

THE UNIVERSITY OF MANITOBA

THE EFFECT OF BEEF TALLOW AND BUTTERFAT
ON SERUM LIPID PATTERNS AND SERUM
CHOLESTEROL SPECIFIC ACTIVITY OF HEALTHY
YOUNG MEN

by

ANN LOUISE WHEELER

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A dissertation submitted to the Faculty of Graduate Studies of
the University of Manitoba in partial fulfillment of the requirements
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A B S T R A C T

The effects of beef tallow and butterfat (fed at about 40% calories) on serum lipid patterns and serum cholesterol specific activity were investigated in ten healthy young men. The 38-day metabolic trial consisted of (1) a 10-day stabilization period (2) a 21-day experimental period and (3) a 7-day final stabilization period. The basic diet consisted of ordinary foods: protein was supplied by textured soy protein, egg albumin and skim milk. The fat source in periods (1) and (3) consisted of a fat mix which simulated the fatty acid composition of the average Canadian diet. Beef tallow or butterfat provided the fat source during the 21-day experimental period. Each subject received only one test fat. Two subjects were assigned to the mixed fat diet for the entire study. Fasting venous blood samples were taken on days 4, 11, 18, 25, 32 and 39.

During the experimental period, subject mean serum total cholesterol level increased by 48 mg/dl ($p < 0.01$) on the butterfat diet, but did not increase significantly (8 mg/dl) on the beef tallow diet. Conversely, subject mean serum triglyceride level decreased by 42 mg/dl

($p < 0.01$) on the butterfat diet, but increased by 32 mg/dl ($p < 0.05$) on the beef tallow diet. Serum lipid phosphorus response was similar to that of cholesterol. Variation among subjects was high for all lipids. The two subjects who received the mixed fat diet for the entire study showed no appreciable change in serum lipids.

Thirty-two days prior to the study, each subject was infused with 50 μ c of 1-2-³H cholesterol, and the disappearance of radioactive cholesterol from the serum was monitored throughout the experiment. A linear ($p < 0.01$) relationship between \log_{10} serum specific activity and serum cholesterol was shown over the 21-day experimental period. No significant differences in the slope of the disappearance curve were noted between beef tallow and butterfat diets; diet was therefore not assumed to have any effect on the disappearance of labelled cholesterol from the serum.

Present data have indicated that beef tallow and butterfat have different effects on serum lipids, despite similarity in saturation. Beef tallow, under the conditions of this experiment, was not hypercholesterolemic, and this may be due to its high content of stearic acid.

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I N T R O D U C T I O N

Atherosclerosis is a major cause of death in the affluent, highly industrialized countries of the world. In Canada, coronary heart disease (CHD) is a leading cause of death. In men aged twenty-five, heart attacks rank fourth as a cause of death, and in men over the age of thirty-five, heart attacks rank as a primary cause of death. Fifty percent of deaths in Canada are related to some form of atherosclerosis (The Committee on Diet and Cardiovascular Disease 1976).

The disease is characterized by a lesion in the vascular intima of large blood vessels, which is composed of lipid, complex carbohydrate, blood components and fibrous tissue (Lawrie and Third 1977). As a result, there is an inadequate blood supply to the heart (Keys 1956). The concentration of blood lipids augmented by the blood pressure may determine the general tendency to infiltrate the arterial intima with atheromatous deposits. However, other factors, such as dynamics of blood flow, calibre of the blood vessel, the integrity of the vascular intima

and fibrin deposition, also influence this process (Kannel et al 1971, Kannel 1971). Atherosclerosis is clinically presented as angina pectoris or myocardial infarction when the coronary arteries are involved (coronary heart disease), or as a stroke when the cerebral arteries are involved.

The atherosclerotic process is a complicated and progressive one, and is influenced by a number of factors, cultural and environmental, as well as genetic (Brusis and McGandy 1971, Grande 1974, Lawrie and Third 1977). This general opinion is largely based on epidemiological studies which have identified certain "risk factors", or conditions which may be found in apparently healthy individuals, but which carry with them, an increased risk of development of CHD with the passage of time (Lawrie and Third 1977). There is ample precedent for the concept that a disease such as CHD can be prevented in advance of clinical manifestation, provided the chain of events in its evolution within a population can be determined (Kannel 1974). Genetic factors, such as race, sex and familial lipid disorders which are associated with an increased risk of CHD,

are not under the control of the individual. However, cultural and environmental factors, such as sedentary living habits, cigarette smoking and dietary pattern, which have been delineated as risk factors for CHD, can be modified to decrease the risk of developing atherosclerotic disease.

It is currently believed that the effect of diet on the development of the atherosclerotic process and its complications (in particular CHD) is mediated through the effect of diet on serum lipid levels (Grande 1974). The lipid hypothesis of atherosclerosis is that an elevation of plasma lipid may lead to more rapid deposition of lipid in the arterial wall, if the vascular tissue is susceptible. The intima of atherosclerotic aortae have been shown to contain about five times as much cholesterol, and also a greater amount of phospholipid than the normal intima (Insull and Bartsch 1966). Moreover, the exchangeability of cholesterol between plasma and atherosclerotic deposits has also been demonstrated *in vivo* (Jagannathan et al 1974). The equilibration between serum and intimal cholesterol suggested to Chobanian and Hollander (1962)

that at least some of the cholesterol deposited in the vessel wall had its origin in the serum. These investigators also noted that the exchange of cholesterol in the serum with that in the intimal tissue was inversely related to the degree of atherosclerosis and cholesterol content of vessels.

Therefore diet, at least insofar as it influences the levels of blood cholesterol and other lipids, may be involved in the pathogenesis of atherosclerotic disease. There is also evidence to indicate that dietary fat, and dietary saturated fat in particular, may have a primary role. The differences in mean serum lipid levels among world population groups are, in general, closely related to differing rates of atherosclerosis and of cardiovascular mortality in these populations (Brusis and McGandy 1971, Grande 1974). The average serum cholesterol level in North America (where the incidence of CHD is high) in men aged fifty, is 230 mg/dl. The average serum cholesterol level of his counterpart in Japan (where the incidence of CHD is rare) is 180 mg/dl (Wynder and Hill 1972). In addition, there is good evidence to associate dietary practices with geographic and cultural

differences. In North America, the average fat intake is 40% of calories. In Japan, the fat content of the average diet is only 11% of calories (Keys et al 1957b). The low level of total fat in diets such as those of Japan, have been suggested by Keys (1956), Keys et al (1958b) to be mainly a reflection of the small quantity of animal fat consumed. In a study of several countries, Keys (1970) found a significant relation between the percentage of calories derived from saturated fat, and both elevation in serum cholesterol level, and deaths from CHD. This finding is substantiated by the increased incidence of CHD in Eastern Finland (in comparison with Western Finland) where the diet is higher in saturated fat (Roine et al 1958, Karvonen 1962). In addition, migrants from areas where CHD is rare, and saturated fat and serum cholesterol levels are low, to areas where the incidence of CHD is high, adopt the dietary pattern of that area. Consequently, serum cholesterol level becomes elevated, and incidence of CHD is increased. This phenomenon has been demonstrated in the Japanese who migrated to the United States (Keys et al 1958b), and in Yemenite Jews who adopted the

eating pattern of European Jews in Israel (Toor et al 1960). Experiments with Japanese coalminers and Minnesotans by Keys et al (1957b) demonstrated that both groups responded similarly to changes in the amounts of the same types of fats in the diet. This indicated that dietary fat was a more important determinant in CHD incidence than were racial differences.

In the past two decades, all the epidemiologic studies have demonstrated a consistent quantitative association between blood lipid levels and risk of coronary and cerebrovascular disease (Brusis and McGandy 1971). No one lipid has been shown to be more potent (Kannel 1971), but the probability of an attack has been related to the antecedent level of each lipid. An elevated serum cholesterol level has been associated with an increased risk for CHD in several studies (Paul et al 1963, Kannel 1971, Carlson and Böttiger 1972, Pelkonen et al 1977). It has been estimated that a serum cholesterol level of > 260 mg/dl poses a risk of CHD five times as great as that associated with a level of < 220 mg/dl (Dawber et al 1962). Also, half of the new CHD events have been

shown to occur among men with a cholesterol level of > 250 mg/dl (Epstein 1972). However, the association extends over the range of cholesterol levels found in adult populations in Western society, and, there is no evidence for a 'safe' or 'threshold' value for serum cholesterol (Brusis and McGandy 1971, Kannel et al 1971). Moreover, it has been suggested that the average serum cholesterol level in the North American population may be much above the 'optimal' level, in view of less CHD incidence in countries where average serum cholesterol levels are lower (Kannel et al 1971, Wynder and Hill 1972). An elevation in serum triglyceride levels has also been associated with an increased risk for CHD (Albrink et al 1961, Carlson and Böttiger 1972). There is some controversy, however, as to whether elevations in serum triglycerides pose an independent risk for CHD (Carlson and Böttiger 1972), or an increased risk only if accompanied by an elevation in serum cholesterol level (Kannel 1971). In general, most risk factors for CHD are additive (Cornfield 1962, Epstein 1972). Obesity has been associated with an increase in serum lipid and blood glucose levels, a decrease

in physical activity (García-Palmieri et al 1972) and elevated blood pressure (Kannel et al 1967b) all of which have been shown to be independent, but additive risk factors for CHD. Even though obesity per se may not pose an independent risk for CHD (Paul et al 1963, Schilling et al 1969, Carlson and Böttiger 1972), it may be associated with other risk factors, and produces an increased workload on the heart, in what may be an already compromised circulatory system and therefore, there is a greater risk of sudden death (Kannel et al 1967a, Rabkin et al 1977).

From experimental observations has evolved the hypothesis that (a) the habitual intake of a diet rich in saturated fats and low in polyunsaturated fats, is a primary causative factor in hypercholesterolemia and subsequent development of atherosclerosis and (b) that modification of the diet to lower serum lipid levels will retard atherogenesis, and prevent or delay complications such as CHD or strokes. It is difficult to test these hypotheses because there is no animal which has the lifespan, metabolic processes, atherogenic phenomena and social and emotional stresses similar to man. Moreover, atherosclerosis develops slowly over a

period of years, and there is no simple way to assess in a living person, the degree of atherosclerosis, and the risk it imposes (Rathman et al 1970). However, there are indications from experiments on sub-human primates and from long term human studies, that the nature of the dietary fat is important, and that specific saturated fatty acids are more thrombogenic than polyunsaturated fatty acids.

There is a considerable body of evidence to suggest that not all predominantly saturated fats have the same effects on serum lipids. The fatty acid composition of the fat, in terms of the chain length of saturated fatty acids present, is thought to be the primary factor affecting serum lipid levels (Keys et al 1957a, 1965c). Fats containing large amounts of saturated fatty acids with 12 to 16 carbons in the chain have been shown to be more hypercholesterolemic than those with <10 carbons or 18 carbons in the chain (Keys et al 1965c, Hegsted et al 1965). However, these saturated fatty acids have little effect on serum triglycerides (Grande et al 1970, 1972). Butterfat has a high proportion of saturated fatty acids of 12 to 16 carbons,

and the hypercholesterolemic effect of diets rich in butterfat have been noted (Beveridge et al 1956, Ahrens et al 1957, Keys et al 1957a). Beef tallow is also a highly saturated fat, which has a large proportion of stearic acid. This fat was not shown to be hypercholesterolemic (Losier 1972) in a previous experiment conducted in this laboratory. The literature related to this subject has concentrated mainly on middle aged, male, hospitalized or institutionalized patients, who very often already have clinical symptoms of CHD. Study design has varied considerably, and both natural and synthetic test fats have been incorporated into formula or mixed diets at different levels of intake.

Current dietary recommendations have been partly based on evidence from primary (Turpeinen et al 1968, National Diet Heart Study Group 1968, Dayton et al 1969), and secondary (Leren 1966) prevention trials. These studies have shown that serum lipid levels, and consequently morbidity and mortality from CHD have been significantly lowered by diets which are high in polyunsaturated glycerides, and low in cholesterol and

saturated glycerides. It is now generally thought desirable to reduce total fat in the diet, and replace saturated fat with polyunsaturated fat as early in life as possible. However, the degree to which saturated fats are restricted remains questionable. The present study has attempted to investigate the effects of two natural, highly saturated fats: beef tallow and butterfat, on serum lipid levels of healthy, free-living young men, who received a diet of ordinary foods. Fats were incorporated at a level of about 40% of total calories, which is the average percentage fat intake of Canadians. It was anticipated that the analysis of serum decay curves of labelled cholesterol would augment information obtained from serum cholesterol analysis, which is limited to a comparatively small fraction of the body masses of cholesterol. More detailed information may then be gained about possible changes occurring in the plasma pool as a consequence of dietary alteration.

R E V I E W O F L I T E R A T U R E

Part I

The Effect of Beef Tallow and Butterfat
on Serum Lipid Patterns

Results reported from early investigations indicated that there were marked differences between saturated and unsaturated fats with respect to effects on serum lipids. Whether this difference was due to the cholesterol inherent in the saturated fats, or to saturation of the fat per se was examined, and the relationships between the effects of each factor on serum cholesterol were reported. Similarly saturated fats demonstrated differences in lipid response, and subsequent research concentrated on the fatty acid composition of the fat in terms of the chain lengths of saturated fatty acids present. The absorption and metabolism of fatty acids of different chain lengths is known to differ, and in addition, may also be influenced by the positions of these fatty acids on the glycerol molecule in the natural fat.

Butterfat and beef tallow are both saturated animal fats, and by virtue of this, they also contain cholesterol. Differences are also apparent in the types and relative percentages of fatty acids present. Butterfat is relatively high in short (C4:0 - C10:0) chain saturated fatty acids, lauric (C12:0) and myristic (C14:0) acid. Both fats are relatively high in palmitic (C16:0) acid, but beef tallow has considerably more stearic (C18:0) acid and is also higher in oleic (C18:1) acid (Table 3).

It is therefore evident that in a discussion of the net effects of beef tallow and butterfat on serum lipid patterns, several variables must be considered. This review refers mainly to studies in which experimental subjects are adult males fed mixed diets, who have been categorized as normolipemic. Despite differences in subject age, experimental design and form of diet employed, the data to date indicates that the chain length of component fatty acids in the fat may be a primary factor of consideration.

"Animal" vs "Vegetable" Fats

Numerous studies began in 1950 to establish the nature of the dietary fat which produced an increase in serum lipid levels. Several investigators demonstrated that the total amount of fat in the diet, whether of animal or vegetable origin, had an effect on the level of plasma cholesterol. Vegetable fat added to essentially cholesterol-free fat-free diets, was shown by Keys (1950) and Hildreth et al (1951) to produce a significant increase in serum cholesterol levels. These investigators also showed that a reduction of total dietary fat produced a significant fall in serum cholesterol.

In contrast to these studies, the replacement of animal fat in the diet with equivalent amounts of vegetable fat, resulted in a decrease in serum lipids. Formula diets containing 80% calories from fat in the form of vegetable oil, decreased serum cholesterol and phospholipids in comparison to equivalent amounts of fat of dairy origin (Kinsell et al 1952). In support of this work, Groen et al (1952) have reported that strict vegetarians have lower serum cholesterol levels than lacto-ovo vegetarians or non-vegetarians, independent

of their total fat intake.

Controversy at this time arose because the importance of maintaining food fat intakes at isocaloric levels was not appreciated (Ahrens 1957). In later experiments, butterfat and beef tallow were incorporated into formula diets which provided 58% calories as fat. An increase in serum cholesterol and phospholipid was reported as compared with a mixed "free choice" diet. Beveridge et al (1956) noted that vegetable fat in the form of corn oil given in the same quantity, and containing an equivalent amount of cholesterol, was not associated with an increased serum cholesterol level. Several other investigators reported similar results when comparisons were made between animal and vegetable fats. Butterfat and beef tallow, fed at 35% calories to Bantu subjects, were hypercholesterolemic compared with 35% or even 70% calories from vegetable fat in the form of olive oil (Bronte-Stewart et al 1956). Isocaloric replacement of 40% of total calories derived from milk fat by corn oil produced a decrease in serum cholesterol, despite the addition of an equivalent amount of cholesterol in the diet (Malmros and Wigand 1957).

Further, the replacement of whole milk and butter by soybean emulsion and polyunsaturated margarine in hospital diets in Finland by Turpeinen et al (1960), was found to significantly lower serum cholesterol levels over a twelve month period.

Thus, to the present day, despite the diversity of diets and subjects studied, and major differences in experimental design, there was general agreement among investigators that the ingestion of animal fats increased serum cholesterol and phospholipid, but that diets containing isocaloric amounts of vegetable fats lowered the serum lipids.

Effect of Cholesterol

The most commonly eaten foods containing saturated fat, which also contain significant amounts of cholesterol, are of animal origin. Several studies have shown that dietary cholesterol has a hypercholesterolemic effect, whether fed in crystalline or natural form. Grande et al (1965) conducted experiments in which crystalline cholesterol, dissolved in oil, was incorporated into diets containing 40% fat, at levels

of up to 3000 mg/day. They formulated a prediction equation which expressed serum cholesterol response as a linear function of the square root of cholesterol in the daily diet. Cholesterol, as a component of butterfat and egg yolk, has also been found to be an important variable in the determination of serum cholesterol level, and a linear relationship has been established between dietary cholesterol and serum cholesterol response. Hegsted et al (1965) estimated that each 100 mg dietary cholesterol added to the diet, would cause an increase in serum cholesterol of 5 mg/dl independent of the effects induced by the dietary fat, fed at either 22% or 38% of total calories. Other investigators, using formula (Mattson et al 1972) and mixed diets (Morris 1977) containing 40% calories from fat, have reported that for each additional 100 mg cholesterol added per 1000 calories, serum cholesterol increased by about 12 mg/dl.

Inter-related effects between cholesterol and saturated fats were hypothesized by Conner et al (1964). They found that in mixed diets containing a variety of

foods controlled in fat content (40% total calories) and composition, that the presence or absence of cholesterol influenced the serum lipid levels in normal and diabetic men. The serum cholesterol raising effect of dietary cholesterol was greater in the presence of saturated fat than with polyunsaturated fat, although this was not significant. Other investigators have confirmed the inter-relationship proposed by Connor et al (1964). Serum cholesterol levels were shown to be greater when either 750 mg crystalline cholesterol (Wood et al 1966), or 200 mg/1000 calories of egg yolk cholesterol (National Diet Heart Study Group 1968) were incorporated into diets rich in saturated fat, than a similar addition of cholesterol to isocaloric amounts of polyunsaturated fat. The effects of added cholesterol were similar despite the difference in diet consistency; formula diets were utilized by Wood et al (1966) and natural mixed diets used in the National Diet Heart Study (1968).

Conversely, the independent effects of both cholesterol and the degree of saturation of the fat were reported by Nestel et al (1975). Diets containing 45%

fat calories were designed to compare the effects of polyunsaturated and conventional ruminant fats at two levels of cholesterol intake, 500 mg and 800 mg per day. The higher cholesterol intake was provided by the addition to the diet of dried egg yolk. The plasma cholesterol was lowered both as the consequence of increasing the polyunsaturated to saturated fatty acid ratio, and by decreasing the cholesterol content of the diets. In confirmation, Anderson et al (1976) found no significant difference in the effect of the addition of 291 mg/day crystalline cholesterol to cholesterol-free diets supplying 35% calories from either polyunsaturated (safflower oil) or saturated fat (palm and coconut oil). Grande et al (1965) had also noted that the serum lipid raising effect of 510 mg/1000 calories of purified dietary cholesterol, was the same in the presence of 32% calories derived from saturated or polyunsaturated fat. Current evidence is therefore in agreement with the proposal suggested by Grande et al (1965), namely that the elevation of serum cholesterol and phospholipid produced by dietary cholesterol is independent of the degree of saturation of the fat.

The investigators proposed that the converse hypothesis is also true.

The effect of dietary cholesterol on serum triglycerides has been shown to be inconsistent and varied (Erickson et al 1964, Grande et al 1965, Hegsted et al 1965, National Diet Heart Study Group 1968, Nestel et al 1975, Morris 1977).

Degree of Saturation

Further studies indicated that the arbitrary division of naming fats as "animal" or "vegetable" was incorrect, and it was shown that isocaloric exchanges of a variety of fats in the diet produced serum lipid changes related to the degree of saturation of the dietary fat (Ahrens 1957). Kinsell and Michaels (1955) demonstrated that of the vegetable fats tested, those high in saturated fatty acids, which were incorporated into both formula and mixed diets at 60 - 80% of total calories, produced the greatest elevation in serum cholesterol and phospholipids. It had also been shown that certain vegetable fats increased serum cholesterol, whereas certain animal fats depressed it (Malmros and Wigand 1957). Animal and vegetable fats were

incorporated at 40% calories in formula diets, and fed to patients with diagnosed atherosclerotic heart disease (Ahrens et al 1957). The effect on serum cholesterol and phospholipids was correlated with the average net unsaturation of the fat. Later work by Gunning et al (1964) indicated that the relationship which best expressed the effects of different fats on serum cholesterol, was between the square root of the average net unsaturation of the fat (or iodine number) and serum cholesterol level. In this study, fats of animal or vegetable origin were incorporated into formula diets at 45% total calories. It was therefore apparent that the analysis of the effects of different fats on serum lipids must be made in terms of the chemical composition of the fat, that is, the degree of saturation of component fatty acids.

The response of serum cholesterol to different amounts of dietary glycerides of unsaturated (S), monounsaturated (M) and polyunsaturated (P) fatty acids, which supplied 9 - 44% of total calories from fats, in diets of constant cholesterol content, was reported by Keys et al (1957a). In this experiment, multiple

correlation analysis showed that serum cholesterol responded to changes of fatty acids in the diet, under the conditions of their experiment. The following equation estimates the predicted changes in serum cholesterol.

$$\Delta \text{Chol} = 1.68 + 2.76\Delta S + 0.05\Delta M - 1.35\Delta P$$

where ΔS , ΔP and ΔM refer to the percentage caloric differences from saturated, polyunsaturated and monounsaturated fatty acids in the diet, respectively, and ΔChol refers to the average change in serum cholesterol (mg/dl). However, the investigators noted that only the coefficients for ΔP and ΔS made significant contributions to the equation.

The only monounsaturated fatty acid present in appreciable amounts in ordinary diets, is oleic acid. Olive oil (containing 80% oleic acid) fed at 35 - 40% total calories, was shown to be hypocholesterolemic in comparison with equicaloric amounts of coconut oil (Malmros and Wigand 1957) or beef tallow (Bronte-Stewart et al 1956), although this hypocholesterolemic effect was not as great as that due to equicaloric amounts of corn oil (Malmros and Wigand 1957). The observation by

Keys et al (1957a) that predicted responses of serum cholesterol to changes of fatty acids in the diet, agreed better with observed values if $M = 0$, led them to further investigate the effect of oleic acid. In this experiment, oleic acid was varied between wide limits in the diet, while the levels of other fatty acids were held constant, and the difference was matched by simple carbohydrate calories (Keys et al 1958a). Diets which differed by as much as 18% of total fat as mono-ene did not show any significantly different effects on the level of serum cholesterol and phospholipids, in comparison with a low fat diet (9% fat calories). Therefore, they concluded that saturated fatty acids have about twice as much effect in elevation serum cholesterol as polyunsaturated fatty acids have in lowering it. This hypothesis was expressed by the equation:

$$\Delta \text{Chol} = 2.7 \Delta S - 1.31 \Delta P$$

These investigators also noted that changes of fatty acids in the diet produce serum cholesterol responses that are also correlated with average net unsaturation of the fats concerned, only when this value happens to be highly correlated with $2.74S - 1.3P$. Solution of the

least squares regression equation obtained from experiments by Hegsted et al (1965) provided confirmation of the above equation, since oleic acid made no significant contribution to the regression equation they had proposed. They noted that the proportions of total saturated fatty acids, when considered alone, accounted for 72% of the total variation in serum cholesterol observed.

However, similarly saturated fats have been shown to behave differently. Butterfat displayed a much greater hypercholesterolemic response than either beef tallow (Beveridge et al 1956, Bronte-Stewart et al 1956), or cocoa butter (Ahrens et al 1957). The indication that medium chain triglycerides were not hypercholesterolemic (Hashim et al 1960) despite the saturation (iodine value < 1) led investigators to believe that the different effects on serum lipids of fats of similar saturation must be attributed to the chain lengths of component saturated fatty acids.

Chain Length of Saturated Fatty Acids

The effect of chain length as a dietary variable was measured by Ahrens et al (1957), who fed subjects with atherosclerotic heart disease, formula diets containing equal amounts (45% fat calories) of butterfat or cocoa butter. These fats are similarly saturated, but butter contains mainly short and intermediate chain saturated fatty acids, and cocoa butter contains predominantly long chain saturated fatty acids, particularly stearic acid (C18:0). Serum cholesterol and phospholipids were significantly higher during butter feeding. They interpreted these results as evidence that fatty acids of short and intermediate chain length increase cholesterol and phospholipid levels to a greater degree than do the long chain fatty acids. Their results confirmed the work of Beveridge et al (1957) who compared the effect of equicaloric amounts (40% fat calories) of various butterfat fractions (obtained by molecular distillation) with corn oil fed at 60% total calories. They noticed that the highest cholesterol levels coincided with the feeding of the volatile fraction of butterfat, which contained mostly saturated, short chain fatty acids.

Grande et al (1961) found that a diet containing glycerides of saturated fatty acids with 12 and 14 carbon atoms, was more hypercholesterolemic than one containing equal amounts of glycerides of saturated fatty acids with 16 and 18 carbon atoms. These fats were incorporated into diets at 40% of total calories, and compared with a basic "house" diet. In a second experiment, carried out with adult male mongrel dogs, Grande (1962) compared the effects on serum cholesterol and phospholipid of glycerides of saturated fatty acids with 8 and 10 carbon atoms, with those of 12 and 14, and 16 and 18 carbon atoms. Diets were fed at 40% total fat calories, and compared with a control low fat diet contributing 4% calories as fat. They reported that glycerides of saturated fatty acids with 12 and 14 carbon atoms produced the greatest elevation, those of 16 and 18 carbon atoms an intermediate elevation, but that glycerides of saturated fatty acids with 8 and 10 carbon atoms, showed very little effect on serum cholesterol and phospholipids. These findings are in agreement with the work of Hegsted et al

(1965). These investigators found that no significant contribution to the regression equation was made by saturated fatty acids of less than 10 carbon atoms.

Fatty acids of 8 to 10 carbon atoms in chain length are the primary components of medium chain triglyceride (MCT) mixtures. MCT was shown to lower cholesterol and phospholipids in comparison with butter (Hashim et al 1960) when these fats were incorporated at 40% calories into formula diets. This further supports the work of Keys et al (1957a) who found that serum cholesterol values for saturated fatty acids of less than 10 carbon atoms were overestimated by the prediction equation, and S was assumed to refer only to saturated fatty acids of greater than 10 carbon atoms.

However, Uzawa et al (1964) found that MCT incorporated at 45% fat calories into formula diets was hypertriglyceridemic in comparison with sunflower oil, coconut oil, or a fat mix of predominantly palmitic and oleic acids. The hypertriglyceridemic effect of MCT was confirmed by McGandy et al (1970) who postulated that this might represent an endogenous type of

hypertriglyceridemia. Longer chain fatty acids were thought to be synthesized in the liver via the portal route after ingestion of MCT. The response of serum triglycerides to ingestion of MCT lends support to the data reported by Antonis and Bersohn (1961). In this experiment, a significant increase in serum triglycerides was noted in South African Bantu and white subjects after fifty-one weeks, when a diet containing 40% fat calories from butter was consumed. The effect of butterfat was compared with a basal low fat diet (15% fat calories). Further, a diet containing coconut oil (rich in lauric and myristic acids) fed at 28% total calories, produced lower serum triglyceride levels than a diet containing isocaloric amounts of beef fat (rich in stearic and palmitic acids). This suggested that longer chain saturated fatty acids may also be responsible for elevations in serum triglycerides (Anderson et al 1967). In addition, it has been demonstrated that stearic acid was associated with decreased serum cholesterol and phospholipid levels, but elevated serum triglyceride levels. Stearic acid replaced the palmitic acid in palm oil and supplied 30% total

calories in the diet (Grande et al 1970).

Until 1965, there seemed to be no reason to distinguish between stearic and palmitic acids in the diet. However, Keys et al (1965c) noted that some discrepancies existed between serum cholesterol values predicted from their equation, and observations of other investigators. Serum cholesterol was overestimated by the equation when either ethyl stearate, cocoa butter or beef tallow were employed as test fats. Horlick and Craig (1957) found that stearic acid was not hypercholesterolemic when fed as ethyl stearate at 40% calories, in comparison with a low fat (4% total fat) diet, and Erickson et al (1964) reported that for cholesterol free formula diets (40% calories from cocoa butter), serum cholesterol level was not significantly different from highly unsaturated fat mixtures. Further, the equation of Keys et al (1957a) overestimated the response of Δ cholesterol when beef tallow was replaced by corn oil at 40% total calories (Ahrens et al 1957). Thus, accounting for the previous evidence cited using cocoa butter (Ahrens et al 1957, Connor et al 1964) and beef tallow (Beveridge et al 1956), the

hypercholesterolemic effect of saturated fatty acids was attributed to lauric (C12:0) (Keys et al 1965c), myristic (C14:0) and palmitic (C16:0) acids, (Hegsted et al 1965, Keys et al 1965c).

Based on previous observations, and more recent data (Grande et al 1970, Grande et al 1972), the evidence indicates that of the saturated fatty acids, those of less than 12 carbons and stearic acid have a minimal effect on serum cholesterol and phospholipids, but produce elevations in serum triglycerides. On the other hand, saturated fatty acids of 12, 14 and 16 carbons, which elevate serum cholesterol and phospholipids, seem to have little effect on serum triglycerides.

The Position of Fatty Acids on the Glycerol Molecule

Similarly saturated natural and synthetic fats, differing only in the chain lengths of the saturated fatty acids, were fed at 30% total calories in the diets of constant cholesterol level. Grande et al (1970) recorded the effects on serum lipids. Four fats were utilized in the test diets: CB (cocoa butter),

ICB (imitation cocoa butter), PO (palm oil) and IPO (imitation palm oil where stearic acid replaced the palmitic acid in palm oil). Results indicated that subjects fed CB and ICB had similar cholesterol and phospholipid levels, despite differing glyceride structures of the two fats. Since the fats were shown to be equally well absorbed, the hypocholesterolemic effect of cocoa butter could not be explained by decreased absorption of the saturated fatty acids. Even taking into account this possibility would still not explain the difference between PO and IPO.

In contrast to this work, lauric and stearic acids were found to be hypercholesterolemic when fed at 38% calories as semi-synthetic fats transesterified to various oils, although less so than myristic or palmitic acids (McGandy et al 1970). The contrast in behaviour of transesterified stearic acid (19% trisaturated triglycerides) to its natural product, cocoa butter (2 - 3% fully saturated triglycerides) suggested that, in addition to both chain length and saturation, the position of the fatty acid on the glycerol molecule also

influences its metabolism. This confirms the hypothesis made by Beveridge et al (1959) who suggested that the unsaponifiable butterfat fraction was hypercholesterolemic, due to the position of the component fatty acids on the glycerol molecule.

Metabolism of Fatty Acids and Relation to Effects on Blood Lipids

Fatty acids of different chain length show a marked variation, not only in absorption, but also in chemical properties, the form of transport in the body, and metabolism (Fritz 1961). Differences ascribed to specific fatty acids in terms of saturation and chain length may be modified by both configuration and relative abundance of free fatty acid species. These differences may be of great significance in accounting for net effects on plasma lipids.

The partition of fatty acids between the oil and water phase in the stomach is well established. It is known that the long chain fatty acids pass into the oil phase, form micelles with monoglycerides and bile salts, are re-esterified to triglycerides, and eventually pass into the lymphatics as chylomicrons (Bloom et al 1951).

Conversely, the short chain fatty acids of less than 12 carbons, which are more polar, pass into the water phase, and are transported as free (unesterified) fatty acids to the liver via the portal route. Fatty acids of shorter chain length than lauric acid (C12:0) are not incorporated into the fat depots (Fritz 1961). The rate of oxidation of fatty acids of 10 or less carbon atoms is more rapid than the long chain fatty acids (Kirschner and Harris 1961). Of the long chain fatty acids, stearic and palmitic acids are esterified almost exclusively in the one or three positions in natural fats. Digestion of the triglyceride by pancreatic lipase yields 2 - monoglycerides which are absorbed intact (Mattson and Volpenhein 1964, Kayden et al 1967) and the fatty acids from the 1 - 3 - positions, which together with bile salts, are absorbed as micelles (Hoffman and Borgstrom 1964). Absorption studies carried out with infants (Filer et al 1969) and chicks (Renner and Hill 1961) have shown that palmitic acid, which is predominantly in the two position in natural lard, is absorbed better than

randomized lard, where palmitic acid is transesterified. Mattson (1959) has also shown that stearic acid of tristearin is not absorbed in rats, whereas the mono and di-configurations are completely absorbed. McGandy et al (1970) have concluded that there is a mechanism for preserving the positional specificity of 2-fatty acids in the subsequent synthesis of triglyceride and phospholipid within the intestinal mucosa, since evidence exists for such positional specificity in these lipid classes of lipoproteins in chyle. Data from their experiment is therefore consistent with the theory that 2-glycerylstearate is more hypercholesterolemic than free stearic acid. Specific "free" long chain fatty acids have been shown to significantly affect the lipoprotein form in which absorbed cholesterol and triglyceride are transported in rat lymph, and in which form they enter the circulating plasma (Ockner et al 1969). These findings, together with the observed differences in rates of removal of different lipoprotein forms from the plasma, suggested to these investigators, that variations in lipoprotein production at the intestinal level may be reflected in

differences in subsequent metabolism of absorbed dietary and endogenous lipids. In support of this hypothesis, Spritz and Mishkel (1969) have suggested that micellar structure and composition may affect the structure of circulating lipoproteins. They have postulated that saturated fatty acids occupy less area than unsaturated fatty acids. They therefore alter the spatial configuration of the lipids into which they are incorporated. A greater number of lipid molecules are then accommodated by the apoprotein of low density lipoproteins (LDL), and the lipid content of the lipoprotein is increased. Further suggestions by Spritz (1965), imply that this hypercholesterolemic effect of saturated fat may indicate that the equilibrium between plasma and tissue cholesterol pools is altered to favour the plasma pool.

Elevated serum lipid levels have been reported in the literature for hyperlipemic and normolipemic males of different ages, in response to a variety of factors present in saturated fats of animal origin, including beef tallow and butterfat. Evidence has been collected from studies differing in experimental design, employing

various amounts of dietary fat, and using subjects of different ages, conditions of health and ethnic background. Results have indicated the independent effect on serum lipids of both the cholesterol content and degree of saturation of the dietary fat. In addition to differences in chain length of component fatty acids, configuration on the triglyceride molecule may also contribute to the observed effects on serum lipids when natural fats, such as beef tallow and butterfat, are fed to healthy individuals.

R E V I E W O F L I T E R A T U R E

Part II

The Effect of Change of Dietary Fat on Rate of
Change of Serum Cholesterol Specific Activity

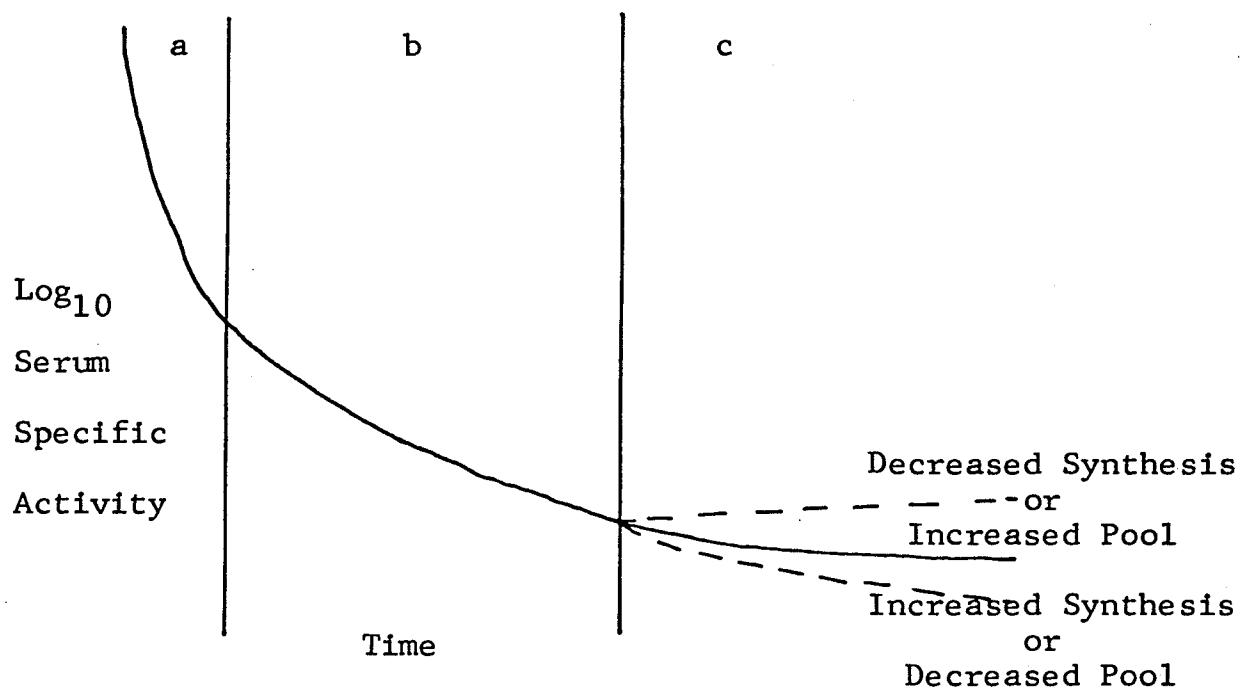
The measurement of serum cholesterol represents only one facet of the body metabolism of cholesterol (Chobanian et al 1962). Changes in the rate of disappearance of cholesterol from the plasma can offer insights into the exchanges between the plasma pool and other exchangeable tissue pools of cholesterol. This exchange may play a necessary part in the determination of alterations in cholesterol metabolism induced by diet, and could lead to speculation as to the location of a derangement in cholesterol metabolism, as may occur in coronary heart disease.

The total amount of new cholesterol which enters body pools each day by absorption and synthesis, or that which is excreted daily from these pools, is termed the metabolic turnover rate of cholesterol (Grundy and Ahrens 1969). The turnover of plasma cholesterol has

been studied in a number of species by administration of isotopically labelled cholesterol, and determination of the specific activity of plasma cholesterol during the ensuing weeks (Dietschy and Wilson 1970a). A constant feature of such studies is that the semilogarithmic plot of cholesterol specific radioactivity vs time, is curvilinear during the first four weeks (Figure 1) and beyond this time, the plot is linear, and shows a constant exponential rate of disappearance (Nestel et al 1965, 1969, Goodman and Noble 1968, 1973, Samuel and Perl 1970, Ho et al 1974). The changing slope of the curve during the first few weeks reflects the different rates of equilibration between cholesterol pools of plasma and various tissues (Chobanian and Hollander 1962). The actual disappearance curve obtained is the average of several curves, each having different rates of exchange of cholesterol. Isotopic equilibration between plasma, and cholesterol in all body tissues outside the nervous system and blood vessels, has occurred by the end of one month (Chobanian and Hollander 1962, Grundy and Ahrens 1966, Oh et al 1976). This equilibration

Figure 1

Schematic Log Specific Activity - Time Curve
 For Serum Cholesterol After Administration of
 a Single Intravenous Dose of Radioactive Cholesterol



a = period of rapid decay (non-linear fall off)

b = period of isotopic equilibrium between ready miscible pools (linear fall off)

c = after change of regimen

(Grundy and Ahrens 1966)

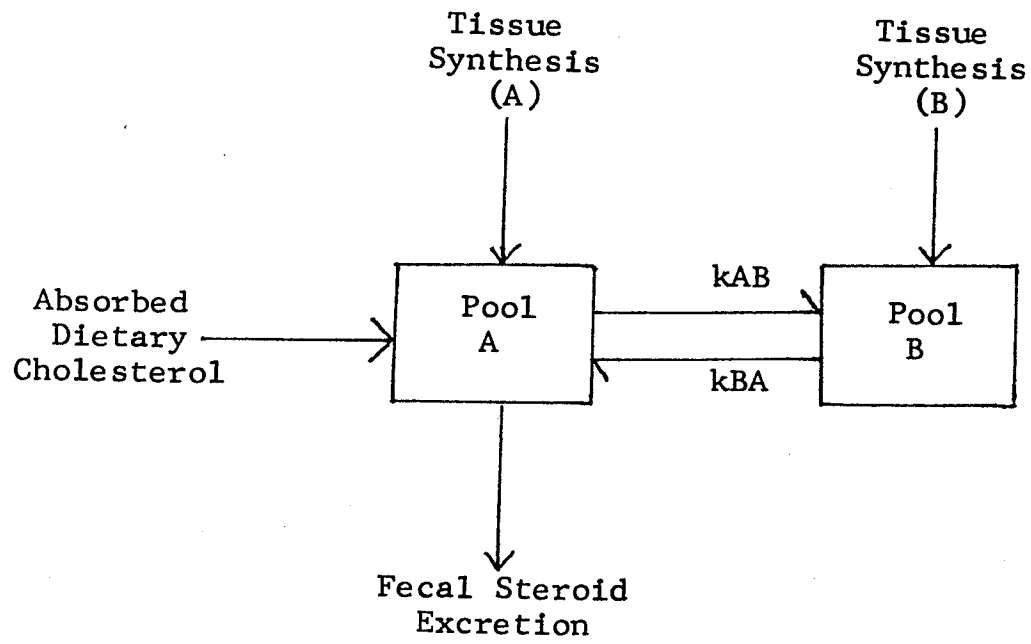
coincides with the beginning of the exponential phase of the disappearance curve (Nestel 1970), which is assumed to reflect the turnover of the slowly miscible pool of exchangeable cholesterol (Chobanian and Hollander 1962, Avigan et al 1962). In addition, this fractional turnover rate is directly related to the total body turnover rate of cholesterol, and bears an inverse relationship to the size of the total pool of body cholesterol (Nestel et al 1965).

Currently perceived models for cholesterol metabolism in humans are based on a two-component serum disappearance curve, for experiments of up to three months duration (Goodman and Noble 1968, Nestel et al 1969, Bhattacharrya et al 1976, Oh et al 1976). Experiments of longer than three months duration have been best described by a three component disappearance curve (Samuel and Lieberman 1973, Goodman et al 1973, Schreibman and Dell 1975, Smith et al 1976). In the present study, the turnover of plasma cholesterol conforms to a two pool model, or two component curve which is the average of two theoretical curves, reflecting two pools of cholesterol. These pools are

referred to as Pool A and Pool B. That is, various tissue pools of cholesterol are classified into two groups, without ascribing any characteristics to them except the rates at which they equilibrate with plasma cholesterol (Goodman and Noble 1968).

Pool A reaches fairly rapid equilibrium with plasma cholesterol, and is comprised of the liver, bile, plasma, erythrocytes, and some cholesterol in the intestine and viscera (Figure 2). Pool B is in slower equilibrium with plasma cholesterol, and relates to the remainder of cholesterol in viscera, skeletal muscle, skin and adipose tissue (Goodman and Noble 1968), and is estimated to contain two-thirds of the total body exchangeable cholesterol. For experiments of longer than three months duration, the two pool model has been modified by the addition of a third pool (Pool C) which is thought to be in very slow equilibrium with plasma cholesterol, and relates to cholesterol in brain or nervous tissue. Implied in all the models derived from studies of tissue cholesterol metabolism, is the knowledge that there is also a pool which is not exchanging with serum cholesterol.

Figure 2
General Two-Pool Model



(Grundy and Ahrens 1969,
after Gurbide et al 1964).



The pools described represent mathematical models and have no true boundaries. The body is actually composed of multiple pools, each with a specific turnover and exchange rate. Some of these pools may be either too small or too slowly exchanging to contribute to the overall shape of the disappearance curve. However, the rates of equilibration with plasma cholesterol are sufficiently similar that the group of tissues behaves as a single pool when analyzed in terms of the disappearance curve of plasma total cholesterol (Goodman and Noble 1968). Each of the multiple pools may, however, be critical in the localized accretion of cholesterol in pathologic states (Dietschy and Wilson 1970a).

In the two pool model, synthesis is assumed to occur in both Pool A and Pool B, and dietary cholesterol enters through Pool A (Figure 2). Cholesterol exchanges freely between the two pools, and exits almost exclusively through Pool A (Goodman and Noble 1968). Labelled cholesterol therefore enters Pool A, and samples are taken from Pool A for measurements of specific radioactivity (Goodman and Noble 1968). It is assumed

that the labelled cholesterol exchanges readily with free cholesterol in plasma lipoproteins, and that homogeneous mixing will occur, so that the labelled cholesterol is metabolized in an equivalent manner to the non-labelled cholesterol (Nestel 1970). The metabolic turnover rate of cholesterol can be estimated by assuming that cholesterol is removed from the body only by way of tissue pools that comprise Pool A. Under these conditions, the metabolic turnover rate is equal to the production rate in Pool A, or the rate of entry of non-labelled cholesterol from any source, by way of synthesis, absorption, or Pool B (Goodman and Noble 1968). However, metabolic turnover of cholesterol is slow, and demands "steady state" conditions (Nestel 1970), that is

- (a) a constant concentration of plasma cholesterol
- (b) unchanging fecal excretion of steroids
- (c) a constant body weight and
- (d) absence of metabolic abnormalities (Grundy et al 1969).

In the steady state, a change in the continuity of the disappearance curve reflects a change in cholesterol metabolism, or more specifically, a change in the turnover rate of cholesterol (Nestel et al 1965). This can be

measured by linearization of the components of the curve. Equilibration between plasma cholesterol, and cholesterol in the tissues which comprise Pool B, requires about thirty days. During the course of the study reported in this thesis, the dietary regimen was changed at day 11, and again at day 32 of the study, so that it was assumed a new equilibration state was not achieved. In addition, changes in the level of serum cholesterol were induced by dietary change, so that steady state conditions were not met. The fractional turnover rate of cholesterol can therefore not be quantitated, but qualitative alterations in the slope of the disappearance curve can offer insights into the mechanisms operative when the size of the plasma cholesterol pool is altered by dietary fat source.

The size of the total body pool of cholesterol can be increased either by increased synthesis and absorption or by decreased excretion of cholesterol (Grundy and Ahrens 1966). After a change in dietary regimen (Figure 1) a decreased slope in the curve reflects a slower fractional turnover rate of cholesterol, and is due to a decrease in endogenous synthesis or absorption, or an

increased total pool size. An increase in the slope reflects a faster fractional turnover rate, and is due to increased synthesis or absorption or a decreased pool size. Hence, the constancy of total body cholesterol depends upon the rapidity and precision by which counterbalancing mechanisms (absorption, synthesis and excretion) compensate for changes that expand or reduce the tissue pools of cholesterol (Grundy et al 1969).

There could be several explanations for changes in the size of the plasma pool of cholesterol, produced by a particular dietary fat.

(a) The absorption of cholesterol may be influenced by variations in the type of dietary fat. The presence of fat per se in the diet has been demonstrated to facilitate cholesterol absorption (Kim and Ivy 1952, Swell et al 1955). Justification for this effect has been postulated as due to (a) the supply of fatty acids necessary for cholesterol esterification (b) stimulation of bile flow (Swell et al 1955) and (c) the capacity of glycerides and free fatty acids to form micelles in conjunction with bile salts (Sylvén and Borgström 1968, Dietschy and Wilson 1970b). The fatty acids in the neutral fat

molecule have been shown to be the active factor (Kim and Ivy 1952, Swell et al 1955). The overall reaction rate of cholesterol esterification has been demonstrated to be dependent upon the composition of the fatty acid mixture available for esterification (Roels and Hashim 1962): esterification is slower for saturated fatty acids and triglycerides (Swell et al 1955, Murthy et al 1961). Free fatty acids are esterified more rapidly than those in triglycerides (Swell et al 1955). The reaction rate for esterification therefore influences the absorption of cholesterol from the gut, and may provide a partial explanation of the mechanism whereby dietary fatty acids influence the concentration of cholesterol in serum, since cholesterol esters are a structural component of lipoproteins (Roels and Hashim 1962). In addition, these investigators have postulated that because short and medium chain fatty acids are absorbed via the portal route, they are therefore not available for cholesterol esterification and transport. Therefore, cholesterol absorption is limited when these fatty acids are present in significant amounts in the diet.

(b) According to Wood and Migicovsky (1956), de novo cholesterol synthesis from ^{14}C - acetate in rat liver homogenates is affected by both chain length and saturation. It has been shown that synthesis is accelerated by an increase in the saturation of the fatty acid. Also, for fatty acids with an even number of carbon atoms in the chain, inhibition of cholesterol synthesis increases for fatty acids with up to twelve carbons in the chain, and then inhibition decreases rapidly. As suggested by Horlick and Craig (1957), this would mean that when the diet contains short chain saturated fatty acids, e.g. butyric acid (C4:0), there is a "relative" acceleration of cholesterol synthesis. Saturated long chain fatty acids with an even number of carbons in the chain, would therefore be expected to have similar effects to butyric acid.

Every mammalian tissue is capable of some degree of de novo cholesterol synthesis, but the highest rate of synthesis has been shown to occur in liver and ileum. Intestinal synthesis is primarily affected by bile flow, but cholesterol synthesis in liver is controlled by the amount of cholesterol absorbed (Grundy et al 1969). It

is therefore apparent that the system will be influenced not only by the amount of cholesterol in the diet, but also by the amount of sterol actually absorbed, the amount of lipoprotein available as a carrier, and therefore, ultimately, the amount of cholesterol actually reaching intra-cellular sites of cholesterol biosynthesis (Dietschy and Wilson 1970c).

(c) Different fatty acids may increase or decrease cholesterol catabolism as a result of the variable rates of breakdown to bile acids of particular types of fatty acid esters of cholesterol in the liver, or, as a result of the influence dietary fatty acids may have on intestinal flora. These micro-organisms may in turn, determine the rate of formation of different catabolic products of cholesterol in the intestinal lumen (Roels and Hashim 1962).

Several investigators have noted that the net gains (or losses) in serum cholesterol could be accounted for almost completely by the changes in fecal excretion of labelled sterols. An increase in plasma cholesterol resulting from an intake of predominantly saturated fat, was accompanied by a decrease in fecal steroid

excretion (Hellman et al 1957, Haust and Beveridge 1958, Wood et al 1966, Grundy and Ahrens 1966, Sodhi et al 1967, Moore et al 1968, Connor et al 1969, Nestel et al 1975). This has been interpreted to indicate a possible net flux of cholesterol from the tissues to the plasma (Schreibman and Ahrens 1976). However, this finding has not been reported consistently among investigators. Others (Spritz et al 1965, Avigan and Steinberg 1965, Grundy and Ahrens 1970) have not noticed any consistent relation between fecal steroid excretion and the concentration of cholesterol in serum. The interpretation was made that the primary action of polyunsaturated fat in lowering serum cholesterol is not to cause an increased output of fecal steroids, but to redistribute cholesterol between the plasma compartment and other tissue storage sites (Spritz et al 1965, Grundy 1975). If the cholesterol which disappears from the plasma compartment enters directly into tissue pools, the increase in tissue cholesterol might accelerate rather than retard atherogenesis (Grundy 1975). The converse hypothesis may therefore be interpreted from the hypercholesterolemic

effect produced by some saturated fats. Although fecal steroid excretion may also reflect changes in endogenous cholesterol synthesis and/or absorption, the analysis of fecal steroids may help to clarify this mechanism.

O B J E C T I V E S

- The purpose of the metabolic study was to
- (a) examine the effects of beef tallow and butterfat on the serum lipids of young men, when these fats provided 40% of total calories in a mixed diet consisting of ordinary foods, and to
 - (b) monitor the disappearance of labelled cholesterol from the serum to gain some insight into possible changes in cholesterol metabolism produced by dietary fats.

E X P E R I M E N T A L M E T H O D S

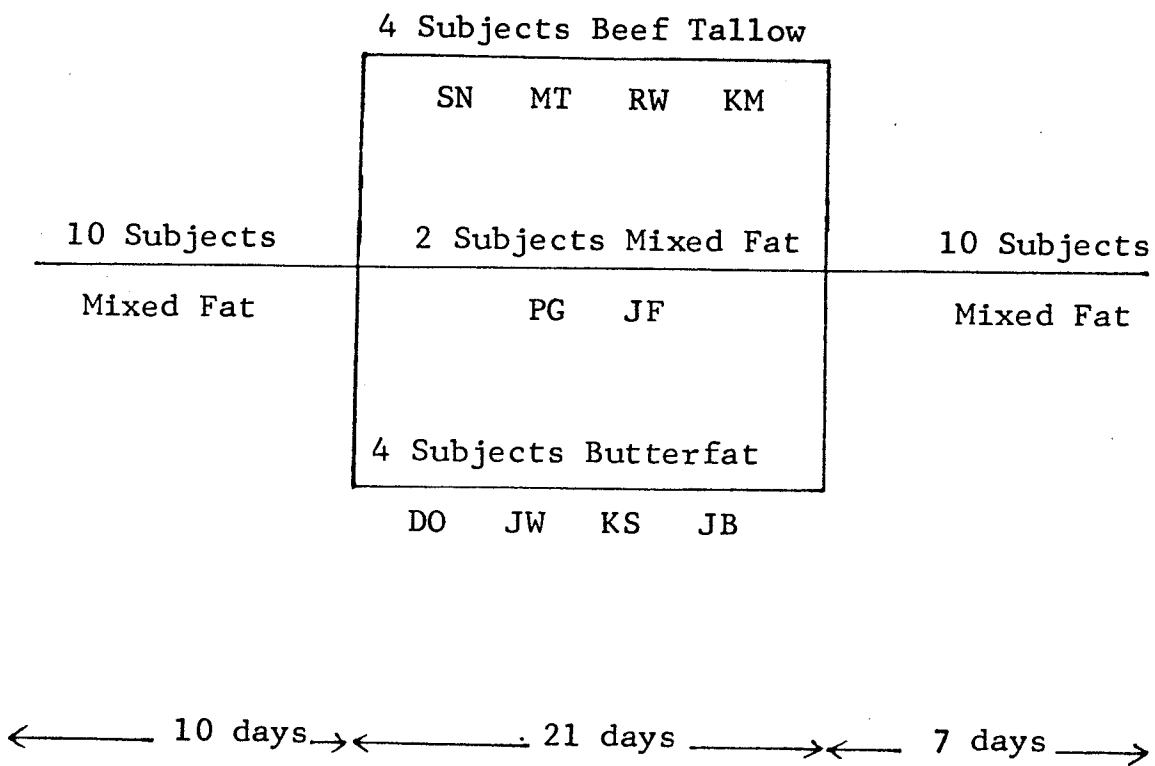
1. Design

The thirty-eight day metabolic study, conducted in October and November 1976, was divided into three dietary treatment periods. Period I lasted ten days, and all subjects consumed the mixed fat (control) diet. During this period, blood lipids stabilized, caloric requirements were determined, and the subjects were introduced to the study procedures. Two subjects were randomly assigned to the mixed fat diet for the entire study. During Period II, four of the remaining eight subjects were randomly assigned to the beef tallow diet, and similarly, four subjects were assigned to the butterfat diet. Throughout Period III, all subjects received the mixed fat diet. The experimental design is shown in Figure 3.

Blood was collected before breakfast, after a ten hour fast, at seven day intervals on days 4, 11, 18, 25, 32 and 39 of the study. The serum was separated for analysis of cholesterol, triglycerides, lipid phosphorus and specific activity, and was stored at -10°C .

Experimental Design

Figure 3



2. Subjects

The subjects were ten healthy male students, nineteen to twenty-nine years of age ($\bar{x} = 24$ years), who were chosen from volunteers responding to posted notices on the campus which advertised the study. Subjects were accepted for the study on the basis of an interview with the project directors, a physical examination, and a willingness to participate after foods had been tasted.

Eight subjects were of average weight for height, and two subjects were 15-20% overweight, according to tables ¹ of normal or desirable weight. Body weights remained within an average of ± 1.2 kg throughout the study. Subject physical data and initial lipid levels are shown in Table 1.

The subjects were all full-time students at the University. They carried out their normal activities and resided in their own homes throughout the study. All meals were served in the Home Economics Building on the University campus. General instructions given to subjects appear in the Appendix Tables 1a and 1b.

¹ Statistical Bulletin, Metropolitan Life Insurance Company 40:3 (Nov.-Dec.) 1959.

Table 1
Subject Physical Data

Subject	Age (yrs)	Height (cm)	Weight (kg)		Initial Serum Lipid Values (mg/dl)		
			Initial	Periods I - III*	Cholesterol	Triglyceride	Lipid Phosphorus
J.B.	19	174	62.50	62.65 ± 0.85	155	96	5.34
J.F.	27	180	63.50	62.82 ± 0.68	150	122	5.96
P.G.	23	171	61.35	61.41 ± 1.08	165	57	5.21
K.M.	21	183	78.36	78.02 ± 1.13	147	60	6.46
S.N.	29	184	103.87	102.17 ± 1.70	283	192	7.63
D.O.	29	168	60.56	60.67 ± 1.02	132	44	4.09
K.S.	20	163	58.40	58.46 ± 0.63	152	110	6.46
M.T.	22	178	75.75	74.67 ± 1.08	182	72	6.80
J.W.	25	184	103.19	102.52 ± 0.91	282	168	6.88
R.W.	22	178	79.61	78.99 ± 0.97	197	59	6.59

* Mean ± S.D. for daily weighings

All subjects successfully completed the study.

3. Isotope Infusion

From September 13 - 15th inclusive, approximately thirty days prior to the study, all subjects were infused with 0.48 to 0.86 microcuries/kg initial body weight ($\bar{x} = 0.70$) of cholesterol - 1-2-³H (N)², (0.32 μ g cholesterol in 1 ml sterile ethanol). The infusion was done in the clinical investigation unit at the Health Sciences Centre, Winnipeg, Manitoba, by Miss Helen Bowan, under the direction of Dr. John Moorhouse. Details of the infusion procedure were described by Cobden (1975).

4. Experimental Diet and Study Procedure

The experimental diet consisted of natural foods, and was designed to resemble a typical Canadian diet, except that the protein was supplied by soy protein of

² Lot No: 919-202. Obtained in July 1976 from New England Nuclear, 549 Albany St., Boston, Massachusetts 02118.

two types ³, spray-dried egg albumin ⁴ and skim milk. The fat content of the basic diet was minimal, (5% of total fat) and the experimental fats which provided 95% of total fat, were incorporated into the diet in an amount which constituted about 40% of total calories. The diets differed only in the type of fat present.

The mixed fat diet was composed of a mixture of butter (15%), corn oil (10%), lard (20%), beef tallow (25%) and hydrogenated soybean oil (30%). The percentage by weight of each constituent fat were derived from domestic fat disappearance figures, supplied by Dr. Paul Sims. ⁵

The menu was planned on a two day rotating basis for each of the test diets. Each menu was comprised of three daily meals plus snacks, provided about 3200 calories daily, and adequately met the nutrient needs of young

³ Promate III Soy Meat Extender and Soy Protein Concentrate GL301, The Griffith Laboratories Ltd., 757 Pharmacy Ave., Scarborough, Ontario.

⁴ Export Packers, Winnipeg, Manitoba.

⁵ Personal Communication, Agriculture Canada, Ottawa, Ontario.

men, based on the 1975 revised Canadian Dietary Standard for males nineteen to thirty-five years of age. The calculated nutrient composition of the diet is compared with recommended values in Appendix Table 2.

The analyzed values for protein, fat and calories were determined, and are compared with calculated values in Table 2. The two day rotating menu is shown in Appendix Table 3.

The two daily entrees provided about 35% of total fat intake. Addition of test fats to cake, cookies, breakfast muffin and vegetables, provided a further 31% of daily fat, and the remainder of fat was used as a spread.

All food servings were weighed, measured and prepared according to standardized recipes. The entrees were prepared, frozen, stored, heated and served in individual foil containers.⁶ The scalloped potatoes were reconstituted from dried potatoes on the day of serving.

⁶ Small Foil Containers with Lids, Reynolds Item, Price Wilson Ltd., 850 Empress St., Winnipeg, Manitoba.

Table 2
 Calculated¹ vs Analyzed² Values for Meals

	Day I			Day II		
	Protein ³	Fat ⁴	Calories ⁵	Protein ³	Fat ⁴	Calories ⁵
Calculated	69	139	3209	70	139	3165
Analyzed	72	133	3352	74	121	3273

- 1 Calculated from USDA Handbook #8, Composition of Foods, (Watt and Merrill, 1963).
- 2 Means of duplicate analyses for each of three experimental diets.
- 3 Determined by the Kjeldahl procedure.
- 4 Analyzed according to the method of Bligh and Dyer (1959).
- 5 Analyzed by Bomb Calorimetry.

Bread was included at each meal to utilize the spread, and to allow subjects to absorb any visible fat remaining on the serving dishes.

Condiments (Worcestershire Sauce, HP Sauce, Ketchup) were available at each meal in moderation, and spices, diet soft drinks, tea and coffee were permitted freely.

On the mixed fat diet, the corn oil was used for the salad, and the butter used as a spread. The hydrogenated soy, lard and tallow were mixed together, and incorporated into menu items. For the beef tallow or butterfat diets, salads were eaten with vinegar, and tallow, margarine or butter were substituted as a spread.

Each subject was weighed daily before lunch, and in order to maintain weight, calories were adjusted by increasing or decreasing bread, fruit, juice, sugar, jam or snack items. The fat intake was correspondingly altered to maintain about 40% calories as fat.

5. Purchase and Storage of Test Fats, Staples and Entrees

Experimental fats were purchased in single lots and stored in closed containers. Beef tallow⁷, tallow margarine⁸, butterfat⁹, hydrogenated soy¹⁰, lard¹¹, and corn oil¹², were stored at 7°C. in a home-style electric refrigerator.

Other staples were similarly bought in single lots, and stored at the appropriate temperature for the form of the product.

7 Bleached, clarified, deodorized. Canada Packers Ltd., Toronto, Ontario.

8 Canada Packers Ltd., Toronto, Ontario.

9 Anhydrous butterfat. New Dundee Co-op, New Dundee, Ontario.

10 Crisco, Proctor and Gamble, Toronto, Ontario.

11 Tenderflake, Maple Leaf Brand, Canada Packers Ltd., Winnipeg, Manitoba.

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

Fresh milk, bread and produce were purchased bi-weekly from a single local source.

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

Duplicate daily composites of all three test diets for days one and two were made of all meals and snacks, and composites were stored at -10°C until chemically analyzed.

6. Meal Analysis

Composites were made of each daily menu for each of the three experimental diets. Previously prepared main entrees and snacks were thawed to room temperature, and the remaining menu items were weighed to the nearest gram on a Sartorius top-loading balance (model 2254)¹³. Composites were homogenized with 200-300 ml distilled water in a Waring commercial blender (model CB-5)¹⁴. The total homogenate was weighed, and a 145-190 gm aliquot lyophilized in a Virtis freeze dryer (model 10-140 MR-BA)¹⁵. The dried sample was crushed to a

¹³ Sartorius-Werke AG, Gottingen, Germany.

¹⁴ Waring Products Co., Winsted, Connecticut.

¹⁵ Virtis Co. Inc., Gardiner, New York, 12525.

fine particle size by pounding, and stored in Whirl-Pak plastic bags (18 oz.)¹⁶ at -10°C for later analysis.

Total lipid was extracted from the lyophilized food samples, according to the method of Bligh and Dyer (1959) using a monophasic mixture of chloroform:methanol: water. The lipid containing chloroform layer was dried for total lipid determination, and an aliquot of the lipid dissolved in petroleum ether, transferred to a screw-top vial, flushed with nitrogen, and stored at -10°C for fatty acid analysis.

Methyl esters of fatty acids were prepared with BF_3 -methanol according to the method of Metcalfe et al (1966), after prior evaporation of petroleum ether and saponification with methanolic NaOH. Fatty acid analyses were completed with a Varian Aerograph gas chromatograph (model 1740-1)¹⁷ equipped with dual columns, flame ionization detectors, Varian Aerograph single pen recorder (model 20)¹⁷ and Varian Aerograph digital integrator (model 477)¹⁷.

¹⁶ Canlab Laboratory Equipment, Winnipeg, Manitoba.

¹⁷ Varian Aerograph, 6358 Viscount Road, Malton, Ontario.

The fatty acids were resolved in 2.7m x 2mm internal diameter stainless steel columns, packed with 10% EGSS-Y on 100/120 mesh GAS CHROM Q¹⁸. Flow rates for the gases were 30 ml/min for helium¹⁹, 25 ml/min for hydrogen¹⁹, and 250 ml/min for air¹⁹.

The columns were operated isothermally at 195°C with injector and detector temperatures of 230°C and 250°C respectively. Individual fatty acids were identified by comparison with linear-log plots of retention time vs carbon number of fatty acid reference standards²⁰. Percent fatty acid methyl esters for each of the test diets are shown in Table 3.

Protein content of the diets was carried out by the boric acid modification (AACC 1962) of the AOAC (1960) Kjeldahl procedure for total nitrogen, except that titanium dioxide was used as the catalyst, as described by Williams (1973). Percentage of protein was determined using the factor of 6.25.

18 Applied Science Lab Inc., P.O. Box 440, State College, Pennsylvania 16801.

19 Welders Supplies, 25 McPhillips St., Winnipeg, Manitoba.

20 Nu Chek Prep Inc., P.O. Box 172, Klysian, Minnesota 56028.

Table 3
 Percent Fatty Acid Methyl Esters¹
 of Experimental Diets

	Tallow	Butterfat	Mixed Fat ⁴
C10 and less	Tr ²	4.8	1.1
C12:0 ³	Tr	4.2	0.7
C14:0	3.1	10.3	3.1
C14:1	0.8	1.8	0.6
C15:0	Tr	1.2	Tr
C15:1	Tr	Tr	Tr
C16:0	24.5	26.5	20.7
C16:1	3.0	3.2	1.9
C17:0	1.3	0.7	1.1
C17:1	0.6	Tr	Tr
C18:0	24.9	12.3	13.9
C18:1	36.0	29.1	37.0
C18:2	4.3	4.6	18.3
C18:3 and C20:0	0.9	1.6	1.5

1 All values are means of duplicate analyses, determined by Gas Liquid Chromatography.

2 "Tr" represents values < 0.5%.

3 Carbon number: number of double bonds.

4 Mixture contained by weight:
 30% hydrogenated soy, 20% lard, 25% beef tallow,
 15% butter oil and 10% corn oil.

Gross energy of diets was determined using a Parr Adiabatic Calorimeter (model 1241)²¹ equipped with a Parr #1541 water heater²¹, and a Parr #1108 oxygen bomb²¹.

Total sterol content of Menu I and Menu II for each experimental diet was determined by the method of Miettinen et al (1965) except that a radioactive tracer was not used to monitor the recovery of sterols from the diets, and the sterols were silylated with BSA²² according to the method of Chambaz and Horning (1968). Recoveries of added cholesterol indicated that losses throughout the procedure were from 5-10%, and data were corrected assuming a 90% recovery level. The analyzed values for the meals in milligrams of cholesterol per day were: beef tallow, 84 mg/day; butterfat, 303 mg/day and mixed fat, 97 mg/day.

21
Parr Instrument Co., 211-53rd St., Moline,
Illinois 61625.

22
BSA = N,O - Bis - (Trimethylsilyl) - Acetamide.

7. Blood Collection and Analysis

Blood was taken from each subject two weeks prior to the study for serum specific activity analysis. During the study, blood samples were taken on Day 4, and at one week intervals thereafter. Samples were taken at 8:00 A.M., following a ten-hour fast.

From each subject, about 50 mls of blood were drawn from the antecubical vein, into three 15 ml BD vacutainer tubes (#4796)²³ and one 7 ml BD vacutainer tube, containing 15% EDTA solution (#4759)²³ which was used for whole blood analysis. Blood used for serum analysis was allowed to clot for one hour in a slanting position at room temperature. The clot was separated from the tube wall, and the three samples centrifuged²⁴ at 1400 x g for ten minutes. Three 2-3 ml and one 5 ml portion of clear sera were pipetted into screw top glass vials, flushed with nitrogen, and stored at -10°C until chemically analyzed.

23 Canlab Laboratory Equipment, Winnipeg, Manitoba.

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

Prior to analysis, sera were thawed for two hours at room temperature. Sera from each subject were analyzed in duplicate for total cholesterol, triglycerides, lipid phosphorus and radioactivity.

Haemoglobin, haematocrit, leucocyte counts and platelet counts of whole blood were monitored at the Haematological Laboratories, Health Sciences Centre, Winnipeg, Manitoba.

8. Analysis of Serum

(a) Cholesterol

Total cholesterol was determined by the Mann (1961) procedure. The optical density of the colour complex was measured at 560 nm in a Coleman Junior Spectrophotometer ²⁵, zeroed by a reagent blank. The micrograms of sterol in the solution were obtained from a standard curve.

(b) Triglycerides

Lipid extraction was carried out by the method of Ryan and Raso (1967). The method was modified to use

²⁵ Model #6A - 36715. Coleman Instrument Inc., Maywood, Illinois.

0.2 ml serum for each determination. Saponification and colour reaction procedures were followed according to Van Handel and Zilversmit (1957) except that sodium bisulphite replaced sodium arsenate, as suggested by Jagannathan (1964). The optical density of the colour complex was measured at 570 nm in a Coleman Junior Spectrophotometer²⁵, zeroed with a reagent blank. The optical density reading for each serum sample was corrected by analyzing an unsaponified serum blank concurrently. Milligrams of triglyceride were determined from a standard curve.

(c) Lipid Phosphorus

The serum lipid phosphorus was analyzed by the method of Chen et al (1956) with some modification to the digestion procedure.

Ashing was carried out by heating in sulphuric acid at 250°C in a heating block (model #120C)²⁶ for one half hour. Hydrogen peroxide was substituted for

26 Hallikainen Instrument, Slaco Division, Richmond, California.

perchloric acid for the second part of the digestion carried out at 150°C. The colour complex produced was measured at 820 m μ in a Unicam SP600 Series 2 Spectrophotometer (model #46511) ²⁷, zeroed with a reagent blank. Micrograms of phosphorus were obtained from a standard curve.

(d) Radioactivity

Total lipid was extracted from two ml of serum, according to the method of Folch et al (1957). The chloroform layer was evaporated under nitrogen in a scintillation vial, and 10 ml of scintillation fluid, prepared by dissolving 5.0 gm PPO ²⁸ and 0.3 gm POPOP ²⁹ in one litre of scintillation grade toluene ³⁰, was added. The samples were stored away from excessive light, and

27 Pye Unicam Ltd., York St., Cambridge CB12X, England.

28 PPO - Diphenyloxazole. Amersham-Searle Corp, 26365 Clearbrook Dr., Arlington Hts., Illinois 60005.

29 POPOP = 1,4 - bis-2 (5-phenylazdy1) benzene
Packard Instruments Corporation Inc., 2200
Warrenville Rd., Downer's Grove, Illinois 60515.

30 Toluene (Scintillation Grade) Fisher Scientific Co., Winnipeg, Manitoba.

counted for twenty minutes or 40,000 counts in a Searle Liquid Scintillation System, Mark III (model 6880) ³¹, equipped with a Silent 700 Electronic data terminal ³², set for the tritium programme.

9. Statistical Analysis

Data from the analysis of serum cholesterol, triglycerides, lipid phosphorus and serum specific activity for the twenty-one day experimental period, were subjected to the analysis of variance technique for a split plot design, as described by Snedecor and Cochran (1967). In order to examine the trends in both plasma lipid levels and serum specific activity over time, the sum of squares for days and day x diet

31 Searle Analytic Inc., 2000 Nuclear Drive, Des Plaines, Illinois 60018.

32 Texas Instruments Inc.

interactions combined, was divided into linear, quadratic and cubic components for each of the three experimental diets. Each sum of squares was tested as a guide to the type of polynomial describing the response curve. The method of Least Significant Difference (LSD) was then used to compare day means for each of the diets.

The analysis of covariance technique was applied to serum specific activity data for all diets during the twenty-one day experimental period, with serum cholesterol as a covariant (Snedecor and Cochran 1967). The presence and nature of a linear relationship between serum cholesterol and serum specific activity was tested.

Within the limitations of experimental design, appropriate orthogonal comparisons were made to compare the slopes of the linear decrease of serum specific activity for beef tallow and butterfat over the twenty-one day experimental period.

RESULTS AND DISCUSSION

Part 1

Serum Lipids

1. Subjects

The subjects remained in good health throughout the entire study. Body weight remained essentially constant during the experiment. Therefore, changes in serum lipid patterns could be attributed to dietary modifications, and not to changes in energy balance. Subjects JW and SN had initial serum lipid levels above the normal range for adult men (Epstein 1972, Wynder and Hill 1972, National Health and Welfare Canada 1973), and both subjects were overweight, according to tables of normal or desirable weight ³³. Kannel (1971) noted that weight gain was associated with a substantial increase in serum cholesterol, particularly in men. Albrink (1973) also reported that obesity is a contributing factor to hypertriglyceridemia. However, Schilling et al (1969) did not find a relationship

33

Statistical Bulletin, Metropolitan Life Insurance Company 40:3 (Nov:Dec) 1959.

between relative weight and plasma lipid levels. Kannel et al (1967a) suggested that subjects with elevations in both serum triglycerides and serum cholesterol, who are also obese, have an increased risk of developing atherosclerosis over the risk associated with either factor alone. The data for these two subjects has therefore, been omitted from group mean values, and will be discussed separately from the other subjects.

Relatively smaller differences existed between the intrinsic levels of the remaining subjects. The nature of response to a change in diet was non-uniform, particularly for serum triglycerides and serum lipid phosphorus. As indicated from the analysis of variance (Appendix Tables 4, 5 and 6), between subject variation was large (MS Error a), but within subject variation was much less (MS Error b) for all lipid measurements. The variability between subjects generally decreased with time.

2. Serum Cholesterol

Individual and group mean values for total serum cholesterol for each of the three diets are shown in Table 4 and described graphically in Figure 4.

The butterfat diet resulted in a significant ($p < 0.01$) increase in mean serum cholesterol (Figure 4) of 48 mg/dl, which was linear. Although the increase was maintained until the end of the experimental period, the rate of increase fell off with time, as indicated by the significant ($p < 0.05$) quadratic response (Appendix Table 4). The difference between the response of serum cholesterol to the test diets was significant ($p < 0.05$) as indicated by the day x diet interaction in the analysis of variance (Appendix Table 4). Subjects who had received the beef tallow diet demonstrated a mean increase in serum cholesterol of 8 mg/dl over the twenty-one day experimental period, which was not significant.

The effect of diet over time was also significant ($p < 0.01$), and was influenced by the subjects who received the butterfat diet. Serum cholesterol values of these subjects appeared to reach a plateau, by the fourteenth day of the experimental period (day 25 of the

Table 4
 Serum Total Cholesterol of Subjects
 In Response to Dietary Fat ¹

Subject	<u>Day of Experiment</u>						
	-13 ³	4	11 ²	18	25	32 ²	39
<u>mg Cholesterol/dl Serum</u>							
<u>Beef Tallow</u>							
S.N. ⁺	248	283	263	245	195	230	290
K.M.	173	147	135	152	162	155	165
M.T.	232	182	182	165	175	175	192
R.W.	198	197	175	165	180	180	220
Group Mean*	201	175	164	161	172	170	192
<u>Mixed Fat</u>							
P.G.	157	165	142	155	160	145	150
J.F.	257	150	160	137	132	137	163
Group Mean	207	158	151	146	146	141	157
<u>Butterfat</u>							
D.O.	125	132	147	165	187	187	165
J.B.	140	155	132	175	175	164	153
J.W. ⁺	262	282	182	210	197	182	242
K.S.	187	152	147	197	208	220	200
Group Mean*	151	146	142	179	190	190	173

- 1 Mean of duplicate analyses.
 2 Days on which dietary fat was changed.
 3 No specified dietary regimen.
 * Excluding S.N. and J.W.
 + Subject excluded from group mean value.

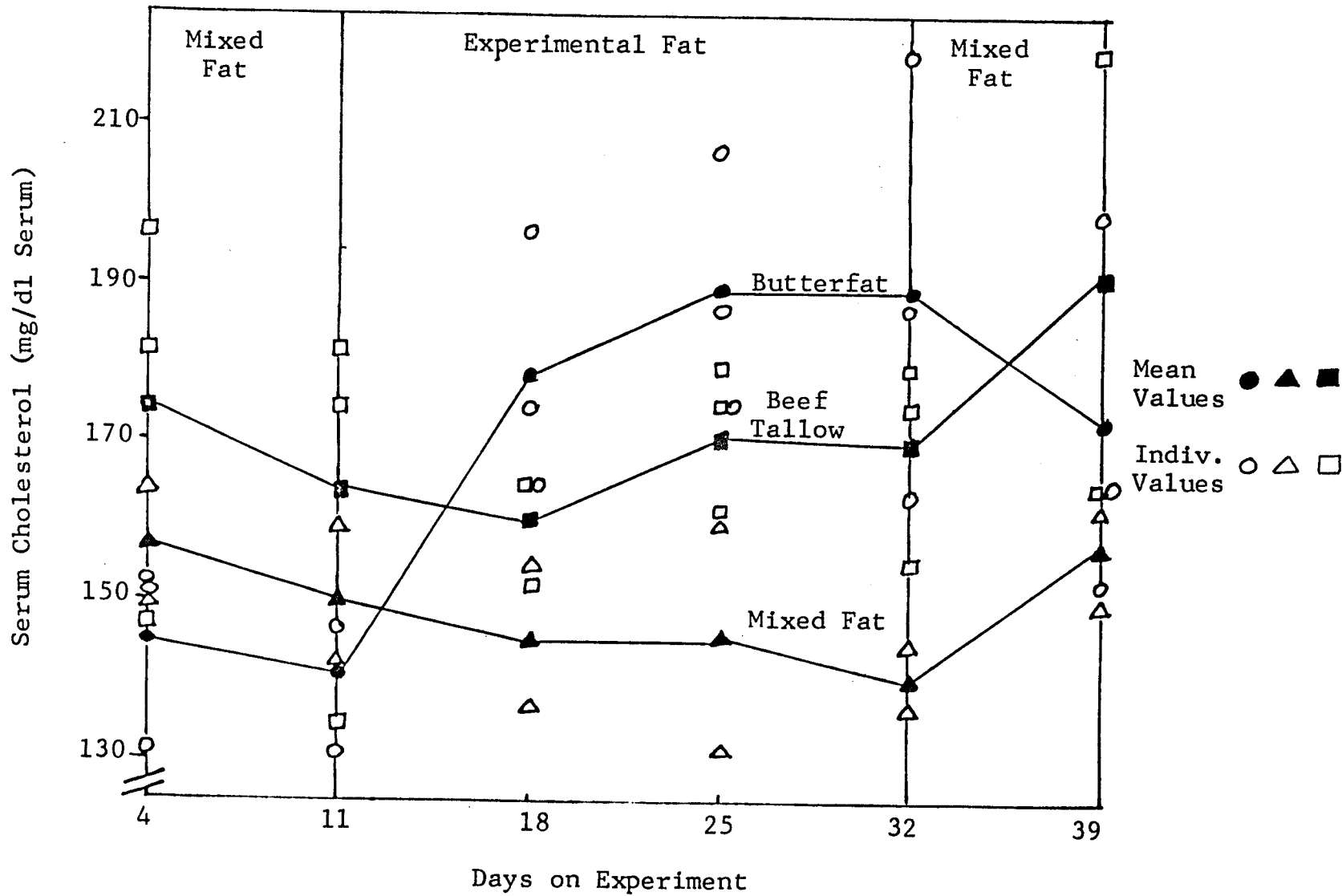


Figure 4: Serum Total Cholesterol Levels of Subjects in Response to Dietary Fat

study). This was confirmed by comparison of day means (Appendix Table 7). The analysis demonstrated a significant difference ($p < 0.01$) between day 11 and days 18, 25 and 32, indicating that a significant response had occurred after seven days on the diet, after which, stabilization occurred. A significant change in serum cholesterol within a week after change of dietary fat was also noted by Keys et al (1957a). By the end of the second week, a relative plateau was reached, and no further significant change was then observed in the next one to two months.

During the seven day stabilization period, serum cholesterol decreased only slightly in most of the subjects. The overall mean decrease during stabilization was 7 mg/dl. In another study conducted in this laboratory (Cobden 1975) a slight decrease in serum cholesterol during stabilization was also noted. It is thought that several factors, including previous meal pattern, and fatty acid composition and cholesterol content of the previous diet, may contribute to this response. Subjects consuming the mixed fat diet throughout the entire experiment, demonstrated similar mean cholesterol

values, which differed by only ± 8 mg/dl. On returning to the mixed fat diet, there was a mean decrease of 17 mg/dl, and a mean increase of 22 mg/dl for subjects who had received the butterfat and beef tallow diets, respectively. Both values were above the pre-experimental level, and this might indicate that one week is not a sufficient length of time for stabilization to occur, following the test fat period.

As indicated in Figure 4, there was considerable between-subject variability. Keys et al (1957a) have also noted that healthy individuals of the same age, and engaged in the same activity, demonstrated differences in serum cholesterol levels when fed an identical diet, and that they also differed in their responsiveness to dietary change. These investigators noted that the average intra-individual standard deviation was large (> 20 mg/dl) and the inter-individual standard deviation was much higher (≈ 45 mg/dl). As a result, significant differences in response to diet for groups of individuals were difficult to prove. Keys et al (1965 b, c), therefore suggested that group mean values should be considered in any comparison of effect of diet on serum cholesterol for a particular group of individuals.

The principal response of serum cholesterol to fat in the diet has been demonstrated by Keys et al (1957a) to be dependent upon the caloric contribution of component fatty acids of the fat. These investigators proposed that under the conditions of their experiment, saturated fatty acids have about twice the effect in elevating serum cholesterol level, as polyunsaturated fatty acids have in lowering it, as expressed by the following equation, derived from least squares multiple correlation analysis.

$$\Delta \text{Cholesterol (mg/dl)} = 1.2 (2 \Delta S' - \Delta P)$$

where $\Delta S'$ and ΔP refer to the percentage caloric differences between saturated and polyunsaturated fatty acids, respectively. Mono-unsaturated fatty acids did not make a significant contribution to the equation, and the neutral effect was confirmed (Keys et al 1958a) in a later experiment. These investigators were able to estimate with reasonable accuracy, the average serum cholesterol response when this equation was applied to data from other investigators. Least squares analysis indicated that stearic acid, and saturated fatty acids with fewer than 12 carbon atoms had little effect

on serum cholesterol in man (Keys et al 1965c). Closer agreement was obtained between predicted and observed values when S' referred only to saturated fatty acids from 12 to 16 carbon atoms.

Although dietary cholesterol was thought to play a minor role (Keys et al 1965a), in diets of differing cholesterol content, the equation was expanded, using the relationship between the effect of cholesterol in the daily diet on cholesterol response, as proposed by Grande et al (1965). The equation thus became:

$$\Delta \text{Chol (mg/dl)} = 1.2 (2 \Delta S' - \Delta P) + 1.5 \Delta Z$$

where ΔZ was the difference in cholesterol content of the diets, expressed as mg/1000 kcals. The effect of cholesterol, and the percentage caloric contributions of fatty acids in the diet were thought to be linearly additive (Keys et al 1965c).

These results were confirmed by Hegsted et al (1965). A regression equation which included only changes of the intake of myristic acid (C14:0), palmitic acid (C16:0), polyunsaturated fatty acids and dietary cholesterol, was adequate to explain 91% of the total variance in serum cholesterol response in the subjects studied. It

was observed, however, that the serum cholesterol response to changes of fat in the diet could be estimated better by expressing the percentage contribution of fatty acids in the fat, rather than by the percentage of total calories supplied by the fatty acids. However, this may have been limited to the fat intakes studied (22% and 38% fat calories). After consideration of the four mentioned variables, the inclusion of other variables did not significantly improve the fit of the regression equation. Stearic acid (C18:0), lauric acid (C12:0), short chain saturated fatty acids and monounsaturates were therefore, not assumed to have any significant correlation with changes in serum cholesterol. These investigators caution that their equations are primarily descriptive of the information from which they were derived, and also have little value in predicting the serum cholesterol response of an individual, due to variation between subjects.

Table 5 shows the percentage of total calories derived from total saturated and polyunsaturated fatty acids and cholesterol in the test fats. The estimated response of serum cholesterol, calculated from the

Table 5
 Percent Total Daily Calories Contributed by Glycerides of Saturated,
 Monounsaturated and Polyunsaturated Fatty Acids and Cholesterol
 for Each Diet ¹

Diet	% Total Daily Calories from Fatty Acids					% Total Fat Calories
	S ²	S ³	M ⁴	P ⁵	Z ⁶	
Mixed Fat	14.23	8.97	13.83	6.94	32	35
Beef Tallow	17.76	9.54	13.33	1.72	28	33
Butterfat	21.02	15.03	11.93	2.17	101	35

- 1 Analyzed values.
- 2 S Total saturated fatty acids.
- 3 S " Total saturated fatty acids minus stearic acid and saturated fatty acids of < 10C.
- 4 M Total monounsaturated fatty acids.
- 5 P Total polyunsaturated fatty acids.
- 6 Z Mg dietary cholesterol/1000 calories.

equation proposed by Keys et al (1965c) is compared with the observed values in Table 6. It can be seen that the response of serum cholesterol to the beef tallow diet is grossly overestimated if stearic acid is not considered to be neutral in its effect. The predicted response for butterfat is less than that observed (Table 6). In a study of similar design, Cobden (1975) also noted that observed serum cholesterol values for a diet containing about 40% calories from lard were higher than those predicted from the equation by Keys et al (1965c). The experimental design and subjects studied by Keys et al (1965c) differed from the present experiment, and that of Cobden (1975). It would therefore, appear that such prediction equations may be of limited application.

In consideration of the above data, the serum cholesterol response of subjects to the test diets may have reflected differences in the fatty acid composition between the test fats and the mixed fat diet. In the case of beef tallow, the non-significant response may have been due to the fact that the decrease in the content of short ($< C_{10:0}$) chain saturated fatty acids, lauric

Table 6

Estimated ¹ vs Observed Response in Serum Cholesterol (mg/dl)
of Subjects Fed Beef Tallow and Butterfat Diets

	<u>Observed</u>	<u>Estimated</u> ²	<u>Estimated</u> ³
Butterfat vs Mixed Fat	48.0 ↑	26.86 ↑	28.61 ↑
Beef Tallow vs Mixed Fat	8.0 ↑	7.08 ↑	14.18 ↑

1 Keys et al 1965c
 $\Delta \text{Chol} = 1.2 (2 \Delta S' - \Delta P) + 1.5 \Delta Z$

2 Using S'' = total saturated fatty acids minus stearic acid and saturated fatty acids of < 10 carbons.

3 Using S = total saturated fatty acids.

(C12:0) and linoleic (C18:2) acids were counterbalanced by the increases in palmitic (C16:0) and stearic (C18:0) acids in beef tallow, so that the net effect of diet change was almost negligible. The dietary change from the mixed fat to the butterfat diet involved increases in short chain saturated fatty acids ($< C10:0$) lauric (C12:0) myristic (C14:0) and palmitic (C16:0) acids, but decreases in oleic acid (C18:1) and linoleic acid (C18:2). It is evident that the response of serum cholesterol to the butterfat diet, in relation to mixed fat, must have been due to the fact that the increase in lauric (C12:0) myristic (C14:0) and palmitic (C16:0) acids and decreases in linoleic acid (C18:2) and oleic (C18:1) acid, more than compensated for the increase in short chain saturated fatty acids ($< C10:0$). Consequently, the serum cholesterol response was increased when butterfat constituted the sole dietary fat source.

The estimated changes in serum cholesterol on the basis of relationships derived between the intake of dietary cholesterol and serum cholesterol response (Hegsted et al 1965, Keys et al 1965c, Mattson et al 1972) are shown in Table 7. From these data, it can be seen

Table 7

Estimated Response in Serum Cholesterol (mg/dl) of Subjects
to Cholesterol in Beef Tallow and Butterfat Diets

	1 Mattson <u>et al 1972</u>	2 Keys <u>et al</u> <u>1965c</u>	3 Hegsted <u>et al</u> <u>1965</u>
Butterfat vs Mixed Fat	8.14 ↑	6.59 ↑	10.3 ↑
Beef Tallow vs Mixed Fat	0.47 ↓	0.56 ↓	0.65 ↓

1 Change in serum cholesterol (mg/dl) = 1.60 + (0.118 dietary cholesterol, mg/1000 k cal.)

2 $\Delta \text{Chol} = 1.5 (Z_2 - Z_1)$ where $Z^2 = \text{mg cholesterol}/1000 \text{ k cal.}$ and Z_1 and Z_2 refer to cholesterol in diets 1 and 2 respectively.

3 Each 100 mg cholesterol added to the diet produces an estimated serum change of 5 mg/dl.

that the total effect on serum cholesterol of the test fats cannot be due to dietary cholesterol alone, since the serum cholesterol responses observed for butterfat and beef tallow were increases of 48 mg/dl and 8 mg/dl, respectively.

The cholesterol content of the butterfat diet was higher (303 mg/day) than that of both mixed fat (97 mg/day) or beef tallow (84 mg/day) diets. Several investigators have demonstrated that cholesterol fed in crystalline or natural form has a significant effect on serum cholesterol (Grande et al 1965, Hegsted et al 1965, Mattson et al 1972, Morris 1977), and that the contribution of fatty acids is minor relative to the response of serum cholesterol to changes in dietary cholesterol (Erickson et al 1964, Connor et al 1964). However, the effects on serum cholesterol produced by both dietary cholesterol and fatty acids have been shown to be independent of each other (Grande et al 1965, Hegsted et al 1965, Nestel et al 1975, Anderson et al 1976).

In the present study, the fatty acid composition of the dietary fat was considered to have had the greatest effect

on serum cholesterol. Although the effect of dietary cholesterol on serum cholesterol response is not disputed, the difference in cholesterol content of the test diets is not considered sufficient to cause the magnitude of the observed changes.

In a previous report from this laboratory (Losier 1972) a significant mean decrease in serum cholesterol of 20 mg/dl was noted in response to beef tallow when fed at about 40% of total calories. The male subjects showed similar serum cholesterol responses to beef tallow as to a diet containing isocaloric amounts of corn oil, for which a significant mean decrease of 48 mg/dl was reported. In this study (Losier 1972) the preceding stabilization diet consisted of ordinary foods, including whole milk and eggs, meats, butter, lard and shortening, and supplied about 40% calories from fat. Mean cholesterol values actually increased slightly during this period. In the present study, such meats and dairy products were replaced by skim milk, soy protein and egg albumin during stabilization as well as during the experimental period. A mixture of fats, supplying about 40% fat calories was added to the stabilization diet, and

resembled the fat composition of a typical Canadian diet. It is suggested that the hypocholesterolemic response of beef tallow noted by Losier (1972) was due to the fatty acid composition of the preceding stabilization diet. This hypothesis is substantiated by the observed hypercholesterolemic effect of lard in a study (Cobden 1972) of similar design to the present study.

The present results indicate that the primary response of serum cholesterol to changes of saturated fats in the diet, is due to the fatty acid composition of the fat, fed at about 40% calories. This confirms the reports of Beveridge et al (1956) and Bronte-Stewart et al (1956), that despite similarity in total saturation, butterfat is more hypercholesterolemic than beef tallow. On the basis of previous reports in the literature, it is concluded that in butterfat, the minimal effect of short chain saturated fatty acids (Hashim et al 1960, Grande et al 1961, Grande 1962, Keys et al 1965c), is more than compensated for by its high content of lauric, myristic and palmitic acids, which are hypercholesterolemic (Keys et al 1965c, Hegsted et al 1965, Grande et al 1961, 1970, 1972).

The non-significant nature of response to a diet containing about 40% beef tallow (high in stearic acid) confirms other reports in the literature using cocoa butter (Ahrens et al 1957, Connor et al 1964, Erickson et al 1964) and ethyl stearate (Horlick and Craig 1957). Present data suggest that the hypercholesterolemic effect of palmitic acid is not manifested due to the greater amounts of stearic acid present in beef tallow. In addition to other investigators (Keys et al 1965c, Hegsted et al 1965, Losier 1972) it is concluded that stearic acid is not hypercholesterolemic. There is some controversy in the literature as to whether the effect is due to the stearic acid per se (Grande et al 1970) or to the position of stearic acid on the triglyceride molecule in the natural fat (McGandy et al 1970), which cannot be determined from this experiment.

3. Serum Triglycerides

Individual subject and group mean values for each of the three diets are shown in Table 8, and depicted graphically in Figure 5.

Table 8
 Serum Triglycerides of Subjects
 in Response to Dietary Fat ¹

Subject	Day of Experiment					
	4	11 ²	18	25	32 ²	39
<u>mg Triglyceride/dl Serum</u>						
<u>Beef Tallow</u>						
S.N. ⁺	192	159	219	189	243	168
K.M.	60	47	72	110	120	101
M.T.	72	96	73	74	70	93
R.W.	59	66	70	77	116	72
Group Mean*	64	70	72	87	102	89
<u>Mixed Fat</u>						
P.G.	57	71	65	57	58	62
J.F.	122	92	81	74	88	124
Group Mean	90	82	73	66	73	93
<u>Butterfat</u>						
D.O.	44	56	70	39	51	41
J.B.	96	119	117	80	65	63
J.W. ⁺	168	126	131	228	204	159
K.S.	110	185	142	93	119	77
Group Mean*	83	120	110	71	78	60

- 1 Mean of duplicate analyses.
 2 Days on which dietary fat was changed.
 * Excluding S.N. and J.W.
 + Subject excluded from group mean value.

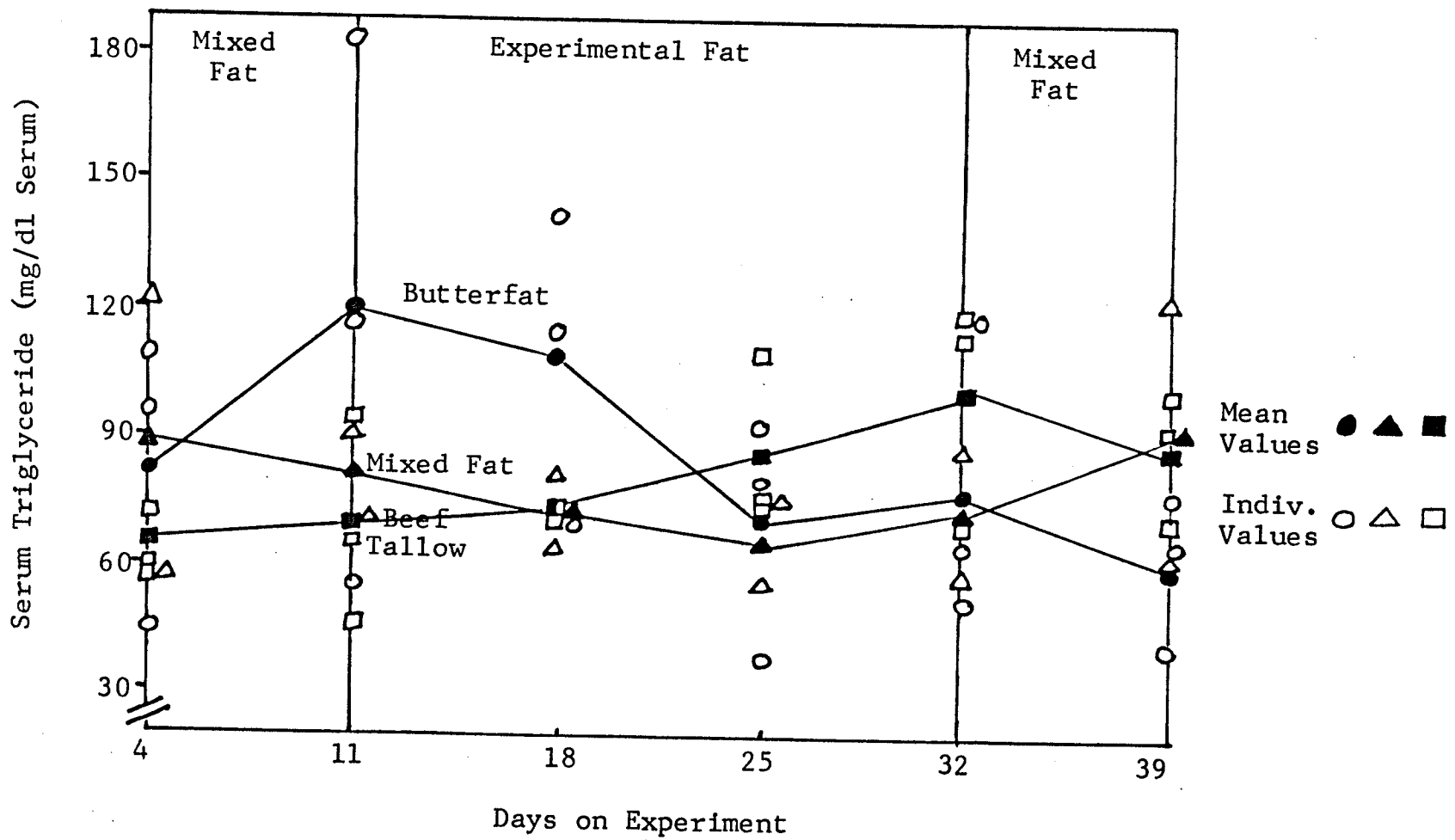


Figure 5: Serum Triglyceride Levels of Subjects in Response to Dietary Fat

Analysis of the response curves for serum triglycerides over the twenty-one day experimental period in subjects receiving the experimental diets, indicated a significant ($p < 0.01$) linear decrease of 42 mg/dl for butterfat, and a significant ($p < 0.05$) linear increase of 32 mg/dl for beef tallow. This contrast in diet effect is reflected in the significant ($p < 0.05$) day x diet interaction in the analysis of variance (Appendix Table 5).

The serum triglyceride response in all subjects was non-uniform, and there was considerable variation between subjects (Appendix Table 5). Other experiments undertaken in this laboratory (Losier 1972) have also suggested a greater variation in serum triglyceride values than serum cholesterol values. A greater coefficient of variation for serum triglycerides (47% as opposed to 21% for serum cholesterol) has been noted by Turpeinen et al (1968). Hegsted et al (1965) also reported a large standard error for triglyceride measurements. Experiments conducted by Ahrens et al (1957) have indicated that the pooled variation of serum triglycerides (± 31 mg/dl) was much larger than that of serum cholesterol (± 9.9 mg/dl). The data

reported in the present study show that the response of serum triglycerides to dietary butterfat and beef tallow are opposite to those responses noted for serum cholesterol.

During the seven day stabilization period, mean serum triglycerides altered only slightly in most subjects. The larger mean response to the mixed fat diet for subjects in the butterfat group was influenced greatly by the response of one subject, K.S. The overall mean increase in serum triglycerides during stabilization was 12 mg/dl. Mean triglyceride values for subjects consuming the mixed fat diet throughout the entire experiment, were similar and differed by only ± 14 mg/dl. On returning to the mixed fat diet, subjects who received the beef tallow diet had a mean decrease of 13 mg/dl, which was above the pre-experimental level. For subjects who received the butterfat diet, there was also a mean decrease of 18 mg/dl, which was influenced by one subject, K.S.

Elevations in the serum triglycerides of Bantu subjects fed a diet containing 40% of calories from butterfat, when compared to a low fat diet, have been

reported by Antonis and Bersohn (1961). Other investigators (Uzawa et al 1964, McGandy et al 1970) have noted elevations in serum triglycerides after ingestion of medium chain triglycerides (of which the primary components are glycerides of 8 to 10 carbons). The above mentioned evidence suggests that short chain fatty acids, which have little effect on serum cholesterol, elevate serum triglycerides. In a study by Anderson et al (1967) coconut oil (containing large percentages of lauric and myristic acids) was shown to be hypotriglyceridemic in comparison with beef fat (containing large percentages of palmitic and stearic acids). Further work by Grande et al (1972) attributed the hypertriglyceridemic effect of long chain saturated fatty acids to stearic acid, although this fatty acid had no detectable effect on serum cholesterol. These investigators also suggested that saturated fatty acids of 12 to 16 carbons were not hypertriglyceridemic when fed at 30% fat calories. Grande et al (1972) have postulated that those saturated fatty acids which elevate serum cholesterol have little effect on serum triglycerides. Conversely, those saturated fatty acids which have little effect on serum cholesterol, elevate serum triglycerides.

Antonis and Bersohn (1961) have suggested that the elevation of serum triglycerides observed in subjects receiving a diet containing butterfat, as compared with isocaloric levels of sunflower seed oil, may reflect differences in the plasma turnover rates of triglycerides of different fatty acid composition. This hypothesis may be applicable to the results of the present study. It may be that the elevation in serum triglycerides in response to the beef tallow diet reflected the high content of stearic acid present, which is cleared from the plasma more slowly than the predominant triglycerides of butterfat. That is, the serum triglyceride elevation produced by beef tallow, may reflect an altered fat clearance rate.

The effect of dietary cholesterol on serum triglycerides has been shown to be inconsistent and varied (Erickson et al 1964, Grande et al 1965, Hegsted et al 1965, National Diet Heart Study Group 1968, Morris 1977).

Results of the present study are in contrast with the work reported by Losier (1972). In the Losier study,

beef tallow, fed at about 40% calories, did not produce a significant change in the level of serum triglycerides, when compared with a mixed fat diet of natural foods, which included animal protein. Grande et al (1972) postulated that saturated fatty acids with less than 12 carbons and stearic acid, have a minimal effect on serum cholesterol, but produce elevations in serum triglycerides. It was also noted that saturated fatty acids of 12 to 16 carbons which elevate serum cholesterol, seem to have little effect on serum triglycerides. Results of the study reported here are in support of this hypothesis.

4. Serum Lipid Phosphorus

Individual and group mean values for subjects fed the three test fats, are shown in Table 9, and described graphically in Figure 6.

There was an elevation of serum lipid phosphorus of 1.83 mg/dl over the twenty-one day experimental period in subjects who received the butterfat diet. Subjects who received the beef tallow diet demonstrated a mean increase of only 0.28 mg/dl which was also not significant.

Table 9
 Serum Lipid Phosphorus ¹ of Subjects
 in Response to Dietary Fat ²

Subject	Day of Experiment					
	4	11 ³	18	25	32 ³	39
<u>mg Lipid Phosphorus/dl Serum</u>						
<u>Beef Tallow</u>						
S.N. ⁺	7.63	7.09	6.80	5.42	6.17	7.30
K.M.	6.46	5.34	5.46	5.42	6.46	6.46
M.T.	6.80	7.21	6.38	6.05	6.38	7.01
R.W.	6.59	6.05	6.67	5.96	6.59	7.01
Group Mean*	6.62	6.20	6.17	5.81	6.48	6.83
<u>Mixed Fat</u>						
P.G.	5.21	4.08	5.42	4.80	4.38	5.34
J.F.	5.96	6.67	4.17	4.92	5.42	5.96
Group Mean	5.59	5.38	4.80	4.86	4.90	5.65
<u>Butterfat</u>						
D.O.	4.09	4.38	4.30	4.71	5.75	4.38
J.B.	5.34	5.42	6.05	5.42	5.34	5.84
J.W. ⁺	6.88	6.80	6.80	5.96	6.30	6.88
K.S.	6.46	6.46	7.00	6.59	7.00	5.63
Group Mean*	5.30	5.42	5.78	5.57	6.03	5.28

- 1 X25 = serum phospholipid (Tietz 1976).
 2 Mean of duplicate analyses.
 3 Days on which dietary fat was changed.
 * Excluding S.N. and J.W.
 + Subject excluded from group mean value.

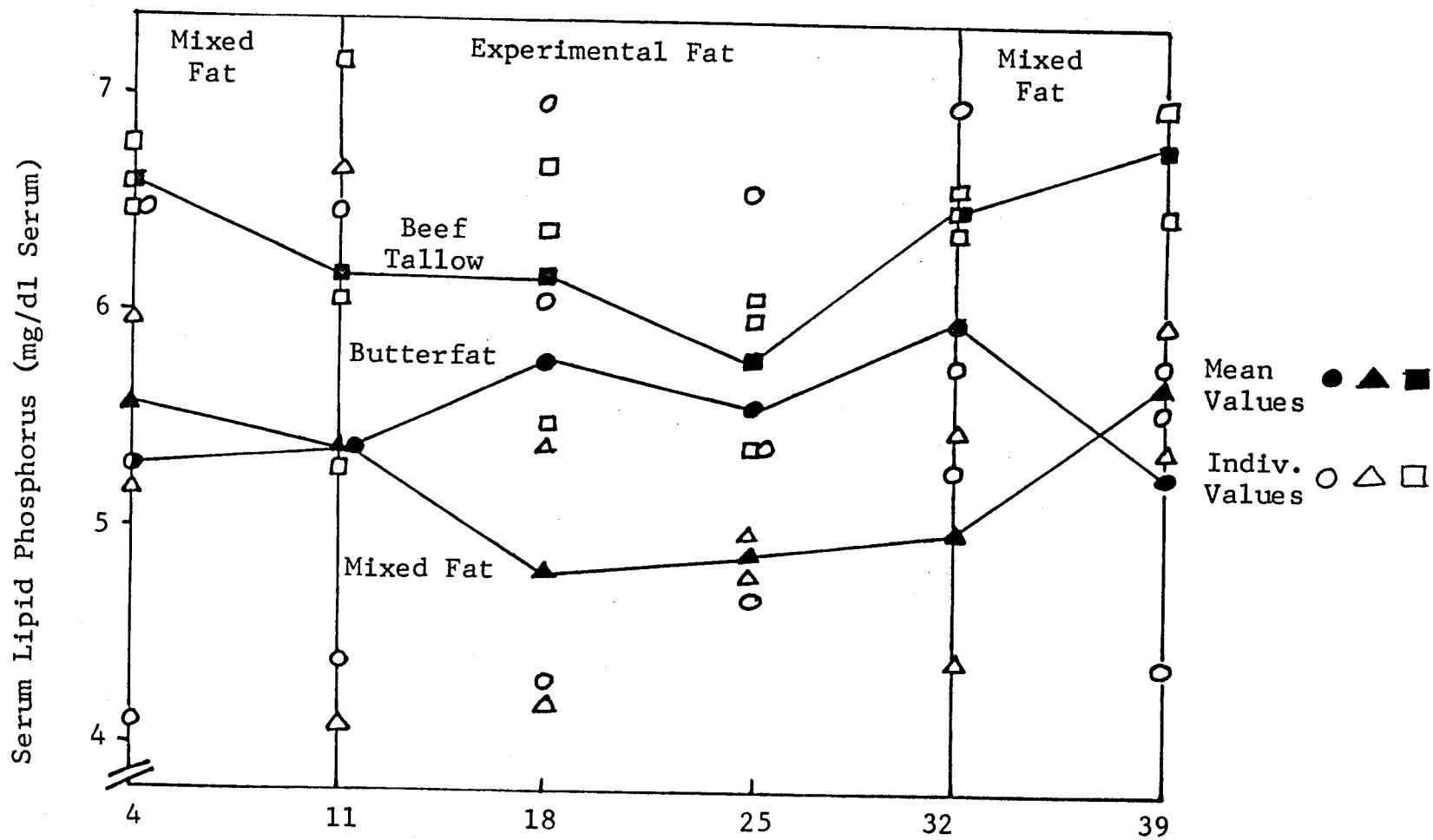


Figure 6: Serum Lipid Phosphorus Levels of Subjects in Response to Dietary Fat

The high between-subject variability (mean square Error a, Appendix Table 8) and non-uniformity of lipid response (Table 9) have prevented any conclusive inference to be drawn, although trends in serum lipid phosphorus are similar to those of serum cholesterol.

During the initial stabilization period, serum lipid phosphorus response was not uniform, but there was a mean change of 0.31 mg/dl for all subjects. Subjects who received the mixed fat diet throughout the entire experiment demonstrated resultant similar mean lipid phosphorus values, which differed by only ± 0.46 mg/dl. On returning to the mixed fat diet, subjects who received the beef tallow diet, demonstrated a mean increase of 0.35 mg/dl to a value slightly above the pre-experimental level. Subjects who received the butterfat diet showed a mean decrease of 0.75 mg/dl to a value slightly below the pre-experimental level.

Turpeinen et al (1968) have reported a 22% coefficient of variation for serum phospholipid response, and Hegsted et al (1965) have noted a relatively large standard error for phospholipid data. Most investigators have found that serum phospholipid values parallel those of serum

cholesterol (Ahrens et al 1957, Keys et al 1958a, Hashim et al 1960, Grande et al 1961, 1970) but that the magnitude of response is much less (Erickson et al 1964, Hegsted et al 1965, Connor et al 1969, McGandy et al 1970). The present data are in general agreement with the above findings.

5. Serum Lipid Response of Subjects JW and SN to Dietary Fat

Serum cholesterol response to experimental fat was associated with a dramatic fall (33 mg/dl) for SN over the twenty-one day experimental period in response to the beef tallow diet. For subject JW, who received the butterfat diet, serum cholesterol was not appreciably changed over the twenty-one day period. The serum cholesterol responses of JW and SN were different to the responses of other subjects (Table 4, Figure 4). Keys et al (1959) have found that, in general, responsiveness to changes in the diet is related to the intrinsic characteristic of the individual, as indicated by the average serum cholesterol level on

a specific diet. These investigators have formulated the following equation:

$$\Delta\% = 1.91 X \% - 91$$

where X% is the serum cholesterol value of an individual expressed as a percentage of the average of a group of men on the same diet, and $\Delta\%$ is that individual's cholesterol response in changing to another diet, expressed as a percentage of the group average response to the same dietary change. From the equation, it can be observed that intrinsic relative hypercholesterolemia is generally associated with a relative hyper-responsiveness to dietary fat changes. Turpeinen et al (1960) in confirmation, have found that the large individual variations in the serum cholesterol response to dietary change, were influenced by the initial serum cholesterol level. The most elevated serum cholesterol levels showed the greatest decreases in response to changes of dietary fat.

The serum triglyceride response of both subjects to the experimental fats were in contrast with the response of serum cholesterol (Table 8). Serum triglycerides increased dramatically ($\bar{x} = 81$ mg/dl)

over the twenty-one day experimental period, regardless of diet. Spritz and Mishkel (1969) have found that the triglyceride concentration is least affected in those subjects with the lowest initial serum values. If the reverse relationship is postulated, this finding offers no explanation for the similar directional change in both subjects, despite the difference in experimental fats. Ahrens et al (1957) have indicated that individual variation is increased, the higher the initial serum triglyceride level, and the results support this observation.

Serum lipid phosphorus response (Table 9) paralleled serum cholesterol changes, but were of a lesser magnitude. The mean decrease of serum phospholipid in these two subjects was 0.71 mg/dl over the twenty-one day experimental period.

Both subjects also demonstrated a much greater magnitude of lipid response to the ingestion of the mixed fat diet, during initial and final stabilization periods.

It appears that further research is needed to clarify the mechanism of lipid response in obese, hypercholesterolemic, hypertriglyceridemic subjects to changes of dietary fat.

R E S U L T S A N D D I S C U S S I O N

Part II

Serum Specific Activity

The disappearance of cholesterol 1-2-³H from the serum was determined by expressing measurements of serum radioactivity (dpm/ml) in terms of serum cholesterol (mg/dl) at periodic time intervals from infusion. Serum specific activity values obtained (dpm/mg serum cholesterol) were adjusted for individual dosage level (50 μ c/kg initial body weight). Subject individual and group mean values for serum radioactivity and serum specific activity are shown in Tables 10 and 11, respectively. Disappearance of labelled cholesterol from the serum (\log_{10} serum specific activity vs time) for each diet is described graphically in Figure 7.

The mean rate of change of \log_{10} serum specific activity for each diet during the twenty-one day experimental period, was linear ($p < 0.01$) (Appendix Table 8). Therefore, it was assumed that isotopic equilibration between plasma, and the tissues in Pool A and Pool B was attained. No significant differences in the slopes of the disappearance curves were noted between

Table 10
 Serum Radioactivity (dpm/ml Serum) of
 Subjects in Response to Dietary Fat¹

Subject	Days From Infusion						
	15 ³	32 ²	39 ²	46	53	60 ²	67
<u>Beef Tallow</u>							
S.N.+	1453	719	628	567	469	467	395
R.W.	-	1050	889	868	757	697	627
M.T.	748	453	416	355	311	277	246
K.M.	1381	766	637	563	523	467	432
Group Mean*	1065	756	647	595	530	480	435
<u>Mixed Fat</u>							
J.F.	2154	1225	1119	862	749	717	615
P.G.	1729	996	857	740	625	553	470
Group Mean	1942	1111	988	801	687	635	543
<u>Butterfat</u>							
J.B.	1774	1013	811	770	695	552	474
K.S.	2427	1251	1068	1044	915	798	582
J.W.+	1072	584	410	366	297	260	248
D.O.	1465	906	849	890	907	865	673
Group Mean*	1889	1057	909	901	839	738	576

- 1 Mean of duplicate analyses.
 2 Days on which dietary fat was changed.
 3 No specified dietary regimen.
 * Excluding S.N. and J.W.
 + Subject excluded from group mean value.

Table 11

Serum Specific Activity of Subjects in

Response to Dietary Fat¹ $\frac{\text{dpm/mg}}{50\mu\text{c/kg}}$

Subject	Days From Infusion						
	15 ³	32 ²	39 ²	46	53	60 ²	67
<u>Beef Tallow</u>							
S.N. ⁺	1218	528	496	481	500	422	283
R.W.	-	849	809	838	669	617	454
M.T.	489	377	346	326	269	240	194
K.M.	1251	817	739	580	506	472	410
Group Mean*	870	681	631	581	481	443	353
<u>Mixed Fat</u>							
J.F.	1065	1038	888	800	721	665	479
P.G.	1351	741	741	586	479	468	385
Group Mean	1208	890	815	693	600	567	432
<u>Butterfat</u>							
J.B.	1586	818	769	551	497	421	388
K.S.	1516	916	848	619	514	424	340
J.W. ⁺	843	427	464	359	310	295	211
D.O.	1419	831	699	653	587	560	493
Group Mean*	1507	870	772	608	533	468	407

1

Mean of duplicate analyses.

2

Days on which dietary fat was changed.

3

No specified dietary regimen.

*

Excluding S.N. and J.W.

+

Subject excluded from group mean value.

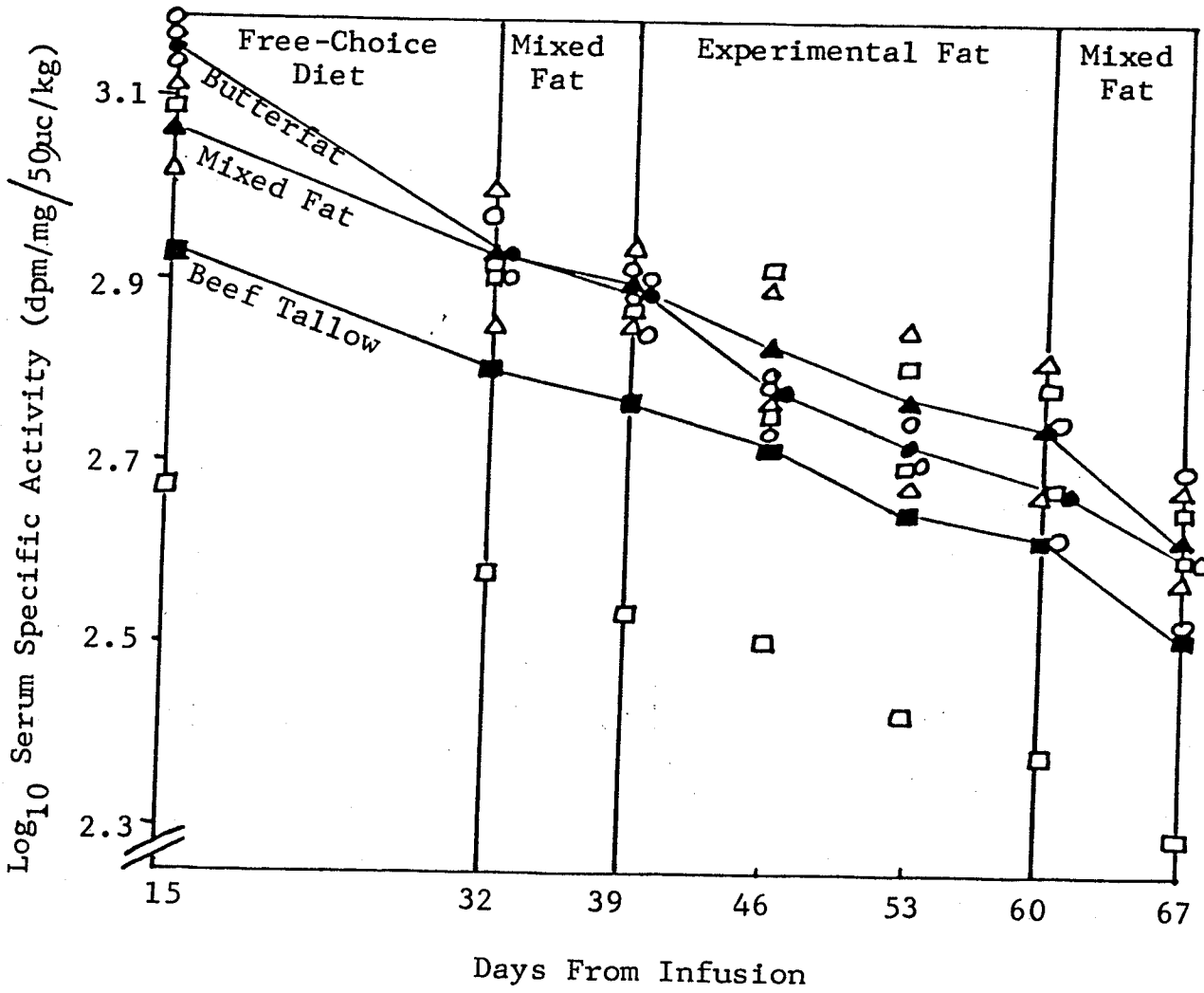


Figure 7: Log_{10} Serum Specific Activity Time-Curve For Subjects in Response to Dietary Fat

Mean Values ● ▲ ■

Indiv. Values ○ △ □

beef tallow and butterfat diets (Appendix Table 9). There appeared to be an increased rate of decline in serum specific activity during the first week of the experimental period, for subjects receiving the butterfat diet. This was undetected by the statistical analysis, due to high between-subject variability (MS Error (a), Appendix Table 8).

When the data for all diets were combined, there was a linear relationship ($p < 0.01$) between \log_{10} serum specific activity, and serum cholesterol during the twenty-one day experimental period. Analysis of covariance (Appendix Table 10) indicated a decrease in \log_{10} serum specific activity of 0.00139 (dpm/mg/50 μ c/kg) for every 1 mg/dl increase in serum cholesterol. Nestel et al (1965) did not find any correlation between serum specific activity and serum cholesterol concentration thirty days after infusion. Due to the limited statistical analysis of specific activity data in the literature, the significance of the relationship found in the present study remains unclear, since serum specific activity can reflect both turnover rate and pool size.

When data for each diet were subjected to covariance analysis, no significant relationship between \log_{10}

serum specific activity and serum cholesterol level was found for any of the diets. This was most probably due to the high between-subject variability in the limited number of subjects studied for each diet.

After infusion of 1-2-³H labelled cholesterol, isotopic equilibration between cholesterol in plasma or serum, and the cholesterol in all body tissues except the nervous system and blood vessels has been shown to occur in approximately one month (Chobanian and Hollander 1962, Grundy and Ahrens 1966, Oh et al 1976). At this point, the disappearance curve becomes essentially linear. Nestel et al (1965) stated that after isotopic equilibration has been achieved, a change in the slope of the log₁₀ plasma specific activity disappearance curve reflected a change in the turnover rate of cholesterol. Quantitation of metabolic turnover is assumed to require 'steady state' conditions (Grundy et al 1969). In the present study, isotopic equilibration had been achieved by the beginning of the experimental period (thirty-nine days after infusion), and this is confirmed by the fact that the slopes for all diets were linear. It was therefore assumed that any changes in cholesterol metabolism produced by diet

may have been reflected in differences between the slopes of the disappearance curves for beef tallow and butterfat. Qualitative alterations in the slopes might then have offered insights into the mechanisms operative when the size of the plasma pool was altered as a result of dietary fat.

When butterfat constituted the dietary fat, there was a significant increase in the concentration of cholesterol in the plasma pool. Most of the change occurred during the first week of the experimental period, and subject mean values stabilized after fourteen days on the diet. A hypercholesterolemic response could result from (a) an increase in the absorption and/or synthesis of cholesterol (b) a decrease in the excretion of cholesterol from the plasma pool (c) a net flux of cholesterol from other tissues to the plasma or (d) a combination of these mechanisms.

Present data have not indicated any differences in the linear slopes of the disappearance curves for beef tallow and butterfat over the twenty-one day experimental period. According to Grundy and Ahrens (1966), changes in the absorption or synthesis of cholesterol as a result of

dietary change, are reflected in the slope of the \log_{10} specific activity-time curve. An increase in either absorption or synthesis of cholesterol causes a dilution of the plasma label, and hence, an increase in slope (Figure 1). Based on this evidence, differences in cholesterol absorption and/or synthesis related to butterfat or beef tallow diets, did not occur in the present study.

A net flux of cholesterol from the tissues to the plasma is likely to cause a hypercholesterolemic effect. The slope of the specific activity disappearance curve will not change significantly if there is an exchange between plasma and other tissues in Pool A, which have the same specific activity as plasma. However, the specific activity of more slowly equilibrating tissues (Pool B) is greater than the tissues of Pool A (Sodhi et al 1973). Consequently, a decreased rate of exchange between plasma and the tissues of Pool B is likely to cause an increase in the slope of the disappearance curve. An increased rate of exchange between plasma and the tissues of Pool B would, conversely, cause a decreased slope (or upswing) in the curve (Sodhi et al 1973).

Present data therefore, suggest that unless a combination of the above mechanisms were operative, the disappearance of labelled cholesterol from the serum is not altered by beef tallow or butterfat. However, analysis of fecal steroids may help to clarify this issue.

Little is known about the changes in the size and kinetics of body pools of cholesterol which result from changes in dietary fat. A diet containing 40% butteroil (Hellman et al 1957, Moore et al 1968) or 60% coconut oil (Avigan and Steinberg 1965) caused a hypercholesterolemic response, but a diet containing 40% corn oil (Hellman et al 1957), 40% safflower oil (Moore et al 1968) or 60% corn oil or safflower esters (Avigan and Steinberg 1965) produced a hypocholesterolemic response. No apparant changes were noted in the slope of the specific activity disappearance curves between diets, from any of the above investigations. Results from a previous experiment in this laboratory (Cobden 1975) did not indicate any apparant change in slope of the \log_{10} specific activity-time response curve between sunflower oil and lard, fed at about 40% of total calories, despite the fact that

the lard diet resulted in hypercholesterolemia. The experimental design in the study reported by Cobden (1975) and the present study were similar. Grundy and Ahrens (1966) noted a transient decreased rate of decline in \log_{10} plasma specific activity when corn oil replaced butter, but the same investigators (1970) did not report any change in slope when corn oil was substituted for butter. In fact, when sunflower oil replaced butter in the diet, there was a slight increase in slope. Wood et al (1966) also noted a more rapid rate of fall in plasma specific activity when a diet containing 45% trilinolein replaced a diet containing 50:50 mixture of palmitate: oleate, fed at an isocaloric level.

Subjects JW and SN. Serum specific activity changes for the obese subjects, JW and SN, were similar to the other subjects (Table 11), despite the marked difference in serum cholesterol response in comparison with the other subjects.

Smith et al (1976) have shown that the major determinant of cholesterol production was body size. An increase in cholesterol synthesis in obesity is well

established (Nestel et al 1973, Schreiberman and Dell 1975, Kudchodkar et al 1977). Subjects with hypertriglyceridemia have also been shown to have enhanced cholesterol synthesis (Miller et al 1976) over and above that associated with hypercholesterolemia (Sodhi and Kudchodkar 1973). The size and amount of cholesterol in Pool B, which includes adipose tissue, has also been correlated with excess weight (Nestel et al 1969, 1973, Miller et al 1976, Smith et al 1976) and with hypercholesterolemia (Smith et al 1976).

Nestel et al (1973) have postulated that the increased rate of turnover noted in obesity may reflect an increased synthesis of cholesterol in adipose tissue, but other investigators (Schreiberman and Ahrens 1976, Kudchodkar et al 1977) postulated that the excess synthesis most probably occurs in liver or intestinal sites, and is related to the known increase in plasma VLDL rather than to an increase in adipose tissue synthesis.

Further, Grundy (1975) has demonstrated that subjects with hypercholesterolemia do not excrete cholesterol equivalent to the decrement in plasma cholesterol

following a change of diet from lard to safflower oil, and suggested that the excess cholesterol may have moved to the tissues.

In the present study, SN demonstrated a marked hypocholesterolemic response to beef tallow. The slope of the disappearance curve for SN was essentially the same as the other subjects, except for a slight upswing in slope during the second week of the experimental diet. The response to beef tallow may have caused a net flux of cholesterol from the tissues of Pool B to the serum, thus causing the upswing in slope. This response could be secondary to the decrement in serum cholesterol which occurred during the ingestion of beef tallow. However, in such a case, steroid excretion would also be expected to increase.

The similarity of the specific activity-time curve of subject JW, despite no appreciable change in serum cholesterol level during the ingestion of butterfat over the twenty-one day experimental period, may be speculated to be due to alternative mechanisms. An increase in the synthesis of cholesterol might have occurred, but serum cholesterol level did not change appreciably. In such a

case, there may have been a net flux of cholesterol out of the serum (by way of an increase in steroid excretion or by a net flux of cholesterol to the tissues) during the ingestion of butterfat. Further data are required on a greater number of subjects before more conclusive inferences can be drawn. However, as suggested by Grundy (1975), the predominant mechanism affecting the plasma cholesterol concentration may depend upon the presence or absence of particular metabolic defects.

The data presented have indicated that the disappearance of labelled cholesterol from the serum is not affected by the dietary fats under investigation. The experimental model may not have permitted a valid comparison to be made between the effects of two different diets over time. Also, comparisons were made on a limited number of subjects who received only one test fat, and demonstrated considerable variability over the same twenty-one day time period. Due to the variability among subjects, and due to the very small changes which may have occurred in the \log_{10} serum specific activity, statistic significance

is difficult to establish. Present results are, however, substantiated by the comparable results for lard and sunflower oil reported by Cobden (1975) in an experiment of similar design.