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The Effects of the Diet Suggested in the United States Dietary Goals on Serum Cholesterol Levels

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THE UNITED STATES DIETARY GOALS ON

SERUM CHOLESTEROL LEVELS

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This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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Abstract

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The effects of the diet suggested in the United States Dietary Goals on serum cholesterol levels was studied by using male Holtzman rats. There were twelve experimental diets. All of the diets contained 28% of total calories as fat. The type of fat was either saturated, unsaturated, or half saturated and half unsaturated. Two sources of carbohydrate, sucrose and cornstarch, and two levels of each, 52% and 62%, were examined. The source of protein was the same in all diets, but varied in amount, 10% or 20% of total calories.

The type of fat did not yield a significant difference in total cholesterol, but HDL cholesterol levels were significantly higher in diets with unsaturated fat. Diets containing sucrose yielded significantly higher total cholesterol levels than cornstarch based diets, especially when the fat was half saturated and half unsaturated. Percent HDL cholesterol levels were significantly higher in the starch diets than in the sucrose diets. Level of protein in the diets produced no significant effects except that total cholesterol and percent HDL cholesterol were highest when the lowest level of protein was combined with saturated fat. As the level of sucrose increased and levels of protein decreased, total cholesterol increased and percent

HDL cholesterol decreased. The effects were just the opposite with starch, total cholesterol decreased and percent HDL increased. From these results, it appears that the source and level of carbohydrate in the diet had the most effect on serum cholesterol levels.

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CHAPTER I

INTRODUCTION

The leading cause of death and disability in the United States and other industrialized nations is coronary heart disease (CHD). More deaths per year are caused by CHD than any other disease, including all forms of cancer combined. In 1983 there were more than one million heart attacks in the United States, more than half with fatal results (1). Many of those who recover remain disabled for the rest of their lives. The personal loss and the economic loss to society is considerable. According to the National Health, Lung, and Blood Institute, deaths from heart attacks annually cost Americans an estimated $60 billion in medical bills, lost wages, and productivity (1).

Because of the high incidence of CHD, much attention has been focused on finding a way to prevent this disease. It has been well established that high levels of total blood cholesterol are associated with an increased risk of CHD. Current interest centers upon examining ways to lower these levels (2-4).

Cholesterol, as well as phospholipids, triglycerides, and other fats, is transported in the plasma bound to protein. These fat transporting particles are called lipoproteins and are commonly broken down into groups according to their density. They are classified as chylomicrons, very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL), low density lipoproteins (LDL), and high density lipoproteins (HDL). The main carriers of cholesterol are LDL and HDL. Many epidemiological studies indicate that an increase in LDL and a decrease in HDL is related to increased incidence of cardiovascular disease and CHD (1-7).

One of the main functions of LDL seems to be the transport of cholesterol to all cells of the body, including cells of arterial walls (3,5). Although body cells need a steady supply of cholesterol to grow and function properly, they need to be protected against an excessive accumulation of cholesterol. Too much cholesterol in artery walls may lead to atherosclerosis, a disease commonly associated with CHD, which may cause complete blockage of the artery. One hypothesis is that HDL functions as a carrier of cholesterol from the body cells to the liver where it can be metabolically altered for excretion. If this hypothesis is correct, it would be a consistent point with observations of the apparent protective effects of HDL against atherosclerosis and CHD (5-7).

Total blood cholesterol (total-C) and the percentage of total cholesterol carried by LDL may be reduced and the percentage of total cholesterol carried by HDL may be increased by modification of the diet. Many studies have been conducted on diets lower in saturated fat and higher in polyunsaturated fat. Although there is some disagreement, results from most studies indicate that diets higher in polyunsaturates reduce total and LDL cholesterol (LDL-C), but decrease only slightly or have no effect on levels of HDL cholesterol (HDL-C) (4,8,9) (Table 1).

The source of protein in the diet has also been the object of some concern to researchers. In several studies, with the level and type of fat held constant, protein from plant sources was found to produce lower blood cholesterol levels than protein from animal sources (9,10). However, a detrimental effect was also reported in one study. The amount of HDL-C was found to decrease when plant protein was increased (10) (Table 1).

Different carbohydrate sources and levels appear to affect cholesterol to varying extents. Simple sugars, such as sucrose, and complex carbohydrates, such as cornstarch, have been shown to have similar effects on total cholesterol (11,12), but sucrose seems to produce a more favorable HDL-C:LDL-C ratio (11). Varying levels of sucrose in the diet may change these ratios; as the amount of sucrose in the diet increases, the HDL-C:LDL-C becomes less favorable (13) (Table 1).

Table 1
Dietary Influences on Cholesterol Levels

	Effect on	
Component of Diet	Total and LDL Cholesterol	HDL Cholesterol
Polyunsaturated Fat	Decrease	No Effect
Saturated Fat	Increase	Decrease
Plant Protein	Decrease	Decrease
Animal Protein	Increase	Decrease
Complex Carbohydrate	No Effect	No Effect
Simple Sugar	No Effect	No Effect
Very High Levels of Simple Sugar	Increase	Decrease

Statement of the Problem

Much research has been conducted on individual effects of carbohydrate, protein, and fat on cholesterol levels. Little has been done on the combination of the variables as suggested in the dietary goals recommended by the United States Senate Select Committee on Nutrition and Human Needs. Recommendations of this committee include protein as 12 percent of total calories, fat as 30 percent, and carbohydrate as the rest of the total calories. Reducing saturated fat to 10 percent of total calories and increasing polyunsaturated and monounsaturated fats to about 10 percent each is also recommended. The committee suggests limiting dietary intake of cholesterol to about 300 milligrams per day and increasing complex carbohydrates to about 48 percent of total calories while decreasing simple sugars to 10 percent (14).

The combination of all of these suggested dietary alterations is likely to influence individual effects of each. There is, therefore, a need to explore how implementation of these dietary goals affect total, LDL, and HDL cholesterol levels.

The purpose of this research was to examine the effects of the diet suggested in the United States Dietary Goals on the levels of blood cholesterol, with particular emphasis on HDL cholesterol and the percent of total cholesterol carried by HDL. Specific objectives of the study were:

1. to assess effects of diets based on the recommendations of the United States Senate Select Committee on Nutrition and Human Needs on total blood cholesterol levels,
2. to assess effects of diets based on the recommendations of the United States Senate Select Committee on Nutrition and Human Needs on HDL cholesterol levels, and
3. to assess effects of diets based on the recommendations of the United States Senate Select Committee on Nutrition and Human Needs on percent HDL cholesterol levels.

Definition of Terms

Throughout the paper, the following definitions of terms and abbreviations will apply.

Coronary Heart Disease (CHD): a general term used to describe many diseases of the heart and its blood vessels.

Atherosclerosis: a type of blood vessel disorder characterized by thickening of artery walls with a loss of elasticity and a build up of fat which may impede or block blood flow, commonly associated with CHD.

Fatty Acids: the main constituents of food fats and oils and of fats stored in the body. They are composed of carbon, hydrogen, and oxygen and are building blocks for a variety of fat and lipid molecules.

Saturated Fat: a fat, obtained mainly from animal sources, that tends to be solid at room temperature. Examples of saturated fatty acids are palmitic acid and stearic acid.

Polyunsaturated Fat: a fat, obtained mainly from plant sources, that tends to be liquid at room temperature. Examples of polyunsaturated fatty acids are linoleic acid and linolenic acid.

Monounsaturated Fat: a fat, obtained mainly from plant sources, which is slightly less fluid than polyunsaturated fat. An example of a monounsaturated fatty acid is oleic acid.

Cholesterol: a substance that is found in every cell of the human body and is essential to life. If accumulated in tissues, it may lead to disease conditions, such as atherosclerosis.

Lipoproteins: a combination of proteins and fats; the major carriers of cholesterol and other fats in the body.

Low Density Lipoproteins (LDL): large, light-weight type of lipoprotein, usually associated with an increased risk of CHD.

High Density Lipoproteins (HDL): the smallest, heaviest type of lipoprotein, often associated with a decreased risk of CHD.

Plasma: the fluid portion of the blood in which blood cells are suspended.

Serum: the clear portion of the plasma that does not contain blood cells, and remains fluid after clotting of the blood.

mg: milligram.

ml: milliliter.

CHAPTER II

REVIEW OF LITERATURE

The purpose of the study was to assess the effects of the diet suggested in the United States Dietary Goals on the levels of blood cholesterol. A considerable amount of research has been done on the relationship of various components of the diet with cholesterol levels. These components include types and amounts of carbohydrates, protein, and fat. Recent literature, as well as some of the ground breaking studies done in the past, will be reviewed on each of these components. To identify the reasons for the importance of conducting research on cholesterol levels, the literature on cholesterol levels and their effects on coronary heart disease (CHD) will be reviewed first.

Cholesterol Levels and Their Effects on Coronary Heart Disease

Coronary heart disease (CHD) is the number one cause of death and disability in the United States and other industrialized nations, and has been the object of much research (1). There are many factors other than blood cholesterol associated with risk for CHD, including family history, smoking, hypertension, obesity, alcohol consumption, behavior patterns, physical activity, diabetes mellitus, gender, age, and race (15). However, high levels of blood cholesterol seem to hold a key position in the development of this disease (16).

As early as the beginning of the century, a study conducted in Russia indicated that cholesterol might have an effect on the development of atherosclerosis, one of the major causes of CHD (17). Experiments were carried out with rabbits fed a high cholesterol diet. Upon examination of the wall of the aorta, atherosclerosis was found to be present. In the control group, fed a diet free from cholesterol, there was no evidence of atherosclerosis. Rabbits have since been found to be a poor choice for this type of study. Their normal diet contains no cholesterol and they are particularly sensitive to cholesterol added to their diet. Nevertheless, this was the beginning of countless investigations on other more suitable animals and humans. Many studies have confirmed this initial observation (18-24).

All body cells need a steady supply of cholesterol (25). It is needed for the formation of cell membranes and is one of the membrane's integral structural components. It is also used to form bile salts, which are essential for proper digestion of fat that is consumed in the diet. Small amounts of cholesterol are used by glands of the body to produce various hormones. A large amount is precipitated in the skin where it makes the skin resistant to absorption of water-soluble substances and the action of many chemical agents. Essentially all of the cholesterol that is required by the body can be produced by body tissues. There is a feedback mechanism that regulates how much is manufactured by the cells. As the amount of cholesterol consumed in the diet increases, the amount produced by the body decreases. This process seems to keep the level of cholesterol in the blood in balance until high levels of cholesterol are consumed. At this point, "abnormally" high serum cholesterol levels can occur (25).

But what is the "normal" level of cholesterol in serum? Much study has been devoted to trying to establish such a level. The issue of level has been examined in several studies of various population groups and were summarized by Dr. Jacob H. Reisel in 1968 (26). Table 2 shows the results of this summary, listing the mean values and ranges considered normal by the various authors.

Table 2
Normal Value of Cholesterol by Different Authors*

Author	Year		Mean value in mg./100 ml.	Range in mg./100 ml.
Bloor	1917		225	190-310
	1922			175-300
Epstein and Lande	1922			160-200
Verhoef	1920		187	161-216
Page et al.	1935			109-376
Sperry	1936			132-392
Peters and Man	1943		194	107-320
Kountz	1945		237 male	-
			196 female	-
Herrman	1947		204	165-410
Steiner	1948		254	214-334
Sperry, Webb	1950		-	150-260
Keys	1950	at age 20	175	100-250
		at age 50	200	150-370
Lewis et al.	1957		216-266	-
Joslin Clinic	1958		230	-
Groen et al.	1950		245-260	135-420

*From <u>Hypercholesterolemia and Clinical Atherosclerosis</u> by J.H. Reisel (26).

More recently, in 1979, Stamler examined five other major investigations (27). From these studies, referred to as the Framingham, Albany, Chicago Gas, Chicago Western Electric, and Tecumseh studies, he observed that serum cholesterol in normal American adults ranges from 140 milligrams (mg) per 100 milliters (ml) to more than

355mg per 100ml. He reported the mean value to be 234mg per 100ml (27).

The values from studies such as Reisel's and Stamler's have led most researchers to agree with Dr. Germain Brisson's interpretation that "66 per cent of adult U.S. citizens would normally have serum cholesterol levels varying between 190 and 280mg per 100ml," (17).

Normal does not necessarily mean optimal, however. In a report by Kannel in 1976, based on data collected in the Framingham study, it was noted that serum cholesterol levels below 200mg per 100ml correlated with a low frequency of CHD. Above 200mg per 100ml CHD frequency increased rapidly until 280mg per 100ml was reached. At 280mg per 100ml the frequency seemed to level off (28). This observation, along with support from many other studies (29-31) has led many to recommend a serum cholesterol level of less than 200mg per 100ml as optimal.

At the beginning of the 1960's researchers began to shift their focus with regard to serum cholesterol and its relationship to CHD. With improved techniques for analysis, it became easier to measure levels of lipoprotein cholesterol in the blood. Because of new methodology, the various lipoproteins began to receive more attention (32).

Cholesterol and other fats are not soluble in the blood and depend on interactions with protein molecules for transport. Cholesterol is transported to the cells of the body attached to lipoproteins, which are complexes of fat and protein. There are four main groups of lipoproteins, classified according to their density.

The groups that are of main concern when considering cholesterol are the low-density lipoproteins (LDL) and the high-density lipoproteins (HDL). In humans, LDL's contain an average of 65 to 70 percent of serum cholesterol whereas HDL's contain about 20 percent (3,32,33).

Because LDL's contain the largest percent of cholesterol, levels of this lipoprotein show high positive correlation with total cholesterol levels (total-C). Results of several studies have shown that when total cholesterol levels are high, LDL cholesterol (LDL-C) levels are also high. When total cholesterol levels are low, LDL-C are also low (5,34). Therefore, the positive correlation between total cholesterol and CHD is believed by many to also apply to LDL-C.

Most of the data on LDL indicates one of its major functions to be that of transporting cholesterol to the body cells where it can be utilized (3,5). But what controls the level of LDL in the blood has been the subject of much recent research. An article published in November 1984 (35) presents the theory of LDL receptors. Scientists at the University of Texas Health Science Center at Dallas are studying a group of specialized proteins that they call LDL receptors. These proteins project from animal cells, bind the LDL particles and take them into the cell. Here the LDL can be broken down and the cholesterol utilized. Because the LDL is being removed from the blood-stream, these receptors help to prevent an accumulation of cholesterol in the blood vessels which could lead to atherosclerosis. The LDL receptor theory states that when the cell's need for cholesterol is low it produces fewer LDL receptors to prevent too much cholesterol from entering the cell. This also decreases the rate at which LDL is removed from the blood, so serum cholesterol levels increase. The authors maintain that if their hypothesis is correct, the human is designed to operate with very low LDL levels and the current high levels in most of the industrialized adult world are a reason to be concerned (35). This theory could provide an explanation for the high correlation of LDL and the incidence of CHD.

Many researchers in the past have noted that atherosclerosis and CHD are found not only in persons with elevated levels of total blood cholesterol but often in those with total blood cholesterol levels considered to be normal. Researchers have observed that people in the latter group usually have a lower than normal level of HDL cholesterol (HDL-C). One resulting conclusion is that HDL may be protective against heart disease (3,5-7).

Too much cholesterol accumulation in the cells can be damaging, therefore cells need to be protected from an excessive amount of cholesterol. Current hypotheses have been put forth indicating that HDL's are the transporters of cholesterol <u>from</u> the body cells to the liver where it can be eliminated (6,7,36). This role of HDL could explain the data received from the Framingham study (7). When HDL-C levels from the subjects of this study were examined, they showed a strong negative association with incidence of CHD. Many other observational and experimental studies support this observation (37-40). Some researchers believe that HDL-C levels are a better indication of risk for atherosclerosis than either LDL-C or total cholesterol (6,7,36). As the percent of HDL-C increases, regardless of the level of total-C, these researchers see a beneficial effect.

Because of the strong evidence that elevated total serum cholesterol levels or low HDL-C levels increase the risk for CHD, much research is focused on finding effective ways to beneficially adjust these levels. Numerous experimental and epidemiological studies have been conducted to determine the effect of modification of the diet on cholesterol levels.

The Effects of Dietary Fat on Cholesterol Levels

A frequent concern of researchers in diet modification studies has been the source and amount of fat in the diet. High levels of dietary fat, especially saturated fat, and dietary cholesterol are suggested by many to be the main dietary factors influencing cholesterol levels. Therefore, much of diet modification research has been aimed at lowering the amount of saturated animal fat which is often accompanied by large amounts of cholesterol, and replacing portions of it with cholesterol free, polyunsaturated fat.

In a study conducted by Schaefer et al in 1981 (4), low cholesterol, high polyunsaturated fat, and low fat diets were examined for serum cholesterol-lowering effects. People with normal serum cholesterol levels and people with high serum cholesterol levels were both studied. People were determined to have high serum cholesterol levels if their total-C and LDL-C levels were two or more standard deviations above the normal mean. Initial levels were taken after subjects were on a "typical" American diet for several days and final levels were taken after being on the experimental diets for at least 14 days. Reduction of dietary cholesterol in the typical American diet with polyunsaturated to saturated fat (P:S) ratio of 0.1 to 0.3 had no significant effect on serum cholesterol levels. When dietary cholesterol was lowered and some of the saturated fat replaced with polyunsaturated fat (P:S ratio of 1.8 to 2.2) there were significant decreases in total cholesterol, LDL-C, and HDL-C in both the normal and the high cholesterol groups. The third diet had low levels of fat with a P:S ratio 0.1 to 0.3 and a cholesterol level even lower than the other two diets. This diet produced significant decreases in total cholesterol, LDL-C, and HDL-C in both groups. The researchers were somewhat concerned about the decrease in HDL-C levels of the last two diets. However, they observed no change in the LDL-C to HDL-C ratio (4).

Several other studies have also shown lower cholesterol levels when the polyunsaturated to saturated fat ratio was increased (8,9, 40-45). Like the Framingham study, Shepherd et al. (41,42) found decreases in HDL-C as well as in total-C and LDL-C. In an effort to understand the mechanisms of this effect, these researchers looked more closely at the composition of each lipoprotein group. They found decreases in the amount of cholesterol carried by each low-density lipoprotein when a high polyunsaturated fat diet (P:S ratio of 4.0 to 1.0) was consumed (41). The cholesterol content of the high-density lipoproteins was not significantly affected. Therefore, some of the reduction in LDL-C could be due to a decreased capacity of LDL to carry cholesterol, the implications of which are not completely understood.

Some studies did not support the finding of a decrease in HDL cholesterol levels (43,44). The studies that showed decreases in HDL-C were usually of short duration, three to five weeks. When longer term studies were conducted the HDL-C levels had risen back to the original level in as little as 8 to 12 weeks (43). One study which was continued for four years even produced a 20 percent increase in HDL-C levels (44).

Although it seems that the research is indicating more favorable serum cholesterol levels from a predominantly polyunsaturated fat diet, there are reasons to question the widespread recommendation of this type of diet. The fat that the human body synthesizes from nutrients supplied in the diet is almost totally saturated. It even seems that the body tries to avoid an accumulation of polyunsaturated fatty acids (PUFA), and, according to Brisson (17), may be trying to protect itself. Polyunsaturated fats easily undergo chemical changes that yield products which are potentially damaging to cells and which may increase the risk for cancer and other diseases. Normally the body can protect itself against these compounds with natural antioxidants such as vitamin E. Risks of cellular damage are increased with high levels of PUFA, however (17). The results of an eight year study conducted in Los Angeles (46) found no effect on total mortality when low saturated fat, high polyunsaturated fat diets were consumed. Although deaths from CHD were significantly lower, deaths from cancer and other diseases were significantly higher. Some researchers do not feel that the magnitude of risk due to increasing PUFA is outweighed by the relatively small decrease in cholesterol level (17).

Because of the risks of increasing the amount of polyunsaturated fat in the diet, some reseachers are of the opinion that lowering the amount of total fat in the diet is a better approach. Currently Americans consume about 40 percent of their calories as fat. Many health professionals feel that a reduction of this level to 30 to 35 percent would be reasonable. Among these are members of the American Heart Association (47), the Senate Select Committee on Human Nutrition (14), and the National Institutes of Health (48).

As a population group, Yemenite Jews generally have a low fat diet and low serum cholesterol levels. The effect of a high fat diet for 12 months on 26 male agriculture workers from this group was investigated (49). These men had moved from Yemen to Israel and provided a good opportunity to study how their new higher fat diet would affect serum cholesterol. They were placed on a high calorie, high fat diet for 7 months. For 3 months they returned to their customary low-caloric diet, and then they went back on the high caloric, high fat diet for 2 months. In the 5th to 7th months and again in the last 2 months, the men showed a significant increase in both total cholesterol and HDL-C, but not a significant change in the ratio of total-C to HDL-C. This study would seem to indicate that even a group with normally low cholesterol levels could be put at risk for CHD when put on a high fat diet. The trial diet also contained a lower P:S ratio and more dietary cholesterol than the regular diet of the men. Both of these factors could, therefore, have also contributed to the rise in serum cholesterol levels (49).

A clinically controlled experiment on the effects of lowering the level of dietary fat from 40 percent to 30 percent was conducted on eleven men from the Veterans Administration Medical Center in San Diego

(50). Two of the men had clinical evidence of coronary heart disease, while the rest were free from symptoms. The men were placed on three different liquid diets. One diet contained 40 percent fat, with 20 percent as saturated fat (S), 10 percent as monounsaturated fat (M), and 10 percent as polyunsaturated fat (P), (S:M:P=2:1:1). The second diet was 30 percent fat with S:P:M=1:1:1. The third diet was also 30 percent fat with S:P:M=2:1:1. None of the diets contained cholesterol. The men were fed each diet for one month. One subject did not receive diet one, and four persons did not receive diet three. The remaining six men received all three diets. Total-C, LDL-C, and HDL-C levels were taken twice each week. As a group there were no significant differences in total-C between the 40 percent fat diet and the 30 percent fat diets. But when the researchers examined individual patients more closely they concluded that most patients who began with total-C levels of over 200mg per 100ml showed a reduction in total-C when fat was reduced to 30 percent. There were no observed differences between the two 30 percent fat diets, which led the researchers to speculate that the P:S ratio at a fat intake of 30 percent may not be a good predictor of cholesterol changes. The LDL-C changes were virtually the same as the total-C changes. However, the HDL-C was significantly less in the lower fat diets, with a greater reduction in the diet with the lower amount of saturated fat. The percent of HDL-C was not significantly different in any of the three diets, however (50).

The effect of dietary cholesterol independent of the type and level of fat in the diet has also been investigated (51-52). In one

1972 study, a group of 44 male inmates of a Philadelphia County Prison were studied (51). All of the men were fed the normal institution diet for 7 days. After that week, the diet was gradually changed to a cholesterol free diet over the next 7 days. They remained on the cholesterol free diet for 21 days, after which time the serum cholesterol levels were determined. The men were then assigned to one of four groups. The groups were matched according to the decrease in serum cholesterol during the cholesterol-free diet as well as body weights and the absolute serum cholesterol level. Each group of men received a diet identical in every respect to each of the other groups except for the level of cholesterol. One group was continued on the cholesterol free diet, while each of the other three groups were fed a cholesterol-containing diet. The three levels of cholesterol used were 106 milligrams cholesterol per 1000 calories of diet, 212 milligrams cholesterol per 1000 calories of diet, and 317 milligrams cholesterol per 1000 calories of diet. The men were on these diets for six weeks. The serum cholesterol levels of the 212mg/1000 calorie group were significantly higher than the cholesterol free group and the serum cholesterol levels of the 317mg/1000 calorie group were approximately 25 percent higher than the cholesterol levels of the men on the cholesterol free diet (51). These results indicate that dietary cholesterol can have a major role in determing serum cholesterol levels.

Not all researchers have seen the same effects from the addition of cholesterol to the diet (52). In one study the addition of cholesterol to a diet produced no significant changes in serum cholesterol levels when compared to the same diet without added cholesterol. Both a saturated fat, coconut oil, and an unsaturated fat, corn oil, were tested.

Whether or not there is an interplay between dietary cholesterol and the composition of dietary fat has also been questioned. Some researchers have found the effects of cholesterol and degree of saturation of fat to be independent (53), but many others have found the effects to be dependent on each other (54-57). Two coordinated studies were conducted in which only the P:S ratio was different (54,55). In each study there were two levels of dietary cholesterol, less that 200mg per day and more than 600mg per day. One group had a high level of polyunsaturated fat while the other group had a low level. In both studies the serum cholesterol levels were higher in the high cholesterol diet than in the low cholesterol diet. However, the serum cholesterol level in the high dietary cholesterol group was significantly greater in the low polyunsaturated fat study than in the high polyunsaturated fat study. This led the researchers to conclude that "the effect of dietary cholesterol is clearly dependent on the type of fat present in the diet" (55).

Studies have also been conducted in free-living populations where dietary cholesterol levels were either increased or decreased as compared to their normal home diets (56,57). These studies also show an increase in serum cholesterol when cholesterol is added to the regular diet. One group of researchers (57) found the effect to be much smaller than that seen among studies of populations under controlled conditions, however.

The Effects of Dietary Carbohydrate on Cholesterol Levels

When the composition of the diet is altered by reducing the percentage of fat, the percentage of some other component must increase. Often that component is carbohydrate. Carbohydrate in turn then becomes a very large part of the diet, 58 percent by the United States Dietary Goals (14). In countries where diets are low in fat and high in carbohydrate, the serum cholesterol levels tend to be low. The dietary carbohydrate of these countries is generally starch rather than the high amount of simple sugars typical of the American diet. Therefore, the source of carbohydrate may contribute to the differences in cholesterol levels.

Low carbohydrate weight reducing diets were very popular in the 1960's and 1970's. Because of their widespread use, a study was conducted under the controlled conditions of the Clinical Research Center at the University of Iowa (58). They studied two categories of subjects. One was a group of four normal weight men and the other was a group of seven obese females. The men were placed on a weight maintenance diet with a low level of carbohydrate, either 12 grams per day or 62 grams per day. The amount of fat and protein in the remainder of the diet varied from month to month for five months, starting out with 70 percent fat and 30 percent protein and ending up with 30 percent fat and 70 percent protein. The last two weeks they were given a normal diet of 10 percent protein, 40 percent fat and 50 percent mixed carbohydrate. While the fat level was over 50 percent, the serum cholesterol of the men rose significantly, with the men on the higher level of carbohydrate experiencing a less significant rise.

After the level of fat went below 50 percent the cholesterol levels fell off and at the end were essentially the same as at the beginning of the experiment. The women were placed on a weight reduction diet low in carbohydrate, 12 grams per day. The fat and protein of this group only varied from 70 percent fat and 30 percent protein to 50 percent fat and 50 percent protein. Their cholesterol levels fell in the first phase of the experiment, but, as with the men, they tended to return to the initial level with the passage of time. This study suggests that a low carbohydrate weight reduction diet may lower the blood cholesterol levels for a short time, while a low carbohydrate weight maintenance diet may raise the blood cholesterol level for a short time (58).

There seems to be wide acceptance of the concept that complex carbohydrates have a cholesterol lowering effect as compared to sucrose. Beginning in the 1960's, a considerable amount of research has been and is still being dedicated to examining this issue. As early as 1955 Portman and his associates (59,60) demonstrated a lower blood cholesterol in rats fed a diet of starch as the source of carbohydrate versus a diet of sucrose.

More recently, a study on rats was conducted where the rats were placed on either a cereal-based stock diet, a purified high starch diet, or a purified high sucrose diet, each with or without brewer's yeast (61). The brewer's yeast produced no significant differences in cholesterol levels. There was no significant alteration in cholesterol levels over the four week experimental period of the rats on the cereal based stock diet. The purified high starch and purified high sucrose diets both showed a plasma cholesterol increase to similar levels. The percentage of LDL-C and HDL-C of the high starch diet were similar to the stock diet, while the percentage of HDL-C was reduced in the high sucrose diet (61).

A similar study was conducted by another group of researchers (62). In this study, rats were placed on a diet of 17 percent protein, 42 percent fat, and 41 percent carbohydrate. They were split into two groups with one receiving its carbohydrate as starch and the other as sucrose. In this experiment the rats were fed these diets for a period of eight weeks, twice as long as the previous study. The results were very similar. The total-C and LDL-C levels were essentially the same in both diets, but the HDL-C levels were significantly lower in the sucrose diet. Both of these studies, because of the changes in HDL-C levels, would indicate a more favorable effect on lipoprotein levels by diets with starch rather than sucrose as the source of carbohydrate (61,62).

This observation of a more favorable effect of starch over sucrose is not universal. Nonhuman primates are often used as subjects when cholesterol levels are examined. In one such study involving cynomolgus monkeys, the effects of carbohydrate type and cholesterol on serum cholesterol levels were tested (63). Twelve male monkeys were fed four different diets for a period of six weeks each. The diets contained 40 percent fat (half butter and half coconut oil), 11 percent protein, and 49 percent carbohydrate. The carbohydrate was either sucrose or starch, with or without 1 mg per kilocalorie cholesterol added. The animals were divided randomly into four groups and each group was placed on a different diet. After the first 6 week period, they were all placed on a chow diet for six weeks. This procedure was continued until all groups had been tested on each diet. Total cholesterol and lipoprotein cholesterol levels were assayed before the diets were administered and weekly thereafter. Total cholesterol levels were slightly higher than initial levels in both the starch and sucrose diets, but there was no significant difference between the two. When cholesterol was added to the diets, both sucrose plus cholesterol and starch plus cholesterol produced a marked rise in total cholesterol levels. The increase was significantly less in the sucrose plus cholesterol diet than the starch plus cholesterol diet. The HDL-C levels were also similar in the starch and sucrose diets, but the starch with added cholesterol diet produced lower HDL-C levels than the sucrose with added cholesterol diet. These findings seem to indicate that when sucrose is the sole source of dietary carbohydrate in cynomolgus monkeys, more favorable levels of serum cholesterol result than when starch is the sole source of dietary carbohydrate (63).

Often diets aimed at lowering total-C and LDL-C levels, such as the low fat diets mentioned previously (49,50), also lower HDL-C. In an effort to find ways to prevent this detrimental effect, experimental research is currently being conducted using diets with various forms of dietary fiber added as part of the carbohydrate source. All dietary fibers do not have the same effect on cholesterol levels. Generally, it seems to be the soluble fibers such as guar gum, pectin, and oat bran, that decrease the total-C and LDL-C while not significantly affecting the HDL-C levels (64,65). The insoluble fibers, such as wheat bran, are usually observed to have no significant effect on any cholesterol level (66). However, in one Swedish study (67) a significant increase in HDL-C levels was observed when a high dose of concentrated wheat bran was added to the diet.

Little et al (68) believe that some people are more susceptible to dietary influences on serum cholesterol levels. They studied 86 male coronary patients and 84 age matched healthy men from the same population. Dietary records were kept on each man for a period of seven days. The diets of the two groups were compared and no important differences were observed. Serum total-C and lipoprotein cholesterol were measured, and correlation coefficients were calculated for each dietary component and the cholesterol levels. All cholesterol levels were higher in the coronary group, and no significant correlation was found between total carbohydrate or sucrose and cholesterol levels of this group. Total carbohydrate and sucrose were negatively correlated with cholesterol levels in the group of healthy men, and there was a significant negative correlation between total carbohydrate and HDL-C. These results led the researchers to conclude that coronary patients appear to be metabolically different than healthy men and that they may be more sensitive to dietary influence on cholesterol levels (68).

Carbohydrate-sensitive subjects were selected by one group of researchers (13). They were fed three levels of sucrose in the diets. The diets were designed to closely resemble those presently consumed by people of the United States. The purpose was to determine if the average level of sucrose in the American diet (15 to 20 percent) contributed to high serum cholesterol levels, and whether these levels can be lowered by decreasing the amount of sucrose in the diet or raised by increasing the amount of sucrose in the diet. Twelve males and twelve females who were determined to be sensitive to sucrose and apparently free from disease were studied. The three diets each contained approximately 44 percent of the calories as carbohydrate, 14 percent as protein, and 42 percent as fat, with high levels of cholesterol and saturated fat, and a low level of dietary fiber. The foods were exactly the same except for a sucrose and/or starch patty that was designed to provide 30 percent of the total calories. The diets contained 5 percent, 18 percent, or 33 percent sucrose. The subjects were divided into six groups, each with two females and two males. All groups were placed on each of the diets for six weeks. Serum was taken weekly and analyzed for total cholesterol and the lipoprotein cholesterols. Total-C, LDL-C, and HDL-C levels all rose significantly when sucrose was increased from 5 percent to 18 or 33 percent. There was no further significant rise in total-C when the sucrose level went above 18 percent. The percent of HDL-C was significantly less in the males on the 33 percent sucrose diet than either of the other two diets (13). These results indicate that a high sucrose diet, even the levels currently consumed by the majority of Americans, could cause potentially harmful increases in serum cholesterol levels of carbohydrate-sensitive people.

The Effects of Dietary Protein on Cholesterol Levels

Probably the least studied dietary component with regard to relationship with cholesterol levels, is dietary protein. In the

1950's there was some interest in this area due to the observation that vegetarians seemed to have lower serum cholesterol levels than non-vegetarians. Besides containing mainly plant sources of protein, the vegetarian diets are almost always lower in total protein and fat content. This has led to research on both level and source of protein in the diet and their relationship to blood cholesterol levels.

In one study, the cholesterol levels of a group of vegetarians were compared to cholesterol levels of a group of non-vegetarians matched by age and sex (69). Total-C and LDL-C were both significantly lower in the vegetarian group. Although total HDL-C was also lower in the vegetarians the percent HDL-C was slightly higher (33 percent compared to 27 percent in the non-vegetarians). The implication from this study is that vegetarian diets may produce serum cholesterol levels that are associated with a decreased risk of CHD. Since this was not a controlled experiment, many other factors could have been partially responsible for the observed effects.

The results of many of the early experimentally controlled studies are conflicting. One study where the fat content was kept constant at 30 percent of calories (70) showed a much lower serum cholesterol level on diets of a very low protein level (4 percent of calories, all of vegetable origin) when compared to a 17 percent animal-origin protein diet. No significant change in cholesterol level was found by another group when diets containing 8.6 and 17.7 percent protein were compared (71). In yet another study of older convalescent patients (72), when a high milk protein supplement was added to their diets, increasing protein content from 14 percent to 19 percent, the serum cholesterol level was usually increased. This last group of researchers suggest that increased levels of protein may decrease serum cholesterol levels during periods of growth, but once animals reach maturity there seems to be less influence (72). They go on to speculate that in some mature individuals high levels of dietary protein may even increase serum cholesterol.

Early studies of the effects of source of protein on cholesterol levels focused mainly on only two types of protein. These were casein (milk protein) as the animal source, and soy protein as the plant source. More recently researchers have been examining the effects of various sources of both animal and plant proteins (73-75).

In a 1980 study, Neves et al looked at the effects of three sources of purified animal protein (casein, egg albumin, and lactalbumin), versus the effects of two sources of pure plant protein (soy and alfalfa) (73). They also examined the effects of crude protein sources (yeast, fish meal, and blood meal). Five male rats were randomly assigned to each of the diets and were fed for 28 days. At the end of this time, blood was taken from each animal and assayed for total-C and HDL-C. The researchers found no consistent differences in either total-C or HDL-C levels among rats fed any of the proteins. They concluded that their data indicated no universal cholesterol lowering effect of plant proteins compared with animal proteins.

The results of another study conducted the same year did indicate a lowering of LDL-C by plant proteins (74). Seven different animal proteins and six different plant proteins were compared in this experiment. Ten female rats were placed on each of the 13 diets.

After being on the diet for 28 days, blood was taken and analyzed for total-C and LDL-C. Although the total-C levels were not significantly different between animal protein groups and plant protein groups, there were significant differences in LDL-C. All of the animal protein diets produced higher LDL-C levels than the plant protein diets (74).

Similar results were obtained from a study conducted on human subjects (75). In a preliminary study (76), researchers could not confirm a cholesterol-lowering effect of soy protein compared to casein. They did observe a higher percentage of HDL-C. The subjects used in the first study were all young people with low initial serum cholesterol levels. In the follow-up study (75), middle-aged people were studied. The researchers tested a less pure soy protein as well as the diets used in the prior study, speculating that some non-protein material associated with the soy could lower the serum cholesterol levels. Fifty-seven human subjects, both males and females, were placed on one of the three different protein diets for 28 days. Blood samples were obtained every two weeks, plus at the beginning and the end of the test period. They were analyzed for total-C and HDL-C. There were no marked effects observed in total-C levels between any of the three diets. The data did show a lowering of LDL-C and a raising of HDL-C in the pure soy protein diet. This result was identical to that seen in their previous study. No such effects were observed in the less pure soy protein diet. This group of researchers could not recommend a moderate replacement of animal protein by plant protein because they found only a small beneficial effect. But, they reasoned that this small favorable effect plus the fact that plant sources are also usually lower in saturated fat and cholesterol, could lead to beneficial changes in serum cholesterol levels (75).

Specific amino acid ratios have been implicated as having more effect on serum cholesterol than the source of the protein. The amino acids which have been studied most are arginine and lysine. Plant protein generally contains a higher level of arginine and a lower level of lysine than animal protein.

In a recent study (10), rats were placed on one of four diets. One contained only plant protein (cottonseed protein), one contained only animal protein (casein), one contained cottonseed protein plus lysine, and the other contained the casein plus arginine. The animals were fed for 28 days and they their serum was analyzed for total-C and HDL-C. Total-C and HDL-C levels were decreased in the casein plus arginine group when compared to the casein only group. The animals fed the cottonseed protein plus lysine diet had higher total-C and HDL-C levels than the animals on the cottonseed protein only diet. Both of the casein diets produced higher cholesterol levels than either of the cottonseed protein diets. The percent HDL-C was not significantly different in any of the diets, however. The results of this study indicate that when the amino acid ratio of the casein diet was altered to resemble cottonseed protein, serum cholesterol levels declined. The authors conclude that the ratio of arginine to lysine may at least partially explain the frequently observed cholesterol-lowering effect of soy protein (10). These results were supported by Sugano, et al (77) in 1984.

Another amino acid, cystine, has been implicated in causing an increase in serum cholesterol levels. In research published in 1984 (78), rats were fed one of six diets. The diets were a control diet, a high cholesterol diet, and a low cholesterol diet, each with or without added cystine. Five percent added cystine increased serum cholesterol levels by 69 percent in the control diet, but it decreased serum cholesterol by 15 percent in the high cholesterol diet. LDL-C levels were markedly increased in all diets when cystine was added; HDL-C was also significantly increased by the addition of cystine.

The amount of protein in the diet was also studied by Neves et al (73). The researchers examined the effects of diets containing 10 percent protein (casein) versus the effects of 20 percent protein (casein) diets on male rats. Total-C and HDL-C were measured. Total-C was not significantly affected; HDL-C was significantly higher in the 20 percent protein diet.

Summary

Over the last thirty years, the research on diet and serum cholesterol has been of such magnitude that only a representative sample of it has been reviewed here. From even this sample it is apparent that there are no completely conclusive findings. Conflicting results can be found with regard to almost every aspect of the cholesterol question. However, some general inferences from the literature reviewed can be made.

1. Blood cholesterol levels are one key indicator of risk for coronary heart disease.

   (a) High levels of total serum cholesterol and LDL cholesterol indicate increased risk for coronary heart disease.

   (b) High levels of HDL cholesterol indicate decreased risk for coronary heart disease.

2. Dietary modification has an effect on serum cholesterol levels.

   (a) Total serum cholesterol and LDL cholesterol levels are:

      (1) higher with high levels of dietary fat,

      (2) higher with low ratios of polyunsaturated to saturated fat in the diet,

      (3) higher when dietary cholesterol is high and the fat is mainly saturated,

      (4) higher with high levels of simple sugar in the diet, and

      (5) higher with dietary protein from animal sources than from plant sources, especially in mature individuals.

   (b) HDL cholesterol levels are:

      (1) lower in low fat diets,

      (2) higher in diets with a high ratio of polyunsaturated fat to saturated fat when continued for long periods of time,

      (3) higher in diets with complex carbohydrate versus simple sugar,

      (4) little affected by source of dietary protein, and

      (5) lower in diets that have very low protein levels.

(c) Percent HDL cholesterol levels (HDL-C x 100/Total-C) are:

(1) little affected by type or amount of dietary fat,

(2) higher in diets of high complex carbohydrate versus high simple sugar, and

(3) higher in diets with protein from plant sources than diets with protein from animal sources.

3. Some people are more susceptible to the effects of dietary modification than others.

(a) Genetic types may affect susceptibility to dietary modification.

(b) Personality types may affect susceptibility to dietary modification.

CHAPTER III

METHODOLOGY AND PROCEDURES

The purpose of this study was to assess the effects of the diet suggested in the United States Dietary Goals on serum cholesterol levels. The study was part of a larger project that was being conducted at the Nutrition and Food Science Department at South Dakota State University.

Experimental Plan and Diets

A total of twelve different diets were used in a 3 x 2 x 2 factorial design experiment. The composition of the diets varied with respect to type of fat, source and amount of carbohydrate, and amount of protein. There were three types of fat: saturated fat (beef tallow), unsaturated fat (corn oil), and a half-and-half combination of the two. The level of fat remained constant in all diets at 28 percent of total calories. Two types of carbohydrates were used, sucrose or cornstarch. The level of carbohydrate varied inversely with the level of protein in the diets. The protein levels were either 10 or 20 percent of total calories, with the carbohydrate levels being 62 or 52 percent, respectively. The diets also included adequate vitamins and minerals for laboratory rats as prescribed by the American Institute of Nutrition (79). Composition of the diets, by percent of total weight and by percent of total energy are included in Tables 3 and 4. The diets were custom mixed by the United States Biochemical Corporation.

Table 3
Composition of Experimental Diets
by Percent of Total Weight

Ingredient	Diet 1	2	3	4	5	6	7	8	9	10	11	12
	%	%	%	%	%	%	%	%	%	%	%	%
Casein	21.1	10.6	21.1	10.6	21.1	10.6	21.1	10.6	21.1	10.6	21.1	10.6
DL-Methionine	0.3	0.2	0.3	0.2	0.3	0.2	0.3	0.2	0.3	0.2	0.3	0.2
Sucrose	55.6	66.3	-	-	55.6	66.3	-	-	55.6	66.3	-	-
Cornstarch	-	-	55.6	66.3	-	-	55.6	66.3	-	-	55.6	66.3
Beef Tallow	6.7	6.7	6.7	6.7	13.3	13.3	13.3	13.3	-	-	-	-
Corn oil	6.7	6.7	6.7	6.7	-	-	-	-	13.3	13.3	13.3	13.3
Fiber-Celufil	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
AIN Mineral Mix	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
AIN Vitamin Mix	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Choline Bitartrate	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2

Table 4
Composition of Experimental Diets
by Percent of Total Energy

Ingredient	Diet 1	2	3	4	5	6	7	8	9	10	11	12
	%	%	%	%	%	%	%	%	%	%	%	%
Fat												
Saturated[1]	14.0	14.0	14.0	14.0	-	-	-	-	28.0	28.0	28.0	28.0
Unsaturated[2]	14.0	14.0	14.0	14.0	28.0	28.0	28.0	28.0	-	-	-	-
Carbohydrate												
Sucrose	52.0	62.0	-	-	52.0	62.0	-	-	52.0	62.0	-	-
Cornstarch	-	-	52.0	62.0	-	-	52.0	62.0	-	-	52.0	62.0
Protein	20.0	10.0	20.0	10.0	20.0	10.0	20.0	10.0	20.0	10.0	20.0	10.0

[1]Beef tallow was used as the source of saturated fat. [2]Corn oil was used as the source of unsaturated fat.

Test Animals

Male albino rats, obtained from the Holtzman Company, Madison, Wisconsin, were used as the subjects for the experiment. Animals that were approximately 28 days old were placed on a commercial rat chow diet for a four day acclimation period. The chow was ground to simulate the texture of the experimental diets. The average weight of the animals when they started the experimental diets was 100 grams ($x=100\pm20$). The weights ranged from 65 grams to 130 grams. After the acclimation period, five rats were randomly chosen for each diet. Because of limited space, labor, and available cages, only two or three experimental groups were in progress at any one time. Each dietary regimen was continued for 28 days. The first group was started in February, 1984, and the last group was sacrificed the end of August, 1984.

The rats were housed individually in clear polycarbonate cages, with alternating 12 hour periods of light and dark. They were allowed ad libitum access to water and to the experimental diets. Food consumption was measured every other day and the animals were weighed twice per week. At the end of each experiment the animals were fasted overnight and, under chloroform anesthesia, were bled by heart puncture. The blood was allowed to coagulate and was then centrifuged at 3000 x g for twenty minutes to obtain the serum. The serum was then immediately frozen at -20 degrees centigrade until determination of the cholesterol concentrations could be made.

Cholesterol Determinations

Total Cholesterol

Total cholesterol of the serum was determined by the enzymatic method of Allain et al (80). The determination of total cholesterol by this method makes use of a single reagent, which was prepared immediately prior to conducting the cholesterol assay. To obtain uniformity, one batch of reagent was used for all of the samples. The samples were kept frozen at -20 degrees centigrade until all were collected and the determinations were done on all samples over a period of two days.

In the assay by this method, 30 microliters of serum were incubated with 3.0 milliliters of the reagent for 10 minutes at 37 degrees centigrade. The change in color, which was due to the amount of cholesterol in the sample, was measured by a DB-GT Beckman spectrophotometer at 500 nanometers. Concentrations of cholesterol in the sera were determined by comparison to a standard curve using known amounts of cholesterol. The total serum cholesterol values are included in Appendix A.

High Density Lipoprotein Cholesterol

Prior to determination of HDL cholesterol, the LDL and VLDL fractions needed to be removed from the serum. This was done by a modification of the method of Warnick and Albers (81). By this method the LDL and VLDL fractions were precipitated out of the serum by a heparin-manganese reagent. The composition of this reagent is shown in Table 5.

Table 5
Formula for Heparin-Manganese Precipitation of LDL and VLDL*

Ingredient	Concentration in One Liter
Sodium Heparin	180,000 Units
Manganese Chloride	0.092 moles
Sodium Chloride	0.150 moles

*Adapted from Warnick and Albers (81).

Twenty microliters of the reagent were added to 200 microliters of serum and thoroughly mixed. After standing at 4 degrees centigrade for 30 minutes, they were centrifuged at 1500 x g for 30 minutes. The clear supernatant portion containing the HDL cholesterol was then analyzed for cholesterol content in the same manner as previously noted for total cholesterol. The HDL cholesterol values are included in Appendix A.

Percent HDL Cholesterol

The percent of total cholesterol that was in the HDL portion was calculated from the results of the previous two tests. The percent HDL cholesterol values are included in Appendix A. The following formula was used to calculate the values:

$$\frac{\text{HDL cholesterol} \times 100}{\text{total cholesterol}}$$

Variables

The diets were grouped factorially by the various dietary components. Table 6 shows the groupings by independent variables and the number of animals that were included in each grouping. Each of these groups was measured for the dependent variables; total serum cholesterol, HDL cholesterol, and percent HDL cholesterol.

Table 6
Independent Variables and Animal Groupings

Independent Variables	Number of Groups per Independent Variable	Number of Animals per Group
Type of Dietary Fat	3	20
Type of Dietary Carbohydrate	2	30
Amount of Dietary Protein	2	30
Types of Dietary Fat and Carbohydrate	6	10
Type of Dietary Fat and Amount of Dietary Protein	6	10
Amount of Dietary Protein and Type of Dietary Carbohydrate	4	15
Types of Dietary Fat and Carbohydrate, and Amount of Dietary Protein	12	5

Hypotheses

The following null hypotheses were developed to be tested and evaluated.

1. There is no significant relationship between total serum cholesterol levels and:

   (a) type of dietary fat,

   (b) type of dietary carbohydrate,

   (c) amount of dietary protein,

   (d) types of dietary fat and carbohydrate,

   (e) type of dietary fat and amount of dietary protein,

(f) amount of dietary protein and type of dietary carbohydrate, and

(g) types of dietary fat and carbohydrate, and amount of dietary protein.

2. There is no significant relationship between HDL cholesterol levels and:

   (a) type of dietary fat,

   (b) type of dietary carbohydrate,

   (c) amount of dietary protein,

   (d) types of dietary fat and carbohydrate,

   (e) type of dietary fat and amount of dietary protein,

   (f) amount of dietary protein and type of dietary carbohydrate, and

   (g) types of dietary fat and carbohydrate, and amount of dietary protein.

3. There is no significant relationship between percent HDL cholesterol levels and:

   (a) type of dietary fat,

   (b) type of dietary carbohydrate,

   (c) amount of dietary protein,

   (d) types of dietary fat and carbohydrate,

   (e) type of dietary fat and amount of dietary protein,

   (f) amount of dietary protein and type of dietary carbohydrate, and

   (g) types of dietary fat and carbohydrate, and amount of dietary protein.

Data Analysis

Results were analyzed by the Statistical Analysis System (81) using factorial analysis of variance between serum cholesterol means for various subgroups as defined by the independent variables. If the factorial analysis of variance yielded a significant F-value ($p<0.05$), a post-hoc test (Fisher's protected LSD test) was conducted for comparison of the treatment means. Pearson product-moment correlations were also conducted between serum cholesterol means and dietary components.

Limitations of The Study

The facilities available for housing of the animals were not adequate for the magnitude of this study. Because of limited space, cages, and labor, three dietary groups were the maximum that could be housed and fed at any one time. This led to a lapse of five months between sacrifice of the first group and sacrifice of the last group. There is some evidence that there are seasonal variations in cholesterol levels, especially the lipoprotein cholesterol levels (82). Although the seasonal variation studies were not conducted on rats, there may be such a trend in rats as well. There was an obvious variation over time observed in this study. A graph illustrating this trend is included in Appendix B. Because there were no true control groups, groups fed commercial chow for the entire four weeks, there was no way to evaluate how much of the difference in means was due to seasonal variations.

There were problems encountered with regard to transport and arrival of the animals. Several times the animals did not arrive on the scheduled date, causing considerable variations in their beginning weights.

Finally, generalizing the findings from this group of animals to other groups of animals (even females of the same species), and especially to people, should be avoided. The majority of serum cholesterol is bound to HDL in rats (10), whereas the majority is bound to LDL in humans. The roles of the various carrier systems are not completely understood in either species, and may vary considerably between the two.

CHAPTER IV

RESULTS AND DISCUSSION

Twelve different diets based on the recommendations of the U.S. Dietary Goals were used to study the effects on serum cholesterol levels. Five laboratory rats were placed on each diet for four weeks. At the end of this time serum total cholesterol, HDL cholesterol, and percent HDL cholesterol were determined. This chapter describes the results obtained from the study.

Computer Analysis of Data

The Statistical Analysis System (81) was used to compute factorial analysis of variance, Fisher's protected least significant difference test, and Pearson product-moment correlations between mean serum cholesterol levels and dietary components.

Relationships Between Serum Cholesterol Levels and Independent Variables

The results of the analysis of variance are included in Appendix C. Mean scores that showed significant F values ($p<0.05$) were further analyzed by Fisher's protected least significant difference (LSD) test. This test was used to determine which of the group means differed significantly.

The Relationship Between Serum Cholesterol Levels and Type of Dietary Fat

Table 7 shows the mean serum cholesterol levels when the diets were grouped by type of dietary fat. No significant difference was found in total serum cholesterol levels due to the type of fat in the diet. Thus the null hypothesis cannot be rejected. This finding differs from many previous studies (4,8,9,40,41,42,43,44) which indicate a significant decrease in total serum cholesterol level in diets where the predominant fat was unsaturated.

Table 7
Mean Serum Cholesterol Levels Grouped By
Type of Dietary Fat

	Mean[1]		
Type of Dietary Fat	Total Serum Cholesterol[2]	HDL Cholesterol	% HDL Cholesterol
50% Saturated, 50% Unsaturated	75.64	[3]51.29[a]	68.73[a]
Saturated	72.34	52.43[a]	71.26[ab]
Unsaturated	80.22	59.82[b]	75.63[b]

[1]All means are reported as mg per 100ml of serum. [2]F-value of this column was not significant. [3]Means within a column without common superscript letters were significantly different according to the Fisher's protected least significant difference test at $p<0.05$.

Levels of HDL cholesterol and percent HDL cholesterol levels were significantly different due to type of dietary fat. Hypotheses describing no relationship between type of dietary fat and HDL cholesterol levels or percent HDL cholesterol levels could, therefore, be rejected.

The results of the LSD tests that were conducted on the mean HDL and percent HDL cholesterol levels are also found in Table 7. Mean HDL cholesterol level was significantly higher (14% higher) in diets containing unsaturated fat (corn oil) than in diets containing either of the other types of fat. Treatment means of percent HDL cholesterol were significantly higher (9% higher) in the unsaturated fat (corn oil) diets than in the 50% saturated (beef tallow):50% unsaturated fat (corn oil) diets. These results are consistent with the findings of Heine, et al (45), and Hjermann, et al (46).

The Relationship Between Serum Cholesterol Levels and Type of Dietary Carbohydrate

Mean serum cholesterol levels when the diets were grouped by type of dietary carbohydrate are shown in Table 8. Total serum cholesterol was significantly ($p<0.05$) related to type of carbohydrate in the diet. Thus the null hypothesis can be rejected. The LSD test shows a significantly higher mean total cholesterol level in the sucrose diets than in the cornstarch diets. This difference was about nine percent.

Table 8
Mean Serum Cholesterol Levels Grouped By
Type of Dietary Carbohydrate

	Mean[1]		
Type of Dietary Carbohydrate	Total Serum Cholesterol	HDL Cholesterol[2]	% HDL Cholesterol
Sucrose	[3]79.53[a]	53.82	67.24[a]
Cornstarch	72.60[b]	55.21	76.51[b]

[1]All means are reported as mg per 100ml of serum. [2]F-value of this column was not significant. [3]Means within a column without common superscript letters were significantly different according to the Fisher's protected least significant difference test at $p<0.05$.

Mean HDL cholesterol levels were not significantly related to type of carbohydrate in the diet. Therefore, the null hypothesis cannot be rejected.

The null hypothesis indicating no relationship between percent

HDL and form of dietary carbohydrate can be rejected. A significant difference was found in percent HDL levels; the cornstarch diets yielded a significantly higher mean percent HDL level than the sucrose diets. The results of this study would comfirm the findings of Hostmark, et al (62,63) who found essentially no change in total cholesterol levels between diets with sucrose and diets with starch, but observed a higher percentage of HDL cholesterol in cornstarch diets.

The Relationship Between Serum Cholesterol Levels and Amount of Dietary Protein

Table 9 shows the mean serum cholesterol levels when the diets were grouped by amount of dietary protein. No significant relationship was found between the amount of dietary protein and any of the mean cholesterol levels. The null hypotheses cannot be rejected.

Table 9
Mean Serum Cholesterol Levels Grouped By Amount of Dietary Protein

	Mean[1]		
Amount of Dietary Protein	Total Serum Cholesterol[2]	HDL Cholesterol[2]	% HDL Cholesterol[2]
20% Protein	74.32	52.51	70.31
10% Protein	77.81	56.52	73.44

[1]All means are reported as mg per 100ml of serum. [2]F-value of this column was not significant.

Keys and Anderson (71) compared diets with levels of protein similar to the levels used in this study. They also observed no significant effect on total cholesterol levels. Neves et al (73) also found no significant effect on total cholesterol but found a significantly higher HDL cholesterol level in 20% protein diets than in 10% protein diets. The HDL cholesterol findings of Neves et al (73) differ from the results of this study.

The Relationship Between Serum Cholesterol Levels and Types of Dietary Fat and Carbohydrate

The effects of the interaction of type of dietary fat and type of dietary carbohydrate on serum cholesterol levels was examined. Table 10 shows the mean serum cholesterol levels when the diets were grouped by this interaction. Mean HDL and percent HDL cholesterol levels were not significantly related to types of dietary fat and carbohydrate. Thus the null hypotheses stating no relationship between mean HDL and percent HDL cholesterol levels and types of dietary fat and carbohydrate cannot be rejected.

Total serum cholesterol was significantly affected by the interaction of type of fat and type of carbohydrate in the diet. But only when the type of fat was 50% saturated:50% unsaturated did the type of carbohydrate make any difference (Table 10). With this type of fat, diets with sucrose as the source of carbohydrate yielded significantly higher (20% higher) mean total cholesterol levels than diets with cornstarch as the carbohydrate.

Table 10
Mean Serum Cholesterol Levels Grouped By
Types of Dietary Fat and Carbohydrate

Types of Dietary Fat and Carbohydrate	Mean[1]		
	Total Serum Cholesterol	HDL Cholesterol[2]	% HDL Cholesterol[2]
50% Saturated, 50% Unsaturated			
Sucrose	[3]83.65[a]	52.89	63.68
Cornstarch	67.63[b]	49.69	73.79
Saturated			
Sucrose	75.66[a]	51.75	65.81
Cornstarch	69.03[a]	53.10	76.72
Unsaturated			
Sucrose	78.29[a]	56.81	72.22
Cornstarch	81.15[a]	62.83	79.03

[1]All means are reported as mg per 100ml of serum. [2]F-value of this column was not significant. [3]Means within a subcolumn without common superscript letters were significantly different according to the Fisher's protected least significant difference test at $p<0.05$.

The Relationship Between Serum Cholesterol Levels and Type of Dietary Fat and Amount of Dietary Protein

All three serum cholesterol levels tested were significantly ($p<0.05$) related to the interaction of type of fat and amount of protein in the diets. Therefore, the null hypotheses indicating no relationship between serum cholesterol levels and type of dietary fat and amount of dietary protein can be rejected. The mean serum cholesterol levels are shown in Table 11.

Table 11
Mean Serum Cholesterol Levels Grouped By
Type of Dietary Fat and Amount of Dietary Protein

Type of Dietary Fat and Amount of Dietary Protein	Mean[1] Total Serum Cholesterol	HDL Cholesterol	% HDL Cholesterol
50% Saturated, 50% Unsaturated			
20% Protein	[2]74.52[a]	52.61[a]	71.05[a]
10% Protein	76.76[a]	49.97[a]	66.42[a]
Saturated			
20% Protein	65.18[a]	43.51[a]	65.30[a]
10% Protein	79.51[b]	61.34[b]	77.23[b]
Unsaturated			
20% Protein	83.26[a]	61.40[a]	74.58[a]
10% Protein	77.18[a]	58.24[a]	76.68[a]

[1]All means are reported as mg per 100ml of serum. [2]Means within a subcolumn without common superscript letters were significantly different according to the Fisher's protected least significant difference test at $p<0.05$.

Upon closer examination with the LSD test, it becomes apparent that only the diets that contained saturated fat showed significant effects due to interaction with the amount of dietary protein. The lower level of dietary protein produced significantly higher means of each of the three cholesterol levels, with the HDL cholesterol being 29 percent higher on the lower protein diets.

The Relationship Between Serum Cholesterol and Amount of Dietary Protein and Type of Dietary Carbohydrate

Both mean total serum cholesterol and mean HDL cholesterol levels were significantly ($p<0.05$) affected by interactions of amount of dietary protein and type of dietary carbohydrate (Table 12). The null hypotheses stating no relationship between these cholesterol levels and this interaction can be rejected.

Table 12
Mean Serum Cholesterol Levels Grouped By
Amount of Dietary Protein and Type of Dietary Carbohydrate

Amount of Dietary Protein, Type of Dietary Carbohydrate	Mean[1]		
	Total Serum Cholesterol	HDL Cholesterol	% HDL Cholesterol[2]
20% Protein			
Sucrose	[3]71.32[a]	48.90[a]	67.72
Cornstarch	77.40[a]	56.12[b]	72.90
10% Protein			
Sucrose	87.82[a]	58.73[a]	66.76
Cornstarch	67.81[b]	54.30[a]	80.13

[1]All means are reported as mg per 100ml of serum. [2]F-value of this column was not significant. [3]Means within a subcolumn without common superscript letters were significantly different according to the Fisher's protected least significant difference test at $p<0.05$.

In the diets with the higher amount of protein, mean total cholesterol was not significantly different between the sucrose and cornstarch diets. The mean HDL cholesterol was significantly higher (13% higher) in the cornstarch diets than in the sucrose diets. With the lower amount of protein, total cholesterol was significantly higher (23% higher) in the sucrose diets while there was no significant difference in HDL cholesterol between the two types of carbohydrate.

The mean percent HDL cholesterol levels were not significantly affected by amount of dietary protein and type of dietary carbohydrate. The null hypothesis cannot be rejected.

The Relationship Between Serum Cholesterol Levels and Types of Dietary Fat and Carbohydrate and Amount of Dietary Protein

When the interaction between type of fat, type of carbohydrate, and amount of protein was examined it was found to significantly ($p<0.05$) influence both HDL cholesterol and percent HDL cholesterol (Table 13). The null hypotheses indicating no relationship between mean HDL and percent HDL cholesterol levels and these interactions can, therefore, be rejected.

Table 13
Mean Serum Cholesterol Levels Grouped By
Types of Dietary Fat and Carbohydrate and Amount of Dietary Protein

Types of Dietary Fat and Carbohydrate and Amount of Dietary Protein	Mean[1] Total Serum Cholesterol[2]	HDL Cholesterol	% HDL Cholesterol
50% Saturated, 50% Unsaturated			
Sucrose, 20% Protein	79.67	[3]56.38[a]	70.89[a]
Sucrose, 10% Protein	87.62	49.40[a]	56.46[b]
Cornstarch, 20% Protein	69.36	48.84[a]	71.20[a]
Cornstarch, 10% Protein	65.90	50.54[a]	76.38[a]
Saturated			
Sucrose, 20% Protein	59.24	33.05[a]	55.25[a]
Sucrose, 10% Protein	92.08	70.45[b]	76.38[b]
Cornstarch, 20% Protein	71.11	53.98[c]	75.35[b]
Cornstarch, 10% Protein	66.94	52.23[c]	78.08[b]
Unsaturated			
Sucrose, 20% Protein	74.81	57.25[a]	77.01[ab]
Sucrose, 10% Protein	83.76	56.36[a]	67.43[a]
Cornstarch, 20% Protein	91.71	65.54[a]	72.15[a]
Cornstarch, 10% Protein	70.59	60.12[a]	85.92[b]

[1]All means are reported as mg per 100ml of serum. [2]F-value of this column was not significant. [3]Means within a subcolumn without common superscript letters were significantly different according to the Fisher's protected least significant difference test at $p<0.05$.

A significant difference in HDL cholesterol levels was found only in diets with saturated fat. The diet with saturated fat, sucrose, and low protein had a significantly higher HDL cholesterol level than the saturated fat, cornstarch diets. The saturated fat, sucrose, and higher amount of protein diet produced a HDL cholesterol level significantly lower than the saturated fat, cornstarch diets. The two saturated fat, sucrose diets also produced significantly different HDL cholesterol levels, with the diet lower in protein having the higher HDL cholesterol level.

The animals on the sucrose, 10 percent protein diet wih 50% saturated:50% unsaturated fat had a significantly lower mean percent HDL cholesterol level than animals on any other diet with the same fat source. The saturated fat (beef tallow), sucrose, 20 percent protein diet produced a significantly lower mean percent HDL cholesterol level than the other three saturated fat diets. The unsaturated fat (corn oil), cornstarch, 10 percent protein diet showed a significantly higher percent HDL cholesterol level than either the cornstarch, higher protein, or the sucrose, lower protein, corn oil diets. None of these three corn oil diets yielded a percent HDL cholesterol level that was significantly different than the unsaturated fat, sucrose, 20 percent protein diet, however. The null hypothesis cannot be rejected since the mean total serum cholesterol levels were not significantly related to types of dietary fat and carbohydrate and amount of dietary protein.

Strength of Relationship Between Cholesterol Levels and Dietary Components

Pearson product-moment correlations were used to describe the strength of relationships between cholesterol levels and dietary fat, carbohydrate, and protein. The strength of relationships between total serum cholesterol, HDL cholesterol, and percent HDL cholesterol were also examined. The Pearson product-moment coefficients (r) and levels of significance are given in Table 14.

Table 14
Correlations Between Cholesterol Levels and Dietary Components

	Total Serum Cholesterol	HDL Cholesterol	% HDL Cholesterol
Dietary Fat	r = .1366 NS*	r = .2924 p<0.05	r = .2471 NS
Dietary Carbohydrate	r = .2533 NS	r = .0590 NS	r = .4072 p<0.01
Dietary Protein	r = .1278 NS	r = .1697 NS	r = .1376 NS
Total Serum Cholesterol		r = .7255 p<0.0001	r =-.1041 NS
HDL Cholesterol			r = .5981 p<0.0001

*Not significant, p<0.05

Perhaps a more meaningful interpretation of the strength of relationship among cholesterol levels and between cholesterol levels and dietary components would be variance. Variance is equivalent to the square of the correlation coefficient, r. The statistically significant r value for the relationship between dietary fat and HDL cholesterol levels is .2924 which would indicate a variance of .085. This means that only 8.5 percent of the variance in HDL cholesterol levels can be explained by the type of fat in the diet.

A significant correlation coefficient of .4072 between dietary carbohydrate and percent HDL cholesterol would be equal to a variance of .165. The type of dietary carbohydrate would explain 16.5 percent of the variance in percent HDL cholesterol level.

Total serum cholesterol and HDL cholesterol were very highly positively correlated ($r = .7255$). For an r value of .7255, the variance is .53 indicating that 53 percent of the variance in total serum cholesterol levels is related to the level of HDL cholesterol. With such a high variance, a direct relationship can be concluded with more certainty. HDL cholesterol level appears to have a strong relationship to total serum cholesterol levels.

As might also be expected, HDL cholesterol levels and percent HDL cholesterol levels were highly positively correlated ($r = .5981$). The variance in this case would be .36, indicating the HDL cholesterol level would explain 36 percent of the variance in percent HDL cholesterol levels. This would also indicate a strong relationship between HDL cholesterol levels and percent HDL cholesterol levels.

CHAPTER V

SUMMARY AND RECOMMENDATIONS

The purpose of the study was to assess the effects of the diet suggested in the United States Dietary Goals on the levels of blood cholesterol. Twelve different diets were tested, with five male rats placed on each diet for a period of four weeks. Serum total cholesterol, HDL cholesterol, and percent HDL cholesterol levels were examined.

Summary

The results of this study indicate that the three cholesterol levels measured were affected by various components of the diets. Although the overall picture of the effects of the diets tested is very complex, certain relationships are apparent.

Total serum cholesterol levels were significantly affected by the type of carbohydrate in the diet. Sucrose diets produced total cholesterol levels significantly higher than cornstarch diets. As the dietary components were narrowed down further, it became clear that when the type of fat was half saturated and half unsaturated, the effects of sucrose in the diet were even more pronounced. When dietary sucrose was fed at its highest level, which was when the diet contained 10 percent protein, the mean total cholesterol level was significantly higher than diets with an equivalent level of cornstarch as the type of carbohydrate. Upon examining the effects of type of fat and amount of protein in the diets, only when the fat source was saturated did the amount of protein have a significant effect on total cholesterol. The lower protein level produced a higher total cholesterol level.

HDL cholesterol levels were significantly and strongly ($p<0.05$) associated with type of dietary fat. Unsaturated fat (corn oil) diets produced significantly higher mean HDL cholesterol levels than the diets with either saturated fat (beef tallow) or a combination of saturated fat and unsaturated fat. When examining the effects of the interaction of type of fat and amount of protein in the diet, the saturated fat, 10 percent protein diets produced mean HDL cholesterol levels significantly higher than the saturated fat, 20 percent protein diets. The mean HDL cholesterol levels of the unsaturated fat diets were not significantly affected by the amount of protein in the diet. The 10 percent protein diets with sucrose as the type of carbohydrate produced mean HDL cholesterol levels significantly higher than 10 percent protein diets with cornstarch. The highest mean HDL cholesterol level was observed in the diet that contained saturated fat, sucrose, and 10 percent protein, while the lowest was in the diet that contained saturated fat, sucrose, and 20 percent protein. In the latter two diets, the diet with the higher level of sucrose, which was the diet with the lower amount of protein, produced a mean HDL cholesterol level that was over twice as high as the mean HDL cholesterol level of the diet with the lower level of sucrose.

Percent HDL cholesterol levels were affected by the type of fat in the diet, with corn oil diets producing mean percent HDL cholesterol levels significantly higher than the half corn oil:half beef tallow diets. Type of carbohydrate was significantly and strongly ($p<0.01$)

correlated to mean percent HDL cholesterol levels, with cornstarch producing the higher levels. In the saturated fat diets, the 10 percent protein diets produced mean percent HDL cholesterol levels significantly higher than the 20 percent protein diets. The unsaturated fat, cornstarch, 10 percent protein diet produced the highest mean percent HDL cholesterol level. The saturated fat, sucrose, 20 percent protein diet produced the lowest level; the mean percent HDL cholesterol level of the half saturated:half unsaturated fat, sucrose, 10 percent protein diet was also low.

Implications

Lower total serum cholesterol levels and higher HDL and percent HDL cholesterol levels are considered to be beneficial in lowering the risk of CHD. Examination of the results of this study would reveal that the diet containing unsaturated fat (corn oil), cornstarch (complex carbohydrate), and 10 percent protein produced the most favorable cholesterol levels. This would support the recommendations of the United States Senate Select Committee on Nutrition and Human Needs. Increases in unsaturated fat and complex carbohydrate and a protein level of 12 percent are recommended by the United States Senate Select Committee in their United States Dietary Goals.

The results of this study indicate that the United States Dietary Goals may be appropriate for attaining more favorable cholesterol levels. The dietary components and interactions that were tested produced several statistically significant relationships. The practical significance needs to be examined a little more closely. In this study the more favorable cholesterol levels were 10 to 15 percent lower in total cholesterol and 10 to 20 percent higher in HDL cholesterol than the least favorable cholesterol levels. According to the Lipid Research Clinics calculations (2), a drop of only about eight percent in total cholesterol and 10 percent in LDL cholesterol and a rise of about 13 percent in percent HDL cholesterol were associated with a 16 to 19 percent reduction in CHD risk. Based on these calculations, it would seem that differences observed in this study are great enough to warrant the dietary modifications suggested in the United States Dietary Goals.

Caution should be taken to avoid generalizing the results of this study on rats to humans. Although rats are a convenient model to use in preliminary studies, one cannot assume that human subjects would be affected in the same way. Neither can one assume that the relationships seen in this study are necessarily cause-and-effect relationships because it is possible that some other unknown factor could be involved.

Recommendations

A vast amount of research is being conducted continually on the effects of dietary modification on serum cholesterol levels. Although we have learned a tremendous amount over the last thirty years, there are many more questions to be answered.

Much of the research has been conducted on animal models, with rats being one of the most widely used. Observations from rat studies have often preceded comparable findings in people. But because of the differences between the two species, there is a need for more human studies. Further research needs to be directed toward the use of humans in more controlled longitudinal studies.

This study indicated that the effects of various dietary components in combination were different from the effects of the dietary component when viewed separately. Many of the studies that have been conducted have examined only one of these components. It would be more beneficial to know how whole foods or complete diets affect serum cholesterol levels than to try to generalize from partial knowledge of their biochemical composition.

High serum total and LDL cholesterol levels and low HDL cholesterol levels are associated with increased risk for coronary heart disease. Because of the magnitude of this disease, a top priority of nutritional research should be examination of ways to effectively alter serum cholesterol levels to decrease this risk.

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APPENDIX A

RAW DATA

Table 15
Raw Data

Diet	Animal Number	Beginning Weight	Final Weight	Food Consumption	Total Serum Cholesterol	HDL Cholesterol	% HDL Cholesterol
1	1	100.5	304.0	421.7	87.41	65.46	74.89
1	2	108.5	342.0	472.8	95.69	65.46	68.41
1	3	107.5	231.5	365.8	68.29	53.61	78.50
1	4	107.5	203.0	324.0	72.31	51.34	71.00
1	5	107.5	255.0	365.7	74.65	46.03	61.66
2	1	98.0	175.0	337.5	83.28	56.40	67.72
2	2	107.5	207.0	498.5	83.62	47.83	57.20
2	3	96.0	159.0	317.5	93.63	55.35	59.12
2	4	103.0	240.0	486.5	93.98	47.83	50.89
2	5	106.5	212.5	445.5	83.97	39.58	47.14
3	1	76.0	228.5	339.1	55.44	50.90	91.81
3	2	107.5	314.0	464.4	70.95	42.53	59.94
3	3	95.0	340.0	516.6	75.00	57.15	76.20
3	4	124.0	407.5	662.5	83.97	39.58	47.14
3	5	104.5	321.0	454.6	61.46	37.95	61.75
4	1	81.0	195.0	451.5	69.17	50.63	73.20
4	2	65.5	162.5	337.5	64.83	59.69	92.07
4	3	65.0	212.0	430.0	72.92	54.94	75.34
4	4	79.0	178.0	335.0	55.08	35.17	63.85
4	5	68.5	136.5	262.5	67.51	52.28	77.44

Table 15 (Continued)
Raw Data

Diet	Animal Number	Beginning Weight	Final Weight	Food Consumption	Total Serum Cholesterol	HDL Cholesterol	% HDL Cholesterol
5	1	121.0	299.0	408.0	50.91	28.07	55.14
5	2	151.0	339.5	485.5	52.52	24.78	47.18
5	3	125.0	324.5	449.0	57.34	32.70	57.03
5	4	141.0	332.5	479.0	71.25	47.33	66.43
5	5	119.0	276.5	366.0	64.18	32.38	50.45
6	1	79.0	145.0	280.0	96.55	77.07	79.82
6	2	80.5	131.6	303.5	93.57	68.78	73.22
6	3	80.5	131.0	303.5	93.93	68.78	73.22
6	4	79.5	150.3	350.0	91.31	68.60	75.13
6	5	69.0	146.5	300.5	85.04	60.30	70.91
7	1	90.0	277.0	415.0	57.00	40.32	70.74
7	2	115.5	342.0	490.5	57.64	42.70	74.08
7	3	99.5	332.5	547.0	80.15	65.64	81.90
7	4	134.0	431.0	597.0	82.37	74.17	90.04
7	5	123.0	340.0	487.5	78.41	47.05	60.01
8	1	112.5	215.0	409.0	66.69	54.64	81.93
8	2	101.0	251.0	462.4	62.82	45.32	72.14
8	3	94.0	219.0	435.5	78.87	60.98	77.32
8	4	99.0	201.0	386.9	54.79	44.32	80.89
8	5	116.0	283.0	513.8	71.51	55.87	78.13

Table 15 (Continued)
Raw Data

Diet	Animal Number	Beginning Weight	Final Weight	Food Consumption	Total Serum Cholesterol	HDL Cholesterol	% HDL Cholesterol
9	1	86.0	342.5	457.5	67.30	51.26	76.17
9	2	71.5	303.0	446.5	66.03	52.91	80.13
9	3	78.5	285.0	408.5	88.95	64.88	72.94
9	4	76.0	297.5	387.0	79.80	51.64	64.71
9	5	77.5	262.0	388.0	71.99	65.58	91.10
10	1	118.0	193.0	338.5	94.84	64.38	67.88
10	2	107.0	224.0	439.5	93.94	62.09	66.10
10	3	125.0	200.0	405.5	56.29	38.31	68.06
10	4	131.5	282.0	504.0	90.05	55.72	61.88
10	5	117.5	178.0	332.0	83.68	61.30	73.26
11	1	79.5	322.0	452.0	98.73	69.04	69.93
11	2	80.5	263.0	364.0	97.01	63.24	65.19
11	3	78.0	308.0	441.0	90.49	64.38	71.15
11	4	83.0	290.0	421.5	96.11	64.38	66.99
11	5	80.5	300.0	439.5	76.20	66.67	87.49
12	1	106.5	251.0	451.5	64.47	50.76	78.73
12	2	120.0	309.0	552.0	87.40	64.74	74.07
12	3	115.5	276.5	502.0	74.34	65.16	87.65
12	4	110.5	224.0	465.0	64.47	62.98	97.69
12	5	111.0	222.5	429.0	62.27	56.95	91.46

Table 16
Mean Serum Cholesterol Levels
by Diet

Diet	Mean ± SEM[1]		
	Total Serum Cholesterol	HDL Cholesterol	% HDL Cholesterol
Diet 1	79.67 ± 5.13	56.38 ± 3.90	70.89 ± 2.88
Diet 2	87.62 ± 2.53	49.40 ± 3.05	56.46 ± 3.56
Diet 3	69.36 ± 5.02	48.84 ± 3.73	71.20 ± 5.87
Diet 4	65.90 ± 3.01	50.54 ± 4.14	76.38 ± 4.56
Diet 5	59.24 ± 3.79	33.05 ± 3.86	55.25 ± 3.29
Diet 6	92.08 ± 1.95	70.45 ± 3.18	76.38 ± 2.18
Diet 7	71.11 ± 5.67	53.98 ± 6.73	75.35 ± 5.09
Diet 8	66.94 ± 4.05	52.23 ± 3.21	78.08 ± 1.71
Diet 9	74.81 ± 4.28	57.25 ± 3.27	77.01 ± 4.34
Diet 10	83.76 ± 7.14	56.36 ± 4.73	67.43 ± 1.83
Diet 11	91.71 ± 4.12	65.54 ± 1.04	72.15 ± 3.98
Diet 12	70.59 ± 4.70	60.12 ± 2.76	85.92 ± 4.27

[1]All means are reported as mg per 100ml of serum ± standard error of the mean.

APPENDIX B

MEAN CHOLESTEROL LEVELS OVER TIME

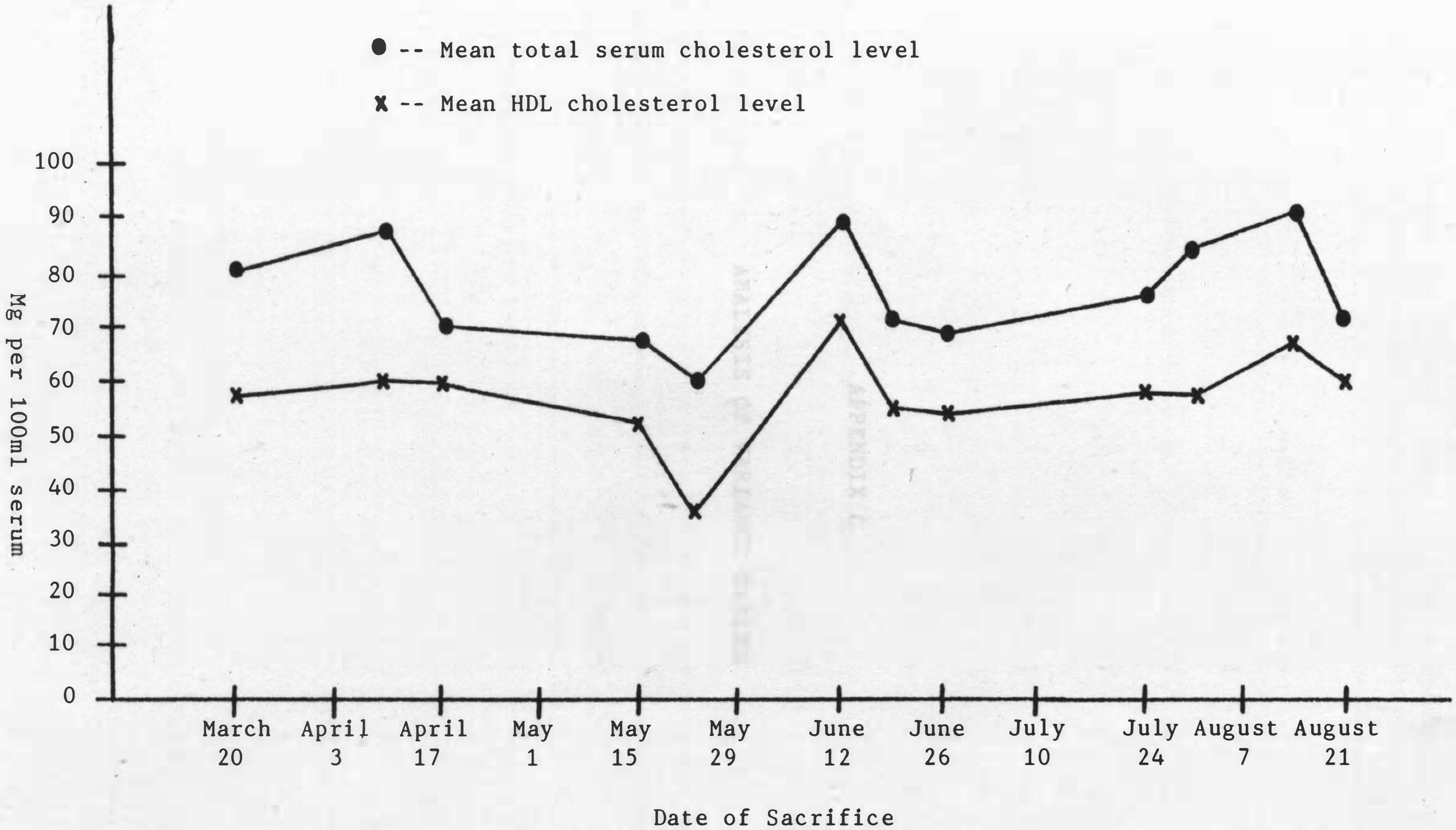

Fig. 1 -- A Comparison of Mean Total Serum Cholesterol Levels and HDL Cholesterol Levels Over Time

APPENDIX C

ANALYSIS OF VARIANCE TABLES

Table 17
Analysis of Variance Summary for Independent Variables and Total Serum Cholesterol

Independent Variable	Degrees of Freedom	Mean Square	F	P
Type of Dietary Fat	2	312.85	3.10	NS[1]
Type of Dietary Carbohydrate	1	720.17	7.15	0.0102
Amount of Dietary Protein	1	183.44	1.82	NS
Types of Dietary Fat and Carbohydrate	2	399.77	3.97	0.0254
Type of Dietary Fat and Amount of Dietary Protein	2	526.94	5.23	0.0088
Amount of Dietary Protein and Type of Dietary Carbohydrate	1	2567.47	25.48	0.0001
Types of Dietary Fat and Carbohydrate and Amount of Dietary Protein	2	219.08	2.17	NS
Residual (Error)	48	100.77		

[1]Not significant, $p<0.05$

Table 18
Analysis of Variance Summary for Independent Variables and HDL Cholesterol

Independent Variable	Degrees of Freedom	Mean Square	F	P
Type of Dietary Fat	2	428.96	5.79	0.0056
Type of Dietary Carbohydrate	1	29.08	0.39	NS[1]
Amount of Dietary Protein	1	240.92	3.25	NS
Types of Dietary Fat and Carbohydrate	2	106.29	1.43	NS
Type of Dietary Fat and Amount of Dietary Protein	2	715.97	9.66	0.0003
Amount of Dietary Protein and Type of Dietary Carbohydrate	1	510.13	6.88	0.0116
Types of Dietary Fat and Carbohydrate and Amount of Dietary Protein	2	762.56	10.29	0.0002
Residual (Error)	48	74.14		

[1]Not significant, $p<0.05$

Table 19
Analysis of Variance Summary for Independent Variables and Percent HDL Cholesterol

Independent Variable	Degrees of Freedom	Mean Square	F	P
Type of Dietary Fat	2	243.24	3.30	0.0453
Type of Dietary Carbohydrate	1	1291.03	17.53	0.0001
Amount of Dietary Protein	1	147.44	2.00	NS[1]
Types of Dietary Fat and Carbohydrate	2	23.55	0.32	NS
Type of Dietary Fat and Amount of Dietary Protein	2	346.62	4.71	0.0136
Amount of Dietary Protein and Type of Dietary Carbohydrate	1	251.22	3.41	NS
Types of Dietary Fat and Carbohydrate and Amount of Dietary Protein	2	667.11	9.06	0.0005
Residual (Error)	48	73.64		

[1]Not significant, $p<0.05$