found a greater initial fall in the slope of the specific activity-time curve when a diet rich in corn oil replaced one high in saturated fats. The data of the present experiment and that of Avigan et al (1965) and Moore et al (1968) indicates a difference in the rate of turnover of cholesterol in response to saturated and polyunsaturated fats in the diet, although the observations of Wood et al (1966) suggests that polyunsaturated fatty acids may have effects on plasma cholesterol other than simply altering the rate of turnover.

Serum lipid phosphorus and serum triglycerides followed similar patterns to serum total cholesterol. Serum triglycerides decreased considerably on the sunflower oil diet, an observation also noted by Kishyakovskaya et al (1973). A slight, but significant, increase in serum tri-

glycerides was observed on the lard diet. Ahrens et al (1959) also found that serum triglycerides increased when lard supplied 40 percent of the calories. In fact, Ahrens et al (1959) found that increases in serum triglycerides were greater when lard was fed than when beef tallow was fed. Losier (1972), on the other hand, found no change in serum triglycerides on a diet rich in beef tallow.

Determination of the fatty acid content of the serum phospholipids indicated that the fatty acid patterns were not markedly influenced by dietary fat source. This is in contrast to the observations by King (1974) and Le Blanc (1973) who found that the fatty acid patterns of the serum phospholipids reflected changes in fatty acid composition when high and low erucic acid rapeseed oils were substituted for mixed fat in diets similar to those used in the present study.

Fidanza (1972) has stated that epidemiological studies have shown that the type and amounts of fat are important factors in the present epidemic of coronary heart disease. Although epidemiological studies have confirmed the relationship between the development of coronary heart disease and the antecedent level of serum cholesterol, there has been little success in relating level of serum cholesterol to patterns of dietary practise for individuals (McGandy et al, 1967). As Altschule (1974b) has pointed out, polyunsaturated fats have increased three-fold in the average American diet "without the slightest decrease in heart