This is to certify that the

thesis entitled

CHANGES IN TOTAL SERUM CHOLESTEROL IN RESPONSE TO A DECREASE IN DIETARY CHOLESTEROL AND MODIFICATION OF THE AMOUNT AND TYPE OF DIETARY FAT: A CONTROLLED DIETARY STUDY

presented by

D. Margaret Ullmann

has been accepted towards fulfillment
of the requirements for

M.S. degree in Nutrition

Sandra Chrouseth
Major professor

Date 2/19/80

CHANGES IN TOTAL SERUM CHOLESTEROL IN RESPONSE TO A DECREASE IN DIETARY CHOLESTEROL AND MODIFICATION OF THE AMOUNT AND TYPE OF DIETARY FAT: A CONTROLLED DIETARY STUDY

By

D. Margaret Ullmann

A THESIS

Submitted to

Michigan State University

in partial fulfillment of the requirements

for the degree of

MASTER OF SCIENCE

Department of Food Science and Human Nutrition

1979

ABSTRACT

CHANGES IN TOTAL SERUM CHOLESTEROL IN RESPONSE TO A DECREASE IN DIETARY CHOLESTEROL AND MODIFICATION OF THE AMOUNT AND TYPE OF DIETARY FAT: A CONTROLLED DIETARY STUDY

By

D. Margaret Ullmann

Thirty-two healthy men (mean age = 24.8 years) were fed a control diet containing 42 to 45% of total calories from fat, a P/S ratio of 0.3 to 0.5, and two eggs/day for ten days. During the next eight weeks, 16 subjects received each of the following diets for four weeks in a crossover design: (1) Diet C-E, the control diet (with two eggs/day or (2) Diet C-ES, the control diet with eggs replaced by a cholesterol-free egg substitute. The remaining 16 subjects received each of the following diets in a similar crossover design: (1) Diet MF-E, a modified fat diet containing 35% of total calories from fat, a P/S ratio ≥1.0, and two eggs/day or (2) Diet MF-ES, the modified fat diet with eggs replaced by a cholesterol-free egg substitute. Diets for each subject were adjusted to maintain body weight. Average serum cholesterol values during the control period ranged from 195 to 288 mg/100 ml. A change from the control period to the modified fat diet produced a decrease in serum cholesterol concentration in 15 of the 16 subjects. In addition, comparison of the mean serum cholesterol concentrations during the control period with average weekly

serum cholesterol values during the experimental diet periods showed a statistically significant decrease ($p<0.5$) when subjects consumed egg substitute versus two eggs/day.

ACKNOWLEDGMENTS

I wish to express my sincere appreciation to the many people who helped to make this study possible. To:

Esperanza Briones for her assistance in menu planning and daily diet modification calculations.

Shirley Ann Mellen and Patricia Smith Brown for their assistance in performing the laboratory analyses.

Dr. Charles Rhoades for the time he contributed generously in serving as the physician for the study.

Dr. J. Gill for statistical advice and assistance.

The men who participated as subjects in the study for their cooperation and compliance.

The members of my thesis committee - Dr. Jenny Bond, Dr. Gilbert Leveille, and Dr. David Rovner for their expertise in research and thesis development.

Dr. Wanda Chenoweth, my major professor, for her guidance, expertise, patience, kindness, criticism, and words of encouragement.

I wish to express gratitude to Standard Brands, Inc., for their support of this project, and particularly to Dr. Ron Simpson.

And last, but not least, I would like to acknowledge my father, Alexander Ullmann, my sister, Sabrina, and my friends for their love and support throughout my graduate school career.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Coronary heart disease (CHD) is the major cause of death in the United States today (Gotto, 1979). There is much documentation in the literature of an association between high serum cholesterol concentration and atherosclerosis (Keys, 1970; Kannel et al., 1971; Carlson and Bottiger, 1972). Epidemiological studies consistently have shown higher serum cholesterol levels and a higher incidence of CHD among populations consuming diets high in cholesterol and fats, particularly saturated fats (Jolliffe and Archer, 1959; Keys, 1970). Although the etiology of CHD is known to be multifactorial, elevated levels of blood cholesterol have been identified as a major risk factor in the development of the disease (Connor and Connor, 1972). Numerous studies in experimental animals and humans have demonstrated a direct influence of diet on serum cholesterol concentrations. Although the studies reported in the literature fail to provide incontrovertible evidence of a causal relationship of serum cholesterol concentration to CHD, collectively they support the theory that dietary manipulations designed to lower serum cholesterol concentrations may be effective in reducing the risk of CHD.

The effects of dietary cholesterol and fats on serum cholesterol concentration have been studied extensively in humans using formula diets as well as diets containing natural foods; the effects of each have been studied independently as well as together. There is general consensus among researchers that reduced intake of saturated fat, increased intake of polyunsaturated fat, and a decrease in total dietary fat will reduce serum cholesterol concentration. There is, however, much controversy regarding the extent to which an increase or decrease in dietary cholesterol may alter serum cholesterol concentration in man.

Recently several studies have been reported indicating that healthy subjects show no change in serum cholesterol in response to ingestion of cholesterol in amounts equivalent to one or two eggs/day (Slater et al., 1976; Porter et al., 1976). Kummerow et al. (1977) and Flynn et al. (1979) reported similar findings. Although the results of these studies suggest that consumption of egg cholesterol in regular meals does not significantly increase plasma or serum cholesterol in man it would seem inappropriate to conclude from them that changes in dietary cholesterol have no effect upon serum cholesterol. The intake of foods other than eggs by these subjects was not controlled. It is possible that variation in cholesterol intake from foods other than eggs or variations in the amount or type of fat may have been sufficient to mask the effect of the amount

of cholesterol provided by one or two eggs/day.

In the experiment described herein, the effects of the restriction of dietary cholesterol and modification of amount and type of fat on serum cholesterol were studied in free-living adult male subjects whose serum cholesterol levels were in the upper normal range. This population group was selected because of the belief that young men with moderately elevated serum cholesterol levels would be likely to benefit from preventive dietary measures designed to lower serum cholesterol levels.

During the winter and spring of 1978 a two-part controlled human feeding study was conducted at Michigan State University to determine the effect on total serum cholesterol of

1) replacing two eggs/day in a "typical" American diet with a cholesterol-free egg substitute.
2) feeding a modified fat diet containing either two eggs/day or an egg substitute.

The major difference between the present study and the previous experiments of the effects on serum cholesterol of adding or deleting one or two eggs/day to the diets of young men in a free-living population is the degree of control over food intake.

REVIEW OF LITERATURE

Historical Approach

The discovery over a century ago that cholesterol is a prominent constituent of atherosclerotic plaque (Vogel, 1847) and that this same substance is also present in the blood has stimulated interest in pursuing a better understanding of the interrelationship between diet, serum cholesterol, atherogenesis and coronary heart disease (CHD). Although it constitutes only a small fraction of total body cholesterol, plasma cholesterol is the only pool of cholesterol incriminated in CHD (Sodhi and Mason, 1977). Initially it was thought that the lipid deposits found within the atheromatous arteries were produced by local synthesis within the arterial walls. It has been demonstrated conclusively, however, through the use of radio-labelled cholesterol that cholesterol from the blood stream does enter into the intimal atheromas and that much of the labelled cholesterol recovered from the atherosclerotic plaque was that which was fed in the diet (Chobanian and Hollander, 1962; Connor and Jackson, 1963).

It has been shown that hypercholesterolemia induced in a variety of ways results in the development of

atherosclerotic lesions in a number of animal species. Anitschkow (1933) produced hypercholesterolemia and the characteristic lesions in the aorta and coronary arteries in rabbits by feeding them 0.5% cholesterol dissolved in highly unsaturated sunflower seed oil. Other investigators have shown that the feeding of a cholesterol-vegetable oil mixture or foods rich in cholesterol and fat induced hypercholesterolemia and atherosclerosis in the guinea pig, chicken, rat, hypothyroid dog, pig and monkey (Katz et al., 1958).

Much criticism has been generated about the applicability of the results from animal studies to humans because of the inherent differences in the susceptibility of various animals to CHD and because of the differences in their serum lipid responses to dietary modifications. Frequently the level of cholesterol fed in animal studies was enormously high in comparison with levels normally consumed by humans. Cholesterol levels in the diet fed to induce atherosclerosis in animals tend to range from 0.5 to 5.0% by weight of dry food, which would be equivalent to feeding 1000 to 10,000 mg of cholesterol/1000 kcal daily (Keys, 1952).

Several longitudinal, prospective studies have demonstrated in a variety of population samples that risk of CHD was directly related to the antecedent serum cholesterol levels (Keys et al., 1963; Chapman and Massey, 1964;

Rosenman et al., 1970; Carlson and Bottiger, 1972). Data from the Framingham study (Kannel et al., 1971) revealed a distinct and striking increment in the risk of CHD proportional to the antecedent serum cholesterol concentration. These data showed that serum cholesterol levels of 260 mg/100 ml or more resulted in a five-times greater incidence of CHD than a level below 200 mg/100 ml in men aged 30-49 years, and that plasma cholesterol concentration was predictive of risk in the development of heart disease. Indirect evidence linking atherosclerosis to serum cholesterol concentration is found in the diseases that are associated with both hypercholesterolemia and premature atherosclerosis, such as diabetes mellitus, nephrosis, and hypothyroidism. Persons with inborn errors of cholesterol metabolism, such as familial hypercholesterolemia and hypercholesterolemia xanthomas, are particularly susceptible to early development of atherosclerotic disease (Stanbury et al., 1972). A parallel relationship also has been shown between the age trend in serum cholesterol concentrations and the age trend in development of atherosclerosis (Keys, 1952).

The theory that diet has a powerful effect on serum cholesterol concentration and on the incidence of CHD has been supported by convincing evidence from several worldwide epidemiologic studies (Keys et al., 1957a; Jolliffe and Archer, 1959; Toor et al., 1960; Connor and Connor, 1972)

which revealed a strong relationship between populations consuming diets containing large quantities of animal products and a high incidence of CHD. As early as 1916 De Langen reported that plasma cholesterol levels in healthy Javanese men were much lower than in the Netherlands, France, or Germany. He observed that CHD was rare among the Javanese population and suggested that this was because their diet was low in cholesterol and other lipids. Toor et al. (1960) reported that Israeli immigrants from Yemen had much lower serum cholesterol levels and were far less prone to CHD than either the eastern European Israeli immigrants who consumed diets that were much higher in dietary fat and cholesterol, or the Yemenites who had immigrated to Israel earlier and had already adopted a higher fat diet. Keys (1970) studied 18 population samples of 40-59 year old men from seven different countries and found that serum cholesterol levels correlated with the consumption of cholesterol and saturated fat and with the incidence of CHD. He concluded that 80% of the serum cholesterol variability among these groups could be explained by differences in the diet.

Other lines of epidemiological evidence linking diet to CHD include the effects of wartime privations (Helwig et al., 1952), geographic migrations (Toor et al., 1960), and social class differences (Logan, 1952), all of which are known to have profound effects on dietary patterns.

That the differences in the incidence of CHD seen in the various population groups studied epidemiologically were not simply racial or genetic variables has been demonstrated in migrants of the same race who changed from a low to a high cholesterol-high fat diet and experienced a corresponding change in their serum cholesterol levels together with a change in their propensity to CHD (Keys et al., 1958). This point is clearly illustrated in a study by Toor et al. (1960) who showed that Yemenites gradually lost their relative protection from CHD as they adopted the dietary pattern of European Jews. Similarly, De Langen (1916) observed the Javanese stewards on Dutch passenger ships who ate Dutch food developed similar blood cholesterol levels to the Dutchmen and developed a higher incidence of CHD. Bersohn and Wayburne (1956) also provided evidence in support of the claim that serum cholesterol concentration is dependent upon the composition of the diet and is not the result of racial or genetic differences. They demonstrated that the cholesterol concentration of blood drawn from the umbilical cord of newborn infants of different populations throughout the world showed no dissimilarity due to race or class and was about 80 mg/ 100 ml.

Epidemiologic studies have made it possible to survey the effects of many different types of diets on serum cholesterol levels in relatively large population samples.

The use of epidemiologic evidence, however, is often belittled because of the arguments that (1) mortality and CHD are influenced by a lifelong exposure to diet while data of this kind often reflect dietary habits over a relatively short period of time, (2) the changes in dietary patterns are usually accompanied by changes in lifestyle and environmental factors, such as stress, level of exercise, calorie intake, smoking habits, sanitation and the incidence of other diseases, and (3) food consumption data are often unreliable in terms of describing individual food consumption.

Although there is much documentation in the literature of an association between high plasma cholesterol levels and atherosclerosis, an etiologic association is more difficult to prove. An association is considered likely to be "causal" if it a) is a consistently strong association and b) precedes the disease by an appropriate period of time. Serum cholesterol concentration meets both of these criteria for causality. Although causality has yet to be proven, it is evident that plasma cholesterol plays a significant, albeit, undefined role in the development of CHD and atherosclerosis.

Dietary Cholesterol

Extensive research has been conducted over the past half-century in the hope of defining the relationship of dietary cholesterol to serum levels in man. The results

of many of the studies have yielded conflicting, questionable, and often inconclusive data (Ahrens, 1976). Instead of providing answers, the results of these studies have generated more confusion and controversy.

The effect of added cholesterol has been studied using formula diets (Kinsell et al., 1952; Beveridge et al., 1960; Erickson et al., 1964; Mattson et al., 1972). Diets containing conventional foods also have been used to study the effects of dietary cholesterol on serum cholesterol concentrations (Messinger et al., 1950; Connor et al., 1964; Grande et al., 1965; Anderson et al., 1976). In some experiments comparisons were made between nearly cholesterol-free diets and diets containing very large quantities of cholesterol (Beveridge et al., 1960; Connor et al., 1961). Purified cholesterol was added to the diet in some studies (Kinsell et al., 1952; Beveridge et al., 1960) while in others frozen egg yolk or whole fresh eggs were used (Messinger et al., 1950; Hegsted et al., 1965; Kummerow et al., 1977). Studies also varied in length of dietary periods from 8 days (Beveridge et al., 1960) to 3 months (Porter et al., 1977). In addition, some studies were conducted under strictly controlled conditions (Connor et al., 1964; Mattson et al., 1972) whereas in others the subjects were asked only to keep food records (Hildreth et al., 1951; Shorey et al., 1974; Porter et al., 1977; Simons et al., 1978). Furthermore, some studies were

conducted using healthy subjects (Hegsted et al., 1965; Slater et al., 1976) while others used hypercholesterolemic and convalescent patients (Steiner et al., 1962; Kummerow et al., 1977). This tremendous variation in experimental design has made interpretation of the often contradictory results nearly impossible.

The results of a number of human studies conducted in the 1950's led several investigators (Keys et al., 1955; 1956; Kinsell et al., 1952; Ahrens et al., 1957) to conclude that restriction of dietary cholesterol was unnecessary and unwarranted because its ingestion failed to alter significantly serum cholesterol levels in man. These investigators believed that the relative homeostasis of serum cholesterol concentration was maintained despite even large increases in cholesterol intake because of negative feedback on cholesterol synthesis in the body. This conclusion was reached by each investigator after feeding crystalline cholesterol in the amounts of 0.5 to 60 gm in special formula diets under strict metabolic conditions. The results of these studies are in agreement with a report from an earlier study (Steiner and Domanski, 1941) in which 10 gm of crystalline cholesterol in 400 cc of milk added to the daily diet of a group of patients for six weeks failed to induce significant elevations in serum cholesterol levels. In a later study, however, Steiner et al. (1962) discovered that the addition of 3 gm of

crystalline cholesterol to a cholesterol-free formula diet containing 95 gm of fat produced a significant elevation in serum cholesterol concentration in a group of hospitalized men and women. Beveridge et al. (1960) demonstrated an increase in serum cholesterol when supplements of highly purified cholesterol in the amounts of 0-1600 mg/950 kcal were added to a fat-free formula diet for a period of 8 days following an 8-day period in which a cholesterol-free, fat-free formula diet was fed. The results of this study have received much criticism because of the lack of fat in the diet, which is known to be essential for cholesterol absorption (Keys et al., 1965b).

Experiments designed to study the effects of added cholesterol in the form of egg yolk and egg yolk powder similarly produced conflicting results. Messinger et al. (1950) reported that the addition of 150 gm of egg yolk powder produced a significant increase in serum cholesterol concentration. Mattson et al. (1972) utilized a formula diet to which various levels of dry egg yolk cholesterol were added. They reported a linear increase in plasma cholesterol within the range of 0 to 317 mg cholesterol/1000 kcal. Connor et al. (1961) tested the effects of adding either egg yolk cholesterol or crystalline cholesterol to formula diets at various levels and found that egg yolk powder produced greater serum cholesterol elevations than did much larger amounts of purified cholesterol, with

or without added fat. Hegsted et al. (1965) similarly showed that addition of egg yolk powder to a diet of ordinary foods under metabolic conditions produced a significant increase in serum cholesterol levels. Mayer et al. (1954), in contrast, found that the addition of approximately 800 mg of cholesterol/day in the form of egg yolk to a diet low in fat and low in cholesterol produced no demonstrable change in plasma cholesterol levels of five healthy male subjects after a period of one week. A lack of serum cholesterol response to increased intake of egg yolk cholesterol was also reported by Keys et al. (1950), who concluded that the serum cholesterol intake of "normal" men is not significantly related to differences in the habitual cholesterol intake over a range of approximately 250-800 mg/day.

Interpretation of many of the early studies in which egg yolk was used as the source of dietary cholesterol is complicated by the fact that the addition of egg yolk also increased the fat content of the diet. The addition of 150 gm of egg yolk powder, for example, would add approximately 95 gm of fat/day to the diet (Mayer et al., 1954).

Several investigators have studied the effects of various levels of dietary cholesterol added to formula and conventional foods diets under strictly controlled conditions in an attempt to describe the relationship of dietary cholesterol to serum cholesterol concentration over a wide

range of intake. From the results of a series of experiments covering a two year period in which schizophrenic patients were fed a natural foods diet to which egg yolk cholesterol was added in amounts ranging from 100-700 mg/day, Hegsted et al. (1965) concluded that serum cholesterol was linearly related to dietary cholesterol. They predicted that an increase of 100 mg of cholesterol would produce a rise in serum cholesterol of approximately 5 mg/100 ml. Similar results were reported by Mattson et al. (1972) who compared the serum cholesterol response of four groups of prisoners to the change from zero dietary cholesterol to 106, 212, 317 mg of egg yolk cholesterol/1000 kcal/day. They concluded that the changes in serum cholesterol were essentially linearly related to intake and were approximately 12 mg for each 100 mg cholesterol/1000 kcal.

Keys and Parlin (1966), in contrast, found that serum and dietary cholesterol were not linearly related, but that the change in serum cholesterol was proportional to the square root of cholesterol intake. These conclusions were based on the results of a series of studies in which schizophrenic patients were fed a natural foods diet containing cholesterol supplements ranging from 125-540 mg/1000 kcal. When the results from this study were analyzed together with those by four other investigators the following prediction formula was derived:

$$\Delta\text{Cholesterol (mg/100 ml)} = 1.5\ (Z_2 - Z_1)$$

where Z is equal to the square root of dietary cholesterol measured as mg/1000 kcal. Keys and Parlin emphasized that this equation applies to groups of people and not to individual subjects.

Several investigators have reported that once a certain level of dietary cholesterol was reached, further increments in intake had little additional effect on serum cholesterol concentration. Beveridge et al. (1960) reported finding good correlation between serum cholesterol concentrations and dietary increments of cholesterol up to intakes of approximately 650 mg/day; dietary cholesterol beyond that level failed to produce additional increases in serum cholesterol. In a similar study Connor et al. (1964) found that the addition of 475 mg cholesterol/day to the diet produced a significant increase in serum cholesterol, but intakes of twice that amount did not cause a greater increase in serum cholesterol.

The results of several recent studies on the effects of added cholesterol in amounts normally consumed in the American diet disagree with the findings of Hegsted et al. (1965), Mattson et al. (1972) and Keys and Parlin (1966). In a study by Slater et al. (1976) three groups of young and middle aged men in free-living populations were asked to eat one or two eggs/day in addition to their usual diets, or to eliminate all eggs. No significant changes in the plasma cholesterol values were observed after the

6-8 week periods on extra eggs compared to periods in which eggs were absent from the diet. Porter et al. (1977) and Flynn et al. (1979) similarly failed to find a significant association between dietary and serum cholesterol levels with the addition or elimination of one egg daily in the customary diets of healthy male subjects. In another study Kummerow et al. (1977) studied the effects of adding two whole fresh eggs incorporated into either a custard or a milk shake to the diets of hospitalized men and women in three separate hospitals. No significant change in serum cholesterol concentration was found in the majority of patients after 54 days of continued consumption of two eggs/day.

Although statistical analyses of the data reported by these investigators support the conclusion that dietary cholesterol in amounts equal to two eggs/day does not significantly influence serum cholesterol concentration, examination of the data for individual subjects revealed a marked variation in individual response of plasma cholesterol to dietary cholesterol challenges in man. It has been shown that some subjects may not show any change; some others may even demonstrate a decrease in their plasma cholesterol when dietary cholesterol is increased (Quintao et al., 1971; Nestel and Poyser, 1976). It has been suggested that the variability in responsiveness of plasma cholesterol to changes in the diet that is shown

to exist among individuals consuming the same diet may reflect changes in other dietary components that necessarily accompany the adjustment of the diet to meet individual needs. Another possible explanation for differences in responsiveness is the existence of genetically determined compensatory mechanisms.

Unlike many animals such as the monkey and the rabbit, which may develop serum cholesterol levels of 600-2000 mg/100 ml following a high cholesterol diet, ingestion of very large quantities by man results in a much smaller increase in serum cholesterol concentration (Connor et al., 1961, 1964). Even when cholesterol intake exceeds the normal turnover rate in man, the rise in serum cholesterol almost never exceeds 100 mg/100 ml (Steiner et al., 1962). Despite the extensive research conducted in the area of cholesterol metabolism over the last few decades, the mechanisms responsible for the relative homeostasis of plasma cholesterol in man have not been clearly defined (Sodhi and Mason, 1977). It is believed that total body cholesterol is regulated by a complex, dynamic interplay between absorption, synthesis and excretion. The following compensatory mechanisms have been proposed to account for this homeostatic phenomenon:

1) Limited absorption of dietary cholesterol. In a recent study, Connor and Lin (1974) demonstrated that the amount of cholesterol absorbed was roughly linear for

different quantities of dietary cholesterol between 110 and 610 mg and that the percentage absorption of cholesterol remained relatively constant at 45% despite the increased intake. Wilson and Lindsey (1965) discovered a maximum daily absorption of approximately 300 mg when 3 g of cholesterol were fed and they concluded that the human intestine has a limited ability to absorb dietary cholesterol. Quintao et al. (1971), on the other hand, demonstrated that absorption is proportional to the amount ingested and that much greater amounts can be absorbed if the intake is high enough. At intakes up to 1 g/day, about half is absorbed; at higher intakes the percent absorbed becomes less.

Several factors are known to limit cholesterol absorption. The requirement for exogenous fat to assist the absorption of dietary cholesterol has been clearly demonstrated (Keys et al., 1965b). Quintao et al. (1971) have shown that the proportion of a large single dose of cholesterol that may be absorbed depends upon the previous intake of cholesterol. When cholesterol intake has been low, a larger fraction of any single dose is absorbed than when the intake has been high. This finding suggests a possible role of the degree of saturation of the intestinal mucosa in controlling absorption of dietary cholesterol. It also has been demonstrated that the amount of absorption varies with the source of dietary cholesterol, with egg yolk

cholesterol being more effectively absorbed than crystalline cholesterol (Cook et al., 1956; Connor et al., 1961).

2) Inhibition of cholesterol biosynthesis. Isotopic studies in man under steady state conditions have shown that biosynthesis of cholesterol in the liver remains relatively constant and that cholesterol biosynthesis is not usually altered by various quantities of dietary cholesterol. Wilson and Lindsey (1965) using a double isotopic steady state technique showed that dietary cholesterol, whether fed in the form of eggs or in a purified state, had little effect on the total rate of endogenous cholesterol synthesis. Quintao et al. (1971), on the other hand, found that increased absorption of cholesterol resulted in a decrease in total body synthesis in five of six patients; however, the amount of suppression in synthesis was extremely variable from person to person.

3) Increased excretion of acidic sterols. Although balance studies carried out in rats by Wilson (1964) indicated that a significant amount of absorbed dietary cholesterol was excreted through the enhanced formation of bile acids, enhanced absorption of dietary cholesterol has not been shown to produce a significant increase in excretion of acidic sterols in man (Quintao et al., 1971). They have shown, however, an increased excretion of endogenous neutral sterols with cholesterol feeding which may have been due to inhibition in reabsorption of endogenous

cholesterol by exogenous cholesterol.

Dietary Fat

The effects of dietary fat on blood cholesterol concentration and its relationship to CHD have been studied extensively in experimental animals and in epidemiological, clinical, and pathological studies in humans using a three-pronged approach: (1) level of dietary fat, (2) degree of saturation of fatty acids, (3) the chain length of the fatty acids in dietary triglycerides.

Level of dietary fat. It has been nearly a quarter of a century since Keys et al. (1955) demonstrated a statistically significant relationship between death rate from CHD, plasma cholesterol, and the proportion of total calories derived from fat. Keys (1952, 1970) and Jolliffe and Archer (1959) have shown that in those countries where death rate from CHD is low, the populations exhibit low serum cholesterol levels and tend to consume less fat than population groups with a higher incidence of CHD.

Although the conclusions from epidemiological studies have generally supported the theory that a change in the level of consumption of dietary fat would produce a concommitant change in serum cholesterol concentration, this has not been consistently demonstrated in controlled human studies. From the results of a study in which groups of schizophrenic patients were fed low-fat diets to which

various test oils were added, Hegsted et al. (1965) reported that the amount of dietary fat, tested at levels equal to 22 to 40 percent of total calories, appeared to be without influence on the level of serum cholesterol. They found no effect on serum cholesterol when various levels of different fats were tested, as long as a constant polyunsaturated:saturated (P/S) fat ratio was maintained. Similar results were attained by Grande et al. (1972) which showed that varying the amount of fat had no effect on serum cholesterol when the composition of dietary fat was such that 2 S- P = 0, where S and P represent, respectively, the percent of total calories from saturated and polyunsaturated fatty acids. Keys et al. (1950), Hildreth et al. (1951) and Mayer et al. (1954), on the other hand, reported that an increase in dietary fat, whether of animal or vegetable origin, led uniformly to higher plasma cholesterol levels. Other investigators (Kinsell et al., 1953; Ahrens et al., 1954: Beveridge et al., 1955) reported increased plasma cholesterol levels following increased intakes of animal fat; similar amounts of vegetable fat, however, produced decreases in serum cholesterol levels. Malmros and Wigand (1957) found, in contrast, no essential difference between vegetable and animal fat. Most of the vegetable oils that they studied produced cholesterol-depressing effects, but coconut oil did not. Milk fat was shown to raise serum cholesterol, but whale oil depressed

it. They concluded that the cholesterol depressing effect of some of the oils was due to their unsaturated fatty acids.

Additional evidence refuting the idea that the level of dietary fat is important irrespective of source is supplied by Hardinge et al. (1962), who demonstrated that strict vegetarians exhibited lower serum cholesterol levels than either lacto-ovo-vegetarians or non-vegetarians in spite of a very liberal intake of vegetable fat. Simons et al. (1978) confirmed reduced plasma cholesterol levels in vegetarians.

Degree of saturation of dietary fat. Numerous investigations have shown reductions in serum cholesterol concentrations in man following the dietary substitution of many vegetable oils for common animal fats or certain tropical oils such as coconut oil (Kinsell et al., 1952, 1953; Ahrens et al., 1954, 1955; Bronte-Stewart et al., 1966; Beveridge et al., 1955, 1956). The relatively high degree of saturation of fats of animal origin and coconut oil had been associated with their cholesterol elevating effect and has led to the development of the concept that saturated fatty acids are hypercholesterolemic and that polyunsaturated fatty acids are hypocholesterolemic (Jolliffe, 1961).

The relationship of the degree of saturation to serum cholesterol concentration has been studied and characterized

according to (1) the iodine number, (2) the P/S ratio, (3) the percent of total calories from saturated and polyunsaturated fatty acids.

Iodine number, a measure of total unsaturation of dietary fats, has been used by some investigators to quantify the effects of dietary fat modifications on serum cholesterol concentrations. Ahrens et al. (1957) demonstrated a rough inverse relationship between serum cholesterol concentration and the iodine number of various oils. They concluded that serum cholesterol response to changes in dietary fat composition was proportional to the average net unsaturation of the fatty acids, and that monosaturated fatty acids were half as effective as linoleic acid, a diene, in reducing serum cholesterol levels. Gunning et al. (1964) found that the plasma cholesterol levels of the four patients they studied were highly correlated with the square root of the iodine number of the total fat in the diet. They concluded that net unsaturation correlated better with plasma cholesterol levels than did either the P/S ratio or the 2S-P formula. Keys et al. (1965a) concurred that cholesterol level does correlate with the iodine value, or its square root, when total dietary fat is constant, provided that the fats contained no significant amounts of fatty acids more unsaturated than linoleic acid and that the proportion of monosaturated fatty acids did not vary widely. They found that changes in serum

cholesterol could be predicted with far greater accuracy from the amounts of saturated and polyunsaturated fatty acids than from total degree of unsaturation because the effects of some fatty acids on serum cholesterol were discrepant from what would be expected from a simple direct iodine number function. It was shown, for example, that the high degree of unsaturation beyond the dienes in fish oils was not reflected in the change in serum cholesterol concentration using iodine number as a predictor (Keys et al., 1965a).

Jolliffe (1961) proposed that the effect of dietary fats on serum cholesterol concentration was related to their P/S ratio rather than to the net unsaturation of the dietary fatty acids, and that the P/S ratio could be used to predict the effect on serum cholesterol of modifying dietary fat. Keys et al. (1957) also showed that quantitatively the P/S ratio was more significant than the quantity of dietary cholesterol in predicting serum cholesterol changes. Other investigators have found that P/S ratio did not always predict serum cholesterol changes accurately. Stamler (1960) observed that when the amount of saturated fat in the diet was controlled, the P/S ratio played a lessened role, particularly when the dietary cholesterol levels were below 400 mg/day. Connor et al. (1964) showed that changing the P/S ratio from 0.2 to 2.6 had no effect on blood cholesterol when subjects consumed

a cholesterol-free diet. Erickson et al. (1964) demonstrated that plasma cholesterol level was unaffected by variations in the P/S ratio between 0.1 and 1.6. Similar findings were reported by Bierenbaum et al. (1961) who found no significant differences in serum cholesterol levels when subjects were fed diets containing 28% of total calories from fat, less than 400 mg cholesterol/day, and a P/S ratio of either 0.34 or 2.6.

Keys et al. (1965d) demonstrated that the relationship between dietary fat and serum cholesterol concentration could be described quantitatively from the percent of total calories as saturated and polyunsaturated fatty acids. From a comprehensive study in which groups of schizophrenic patients were fed a series of 40 different diets under metabolic conditions it was shown that saturated fatty acids had a cholesterol-raising effect that was twice the cholesterol-lowering effect of polyunsaturated fatty acids. It was also shown that monosaturated fatty acids were essentially neutral in their effect on serum cholesterol.

Multiple regression equations were developed to relate the change in serum cholesterol to dietary content of saturated (S), monosaturated (M), and polyunsaturated (P) fatty acids and the following prediction equation was derived.

ΔCholesterol (mg/100 ml) = -1.68 + 2.76 ΔS + 0.05 ΔM - 1.35 ΔP in which ΔS, ΔM, and ΔP were the change in the

calories contributed by each class of fatty acids. The constant -1.68 and the coefficient for ΔM of 0.05 did not contribute significantly to the equation which was modified to

ΔCholesterol (mg/100 ml) = 2.74 ΔS - 1.31 ΔP

indicating that the relationship of saturated and polyunsaturated fatty acids to serum cholesterol could be described as a function of 2S -P.

Hegsted et al. (1965) conducted a series of studies that were similar in design to those of Keys et al. Groups of schizophrenic patients were fed a low-fat, natural foods diet to which various test oils were added at levels varying from 22 to 38% of total calories from fat for four week diet periods. The following equation was derived to relate the changes in serum cholesterol to changes in dietary fat and cholesterol

ΔCholesterol (mg/100 ml) = 2.16 ΔS - 1.65 ΔP + 6.66 ΔC - 0.53 where S and P are the changes in the percent of dietary calories derived from saturated and polyunsaturated fatty acids, respectively; C is the change in cholesterol intake in 100 mg/day.

Both the equations by Hegsted et al. (1965) and Keys et al. (1965d) suggest that saturated fatty acids have, per weight basis, approximately twice the effect on the change in serum cholesterol as do the polyunsaturated fats, which act in the opposite direction.

Based on the small coefficient for monosaturated fatty acids Keys et al. concluded that monosaturated fatty acids have no effect on serum cholesterol. Although the data of Hegsted et al. also yielded an equation with a small coefficient for monosaturated fatty acids, they were reluctant to conclude that monosaturated fatty acids were neutral and could be isocalorically exchanged for starch in the diet without affecting serum cholesterol. This conclusion was based on the fact that significant regression coefficients for monosaturated fatty acids were found when the data for each of the different trial diets were analyzed separately, as opposed to analyzing all the data from the different trial diets together.

Chain length of fatty acids. There is considerable evidence that saturated fatty acids may vary substantially in their hypercholesterolemic effects (McGandy and Hegsted, 1975). Evidence that short chain fatty acids have much less influence on serum cholesterol than the longer chain fatty acids has been reported in the literature. Keys et al. (1965d) demonstrated that saturated fatty acids containing fewer than 12 carbon atoms had little or no effect on serum cholesterol in man. They attributed this lack of effect to the fact that fatty acids with fewer than 12 carbon atoms in the chain are more polar and less hydrophobic than the other fatty acids and appear to be metabolized differently, being absorbed via the intestinal

capillaries and the hepatic portal system rather than via the lymphatics.

The relative effects of the different fatty acids have been studied by various investigators. Based on the data available from their multiple regression equations Hegsted et al. (1965) concluded that, aside from linoleic acid, the only other fatty acids clearly influencing the level of serum cholesterol were myristic (S_{14}) and palmitic (S_{16}) acids; lauric (S_{12}) and stearic (S_{18}) acids, saturated fatty acids with 10 carbon atoms or fewer, and monosaturated fatty acids had little effect on serum cholesterol concentration. Keys et al. (1965d) in contrast, found that the cholesterol-promoting effect of saturated fatty acids was due to lauric, myristic, and palmitic acids. They concluded that since palmitic acid was much more abundant than lauric and myristic acids in most diets, it was primarily responsible for the contribution of dietary fat to the serum cholesterol level in man. They similarly reported a lack of effect on serum cholesterol level by stearic acid. This confirmed the findings of Connor et al. (1964) that when diets containing substantial amounts of cocoa butter, which has a high content of stearic acid, were fed an unexpectedly low serum cholesterol level was observed.

Several mechanisms have been proposed to explain the cholesterol lowering effect of polyunsaturated fats. The

literature contains evidence both for and against each of the proposed mechanisms. To date no single mechanism has been shown to apply in all cases. Grundy and Ahrens (1970) provide a good review of the effects of polyunsaturated fats on absorption, excretion, synthesis, and the distribution of cholesterol in man. The mechanisms are summarized briefly below:

Fecal excretion of neutral steroids and/or bile acids. Polyunsaturated fat has been reported to cause both an increase (Lewis, 1958) and a decrease (Ali et al., 1966) in bile acid excretion; Grundy and Ahrens (1970) reported no change. An increase in bile acid excretion may be the result of decreased reabsorption of bile acids or increased conversion of cholesterol into bile acids.

Cholesterol absorption. Several recent studies (Nestel et al., 1976; Grundy and Ahrens, 1970) have shown no difference in the absorption of dietary cholesterol when high polyunsaturated fat diets were consumed. This finding conflicts with an earlier report by Wood et al. (1966) that showed a reduction in the absorption of cholesterol following a diet high in polyunsaturated fat.

Cholesterol synthesis. It has been proposed that a decrease in the rate of cholesterol synthesis in the liver may contribute to the lowering of plasma cholesterol that follows the feeding of diets high in polyunsaturated fat. There have been no direct measurements of cholesterol

synthesis in vivo in man. Indirect measurements, however, in normolipemic (Nestel et al., 1976) and in hypercholesterolemic subjects (Grundy and Ahrens, 1970) have shown no significant changes in cholesterol synthesis following ingestion of diets high in polyunsaturated fat.

Distribution of cholesterol between plasma and tissue. Grundy and Ahrens (1970) proposed that polyunsaturated fat may lower serum cholesterol by causing its redistribution from plasma to one or more tissue compartments. Direct evidence for the transfer of cholesterol to tissues during polyunsaturated fat feeding in man is lacking.

Summary

Coronary heart disease is a major health problem in the United States as well as in many other countries. The results of epidemiologic, experimental, and clinical investigations have implicated elevated serum cholesterol levels as an important risk factor in the development of atherosclerosis. Although no causal relationship has been proven between serum cholesterol concentration and CHD, there is abundant evidence indicating that the risk of developing CHD is positively correlated with the level of cholesterol in the blood.

A need exists for well-controlled research to study the effects on serum cholesterol of reducing cholesterol intake and modifying the amount and type of fat that is

normally consumed in the American diet. In order for the diet modifications to be meaningful they must be economically feasible and easily incorporated into daily food preparation. The modified diet must be palatable and easily adapted to the individual's lifestyle.

METHODS AND PROCEDURES

Subject Recruitment

Male subjects were recruited to participate in a 10-week feeding study by advertising in the campus newspaper. A total of 32 subjects was needed to participate in the two-part study: 16 in the Winter term and 16 in the Spring term. The same protocol was used in both terms. The criteria for eligibility in the study which were established in the protocol stated that the subjects should

i) like eggs, habitually consume eggs, and be willing to eat two eggs/day

ii) have serum cholesterol levels greater than 200 mg/100 ml

iii) agree to eat only the foods provided by the study at the arranged facilities

iv) be within ± 20% of their desirable body weight and be willing to maintain this weight throughout the study

v) be willing to maintain a relatively constant pattern of physical activity.

Screening Procedures

A total of 107 men were screened for total serum cholesterol following a 14-hour overnight fast. Forty-seven men were found to have serum cholesterol values ≥ 200 mg/100 ml. Ultimately several people with screening serum cholesterol values in the 190 mg/100 ml range were invited to participate in the study because some of the men with higher serum cholesterol levels were unwilling to make a complete commitment to the study.

The subjects who were selected received a physical examination and routine laboratory tests that included blood glucose, electrolytes, SGPT, SGOT, hematologic tests, and urinalysis. All screening laboratory tests were performed by a commercial medical laboratory that serves many of the local hospitals, the Student Health Center, and many of the research projects conducted at Michigan State University.

The subjects were interviewed to obtain information about normal eating patterns, food allergies, food dislikes, coffee and tea consumption, smoking habits, and family medical history. The men were asked to keep a diet record on three consecutive days for the purpose of estimating energy requirements. They were given specific verbal and written directions in a cover letter accompanying the food record form instructing them to list all foods consumed, portion sizes, food preparation method and time of

consumption. An example of a complete diet record was provided. Despite the specific directions which were given, the diet records were returned in such an incomplete state that they were of limited value in predicting energy requirements. They did prove useful, however, in providing information, albeit sketchy, on the frequency of consumption of eggs, butter, milk and meat.

Subject Characteristics

Age, Race and Marital Status. With the exception of one subject who was 69 years old, subjects ranged in age from 19 to 42 years, with a mean age of 24.8 years (Table 1). Thirty subjects were Caucasian; two were Black. There were two sets of brothers participating in the study (subjects 3 and 4; 9 and 10); two of the brothers were fraternal twins. Three of the subjects were married and two had children. All but three subjects were students at Michigan State University. The three non-student subjects worked adjacent to the university campus.

Body Weight and Anthropometric Measurements. The body weights of the subjects measured at the time of their physical examinations ranged from 84 to 134% of the average weight/height for men of medium frame according to the 1960 Height-Weight chart of the Metropolitan Life Insurance Company.

Lean body mass (LBM) and body fat (BF) were estimated using measurements of flexed biceps circumference, height,

Table 1. Summary table of subject characteristics[a]

Subject	Age	Height (cm)	Weight (kg)	Biceps Diameter (cm)	Lean Body Mass (kg)	Body Fat[c] (%)	Screening Serum Cholesterol[d] (mg/100 ml)	Family Medical History[e]
1	25	177.8	70.6	34.8	65.8	7	232	D
2	29	179.7	75.8	37.3	72.1	5	232	HA
3	21	171.5	64.9	31.3	55.4	15	207	D
4	21	170.8	70.6	33.0	58.2	18	227	D
5	22	178.1	87.1	35.2	66.9	23	206	H
6	32	186.6	93.9	34.6	71.9	23	217	D,O
7	22	179.7	66.7	31.4	60.6	9	259	HA
8	25	180.3	75.6	33.8	65.4	14	237	
9	43	164.5	69.2	34.4	57.2	17	293	
10	31	165.4	81.4	37.3	63.0	23	226	
11	27	181.3	67.9	28.4	56.4	17	260	HA
12	22	182.9	76.5	31.9	63.3	17	212	HA
13	21	186.7	76.2	33.0	67.7	11	233	D
14	24	186.7	81.7	31.3	64.5	21	204	HA,H
15	22	186.1	74.9	31.8	64.9	13	223	
16	22	181.0	67.9	32.2	62.8	8	208	HA,D
17	29	185.4	85.5	36.4	73.6	14	197	HA,H
18	22	177.8	65.0	30.8	58.3	10	210	
19	24	182.9	58.2	32.6	64.6	5	252	HA,H,S
20	34	179.7	82.3	35.2	67.8	18	323	H
21	28	175.3	70.9	31.6	58.3	18	192	S
22	23	193.7	81.4	34.3	74.3	9	202	
23	22	190.5	81.8	33.0	69.9	14	194	
24	24	173.4	71.4	29.0	52.6	26	204	
25	19	180.3	70.5	27.9	54.9	22	233	

Table 1. (cont'd.).

Subject	Age (yr)	Height (cm)	Weight (kg)	Biceps Diameter (cm)	Lean Body Mass (kg)	Body Fat[c] (%)	Screening Serum Cholesterol[d] (mg/100 ml)	Family Medical History[e]
26	23	175.9	71.4	35.6	66.3	7	246	HA,D
27	21	177.2	83.4	37.0	70.0	16	271	H
28	27	169.5	75.7	33.0	57.4	24	199	HA,H,O
29	22	196.4	79.3	35.8	78.1	2	199	H
30	22	175.9	93.2	35.9	66.9	28	237	
31	22	172.7	60.5	30.4	54.6	10	210	
32	69	163.2	62.3	29.8	47.9	23	218	

[a]Measurements of height, weight, and biceps circumference for each subject were taken on the same day during the second and third weeks of the study.

[b]Lean body mass was calculated from the equation by Wright and Behnke (1974) using height, weight, and biceps circumference.

[c]% body fat was calculated by subtracting lean body mass from total body weight.

[d]Screening serum total cholesterol concentration.

[e]Incidence of the conditions reported in parents, grandparents, siblings, aunts or uncles: HA=heart attack before age 55 years; H=hypertension; S=stroke; D=diabetes; O=tendency to overweight.

and weight. All measurements were taken by one technician before breakfast during the second or third weeks of the study. Two consecutive measurements of biceps circumference were taken at the fullest point of the fully flexed dominant arm using a plastic coated tape measure and the mean value was reported. Height was measured with the subjects in stocking feet, eyes straight ahead, with heels and shoulders touching a vertical surface to which a Lufkin steel tape was attached. Weight was measured on a beam scale by the subjects and later recorded by the technician. The following prediction equation (Wright and Wilmore, 1974) was used to calculate LBM:

$$\text{LBM (kg)} = -74.58 + 0.5949 \times \text{height (cm)} + 0.0286 \times [\text{biceps circumference (cm)}]^2$$

Percent body fat was calculated from the difference between LBM and total body weight.

The 32 subjects who were selected were free from detectable disease. The results of the laboratory tests performed at the time of the physical examination were within the normal range except for serum triglyceride values of 311, 225, and 175 mg/100 ml is subjects 9, 20 and 30, respectively. Total serum cholesterol was found to be greater than 250 mg/100 ml in six subjects, thus exceeding the laboratory's range for normal values (Table 1).

The 16 subjects from each term were assigned to one of four groups so that the average age, body weight, and screening serum cholesterol levels were approximately the

same for all groups.

Orientation

An orientation meeting was held prior to the start of the study to introduce subjects to the staff and their fellow volunteers, to familiarize them with the surroundings, to review the procedures, and to answer any questions that may have arisen. The subjects were reminded to avoid taking any medication (including aspirin) or vitamin supplements unless prescribed by the consultant physician for the study. They were instructed to notify us and to call the physician in the event of illness.

We stressed the point that each subject would serve as his own control and hence it was important that he maintain a relatively steady pattern of activity and that his body weight remain stable. They were asked to be sure to eat two eggs/day if they were not already doing so, so that the 10 day control period would approximate as closely as possible a diet to which they were accustomed.

Informed Consent

The project was reviewed and approved by the University Committee on Research Involving Human Subjects. The purpose and the nature of the study and the possible risks were explained to the prospective subjects and a consent form for the screening blood test was signed. The consent form explained that the subject could withdraw from the study at

any time without penalty and that results would be treated in strict confidence (Appendix A-1). A second consent form for participation in the study was signed at the orientation session by those subjects selected to be in the study (Appendix A-2).

Experimental Diet Plan

During the first ten days of the study all subjects consumed a control diet. The control diet was designed to represent a "typical" American diet with two eggs/day, 42 to 45% of total calories as fat, and a polyunsaturated: saturated fat (P/S) ratio of 0.3 to 0.5. Other foods in the diet were controlled to provide an average of 350 mg of cholesterol. The control period allowed for estimation of energy needs to maintain weight, adjustment of subjects to experimental procedures, and collection of baseline data. During the next eight weeks, half of the subjects (8 subjects/ Winter and Spring terms) received each of the following diets for four weeks in a crossover design (Table 2): 1) Diet C-E: the control or typical American diet described above; 2) Diet C-ES: the same diet as the control diet except that two eggs were replaced by a cholesterol-free egg substitute. The remaining half of the subjects received each of the following diets in a similar crossover design: 1) Diet MF-E: a modified fat diet similar to the one recommended by the Inter-Society Commission for Heart Disease Resources (1972) except that it included two eggs/day,

Table 2. Experimental diet plan[a]

Group	Weeks		
	1-2	3-6	7-10
1	C-E	C-E	C-ES
2	C-E	C-ES	C-E
3	C-E	MF-E	MF-ES
4	C-E	MF-ES	MF-E

[a]Diet designations are as follows:

C-E: Control or typical American diet with 2 eggs daily and 42 to 45% of total calories from fat, P/S ratio ∿ 0.3 - 0.5.

C-ES: Control diet using cholesterol-free egg substitute to replace eggs.

MF-E: Modified fat diet providing 32 to 35% of total calories from fat, P/S ratio ≥ 1.0. Diet included 2 eggs/day.

MF-ES: Same as diet MF-E except that egg substitute was used to replace the eggs.

approximately 35% of the total calories as fat with a P/S ratio ≥ 1.0. 2) Diet MF-ES: this diet was the same as the modified fat diet except that the egg substitute was used instead of eggs.

Menus

A two-week cycle of basal menus with 2800 kcal/day was planned for each of the control and modified fat diets (see Appendix B for sample menu). The amounts of carbohydrate, protein, and calories in the diets were calculated using the 1975 USDA Handbook No. 456 (Adams, 1975) for all foods except for dairy products, the values for which were taken from USDA Handbook 8-I (Posati and Orr, 1976). Values for total lipids, saturated fatty acids, polyunsaturated fatty acids and monosaturated fatty acids were taken from a series of tables prepared by the USDA Agricultural Research Service (Anderson, 1976; Anderson et al., 1975, 1977; Brignoli et al., 1976; Exler and Weihrauch, 1976; Fristrom and Weihrauch, 1976; Posati et al., 1975; Weihrauch et al., 1976). Values for cholesterol were taken from the table prepared by Feeley et al. (1972). Nutrient composition values supplied by the food manufacturers were used in menu calculations when applicable.

The basic menus were planned so that as many of the foods as possible could be used in all four diets. Calorie adjustments and allowances for food preferences

for individual subjects were accomplished by adding or subtracting food portions to obtain the desired calorie level while maintaining the specified percent of the calories from fat and the appropriate P/S ratio. The subjects weighed themselves daily. Adjustments in caloric intake were made at regular intervals if subjects were gaining or losing weight. Adjustments for the change from the control diet to the modified fat diet were accomplished primarily by substituting margarine for butter and skim milk for whole milk. The reduction in calories resulting from the decrease in fat in the modified fat diet was compensated for by increasing fruit, vegetable, and bread servings. The items allowed ad libitum in all four diets were coffee, tea, and spices. Subjects were given the choice between whole wheat, cracked wheat, and white bread.

The amount of commercial cholesterol-free egg substitute used was comparable in nutrient composition to the equivalent of two large whole eggs. The egg substitute was made from egg whites (82%), liquid corn oil (10%), nonfat dry milk (7%), emulsifiers, preservatives, coloring and flavor additives (Table 3).

Meals

Meals were served in the dining room of the Student Health Center. Three meals were served each day except on Sunday when only brunch and dinner were served. The meal

Table 3. Comparison of the nutrient composition of two large whole eggs and an equivalent serving portion of the cholesterol-free egg substitute used in this study

	Egg Substitute[1]	Whole Eggs
Serving size, portion	1/2 cup	two large
, g	120	105
Calories,	180	160
Protein, g	14	14
Carbohydrate, g	6	2
Fat, total g	12	12
polyunsaturated g	6	2
saturated g	2	2
Cholesterol, mg	0	480
Sodium, mg	260	140

[1]Egg Beaters, Standard Brands, Inc., Stamford, Conn.

hours were: breakfast 7:00 - 9:00 a.m.; lunch 11:00 - 1:00; and dinner 4:30 - 6:30. Trays bearing name cards were set up in advance in refrigerated carts so that when the subjects came in for meals the cook only had to add the foods from the hot cart and double-check the food on the tray with the diet sheets. For those subjects who expressed the desire for an early morning snack on Sundays, a box of cereal, a carton of milk, and a piece of fruit were provided to take home Saturday evening. Occasionally meal hours were extended to accommodate problems arising from time conflicts. In some instances field trips or lunch hour meetings necessitated packing a meal to go. For one subject a bag lunch was provided on Mondays because of a lunchtime schedule conflict. All meals that were packed and taken from the Health Center were recorded.

Occasionally friends and family members purchased food from the Health Center cafeteria line and joined the subjects for meals. It was not unusual on a Saturday night to see several guests sitting at the subjects' table next to their dates.

Food Preparation

The majority of foods served in the study were purchased through the University Food Stores. The meat, fish and poultry were purchased in large quantities at the beginning of each term and weighed into approximate portion

sizes by the Food Stores and frozen to ensure uniformity of source. Eggs, milk and bread were purchased as needed from the same suppliers throughout the study. Bread and angel cake were the only two baked goods not prepared in our kitchen. Foods containing appreciable amounts of fat (e.g. salad dressings, peanut butter, cheese, nuts, and meats) were weighed on a Toledo scale to the nearest gram, whereas foods containing little or no fat (e.g. cooked and salad vegetables, juices, and canned fruit) were served by measurement or by commercially available preportioned servings (e.g. ketchup, jelly, raisins, sugar and prepared cereal). Preportioned pats of butter and margarine and cartons of milk were used. They were weighed randomly and found to be of consistent weight. Similar procedures were used to weigh or measure ingredients for recipes prepared during the study. Many of the recipes were prepared individually, particularly when appreciable amounts of fat and cholesterol were involved, rather than in large quantities to be divided into individual portions. For example, a vegetarian chili recipe was prepared in a large kettle and divided into the appropriate number of servings. Then to each serving of chili was added a carefully weighed portion of cooked ground beef. Whenever possible recipes were portioned and cooked in individual pans. At breakfast the eggs and egg substitute were prepared in separate frying pans.

As many of the entrees as possible were prepared and frozen before the beginning of the Winter and Spring terms (e.g. macaroni and cheese, chili, chicken cashew, potato kugel) to reduce the amount of daily preparation and facilitate serving. Weighed or measured portions of several kinds of cookies, cakes and muffins as well as cherry tarts, pumpkin pies, pizza, waffles, and french toast also were prepared in advance and frozen. Nearly all of the recipes for these products were made with egg substitute, margarine, and corn oil, so that they could be used on both the control and modified fat diets. All ingredients in these recipes were weighed except for spices and flavorings.

Food Intake Records

Careful food intake records were kept for each subject. All trays were checked after meals for food that may have been left. The few food mistakes that occurred in serving food were recorded and the daily nutrient calculations were corrected when the mistakes involved any appreciable amounts of fat. Occasionally extra servings of certain food items (e.g. raw or cooked vegetables) were given if requested; records of these foods were also kept.

Subjects who were accustomed to consuming alcoholic beverages on a regular basis were given the option of having 12 ounces of beer (to be supplied by the subject) included in their daily diet plan. Six subjects chose to do so.

The rest of the subjects were told that they could substitute one 12 ounce beer (or two light beers) for two slices of bread on any day except immediately prior to the blood tests. Alcohol substitutions were recorded.

The daily intake of calories, carbohydrate, protein, total fat, saturated fatty acids, polyunsaturated fatty acids, monosaturated fatty acids and cholesterol were recorded for each subject. From this the percent of total calories from fat, the percent of calories from protein, and the P/S ratio were calculated.

Blood Tests

Blood samples for serum cholesterol determinations were drawn before breakfast following an overnight fast on the first day of the study, on Friday of each week, and on Tuesday at the end of each experimental diet period (weeks 2, 6, and 8) (Table 4). The blood samples were centrifuged, and the serum divided into two portions and frozen for subsequent serum cholesterol analysis by our laboratory and by the commercial medical laboratory.

Total serum cholesterol was determined in our laboratory by a colorimetric method using ferrous sulfate (Searcy and Berquist, 1960) and a chloroform-methanol extraction (Leveille et al., 1962). Samples were analyzed in triplicate and the mean value was used. See Appendix C for laboratory method.

Table 4. Schedule of blood tests

	Weeks	Tuesday	Friday
Control Period	1	Chol[a]	Chol
	2	Chol	Chol
Experimental Period 1	3		Chol
	4		Chol
	5		Chol
	6	Chol	Chol
Experimental Period 2	7		Chol
	8		Chol
	9		Chol
	10	Chol	Chol

[a]Chol=total serum cholesterol

At the end of Winter and Spring terms an aliquot of the frozen serum samples was sent to the commercial laboratory for determination of serum cholesterol using an automated Hycel Super Seventeen™ method, which is basically the Liebermann-Burchard reaction[1]. The automated procedure made it possible to analyze all samples at one time.

Statistical Design

The statistical design of the study was that of a split-plot design with crossover treatments for the subjects. The values for total serum cholesterol were averaged within experimental diet periods and the differences between the average cholesterol values for each of the two experimental diet periods and the average control period value were calculated for each of the parameters. Averaging was done in two ways: (1) averaging all the values within each diet period and (2) averaging only the last two values from each diet period, that is, those values from blood samples drawn on Tuesday and Friday at the end of each diet period.

[1]Hycel, Inc., Houston, TX.

RESULTS

Body Weights

Weekly body weight values were calculated by averaging daily weights from Friday through Thursday so that the weekly averages corresponded to the weekly serum cholesterol values for bloods drawn each Friday. See Appendix D for weekly body weight values for individual subjects.

The average change in body weight that occurred within each of the 4-week experimental diet periods and the control periods was calculated for the two groups of subjects participating in the Winter and Spring Terms (Table 5).

The greatest weight change within a given diet period was a 3.6 kg decrease in one subject (#4) during the first experimental diet period of the Winter term while consuming the control diet with eggs, followed by a 2.3 kg decrease in the second experimental diet period while consuming the same diet with egg substitute. This subject, as well as other subjects from the Winter term, claimed to have gained weight during the preceding Christmas holiday season. This weight loss may have, in part, reflected a reversion to his normal body weight. A second subject (#15) experienced a 2.7 kg decrease during the first experimental diet period

Table 5. Average change in body weight within the control period (weeks 1-2) and each of the experimental diet periods (weeks 4-6, 7-10)[a]

	Winter term	Spring term
	kg	kg
Control Period	-0.54	-0.09
Experimental Period 1	-1.00	-0.27
Experimental Period 2	-1.23	-0.77

[a]The average body weight change for the control period was found by calculating the changes in body weight between weeks 1 and 2 for each subject and computing the mean value. The average body weight change for the first experimental diet period was calculated by comparing the weekly weights from week 6 (the end of the first experimental diet period) with the weekly weights from week 2 (the end of the control period). The average body weight change for the second experimental diet period was determined by comparing the weekly weights from week 10 (the end of the second experimental diet period) with the weekly weights from week 6.

while consuming the control diet with egg substitute, followed by a 2.3 kg decrease during the second diet period when eggs replaced the egg substitute. Prevention of this weight loss was a perplexing problem because the caloric intake of this individual was increased by increments of 100 to 300 calories each week, yet he continued to lose weight even while consuming 4000 calories/day.

During the last four weeks of the Spring term one subject (#27) experienced a 2.3 kg decrease in average body weight while consuming the modified fat diet with eggs; another subject (#30) experienced a 2.7 kg decrease while consuming the modified fat diet with egg substitute. Whereas the body weights of the subjects in the Spring term were relatively stable during the first five weeks, there tended to be a gradual decrease in body weight that began during the sixth week of the study. This decrease may have reflected the arrival of spring and the shredding of layers of heavy winter clothing. The subjects were instructed to weigh themselves in their normal indoor clothing after removing their shoes/boots. During the winter months normal indoor clothing included long underwear, sweaters, and extra heavy socks not worn during the warmer months.

Nutrient Intake

The daily intake of calories, carbohydrate, protein, total fat, polyunsaturated/saturated fat (P/S), and

cholesterol were tabulated for each subject and the average intakes for diet periods were calculated. See Appendix E for individual data. Table 6 is a compilation of the ranges and averages of protein, fat, and cholesterol intake for subjects by diet.

The subjects consumed between 2600 and 4000 kcal/day. The average protein intake, percent of total calories from protein, intake of total fat, and percent of total calories from fat were nearly the same for each of the control diet groups (subjects on the control diet with eggs or egg substitute) and for each of the modified fat diet groups (subjects on the modified fat diet with eggs or egg substitute). Because the egg substitute had a higher content of polyunsaturated fat, replacement of eggs with the egg substitute resulted in a slightly higher P/S ratio. The average P/S ratios of the control diet with eggs or egg substitute were 0.45 and 0.57, respectively; the average P/S ratios of the modified fat diets with eggs or egg substitute were 1.10 and 1.34, respectively.

The difference between the average cholesterol intakes on the control and modified fat diets reflect the decrease in dietary cholesterol that would naturally accompany the reduction in dietary fat. The average decrease in dietary cholesterol seen in the control diet when eggs were replaced by the egg substitute is approximately equal to the reduction in dietary cholesterol when eggs were replaced

Table 6. Dietary intake of protein, fat, and cholesterol

	Diets			
	C-E[a]	C-ES[a]	MF-E[b]	MF-ES[b]
Protein, g				
Range	89-167	90-166	89-163	98-205
Average	120.1	119.2	124.8	126.0
Protein-% kcal				
Range	12-22	12-23	12-21	12-21
Average	16.0	16.1	16.5	16.5
Fat, g				
Range	121-185	122-175	96-153	94-160
Average	145.0	145.1	116.9	119.7
Fat-% kcal				
Range	39-47	33-47	32-37	31-47
Average	43.8	44.0	34.6	35.0
P/S				
Range	0.18-0.66	0.26-0.89	0.77-1.68	0.98-1.74
Average	0.45	0.57	1.10	1.34
Cholesterol, mg				
Range	751-1033	207-491	553-952	97-394
Average	894	339	728	186

[a]Values represent intake of subjects fed diets C-E and C-ES. Eight subjects consumed diet C-E during the first experimental diet period (weeks 3-6) and diet C-ES during the second experimental period (weeks 7-10). The remaining eight subjects consumed the diets in the reverse order.

[b]Values represent intake of subjects fed diets MF-E and MF-ES following diet C-E in the control period. Eight subjects consumed diet MF-E during the first experimental period and diet MF-ES during the second experimental period. The remaining eight subjects consumed the diets in the reverse order.

by egg substitute on the modified fat diet. These average decreases in dietary cholesterol are approximately equal to the cholesterol content of two large whole eggs (500 mg).

The wide range in caloric intake among subjects explains in part the large group ranges seen here in the amount of protein, fat, and cholesterol consumed. The day-to-day variation for individual subjects tended to be less, particularly for those subjects whose caloric requirements remained relatively constant throughout the study. It is nearly impossible when using a diet of conventional foods to increase calorie intake without increasing cholesterol if the same percent fat and P/S ratio are maintained.

It is important to note that although nutrient intake did vary somewhat from day-to-day within the two-week cycle of menus, unless calorie requirements changed, each subject received the same menu every two weeks, and hence the same nutrient intake.

Total Serum Cholesterol

Serum cholesterol values from our laboratory tended to be lower than the values reported by the commercial laboratory because of differences in methodology; however, the trends in the response of serum cholesterol to dietary changes and the magnitude of response tended to be similar for both laboratories. The commercial laboratory had the

advantage of being able to run all the serum cholesterol determinations at one time by an automated method. Only the values for serum cholesterol from the commercial laboratory will be reported here.

The average cholesterol values for individual subjects during each diet period are presented in Tables 7 to 10. These values represent the average of the two cholesterol values for blood samples drawn on Tuesday and Friday of the last week of each diet period. (See Appendix F for the complete listing of all values).

Six of the eight subjects who continued on the control diet with two eggs/day for four weeks following the 10-day control period experienced decreases in serum cholesterol ranging from 3 to 30 mg. One subject demonstrated a 1 mg increase and the eighth subject showed an average increase of 8 mg. The average change in serum cholesterol for this group during this period was a 10.5 mg decrease. The percent change ranged from an 8.3% decrease to a 3% increase, with an average change of a 4.3% decrease from the average control period value.

When eggs were removed from this diet six of the eight subjects demonstrated an additional decrease in serum cholesterol ranging from 6 to 52 mg. One subject experienced a 7 mg increase and one a 15 mg increase. The average change in serum cholesterol between the first and second diet periods when eggs were removed from the diet was a

Table 7. Concentration of total serum cholesterol and percentage change in subjects who continued on the control diet with 2 eggs/day during the first 4-week experimental diet period, followed by egg substitute during the last 4-week experimental diet period[a]

Subject	Serum Cholesterol			Change in Serum Cholesterol					
Diet Period / Diet	C C-E	1 C-E	2 C-ES	C→1	1→2	C→2	C→1	1→2	C→2
	mg/100 ml			mg			%		
1	262	270	218	+ 8	-52	-44	+ 3.1	-19.2	-16.8
4	262	232	226	-30	- 6	-36	-11.5	- 3.6	-13.7
10	218	204	219	-14	+15	+ 1	- 6.4	+ 7.3	+ 0.5
13	195	192	199	- 3	+ 7	+ 4	- 1.5	+ 3.6	+ 2.1
19	224	225	181	+ 1	-44	-43	+ 0.4	-19.6	-19.2
20	288	264	246	-24	-18	-42	- 8.3	- 6.8	-14.6
24	230	211	190	-19	-21	-40	- 8.3	- 9.9	-17.4
25	244	240	226	- 3	-14	-17	- 1.6	- 5.8	- 7.4
Mean	240	230	213	-10.5	-16.6	-27.1	- 4.3	- 6.6	-10.8

[a]These values represent the average of the two cholesterol values from the last week of the control diet period (C) and the experimental diet periods 1 and 2.

Table 8. Concentration of total serum cholesterol and percentage change in subjects who consumed the control diet with egg substitute during the first 4-week experimental diet period, followed by eggs during the last 4-week experimental diet period[a]

Subject	Serum Cholesterol			Change in Serum Cholesterol					
Diet Period	C	1	2	C→1	1→2	C→2	C→1	1→2	C→2
Diet	C-E	C-ES	C-E						
	mg/100 ml			mg			%		
7	264	236	294	-28	+58	+30	-10.6	+24.6	+11.4
8	232	200	240	-32	+40	+ 8	-13.8	+20.0	+ 3.4
14	232	206	196	-26	-10	-36	-11.2	- 4.8	-15.5
15	195	164	206	-31	+42	+11	-15.9	+25.6	- 5.6
17	200	180	196	-20	+16	- 4	-10.0	+ 8.9	- 2.0
18	204	180	180	-24	0	-24	-11.8	0	-11.8
23	214	208	226	- 6	+18	+12	- 2.8	+ 8.6	+ 5.6
31	224	201	238	-23	+37	+14	-10.3	+18.4	+ 6.2
Mean	221	197	222	-23.8	+25.1	+1.4	-10.8	+13.3	+ 1.0

[a]These values represent the average of the two serum cholesterol values from the last week of the control period (C) and the experimental diet periods 1 and 2.

Table 9. Concentration of total serum cholesterol and percentage change in subjects who consumed the modified fat diet with 2 eggs/day during the first 4-week experimental diet period, followed by egg substitute during the last 4-week experimental diet period[a]

Subject	Serum Cholesterol				Change in Serum Cholesterol				
Diet Period / Diet	C / C-E	1 / MF-E	2 / MF-ES	C→1	1→2	C→2	C→1	1→2	C→2
	mg/100 ml				mg			%	
3	200	153	178	-47	+25	-22	-23.5	+16.3	-11.0
5	215	200	200	-15	0	-15	- 7.0	0	- 7.0
11	246	212	186	-34	-26	-60	-13.8	-12.2	-24.2
12	229	198	180	-31	-18	-49	-13.5	- 9.1	-21.4
22	236	188	188	-48	0	-48	-20.3	0	-20.3
27	264	234	217	-30	-17	-47	-11.3	- 7.3	-17.8
29	196	172	170	-24	- 2	-26	-12.2	- 1.2	-13.3
32	218	222	199	+ 4	-23	-19	+ 6.4	-10.4	- 8.7
Mean	225	197	190	-28.1	- 7.6	-35.8	-12.5	- 3.0	-15.5

[a]These values represent the average of the two serum cholesterol values from the last week of the control diet period (C) and the experimental diet periods 1 and 2.

Table 10. Concentration of total serum cholesterol and percentage change in subjects who consumed the modified fat diet with egg substitute during the first 4-week experimental diet period, followed by eggs during the last 4-week experimental diet period[a]

Subjects	Serum Cholesterol			Change in Serum Cholesterol					
Diet Period / Diet	C C-E	1 MF-ES	2 MF-E	C→1	1→2	C→2	C→1	1→2	C→2
	mg/100 ml			mg			%		
2	207	142	164	-65	+22	-43	-31.4	+15.5	-20.8
6	208	174	200	-34	+26	- 8	-16.3	+14.9	- 3.8
9	273	247	264	-25	+13	- 8	- 9.4	+ 5.3	- 3.3
16	204	170	189	-34	+19	-15	-16.7	+11.2	- 7.4
21	195	160	172	-35	+12	-23	-17.9	+ 7.5	-11.8
26	240	186	208	-54	+22	-32	-22.5	+11.8	-13.3
28	206	164	195	-42	+31	-11	-20.4	+18.9	- 5.3
30	236	196	204	-40	+ 8	-32	-16.9	+ 4.1	-13.6
Mean	221	180	200	-41.1	+19.1	-21.5	-19.0	+11.2	- 9.9

[a]These values represent the average of the two serum cholesterol values from the last week of control diet period (C) and the experimental diet periods 1 and 2.

16.6 mg decrease. This represents a 6.6% decrease from the average values from the first experimental diet period. The serum cholesterol values during the egg-free period was on the average 27.1 mg below the control period values, with decreases ranging from 17 to 44 mg in six of the subjects. One subject had a final serum cholesterol value 1 mg above his control period value, while the eighth subject's cholesterol level was 4 mg above his control period level.

When subjects were placed on the egg-free control diet for four weeks following the 10-day control diet with two eggs/day, all eight subjects experienced a decrease in serum cholesterol ranging from 6 to 32 mg, with an average decrease of 23.8 mg for the group (Table 8). This represents an average decrease of 10.8% below the average control period value.

When eggs were added back to this diet six of the eight subjects demonstrated an increase in serum cholesterol levels ranging from 16 to 58 mg. One subject showed an additional 10 mg decrease in serum cholesterol when eggs were added back to the diet, and one subject showed no change. The average serum cholesterol value for this group during the last week of the four week diet period was 25.1 mg/100 ml higher than the value for the egg-free diet period of the previous four weeks. This change represents an average increase of 13.3% above the average values

from the egg-free diet period. Five of the eight subjects had higher serum cholesterol values when eggs were added back to the diet than they had when consuming the same diet eight weeks previously.

When placed on the modified fat diet with two eggs/day following the 10-day control diet with two eggs/day, seven of the eight subjects experienced a decrease in serum cholesterol ranging from 15 to 48 mg (Table 9). The eighth subject demonstrated a 4 mg increase. The average change for the group was a 28.1 mg or 12.5% decrease below the control period value.

When eggs were replaced by an egg substitute during the last four weeks of the modified fat diet, five of the eight subjects experienced an additional decrease in serum cholesterol ranging from 2 to 33 mg. Two subjects showed no change, and one subject demonstrated a 25 mg increase when eggs were removed from the diet. The average change in serum cholesterol when eggs were removed from the modified fat diet was an 8.9 mg or 3.5% decrease. The average serum cholesterol level during the last week of the final experiment diet period on the egg-free modified fat diet was 35.8 mg below the average serum cholesterol value during the 10-day control period.

When subjects were placed on the egg-free modified fat diet for four weeks following the 10-day control diet with two eggs/day, all eight subjects experienced a decrease in

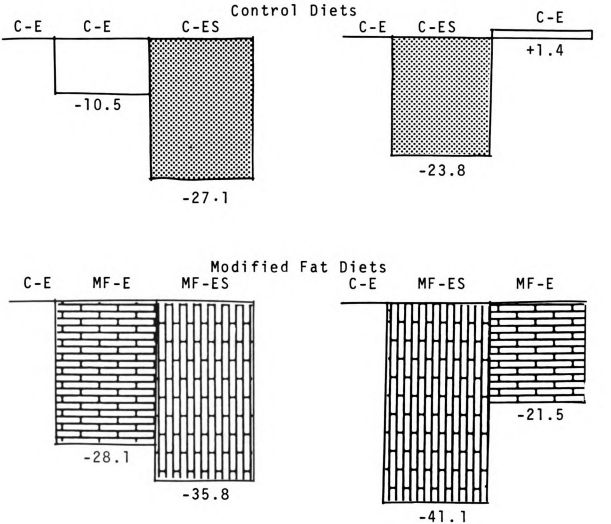
serum cholesterol ranging from 25 to 65 mg, with an average decrease of 41.1 mg or 19.0% (Table 10). When eggs were added back to this modified fat diet for four weeks all eight subjects experienced an increase in serum cholesterol ranging from 8 to 31 mg, with an average increase of 18.9 mg, or 11.2%. The average group serum cholesterol level during the last week of this final experimental period was 21.5 mg below the average value during the 10-day control period when subjects were consuming a diet with more total fat, saturated fat and cholesterol.

Figure 1 summarizes the changes in serum cholesterol concentration relative to the control period values for the four diet groups. Replacement of eggs with egg substitute resulted in a decrease in serum cholesterol in both the control and modified fat diets groups. The addition of eggs back to the egg-free control and modified fat diets produced an increase in serum cholesterol concentration in both groups. The decrease in serum cholesterol concentration that occurred when subjects were placed on the modified fat diet was greater than the decrease in serum cholesterol that occured when eggs were replaced by egg substitute in the control diet.

It is not known why the 8 subjects who continued on the same control diet with 2 eggs/day during the first experimental diet period demonstrated a mean reduction in serum cholesterol concentration of 10.5 mg, or 4%. Four of the

Figure 1. The changes in total serum cholesterol concentration during the two 4-week experimental diet periods relative to the control period were found for each of the four diet groups (8 subjects/group) by subtracting the average values of the last two serum cholesterol values for the experimental diet periods from the mean value of the last two serum cholesterol values of the 10-day control period. The symbols used are as follows:

C-E =the control diet with 2 eggs/day
C-ES =control diet with egg substitute replacing eggs
MF-E =modified fat diet with 2 eggs/day
MF-ES=modified fat diet with egg substitute replacing eggs


Control Diets
C-E
C-E
C-ES
-10.5
-27.1
C-E
C-ES
C-E
+1.4
-23.8
Modified Fat Diets
C-E
MF-E
MF-ES
-28.1
-35.8
C-E
MF-ES
MF-E
-21.5
-41.1

subjects in this group experienced a decrease in serum cholesterol concentration, with the greatest decrease equal to 30 mg. One subject experienced an 8 mg increase in serum cholesterol and 3 subjects showed essentially no change. This group demonstrated the greatest variability in response to dietary changes. The reason for this is unclear.

Analysis of variance of the serum cholesterol values obtained by the commercial laboratory revealed significant diet and treatment effects at the 5% level, indicating that the changes in serum cholesterol seen in this study were related to the type of diet (control vs modified fat diet) and to treatment with either eggs or egg substitute (Table 11). No significant group or seasonal effect was found, thus allowing us to combine the data for subjects from both the Winter and Spring terms. No significant period effect was seen, indicating that the changes in serum cholesterol seen in this study were not related to the sequencing of the experimental diets (i.e. eggs→egg substitute vs egg substitute→eggs). The same level of significance was found when the data were analyzed by averaging all the serum cholesterol values within a diet period or when averaging only the last two values of each diet period.

Correlation between average weekly body weights and weekly serum cholesterol levels was computed for the entire group of 32 subjects. No correlation was found at the 5%

Table 11. Analysis of variance of the data for total serum cholesterol[a]

Sources of Variation	DF	F_1	F_2	CV
Seasons	1	0.09	0.09	
Diets (Control vs MF)	1	13.77*	12.23*	4.20
SXD	1	3.25	0.08	
Error (Subject/SD)	28			
Periods	1	1.78	2.48	
Treatments	1	12.88*	28.19*	
SXT	1	2.89	0.02	4.21
DXT	1	0.47	1.24	
SXDXT	1	0.21	0.01	
$Error_2$ (Residual)	27			
	63			

*Significant at $p<0.05$.

[a]Symbols used are as follows:
DF=degrees of freedom
CV=critical value
S=Seasons (Winter vs Spring terms)
D=Diets (Control vs Modified Fat)
T=Treatment (Eggs vs Egg Substitute)
F_1=f ratio for the average of all values
F_2=f ratio for the average of the last two values

significance level (r=0.66).

DISCUSSION

The purpose of this study was to compare the effects on total serum cholesterol of replacing 2 eggs/day in the diet with a cholesterol-free egg substitute and changing from a typical American diet to a modified fat diet that is lower in total and saturated fat and higher in polyunsaturated fat. The modified fat diet utilized was similar to the diet recommended in the Report of the Inter-Society Commission for Heart Disease Resources (1972). The menu was made up entirely of natural foods that are customarily consumed by the United States population. The amount and type of dietary fat and cholesterol fed in the study were within the normal range of consumption in the United States (Keys et al., 1974). Unlike other studies reported in the literature the subjects were not adapted to a cholesterol-free diet, or to a diet to which they were not accustomed. Attempts were made to individualize the menu for each subject in order to accommodate food dislikes and preferences, to maintain weight, and to provide a diet that approximated as closely as possible the customary diets of the subjects.

The Design of the Study

The split-plot design of this study allowed for analysis of the effects of various factors (e.g., diet, treatment) as well as of their combined effects (e.g., diet x treatment). The crossover design in which each subject served as his own control eliminated the possibility of factors other than those related to diet influencing the results.

The study was designed to correspond to the 10-week academic term. The study was planned to terminate before the week of final exams in order to avoid the stress that often accompanies studying for exams. The influence of stress on serum cholesterol levels has been documented by Dreyfus and Czaczkes (1959), who have shown an increase in serum cholesterol concentration in medical students at exam time.

Since one of the criteria for eligibility in the study stated that subjects must like and habitually consume eggs, the control period was assumed to be an extension of the subjects' customary diets. A 10-day control period was believed to be adequate for the estimation of caloric needs, collection of baseline data, and adjustment of subjects to experimental procedures. Keys et al. (1974) pointed out that all, or nearly all, of the dietary fat-serum cholesterol response of man is exhibited in two or at the most three weeks. In view of this fact, four weeks

was determined to be an adequate period of time for the experimental diet periods.

Subject Selection

Young male subjects with moderately elevated serum cholesterol levels were selected to participate in the study because they are considered to be at high risk for CHD and a segment of the population who would benefit from preventive dietary measures. Enos et al. (1955) provided pathological data on young American soldiers killed in the Korean war that demonstrated that atherosclerosis was already present in young men in their 20's. It has been assumed that elimination of the risk factors for atherosclerotic heart disease (of which serum cholesterol is one) at an early age would be more effective than after atherosclerosis is in an advanced state. In the Oslo diet-heart study, Leren (1970) showed that the reduction in the recurrence of heart attacks after dietary modification was evident only in the younger men in the study.

Because of the relationship of serum cholesterol levels to the menstrual cycle, female subjects were not included in the study. Serum cholesterol levels have been shown to decrease in women at the time of ovulation (Oliver and Boyd, 1953).

Subject Compliance

All 32 subjects completed the 10-week study. There were very few food rejections or complaints. The exceptionally high subject compliance that was seen in this study was probably related to their genuine concerns, in view of their family histories, about the risk of CHD. Also, many of the subjects had participated in or were involved in their own research and they were aware of the importance of reliable information. It would seem, too, that the gourmet menu contributed, at least in part, to the adherence to the diet. At the end of the ten weeks, even after having seen the same menu five times, many of the subjects requested copies of the recipes.

Body Weight

Subjects were weighed daily and adjustments in caloric intake were made regularly because it has been shown that rapid weight gain or loss was paralleled by elevations or depressions, respectively, in serum cholesterol levels (Anderson et al., 1957). Brunner et al. (1979) reported similar findings. Although several subjects in the present study experienced some fluctuation in body weight, the changes were not rapid or extreme, and no correlation was found at the 5% significance level between weekly body weight values and weekly serum cholesterol concentrations for the total group of subjects ($r = 0.66$).

Alcohol Consumption

One 12 ounce can of beer was included in the daily diet plan of six subjects. Other subjects occasionally substituted 12 ounces of beer for two slices of bread, which are approximately comparable in calorie content. There was no apparent difference between the subjects who consumed beer on a daily basis and the subjects who consumed no alcohol during the 10-week study in either the direction or the magnitude of response of serum cholesterol to diet modifications. Similarly, there was no apparent difference in serum cholesterol response to diet modifications in the subjects who, on occasion, substituted beer for bread. These subjects were asked to refrain from substituting beers on the days preceeding blood tests. It was believed that the beer would be fully metabolized before bloods were drawn and that such small quantities of alcohol consumed on an occasional basis would have little or no effect on total serum cholesterol levels.

Although the short term effect of alcohol on serum lipid levels has been studied experimentally in man the methodology is often questionable. Many of the participants in such research were alcoholics who were temporarily deprived of alcohol before the experiments. Also the number of subjects in each study generally was small. Friedman et al. (1965) demonstrated no short term effect of alcohol on serum cholesterol levels in daily drinkers

who continued their usual pattern of imbibing. Carey et al. (1971) showed that "moderate" alcohol intake, 20% of total calories as alcohol, ingested during the evening for 12 consecutive days, had no noticeable effect on cholesterol. Ostrander et al. (1974), in contrast, found that frequency of alcohol ingestion was significantly related to serum cholesterol concentration among the 202 male participants in the Tecumseh epidemiological study. These findings were based on data obtained from questionnaires on the drinking habits of each subject, followed by a blood test for serum lipid determinations. Again, the methodology to provide this type of data is questionable because of the possibility of the subjects' reports of alcohol intake being influenced by their concept of society's view on alcohol consumption.

Changes in Serum Cholesterol

The results reported here indicate that dietary cholesterol may influence serum cholesterol concentration in healthy males. Addition or deletion of 2 eggs/day from the diet generally produced parallel changes in total serum cholesterol concentration. Replacement of eggs with an equivalent serving of cholesterol-free egg substitute resulted in average reductions in serum cholesterol of approximately 17 and 24 mg/100 ml, or 7 and 11%, respectively, in each of the two diet groups consuming the high saturated fat diets. The addition of eggs to the diet

of the subjects who consumed the egg substitute in the first experimental diet period resulted in a 25 mg/100 ml or 13% increase in serum cholesterol.

These findings conflict with those of Slater et al. (1976), Porter et al. (1977) and Flynn et al. (1979), who found a lack of association between egg consumption and serum cholesterol concentration in healthy subjects. The intake of foods other than eggs, however, was not controlled in these studies. It is conceivable that the variation in cholesterol intake from other foods and the variation in the amount and type of fat may have been sufficient to mask the effect of adding or deleting two eggs from the diet.

During the control period with 2 eggs/day subjects were consuming approximately 270 mg of cholesterol/1000 kcal compared to approximately 105 mg cholesterol/1000 kcal on the control diet with egg substitute. According to the following prediction equation proposed by Keys et al. (1965b)

$$\Delta\text{Cholesterol (mg/100 ml)} = 1.5\ ((\Delta\text{dietary cholesterol mg/1000 kcal})^{1/2})$$

one would expect a 19 mg decrease in total serum cholesterol concentration with the removal of 2 eggs/day from the control diet. The results of this study are in agreement with the predicted value from the Keys equation.

Based on the results of a comprehensive study on schizophrenic patients under metabolic conditions, Hegsted et al. (1965) found that for each 100 mg change in dietary cholesterol one could predict a change in total serum cholesterol of approximately 5 mg/100 ml. Using this equation one would expect a 27 mg decrease in serum cholesterol concentration following the removal of 2 eggs/day from the control diet. The observed value was somewhat lower than the predicted value.

A prediction equation was developed by Mattson et al. (1972) which related changes in dietary cholesterol to serum cholesterol levels. This equation predicted a 12 mg/100 ml change for each 100 mg change in dietary cholesterol/1000 kcal, or a 20 mg decrease in serum cholesterol concentration for subjects on the control diet following the removal of eggs from the diet.

Based on the evaluation of the combined results of 19 sets of experiments by several different investigators, Keys et al. (1974) predicted that the serum cholesterol response to the addition of two eggs/day to the diet would be an increase of 10 or 12 mg. In the present study mean increases in serum cholesterol concentration of 25.1 and 19.1 mg were observed when eggs were added back to the control and modified fat diets, respectively, following the 4-week diet period in which eggs were replaced by egg substitute. These increases in serum cholesterol

concentration are approximately twice as large as the values predicted by Keys et al.

When the percent of total calories from fat was reduced from 45 to 35 and the P/S ratio was increased from 0.45 to 1.10 on the modified fat diet the subjects demonstrated a statistically significant decrease ($p<.05$) in total serum cholesterol. When eggs were replaced by egg substitute in the modified fat diet an additional decrease in serum cholesterol was seen.

The decrease in serum cholesterol concentration that occurred when subjects were placed on the modified fat diet was greater than the decrease in serum cholesterol that occurred when eggs were replaced by egg substitute. These findings are in agreement with those of Keys et al. (1965) and McGandy and Hegsted (1975), who found that the potential for altering serum cholesterol was greater by the modification of dietary fats than by restriction of dietary cholesterol. The results of the present study disagree with the conclusion by Mattson et al. (1972) that a change in the intake of cholesterol may be more important than a change in dietary fat in reducing serum cholesterol levels.

The degree of responsiveness of serum cholesterol levels to removal of eggs from the diet did not appear to be related to the subjects' initial serum cholesterol levels or to the order in which dietary changes were

initiated. For example, subject 19, whose control period cholesterol level was 224 mg/100 ml experienced a 44 mg decrease (19.6%) in cholesterol when eggs were removed from the diet (Table 7). Subject 4, on the other hand, whose cholesterol level during the control period was 262 mg/100 ml, experienced only a 6 mg or 2.6% decrease when eggs were removed from the diet. For both of these subjects eggs were replaced by egg substitute during the last four weeks of the study. The same lack of correlation was seen between initial serum cholesterol levels and magnitude of response to removal of eggs from the diet when the eggs were replaced by egg substitute immediately following the control period. Subject 31 whose control period serum cholesterol level was 224 mg/100 ml experienced a 23 mg or 10.3% decrease in serum cholesterol when eggs were replaced by egg substitute (Table 8). Subject 7 whose control period serum cholesterol level was 262 mg/100 ml demonstrated a 28 mg decrease (10.6%) when placed on the egg-free diet.

Similarly, the magnitude of response to cholesterol intake on the modified fat diets did not appear to be related to the subjects' initial serum cholesterol levels or to the order in which eggs were added or deleted from the diet. Subject 22 whose control period serum cholesterol level was 236 mg/100 ml showed a 48 mg or 20.3% decrease in total serum cholesterol after consuming the modified fat

diet with two eggs/day for four weeks (Table 9). In contrast, subject 27 from the same diet group experienced a 30 mg or 11.3% decrease from his control period value of 264 mg/100 ml. When eggs were removed from their diet during the last four weeks of the study, subject 22 experienced no additional decrease in serum cholesterol whereas subject 27 experienced a 17 mg or 7.3% decrease. The degree of responsiveness of serum cholesterol concentrations to changes in the diet did not appear to be related to family history of heart disease, hypertension, diabetes, or to tendency to overweight.

CONCLUSIONS

The results of this study indicate that mean reductions in serum cholesterol of approximately 5 to 10% can be achieved in healthy young men when two eggs/day are replaced by a cholesterol-free egg substitute in a typical American diet. A 10 to 12% reduction in serum cholesterol was achieved when subjects consumed diets lower in total and saturated fat, and higher in polyunsaturated fat; when eggs were replaced by the egg substitute in this modified fat diet a 15 to 19% mean reduction in serum cholesterol was achieved. These findings are in agreement with those reported by Christakis et al. (1966), the National Diet-Heart Study Research Group (1968), Turpeinen et al. (1968) and Leren (1970), who demonstrated mean reductions in blood cholesterol of 10 to 15% with diet modifications in men participating in longitudinal studies. These studies have shown that with continued diet modification these reductions in serum cholesterol can be maintained for years.

Although a causal relationship between serum cholesterol concentration and coronary heart disease has yet to be proven, strong evidence exists that suggests that

elevated serum cholesterol is an important risk factor in the development of atherosclerosis. The results of this study have shown that it is possible to decrease serum cholesterol level by diet modification. Whether diet modification will lead to a reduction in the prevalence of CHD remains to be seen. It is only logical, however, that an attempt should be made to reduce or remove the risk factors over which man has control.

SUGGESTIONS FOR FURTHER RESEARCH

The results of the present study indicate that addition or deletion of two eggs/day and modification of the amount and type of fat in the diet do influence the concentration of cholesterol in the serum of healthy, young men with moderately elevated levels of serum cholesterol. A similar study conducted on larger population samples would enable one to more accurately quantitate the effects of these two types of diet modifications on serum cholesterol. It would seem highly desirable to increase the length of the experimental diet periods to determine whether further changes or adaptation of serum cholesterol levels would occur with time.

APPENDICES

APPENDIX A

APPENDIX A-1

Consent form for screening serum cholesterol determination

Michigan State University
Department of Food Science and Human Nutrition

Human Cholesterol Study
1977

CONSENT FORM

I, _____, agree to participate in a screening test for a study of the effect of the dietary intake of eggs and an egg substitute on serum cholesterol. The purpose and the nature of the study have been explained to me by _____, and I have been given an opportunity to ask questions. Criteria for selection of participants in the study have been described and I agree to the preliminary blood tests and physical examination, realizing that they will be used to determine my eligibility for further participation. I understand that my participation in these preliminary tests does not guarantee that I will be selected as a subject for this study. I understand that I can withdraw from the study at any time without penalty and that results will be treated in strict confidence.

Signed:_____

Date:_____

APPENDIX A-2

Consent form for participation in the cholesterol study

DEPARTMENT OF FOOD SCIENCE AND HUMAN NUTRITION

MICHIGAN STATE UNIVERSITY

EAST LANSING, MICHIGAN

I, _____, agree to participate in a study of the effect of the dietary intake of eggs and an egg substitute on serum cholesterol. The purpose and nature of the study have been explained to me by _____, and I have been given an opportunity to ask questions. Criteria for selection of participants in the study have been described and I agree to the preliminary blood tests and physical examination, realizing that they will be used to determine my eligibility for further participation. If I am selected for the study I am aware of the potential risks which may be involved as well as any possible benefits. However, I understand that my participation in the study does not guarantee any beneficial results to me as an individual. My participation in this experiment can be terminated by me at any time without penalty. I understand that the results of the study will be treated in strict confidence and that I will remain anonymous. A summary of the results of this experiment will be provided to me at my request.

Signed:_____

Date:_____

APPENDIX B

APPENDIX B

Two week sample menu for subjects on the control diet with 2 eggs/day[a]

Week 1

	BREAKFAST	LUNCH	DINNER
MON	Pineapple juice Eggs English muffin Butter, jelly Milk	Macaroni & cheese Tossed salad Kraft cr. Russian drsg Canned peaches Peanut butter cookies	Baked pork loin (170 g)/cr. mushroom soup Stovetop dressing Brussel sprouts Spicy applesauce Cherry bavarian Bread;butter
TUES	Apricot juice Eggs Toast Butter, jelly Milk	Hamburger (180 g)/bun Potato chips Raw spinach salad/creamy Russian dressing Sherbet	Baked fish (170 g)/dill and wine sauce Baked potato/sour cream Cut green beans Tossed salad/blue cheese drsg Angel cake/frozen strawberries Bread, butter Milk
WED	Orange juice Cold cereal Banana Toast Butter, jelly Milk	Beef noodle Cup-A-Soup Egg salad/tomato & cucumber slices Rye bread Oatmeal cookies Milk	Beef kabobs (170 g)/green pepper, onion, mushrooms Rice Carrot sticks Baked custard/grape-nuts Bread, butter Milk
THURS	Grapefruit juice Eggs Toast Butter, jelly Milk	Sliced ham (80 g)/Syrian bread Potato salad Dill pickle spear Baked apple/dates, cinnamon & brown sugar Milk	Chicken cashew - 1 serving Chow mein noodles Broccoli Coconut mandarin salad Lemon bisque Bread, butter Milk

	BREAKFAST	LUNCH	DINNER
FRI	Canned peaches Cold cereal Raisins Date nut muffin Butter, jelly Milk Sugar	Roast beef sandwich (120 g) Celery sticks Banana Sugar cookies Butter Milk	Omelet/mushrooms French fries Sausage links Tossed salad/French dressing Chocolate cake Bread, butter Milk
SAT	Apple juice Fried eggs Toast Butter, jelly Milk	Turkey (113 g)/ sandwich lettuce, tomato, mayonnaise Coleslaw Jello/peach & pear halves Milk	Swiss steak (170 g) Parsley potato Pea pods Cottage cheese/pine-apple ring Spiced apple cake Bread, butter Milk
SUN	Snack Milk Cereal Fruit	Orange juice Eggs Canadian bacon (85 g) French toast/sirup Butter Milk	Baked pork loin (170 g)/barbecue sauce Oven fried potato Peas Waldorf salad Ice cream/chocolate sauce, pecans Banana Bread, butter Milk

[a]Replacement of eggs with egg substitute was the only change made in the diet when subjects were placed on their respective egg-free diets.

The portion sizes of many food items (e.g. juice, bread, vegetables, fruit, baked goods, butter/margarine, salad dressings, and milk) varied from subject to subject according to type of diet (Control vs Modified Fat), energy requirements, and food preferences.

Coffee and tea were available at each meal and were allowed ad libitum.

Week 2 - Control

	BREAKFAST	LUNCH	DINNER
MON	Grapefruit half Eggs Toast Butter, jelly Milk	Chili con carne Crackers Tossed salad/Italian dressing Oatmeal cookies Butter Milk	Baked veal cutlet (142 g) Rice Cut green beans Cucumber & onion/ mock sour cream Yellow cake/praline topping Bread, butter Milk
TUES	Pineapple juice Eggs Bran muffins Butter, jelly Milk	Fish fillets (135 g)/bun Tartar sauce Carrot & celery sticks Peanut butter cookies Apple Milk	Broiled steak (170 g) Baked potato Zucchini vegetable medley Tomato juice Sherbet Bread, butter Milk
WED	Banana Orange juice Cold cereal Toast Butter, jelly Milk	Hamburger (180 g)/ bun Carrot sticks Pineapple upside down cake Milk	Quiche/cheese, Bac0-bits Broccoli Tossed salad/French dressing Fresh mixed fruit cup Bread, butter Milk
THURS	Cranapple juice Eggs Date nut muffin Butter, jelly Milk	Chicken noodle Cup-A-Soup Peanut butter/jelly sandwich Potato chips Orange Sugar cookies Milk	Meatloaf - 1 serving Potato kuegel Peas/mushrooms Pear/shredded cheese on endive Ice cream Bread, butter Milk
FRI	Apricot juice Eggs Toast Butter, jelly Milk	Hot dog (91 g)/bun catsup, mustard, pickle relish French fries Celery sticks Brownie Milk	Oven baked chicken (120 g) Acorn squash/brown sugar 3 bean salad Cherry cobbler Bread, butter Milk

	BREAKFAST	LUNCH	DINNER
SAT	Grapefruit juice Eggs Toast Butter, jelly Milk	Ham (113 g) sandwich/ lettuce, mustard Potato salad Apple crisp Milk	Pizza/sausage-1 serving Tossed salad/Italian dressing Canned peaches Sugar cookies Milk
SUN	Snack Milk Cereal Banana	Orange juice Eggs Sausage (41 g) Waffles/sirup & butter Milk	Roast beef (150 g ck) Mashed potatoes/gravy Green beans Tossed salad/French dressing Pumpkin pie Bread, butter Milk

Two week sample menu for subjects on the modified fat diet with 2 eggs/day[a]

Week 1

	BREAKFAST	LUNCH	DINNER
MON	Pineapple juice Eggs English muffins Jelly, margarine Skim milk	Macaroni & cheese (skim milk) Tossed salad/creamy Russian dressing Canned peaches Peanut butter cookie Skim milk	Baked pork (142 g)/ cr mushroom soup Stovetop dressing Brussel sprouts Spicy applesauce Cherry bavarian Bread, margarine Skim milk
TUE	Apricot juice Eggs Toast Margarine, jelly Skim milk	Hamburger (120 g)/bun Potato chips Raw spinach salad/ creamy Russian dressing Sherbet Margarine Skim milk	Baked fish (170 g)/ dill & wine Baked potato/margarine Cut green beans Tossed salad/blue cheese dressing Angel cake/frozen strawberries Bread, margarine Skim milk
WED	Orange juice Cold cereal Banana Toast Margarine, jelly Skim milk Sugar	Beef noodle Cup-A-Soup Egg salad/ tomato & cucumber slices Rye bread Oatmeal cookies Skim milk English walnuts	Beef kabobs (142 g)/ green pepper, onion, mushrooms Rice Carrot sticks Baked custard (skim)/ grapenuts Bread, margarine Skim milk
THURS	Grapefruit juice Eggs Toast Margarine, jelly Skim milk	Pineapple juice Ham (80 g)/Syrian bread Potato salad Dill pickle spear Baked apple/dates, brown sugar, Eng walnuts, cinnamon Skim milk	Chicken cashew - 1 serving Chow mein noodles Broccoli Coconut mandarin salad Lemon bisque (skim) Bread, margarine Skim milk

	BREAKFAST	LUNCH	DINNER
FRI	Canned peaches Cold cereal Bran muffin Margarine, jelly Skim milk	Roast beef (120 g) sandwich Celery sticks Banana Sugar cookies Skim milk	Omelet (skim, margarine)/mushrooms Sausage links French fried potatoes Tossed salad/French dressing Chocolate cake Bread, margarine Skim milk
SAT	Apple juice Fried eggs Toast Margarine, jelly Skim milk	Turkey (113 g) sandwich/lettuce, tomato, mayonnaise Coleslaw Jello/peach & pear halves Skim milk	Swiss steak (170 g) Parsley potato Pea pods Cottage cheese (low fat)/pineapple ring Spiced apple cake Bread, margarine Skim milk
SUN	HS snack Cold cereal Skim milk Apple	Orange juice Eggs Morningstar breakfast slices French toast/sirup Margarine Skim milk Apricots	Baked pork loin (114 g)/barbecue Oven fried potatoes Peas Tossed salad/French dressing, ½ hard cooked egg Sherbet/chocolate sauce/pecans Banana Bread, margarine Skim milk

Week 2 - Modified

	BREAKFAST	LUNCH	DINNER
MON	Grapefruit half Eggs Toast Margarine, jelly Skim milk	Apple juice Chili con carne Crackers Tossed salad/Italian dressing Oatmeal cookies Rosano cheese (chol-free) Skim milk	Baked veal cutlet (142 g) Rice Cut green beans Cucumber & onion/mock sour cream Yellow cake/praline topping Bread, margarine Skim milk
TUE	Pineapple juice Eggs Bran muffins Margarine, jelly Skim milk	Fish fillets (100 g)/ bun Tartar sauce Tossed salad/French dressing Peanut butter cookie Apple Skim milk	Broiled steak (142 g) Baked potato Zucchini vegetable medley Tomato juice Sherbet/chocolate sauce Margarine Skim milk Snack: fruit
WED	Banana Orange juice Cold cereal Toast Margarine, jelly Skim milk	Hamburger (90 g)/bun catsup, mustard Carrot sticks Pineapple upside down cake Skim milk	Quiche (skim)/BacObits Broccoli Tossed salad/French dressing Fresh mixed fruit cup Bread, margarine Skim milk
THURS	Cranapple juice Eggs Date nut muffin Margarine, jelly Skim milk	Chicken noodle Cup-A-Soup Peanut butter/jelly sandwich Orange Sugar cookies Skim milk	Meatloaf-1 serving Potato kuegel Peas/mushrooms Pear/shredded cheddar cheese on endive Ice cream Bread, margarine Skim milk
FRI	Apricot juice Eggs Toast Margarine, jelly Skim milk	Hot dog (91 g)/bun catsup, mustard, pickle relish Celery sticks Brownie Skim milk	Oven baked chicken (120 g) Acorn squash/brown sugar 3 bean salad Cherry cobbler Bread, margarine Skim milk Snack apple

	BREAKFAST	LUNCH	DINNER
SAT	Grapefruit juice Eggs Toast Margarine, jelly Skim milk	Ham (113 g) sandwich/ lettuce, mustard Potato salad Apple crisp Skim milk	Pizza/Morningstar sausage Tossed salad/Italian dressing Canned peaches Sugar cookies Skim milk
SUN	HS snack Skim milk Cold cereal Banana Sugar packet	Orange juice Eggs Sausage (41 g) Waffles/sirup & butter Skim milk	Roast beef (150 g ck) Mashed potatoes/ gravy Green beans Celery sticks Pumpkin pie Bread, margarine Skim milk

APPENDIX C

APPENDIX C-1

Serum Cholesterol Determination by the Laboratory at Michigan State University

Reagents

1. $FeSo_4:CH_3COOH$

   Place about 60 g ferrous sulfate ($FeSO_4:7H_2O$) in 1 liter glacial acetic acid. Allow to stand for at least 3 hours. Filter and store in glass bottle at room temperature. Solution remains stable for several months (Do not use if solution has any color).

2. Stock Cholesterol Standard

   5 mg cholesterol/ml chloroform

   Store in refrigerator

Procedure

1. A series of working standards were prepared daily using the stock cholesterol standard and chloroform methanol.

mg/100 ml	Stock Cholesterol Standard	$CHCL_3$
100	0.2	0.8
200	0.4	0.6
300	0.6	0.4
500	1.0	0.0

2. With micropipeter, transfer an aliquot of serum or standard (0.1 ml) to small test tube. For blank, use chloroform.
3. Add 2.5 ml chloroform-methanol solution (2:1).
4. Mix.
5. Cover test tubes with marbles and place in hot water bath (50-60) for 15 min.
6. Cool.
7. Mix.
8. Centrifuge at 3000 rpm for 15 min.
9. With automatic dilutor, remove 1.0 ml of the supernatant and add to it 6.0 ml $FeSO_4 \cdot CH_3COOH$ in large test tube.
10. Add 2.0 ml H_2SO_4 (conc.) and immediately mix thoroughly.
11. Cool.
12. Read Optical Density at 490 mμ.

Principles of the Procedures for Quantitative Determination of Cholesterol in Serum by the Hycel Super Seventeen Method

1. Extraction: Cholesterol and cholesterol esters are soluble in organic solvents but almost insoluble in aqueous solvents.

2. Color Reaction: Cholesterol is dehydrated in an acidic medium to yield 3,5-cholestadiene. There is some question about the reactions that follow which produce the green color measured at 625 nm. It has been thought that the cholestadiene is dimerized to bis-3,5-cholestadiene which in turn reacts with sulfuric acid to form cholestadiene-3-monosulfonic acid. Recently, however, evidence has been presented which indicates that the cholestadiene is oxidized by sulfuric acid to yield a pentaenylic cation which is the colored species.

APPENDIX D

APPENDIX D

Average weekly body weights (lbs) for individual subjects[a]

	Weeks									
Subject	1	2	3	4	5	6	7	8	9	10
1	158	158	157	156	155	155	155	155	154	153
2	171	169	168	168	168	167	166	165	165	164
3	145	145	144	144	142	142	141	140	140	139
4	162	160	158	157	153	152	151	150	149	147
5	193	192	191	192	192	192	192	192	192	192
6	210	209	208	208	207	206	204	204	204	203
7	150	148	148	148	147	147	146	145	144	144
8	170	168	165	166	168	169	168	168	168	169
9	154	154	153	152	150	151	149	149	148	147
10	178	178	179	180	178	180	181	180	181	182
11	156	154	155	154	150	150	150	151	150	151
12	170	169	169	169	167	166	164	164	164	163
13	173	171	170	169	169	169	167	167	166	165
14	182	181	181	181	181	180	178	178	178	177
15	172	172	170	166	166	166	163	163	163	161
16	155	155	154	152	151	150	150	149	149	147
17	186	187	188	188	188	187	188	189	188	188
18	144	144	143	143	142	142	142	141	142	142
19	149	149	149	149	149	149	149	149	150	150
20	176	180	180	180	180	180	180	179	179	179

APPENDIX D (cont'd.).

Subject	Weeks									
	1	2	3	4	5	6	7	8	9	10
21	-	158	158	158	158	157	157	157	155	154
22	184	181	180	180	181	180	181	180	180	178
23	182	183	182	181	179	180	180	180	177	176
24	160	158	158	158	157	157	158	156	156	155
25	155	157	156	155	158	156	155	156	154	153
26	156	154	155	156	157	156	157	157	156	157
27	186	186	186	184	184	183	181	181	180	178
28	162	164	165	166	166	167	167	167	167	166
29	174	172	171	172	172	170	171	171	171	170
30	209	207	206	206	206	205	203	202	201	199
31	135	133	134	135	135	135	136	136	135	135
32	-	137	137	138	137	137	137	136	134	134

[a]Average weekly body weights were found by calculating the mean value for daily body weights measured on Friday through Thursday of each week.

APPENDIX E

APPENDIX E-1

Average nutrient intake for subjects fed the control diet with 2 eggs/day (C-E) during the first experimental diet period, and egg substitute (C-ES) during the second experimental diet period.

	Protein (g)		% Protein		Total Fat (g)		% Fat		P/S		Cholesterol (mg)	
Subject	C-E	C-ES	C-E	C-ES	C-E	C-ES	C-E	C-ES	C-E	C-ES	C-E	C-ES
1	117.2	116.7	16.6	16.4	138.3	141.0	44.1	44.6	0.46	0.59	881	329
4	117.2	117.4	16.6	16.1	138.3	143.1	44.1	44.1	0.48	0.58	881	332
10	116.7	114.6	15.4	15.6	143.2	141.2	42.5	43.2	0.44	0.55	885	327
13	127.2	126.2	15.8	15.7	153.5	154.8	42.8	43.2	0.44	0.56	923	368
19	117.3	117.8	16.1	16.0	140.4	142.9	43.4	43.8	0.45	0.58	881	332
20	117.4	116.9	16.6	16.5	137.3	140.5	43.7	44.5	0.47	0.59	888	328
24	117.2	117.4	16.6	16.6	138.2	140.6	44.0	44.8	0.44	0.54	884	338
25	108.7	109.6	16.6	16.6	129.5	132.6	44.4	45.2	0.49	0.64	844	299

EPPENDIX E-2

Average nutrient intake for subjects fed the control diet with egg substitute (C-ES) during the first experimental diet period, and 2 eggs/day (C-E) during the second experimental period.

	Protein (g)		% Protein		Fat (g)		% Fat		P/S		Cholesterol (mg)	
Subject	C-ES	C-E	C-ES	C-E	C-ES	C-E	C-ES	C-E	C-ES	C-E	C-ES	C-E
7	112.8	116.3	16.7	15.9	135.2	140.7	43.4	43.4	0.58	0.44	306	885
8	125.6	122.6	16.6	16.2	149.2	146.6	44.2	43.6	0.56	0.44	360	906
14	116.2	115.9	16.4	16.4	140.4	138.5	44.7	44.1	0.60	0.46	329	880
15	129.9	134.0	15.3	14.8	161.4	171.3	42.8	42.6	0.54	0.42	378	948
17	126.8	127.1	15.8	15.8	157.8	155.3	43.5	43.4	0.54	0.44	369	918
18	113.1	119.5	16.2	15.6	136.6	146.1	44.2	48.9	0.56	0.44	329	895
23	128.2	129.1	15.9	15.6	159.1	160.3	44.3	43.6	0.54	0.43	367	926
31	118.5	117.1	15.7	15.6	145.0	142.7	43.3	42.6	0.57	0.45	336	885

APPENDIX E-3

Average nutrient intake for subjects fed the modified fat diet with 2 eggs/day (MF-E) during the first 4-week experimental diet period and egg substitute (MF-ES) during the second 4-week experimental diet period.

	Protein (g)		% Protein		Total Fat (g)		% Fat		P/S		Cholesterol (mg)	
Subject	MF-E	MF-ES	MF-E	MF-ES	MF-E	MF-ES	MF-E	MF-ES	MF-E	MF-ES	MF-E	MF-ES
3	115.4	106.2	17.5	16.0	103.0	106.0	35.2	36.0	1.06	1.30	730	178
5	132.1	131.8	16.4	16.3	123.2	125.3	34.5	34.9	1.12	1.36	737	187
11	117.9	122.0	17.3	16.7	105.7	114.0	34.9	35.9	1.08	1.34	731	181
12	121.3	128.2	16.6	16.3	114.9	123.0	35.2	35.6	0.96	1.18	729	184
22	135.1	136.9	15.2	15.2	132.5	133.2	33.6	33.8	1.17	1.35	740	188
27	129.8	129.8	16.7	16.7	121.3	121.6	35.0	35.2	1.14	1.27	712	185
29	138.2	157.0	15.5	15.6	138.7	154.8	34.8	34.7	1.17	1.36	742	215
32	114.6	114.2	18.0	19.4	101.8	103.2	34.9	35.2	1.08	1.32	728	178

APPENDIX E-4

Average nutrient intake for subjects fed the modified fat diet with egg substitute (MF-ES) during the first experimental diet period and 2 eggs/day (MF-E) during the second experimental diet period.

	Protein (g)		% Protein		Fat (g)		% Fat		P/S		Cholesterol (mg)	
Subject	MF-ES	MF-E	MF-ES	MF-E	MF-ES	MF-E	MF-ES	MF-E	MF-ES	MF-E	MF-ES	MF-E
2	130.0	132.4	16.7	16.4	121.4	123.4	35.1	34.4	1.37	1.13	189	732
6	132.6	133.3	16.5	16.0	123.7	127.3	34.6	34.4	1.39	1.15	189	733
9	114.5	112.0	17.3	17.1	104.5	102.6	35.6	35.2	1.35	1.06	181	726
16	117.8	120.6	16.6	16.4	110.7	111.3	35.2	34.1	1.36	1.09	183	730
21	122.8	122.6	16.8	16.8	114.3	111.6	35.2	34.4	1.36	1.09	182	719
26	129.2	130.8	16.3	16.2	123.1	121.4	34.8	34.0	1.36	1.11	185	721
28	121.7	120.6	17.2	17.1	111.8	109.8	35.5	34.9	1.34	1.08	181	719
30	122.0	120.8	14.6	14.6	124.5	121.6	33.6	33.0	1.36	1.10	184	720

APPENDIX F

APPENDIX F-1

Table of all serum cholesterol values (mg/100 ml) for subjects fed the control diet with 2 eggs/day (C-E) during the first experimental diet period, and egg substitute (C-ES) during the second experimental diet period.

	Control Period: C-E				Experimental Period 1: C-E					Experimental Period 2: C-ES				
Subject	Blood Sample Number													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	219	-	267	258	263	248	282	257	283	217	263	228	225	211
4	224	-	274	251	220	206	219	238	226	217	222	219	242	211
10	194	-	216	221	216	211	206	204	205	192	213	218	217	221
13	184	-	196	194	198	196	195	192	192	161	193	199	197	201
19	211	236	220	229	203	226	230	222	228	198	194	185	170	192
20	293	307	289	288	272	283	259	256	272	223	217	241	236	257
24	224	239	233	226	210	223	208	214	208	201	188	183	181	198
25	270	252	250	237	235	226	225	239	241	198	202	185	221	232

APPENDIX F-2

Table of all serum cholesterol values (mg/100 ml) for subjects fed the control diet with egg substitute (C-ES) during the first experimental diet period, and 2 eggs/day (C-E) during the second experimental diet period.

	Control Period: C-E				Experimental Period 1: C-ES					Experimental Period 2: C-E				
Subject	Blood Sample Number													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
7	-	-	269	259	236	263	272	244	228	254	279	288	292	297
8	256	-	242	221	188	252	221	211	189	195	236	218	245	236
14	216	-	238	226	208	215	221	210	202	191	204	199	198	193
15	220	-	199	191	155	193	182	169	158	189	217	200	210	203
17	181	196	194	205	191	175	183	180	181	201	194	186	181	212
18	227	221	215	193	197	172	167	184	175	194	181	186	164	195
23	242	233	217	211	199	220	211	209	208	217	212	206	232	220
31	233	253	219	226	210	234	219	208	194	217	231	224	233	243

APPENDIX F-3

Table of all serum cholesterol values (mg/100 ml) for subjects fed the modified fat diet with two eggs/day (MF-E) during the first experimental diet period, and egg substitute (MF-ES) during the second experimental diet period.

	Control Period: C-E				Experimental Period 1: MF-E					Experimental Period 2: MF-ES				
Subject	Blood Sample Number													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
3	221	-	199	201	185	195	188	146	160	164	183	169	181	175
5	236	-	219	211	198	201	212	210	191	155	204	207	201	200
11	267	-	242	249	218	203	197	219	205	165	184	185	190	182
12	236	-	225	233	209	211	215	205	192	158	180	182	181	180
22	239	247	234	237	208	-	219	191	184	183	191	202	183	193
27	259	295	275	253	250	236	234	237	231	201	212	211	210	224
29	180	190	203	188	174	190	175	175	170	164	162	156	167	172
32	-	-	-	218	225	222	219	228	215	183	193	185	190	208

APPENDIX F-4

Table of all serum cholesterol values (mg/100 ml) for subjects fed the modified fat diet with egg substitute (MF-ES) during the first experimental diet period, and 2 eggs/day (MF-E) during the second experimental diet period

	Control Period: C-E				Experimental Period 2: MF-ES					Experimental Period 2: MF-E				
Subject	Blood Sample Number													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
2	204	-	213	201	152	159	157	134	150	164	175	161	165	162
6	210	-	212	204	190	195	197	172	175	175	186	199	201	198
9	272	-	-	273	243	308	250	255	239	228	272	271	265	263
16	233	-	203	206	174	191	187	181	158	171	174	178	185	193
21	-	-	194	196	175	178	167	159	160	169	153	172	167	177
26	230	264	235	245	188	167	174	186	186	183	191	207	197	220
28	170	202	211	202	172	167	164	161	167	160	175	161	215	175
30	253	270	243	228	188	196	195	194	198	208	202	205	198	211

LIST OF REFERENCES

LIST OF REFERENCES

Adams, C.F. Nutritive Value of American Foods in Common Units. No. 456. Agricultural Research Service. United States Department of Agriculture. Washington, D.C. November, 1975.

Ahrens, E.H., D.H. Blackenhorn and T.T. Tsaltas. Effect of human serum lipids of substituting plant for animal fat in the diet. Proc. Soc. Exp. Biol. Med. 86:872-878, 1954.

Ahrens, E.H., T.T. Tsaltas, J. Hirsch and W. Insull. Effects of dietary fats on the serum lipids of human subjects. J. Clin. Invest. 34:918, 1955 (abstr.).

Ahrens, E.H., J. Hirsch, W. Insull, T.T. Tsaltas, R. Blomstrand and M.L. Peterson. The influence of dietary fats on serum-lipid levels in man. Lancet 1:943-953, 1957.

Ahrens, E.H. The management of hyperlipidemia: Whether, rather than how. Ann. Intern. Med. 85:87-93, 1976.

Ali, S.S., A. Kukis and J.M.R. Beveridge. Excretion of bile acids by three men on corn oil and butterfat diets. Can. J. Biochem. 44:1377-1388, 1966.

Anderson, B.A. Comprehensive evaluation of fatty acids in foods. VII. Pork products. J. Am. Diet. Assoc. 69: 44-49, 1976.

Anderson, B.A., G.A. Fristrom and J.I. Weihrauch. Comprehensive evaluation of fatty acids in foods. X. Lamb and Veal. J. Am. Diet. Assoc. 70:53-58, 1977.

Anderson, B.A., J.A. Kinsella and B.K. Watt. Comprehensive evaluation of fatty acids in foods. II. Beef products. J. Am. Diet. Assoc. 67:35-41, 1975.

Anderson, J.T., A. Lawler and A. Keys. Weight gain from simple overeating. II. Serum lipids and blood volume. J. Clin. Invest. 36:81-88, 1957.

Anderson, J.T., F. Grande and A. Keys. Cholesterol-lowering diets. J. Am. Diet. Assoc. 62:133-142, 1973.

Anderson, J.T., F. Grande and A. Keys. Independence of the effects of cholesterol and degree of saturation of the fat in the diet on serum cholesterol in man. Am. J. Clin. Nutr. 29:1184-1189, 1976.

Anitschkow, N. Experimental arteriosclerosis in animals. In: Arteriosclerosis. Cowdry, E.V., New York: McMillan Co. 1933, pp. 271-322.

Bersohn, J. and S. Wayburne. Serum cholesterol concentration in new-born African and European infants and their mothers. Am. J. Clin. Nutr. 4:117-123, 1956.

Beveridge, J.M.R., W.F. Connell, G.A. Mayer, J.B. Firstbrook and M.S. DeWolfe. The effects of certain vegetable and animal fats on the plasma lipids of humans. J. Nutr. 56:311-320, 1955.

Beveridge, J.M.R., W.F. Connell and G.A. Mayer. Dietary factors affecting the level of plasma cholesterol in humans: the role of fat. Canad. J. Biochem. 34:441-455, 1967.

Beveridge, J.M.R., W.F. Connell, G.A. Mayer and H.L. Haust. The response of man to dietary cholesterol. J. Nutr. 71:61-65, 1960.

Bierenbaum, M.L., D.P. Green, A.B. Caldwell and E.H. Kelly. Blood cholesterol and coronary heart disease: Preliminary report. J. Med. Soc. New Jersey 58:134-138, 1961.

Brignoli, C.A., J.E. Kinsella and J.I. Weihrauch. Comprehensive evaluation of fatty acids in foods. V. Unhydrogenated fats and oils. J. Am. Diet. Assoc. 68 224-229, 1976.

Bronte-Stewart, B., A. Antonis, L. Eales and J.F. Brock. Effects of feeding different fats on serum cholesterol level. Lancet 1:521-526, 1966.

Brunner, D., J. Weissbort, M. Fischer, J.E. Bearman, K. Loebl, S. Schwartz and S. Levin. Serum lipid response to a high-caloric, high fat diet in agricultural workers during 12 months. Am. J. Clin. Nutr. 32: 1342-1349, 1979.

Carey, M.A., J.D. Jones and C.F. Gastineau. Effect of moderate alcohol intake on blood chemistry values. J. Am. Med. Assoc. 216:1766-1769, 1971.

Carlson, L.A. and S. Bottiger. Ischaemic heart disease in relation to fasting values of plasma triglycerides and cholesterol. Stockholm prospective study. Lancet 1:865-868, 1972.

Chapman, J.M. and F.J. Massey. The interrelationship of serum cholesterol, hypertension, body weight and risk of coronary heart disease. Results of the first ten years follow-up in the Los Angeles heart study. J. Chron. Dis. 17:933-949, 1964.

Chobanian, A.V. and W. Hollander. Body cholesterol metabolism in man. 1. The equilibration of serum and tissue cholesterol. J. Clin. Invest. 41:1732-1736, 1962.

Christakis, G., S.H. Rinzler, M. Archer, G. Winslow, L. Jampel, J. Stephenson, G. Friedman, H. Fein, A. Kraus and G. James. The anti-coronary club. A dietary approach to the prevention of coronary heart disease--a seven year report. Am. J. Publ. Hlth. 56:299-314, 1966.

Connor, W.E., R.E. Hodges and R.E. Bleiler. The serum lipids in men receiving high cholesterol and cholesterol-free diets. J. Clin. Invest. 40:894-901, 1961.

Connor, W.E. and C.S. Jackson. The regression of cholesterol-4-C^{14} from the blood and tissues of animals: The effect of dietary corn oil compared with a low fat diet. Circulation 28:653, 1963 (abstr).

Connor, W.E., D.B. Stone and R.E. Hodges. The interrelated effects of dietary cholesterol and fat upon human serum lipid levels. J. Clin. Invest. 43:1691-1696, 1964.

Connor, W.E. and S.L. Connor. The key role of nutritional factors in the prevention of coronary heart disease. Prev. Med. 1:49-83, 1972.

Connor, W.E. and D.S. Lin. The intestinal absorption of dietary cholesterol by hypercholesterolemic (type II) and normocholesterolemic humans. J. Clin. Invest. 53: 1062-1070, 1974.

Cook, R.P., D.C. Edwards and C. Ridell. Cholesterol metabolism. 7. Cholesterol absorption and excretion in man. Biochem. J. 62:225-235, 1956.

De Langen, C.D. Cholesterike-stoffwisseling en rassenpathologie. Geneesk Tijdschrift Nederl-Indie 26:1-34, 1916.

Dreyfus, F. and J. Czaczkes. Blood cholesterol and uric acid of healthy medical students under stress of examination. Arch. Intern. Med. 103:708-711, 1959.

Enos, W.F., J.C. Beyer and R.H. Holmes. Pathogenesis of coronary disease in American soldiers killed in Korea. J. Am. Med. Assoc. 158:912-914, 1955.

Erickson, B.A., R.R. Coots, F.H. Mattson and A.M. Kligman. The effect of partial hydrogenation of dietary fats, of the ratio of polyunsaturated to saturated fatty acids, and of dietary cholesterol upon plasma lipids in man. J. Clin. Invest. 43:2017-2025, 1964.

Exler, J. and J.L. Weihrauch. Comprehensive evaluation of fatty acids in foods. VIII. Finfish. J. Am. Diet. Assoc. 69:243-248, 1976.

Feeley, R.M., P.E. Criner and B.K. Watt. Cholesterol content of foods. J. Am. Diet. Assoc. 61:134-149, 1972.

Flynn, M.A., G.B. Nolph, T.C. Flynn, R. Kahrs and G. Krause. Effect of dietary egg on human serum cholesterol and triglycerides. Am. J. Clin. Nutr. 32:1051-1057, 1979.

Friedman, M., R.H. Rosenman and S.O. Byers. Effect of moderate ingestion of alcohol upon serum triglyceride responses of normo- and hyperlipemic subjects. Proc. Soc. Exp. Biol. Med. 120:696-698, 1965.

Fristrom, G.A. and J.L. Weihrauch. Comprehensive evaluation of fatty acids in foods. IX. Fowl. J. Am. Diet. Assoc. 69:517-522, 1976.

Gotto, A.M. Introduction. In: Atherosclerosis Reviews, Vol. 5. Paoletti, R. and A.M. Gotto. New York: Raven Press, 1979, pp. vii-x.

Grande, F., J.T. Anderson, C. Chlouverakis, M. Proja and A. Keys. Effect of dietary cholesterol on man's serum lipids. J. Nutr. 87:52-62, 1965.

Grande, F., J.T. Anderson and A. Keys. Diets of different fatty acid composition producing identical serum cholesterol levels in man. Am. J. Clin. Nutr. 25:53-60, 1972.

Grundy, S.M. and E.H. Ahrens. The effects of unsaturated dietary fats on absorption, excretion, synthesis, and distribution of cholesterol in man. J. Clin. Invest. 49:1135-1152, 1970.

Gunning, B.E., K. Imaichi, S.D. Splitter and L.W. Kinsell. Effects of different fats on plasma lipid levels. Lancet 2:336-341, 1964.

Hardinge, M., H. Crooks and F. Stare. Nutritional studies of vegetarians. IV. Dietary fatty acids and serum cholesterol levels. Am. J. Clin. Nutr. 10:516-524, 1962.

Hegsted, D.M., R.B. McGandy, M.L. Myers and F.J. Stare. Quantitative effects of dietary fat on serum cholesterol in man. Am. J. Clin. Nutr. 17:281-295, 1965.

Helwig, L., P.H. Hofmeyer and J. Kieler. Famine disease in German concentration camps: complications and sequels. Acta. Med. Scand. 144 (suppl. 274):3-460, 1952.

Hildreth, E.A., S.M. Mellinkoff, G.W. Blair and D.M. Hildreth. The effect of vegetable fat ingestion on human serum cholesterol concentration. Circulation 3: 641-646, 1951.

Jolliffe, N. and M. Archer. Statistical associations between international coronary heart disease death rates and certain environmental factors. J. Chron. Dis. 9:636-652, 1959.

Jolliffe, N. Dietary factors regulating serum cholesterol. Metabolism 10:497-513, 1961.

Kannel, W.B., W.P. Castelli, T. Gordon and P.M. McNamara. Serum cholesterol, lipoproteins, and the risk of coronary heart disease. Ann. Intern. Med. 74:1-12, 1971.

Katz, L.N., J. Stamler and R. Pick. Nutrition and Atherosclerosis. Philadelphia: Lea and Febiger, 1958.

Keys, A., O. Mickelsen, E.V.O. Miller and C.B. Chapman. The relation in man between cholesterol levels in the diet and in the blood. Science 112:79-81, 1950.

Keys, A. Human atherosclerosis and the diet. Circulation 5:115-118, 1952.

Keys, A., J.T. Anderson, F. Fidanza, M.H. Keys and B. Swahn. Effects of diet on blood lipids in man: particularly cholesterol and lipoproteins. Clin. Chem. 1:34-52, 1955.

Keys, A., J.T. Anderson, O. Mickelson, S.F. Adelson and F. Fidanza. Diet and serum cholesterol in man. Lack of effect of dietary cholesterol. J. Nutr. 59: 39-56, 1956.

Keys, A., J.T. Anderson and F. Grande. Prediction of serum cholesterol responses of man to changes in fats in the diet. Lancet 2:959-966, 1957a.

Keys, A., J.T. Anderson and F. Grande. Essential fatty acids, degree of unsaturation, and effect of corn (maize) oil on the serum cholesterol level in man. Lancet 1:66-68, 1957b.

Keys, A., H.L. Taylor, H. Blackburn, J. Brozek, J.T. Anderson and E. Simonson. Coronary heart disease among Minnesota business men and professional men followed 15 years. Circulation 28:381-395, 1963.

Keys, A., J.T. Anderson and F. Grande. Serum cholesterol response to changes in the diet. I. Iodine value of dietary fat versus 2S-P. Metabolism 14:747-758, 1965a.

Keys, A., J.T. Anderson and F. Grande. Serum cholesterol response to changes in the diet. II. The effect of cholesterol in the diet. Metabolism 14:759-765, 1965b.

Keys, A., J.T. Anderson and F. Grande. Serum cholesterol response to changes in the diet. III. Differences among individuals. Metabolism 14:766-775, 1965c.

Keys, A., J.T. Anderson and F. Grande. Serum cholesterol response to changes in the diet. IV. Particular saturated fatty acids in the diet. Metabolism 14: 776-787, 1965d.

Keys, A. and R.W. Parlin. Serum cholesterol response to changes in dietary lipids. Am. J. Clin. Nutr. 19: 175-180, 1966.

Keys, A. Coronary heart disease in seven countries. Circulation 41 (Suppl. I), 1-19, 1970.

Keys, A., F. Grande and J.T. Anderson. Bias and misrepresentation revisited: Perspective on saturated fat. Am. J. Clin. Nutr. 27:188-212, 1974.

Keys, A. Coronary heart disease--the global picture. Atherosclerosis 22:149-192, 1975.

Kinsell, L.W., J. Partridge, L. Boling, S. Margen and G. Michaels. Dietary modification of serum cholesterol and phospholipid levels. J. Clin. Endocr. 12:909-913, 1952.

Kinsell, L.W., G.D. Michaels, J.W. Partridge, L.A. Boling, H.E. Balch and G.C. Cochrane. Effect upon serum cholesterol and phospholipids of diets containing large amounts of vegetable fat. J. Clin. Nutr. 1: 224-230, 1953.

Kummerow, F.A., Y. Kim, M.D. Hull, J. Pollard, P. Illinov, D.L. Dorossiev and J. Valek. The influence of egg consumption on the serum cholesterol level in human subjects. Am. J. Clin. Nutr. 30:664-673, 1977.

Leren, P. The Oslo diet heart study. Eleven year report. Circulation 42:935-942, 1970.

Leveille, G.A., J.W. Shockley and H.E. Sauberlich. Influence of dietary protein level and aminoacids on plasma cholesterol of growing chick. J. Nutr. 76:321-323, 1962.

Lewis, B. Effect of certain dietary oils on bile-acid secretion and serum cholesterol. Lancet 1:1090-1092, 1958.

Logan, W.P.D. Mortality from coronary and myocardial disease in different social classes. Lancet 1:758-759, 1952.

Malmros, H. and G. Wigand. The effect on serum cholesterol of diets containing different fats. Lancet 2:1-7, 1957.

Mattson, F.H., B.A. Erickson and A.M. Kligman. Effect of dietary cholesterol on serum cholesterol in man. Am. J. Clin. Nutr. 25:589-594, 1972.

Mayer, G.A., W.F. Connell, M.S. DeWolfe and J.M.R. Beveridge. Diet and plasma cholesterol levels. Am. J. Clin. Nutr. 2:316-321, 1954.

McGandy, R.B. and D.M. Hegsted. Quantitative effects of dietary fat and cholesterol on serum cholesterol in man. In: The Role of Fats in Human Nutrition. Vergroesen (ed.), New York: Academic Press, 1975.

Messinger, W.J., Y. Porosowska and J.M. Steele. Effect of feeding egg yolk and cholesterol on serum cholesterol levels. Arch. Intern. Med. 86:189-195, 1950.

National Diet-Heart Study Research Group. The national diet heart study: final report. Circulation 37 (Suppl. I):1-203, 1965.

Nestel, P.J. and A. Poyser. Changes in cholesterol synthesis and excretion when cholesterol intake is increased. Metabolism 25:1591-1599, 1976.

Oliver, M. and G. Boyd. Changes in plasma lipids during menstrual cycle. Clin. Sci. 12:217-222, 1953.

Ostrander, L.D., D.E. Lamphier, W.D. Block, B.J. Johnson, C. Ravenscroft, F.H. Epstein. Relationship of serum lipid concentrations to alcohol consumption. Arch. Intern. Med. 134:451-456, 1974.

Porter, M.W., W. Yamanaka, S.D. Carlson and M.S. Flynn. Effect of dietary eggs on serum cholesterol and triglyceride of human males. Am. J. Clin. Nutr. 30: 490-495, 1977.

Posati, L.P., J.E. Kinsella and B.K. Watt. Comprehensive evaluation of fatty acids in foods. I. Dairy products. J. Am. Diet. Assoc. 66:482-488, 1975.

Posati, L.P., J.E. Kinsella and B.K. Watt. Comprehensive evaluation of fatty acids in foods. III. Eggs and egg products. J. Am. Diet. Assoc. 67:111-115, 1975.

Posati, L.P. and M.L. Orr. Composition of foods. Dairy and egg products. Raw-Processed-Prepared. Agricultural Handbook No. 8-I, Agricultural Research Service, United States Department of Agriculture, Washington, D.C., November 1976.

Quintao, E., S.M. Grundy and E.H. Ahrens. An evaluation of four methods for measuring cholesterol absorption by the intestine in man. J. Lipid. Res. 12:221-232, 1971.

Quintao, E., S.M. Grundy and E.H. Ahrens. Effects of dietary cholesterol on regulation of total body cholesterol in man. J. Lipid. Res. 12:233-247, 1971.

Report of the Inter-Society Commission for Heart Disease Resources. Primary prevention of atherosclerotic diseases. Circulation 42:A55-A95, 1970 (Revised April 1972).

Rosenman, R.H., M. Friedman, R. Straus, C.D. Jenkins, S.J. Zyzanski and M. Wurm. Coronary heart diseast in the Western Collaborative Group Study. J. Chron. Dis. 23:173-190, 1970.

Searcy, R.L. and L.M. Berquist. A new color reaction for the quantification of serum cholesterol. Clin. Chim. Acta. 5:192-196, 1960.

Shorey, R.L., K. Brewten, B. Sewell and M. O'Brien. Alteration of serum lipids in a group of free-living adult males. Am. J. Clin. Nutr. 27:268-275, 1974.

Simons, L.A., J.C. Gibson, C. Paino, M. Kosking, J. Bullock and J. Trim. The influence of a wide range of absorbed cholesterol on plasma cholesterol levels in man. Am. J. Clin. Nutr. 31:1334-1339, 1978.

Slater, G., J. Mead, G. Dopeshwarkar, S. Robinson and R.B. Alfin-Slater. Plasma cholesterol and triglycerides in men with added eggs in the diet. Nutr. Rep. Intl. 14: 249-259, 1976.

Sodhi, H.S. and D.T. Mason. New insights into the homeostasis of plasma cholesterol. Am. J. Med. 63:325-327, 1977.

Stamler, J. Current status of the dietary prevention and treatment of atherosclerotic coronary heart disease. Progr. Cardiovas. Dis. 3:56-85, 1960.

Stanbury, J.B., J.B. Wyngaarden and D.S. Fredrickson. The Metabolic Basis of Inherited Disease. 3rd ed. New York: McGraw Hill, 1972, pp. 577, 600.

Steiner, A. and B. Domanski. Dietary hypercholesterolemia Am. J. Med. Sci. 201:820-824, 1941.

Steiner, A., E.J. Howard and S. Akgun. Importance of dietary cholesterol in man. J. Am. Med. Assoc. 181: 186-189, 1962.

Torr, M., A. Katchalsky, J. Agmon and D. Allalouf. Atherosclerosis and related factors in immigrants to Israel. Circulation 22:265-279, 1960.

Turpeinen, O., M. Miettinen, M.J. Karvonen, P. Roine, M. Pekkarinen, E.J. Lehtosuo and P. Alivirta. Dietary prevention of coronary heart disease: Long-term experiment. Am. JL. Clin. Nutr. 21:255-276, 1968.

Vogel, J. The Pathological Anatomy of the Human Body. 1st ed. Philadelphia: Lea and Blanchard, 1847, p. 531.

Weihrauch, J.I., J.E. Kinsella and B.K. Watt. Comprehensive evaluation of fatty acids in foods. VI. Cereal products. J. Am. Diet. Assoc. 68:335-340, 1976.

Wilson, J.D. The quantification of cholesterol excretion and degradation in the isoptopic steady state in the rat: the influence of dietary cholesterol. J. Lip. Res. 5:409-417, 1964.

Wilson, J.D. and C.H. Lindsey. Studies on the influence of dietary cholesterol on cholesterol metabolism in isotopic steady state in man. J. Clin. Invest. 44:1805-1814, 1965.

Wood, P.D.S., R. Shioda and L.W. Kinsell. Dietary regulation of cholesterol metabolism. Lancet 2:604-607, 1966.

Wright, H.F. and J.H. Wilmore. Estimation of relative body fat and lean body weight in a U.S. Marine population. Aerospace Med. 45:301-306, 1974.