THE BLOOD SERUM CHOLESTEROL OF OVERWEIGHT COLLEGE WOMEN ON A CONTROLLED WEIGHT REDUCTION DIET

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THE BLOOD SERUM CHOLESTEROL OF OVERWEIGHT COLLEGE WOMEN ON A CONTROLLED WEIGHT REDUCTION DIET

By

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INTRODUCTION

Cholesterol has been known since the eighteenth century as the chief constituent of human gall stones. It is the characteristic sterol of higher animals and is present in all cells of the animal organism, in the brain and nerve tissue, the suprarenal glands and in egg yolk.

A possible physiological function for cholesterol in blood was suggested by Kelsey (1941) who believed that blood cholesterol was associated with the transport of unsaturated fatty acids. Studies with deuterium as a trace element have demonstrated that cholesterol is converted by the body to other biologically active substances, including both bile salts and hormones. (Schoenheimer, Rittenberg, and Graff 1935).

There is general agreement among several authors that the concentration of cholesterol in the blood of any person remains constant and is not easily changed by diverse physiological conditions (Kirk, Page, Lewis, Thompson, Van Slyke 1935, Sperry 1936, Offenkrantz 1938). Any change in the concentration of blood cholesterol, therefore, would be expected to result only from a disturbance of some metabolic function.

The relationship of blood cholesterol to diseased conditions has been the subject of both clinical and experimental investigations. Since cholesterol is present in high quantities in atherosclerotic lesions, several investigations have been directed toward studies of factors influencing the concentration of blood cholesterol in atherosclerosis. Since the incidence of atherosclerosis is relatively high among obese individuals, and since the metabolism of cholesterol appears to be allied closely with the lipid metabolism, studies of the blood cholesterol of

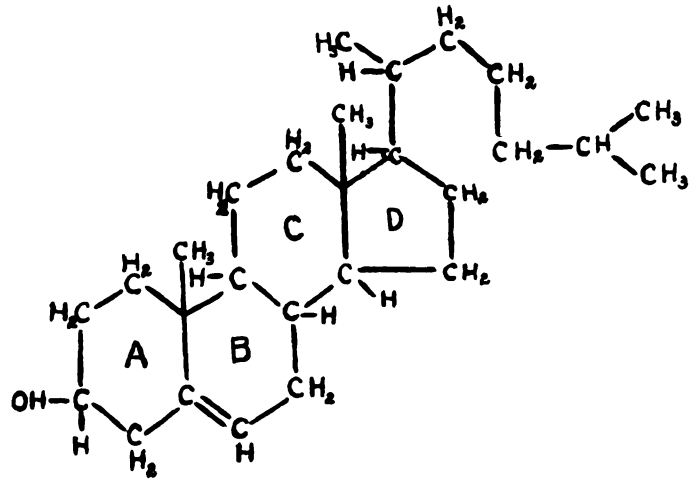
overweight individuals may be helpful in understanding the physiology of obesity. The following study was conducted to investigate the possible changes in the blood serum cholesterol of overweight college women on a controlled weight reduction diet and the possible relationship between excess body weight and serum cholesterol values.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Chemical Structure of Cholesterol.

Cholesterol is one of the primary constituents of body cells. It is a monatomic alcohol containing one double bond and possesses the formula $C_{27}H_{45}OH$. The structure is as follows:



CHOLESTEROL

The four rings A, B, C, and D form the cyclopentanoperhydrophenanthrene nucleus, which is characteristic not only of cholesterol but also of other plant and animal sterols and a wide variety of naturally occurring compounds. Some of these compounds have important physiological significance. These

compounds include the bile salts, the steroid hormones, the sterol vitamins, the aglycone portion of the cardiac glycosides, the saponins derived from the plant saponin, and the carcinogenic hydrocarbons of the phenanthrene type (Feiser, Feiser 1949). The possible relationship of the varied compounds whose chemical structure are fundamentally similar has attracted considerable attention.

Concentration of Cholesterol in Blood.

Values reported by various investigators for whole blood cholesterol for apparently healthy adults are given in Table 1. The total blood cholesterol ranged from 107.5 to 392 milligram per 100 cubic centimeters. Values for free cholesterol ranged from 24.3 to 110 milligram per 100 cubic centimeters.

Cholesterol contains an alcohol group and therefore is capable of forming esters; in the blood, a large proportion of cholesterol is combined with fatty acids. Sperry (1936) found that the ratio of free to total cholesterol in the blood was remarkably constant for humans. The amount of free in the total cholesterol varied within narrow limits of 24.7 to 30.1 per cent with an average of 26.9 per cent. In an earlier report, Kirk and co-workers (1935) had stated that the ratios of free to total cholesterol varied widely between individuals. Values were reported which ranged from 22 to 72 per cent. The reasons for the differences between the work of Kirk and that of Sperry were not evident although both investigators presented ranges for the concentration of total cholesterol which were approximately the same. Subsequent studies from other laboratories have confirmed the work of Sperry and have demonstrated the constancy of

Table 1

CONCENTRATION OF CHOLESTEROL IN HUMAN BLOOD AS REPORTED BY VARIOUS INVESTIGATORS

Investigator	Subjects	Blood Cholesterol	
		total	free
		mg/100 cc	mg/100 cc
Gardner, Gainsborough, 1927	22	120-210	39-71
Okey, Boyden, 1927	16 women students	162-258	
Man, Peters, 1933	12	178-236	
Boyd, 1935	8 women	177	52
Schoenheimer, Sperry, 1934	46	107.5-150.7	37.5-49.5
Kirk, et. al., 1935	65 men; 20-100 yrs.	109-376	
Sperry, 1936	91 adults; 19-43 yrs.	131-392	24.3-29.5
Kornerup, 1942		175-259	45-71
Vitaliano, 1943		123-218	66-110
Oppenheimer, Bruger, 1943	7 lab. technicans	166-(obese 196)	
Sobel, Mayer, 1945	10	160-267	41-73
Saifer, Kammerer, 1946	50 men; 20-35 yrs.	190-270	

Table 1 (continued)

CONCENTRATION OF CHOLESTEROL IN HUMAN BLOOD AS REPORTED BY VARIOUS INVESTIGATORS

Investigator	Subjects	Blood Cholesterol	
		total	free
		mg/100 cc	mg/100 cc
Kingsley, Schaffert, 1949	50 adults	159-260	
Groen, et. al., 1950	152 adults	245	
Keys et. al., 1950	312 men; 18-55 yrs.	250-800	

the ratio of free to total blood cholesterol in a variety of conditions. (Offenkrantz and Karshan 1936, Offenkrantz 1938, Smith and Marble 1937, Gardner, Gainsborough, and Murray 1938, Peters and Man 1943).

The remarkable constancy of the ratio between free and combined cholesterol may indicate the presence of an enzyme system which regulates the proportions of the two substances in the blood. Sperry (1936) found that esterification of free cholesterol occurred in the serum. Thus the ratio may be maintained through continuous and simultaneous esterification of free cholesterol.

Bugnard (1930) observed that the venous plasma of dogs contained more cholesterol than arterial plasma, whereas the concentration in whole venous blood did not differ from that of arterial blood. He concluded that there was an exchange of cholesterol between the corpuscles and the plasma which was dependent upon the pH of the circulating blood. According to this theory, when carbon dioxide is given up by the blood in the lungs, and the blood becomes relatively alkaline, cholesterol migrates from the plasma to the corpuscles. The reverse shift takes place as the blood becomes saturated with carbon dioxide. Bugnard found also <u>in vitro</u> experiments that the saturation of whole arterial blood with oxygen resulted in lower plasma cholesterol values than saturation of the blood with carbon dioxide. Levine and Soskin (1939) also found an inverse relationship between the total serum lipids and the carbon dioxide combining power of the blood.

Shope (1928) reported that the concentration of cholesterol esters in heparinized plasma did not differ from that in the serum. When sodium citrate or potassium oxalate were used as anti-coagulants, the plasma contained less bound cholesterol than the serum. Sperry and Schoenheimer (1935, 1937) found that oxalated plasma contained significantly smaller amounts of total and free cholesterol than serum or heparinized plasma. These workers considered that the lower values resulted from the effect of the oxalate in altering the serum and plasma volumes. Gardner, Gainsborough, and Murray (1938) stated that the use of potassium oxalate resulted in plasma cholesterol values which were about four per cent lower than heparinized plasma.

Factors Influencing Blood Cholesterol.

The wide range of values for blood cholesterol which are given in Table 1 raises the question of whether the variations represent differences between individuals or variations in a single individual over a period of time. Sperry (1937) and Turner and Steiner (1939) agreed that the concentration of cholesterol in the blood remained remarkably constant for each person. Man and Gildea (1937) and Schube (1936) reported that variations occurred in blood cholesterol concentration of individuals although the values which were reported were within fifteen per cent of the average concentration. A review of the factors which may influence the cholesterol values of blood follows:

Sex. Peters and Man (1943) found no distinction between serum lipids of normal male and female adults but Hornerup (1950) observed

that there was a tendency toward higher serum lipid values in females than in males.

Age. Sperry (1936) found that the concentration of blood cholesterol was low at birth and that a rapid increase in cholesterol content occurred during the first four days of life. After the fourth day, the values for blood cholesterol appeared to remain constant during infancy. The range of plasma cholesterol values for infants four to 25 days old ranged from 71 to 192 milligram per 100 cubic centimeters of plasma. The ratio of cholesterol ester to total cholesterol varied from 51 to 72 per cent for infants. Thus, the constancy of the ratio of free to total cholesterol which was observed for adults was not found for infants. Mulbock and Kaufmann (1938) obtained values that were in agreement with those of Sperry.

A gradual but slight increase of cholesterol with age throughout childhood was reported by Baylac and Sendrail (1928) and Ward (1931); however, Offenkrantz and Karshan (1936) reported values for blood cholesterol for children which were within the same range as for adults. Kirk and his co-workers (1935) studied a group of men who ranged from 21 to 91 years of age. These workers found that age did not have a significant effect either on the concentration of blood cholesterol or on the ratio of total to free cholesterol.

Keys and co-workers (1950) found that the values of serum cholesterol for men and women were not significantly different in the age group of 17 to 30 years. However, above this age, there was an average increase per year of 2.2 milligrams of total cholesterol per 100 cubic centimeters of serum. For the age range 17 to 78 years in men,

Keys and co-workers found a pronounced curvilinear relationship between age and serum cholesterol concentration with a maximum in the sixth decade.

Menstruation and Pregnancy. Okey and Boyden (1927) reported that exceptions to the usual constancy of blood cholesterol occurred during menstruation. The study included measurements of sixteen healthy women over a period of 26 menstrual cycles. The usual fluctuations in the concentration of blood cholesterol during the menstrual cycle involved a slight premenstrual rise, followed by a distinct fall immediately before or during menstruation, then a sudden rise during or slightly after the bleeding phase followed by a gradual decline to the average value. Offenkrantz (1938) observed that the fluctuations during menstruation were within the limits of variations of adult men. Both Okey and Offenkrantz found that the cholesterol concentration in blood of normal women during the intermenstrual period was constant.

The cholesterol content of blood apparently increases during pregnancy. Grigaut (1913) and Hermann and Neumann (1912) found no change in the first trimester; however a gradual rise in all lipid constituents occurred in the fourth month. The concentration remained high during the rest of pregnancy, and through puerperium, then declined gradually to pre-pregnancy values. Boyd (1934) showed that this change occurred only in the plasma, and that the erythrocyte lipids remained constant.

Fasting and Starvation. The effect of fasting on blood cholesterol was first studied by Gardner and Landen (1915) who observed

considerable increases in free and esterified cholesterol during periods of hunger. Shope (1927) observed that hypercholesteremia occurred during periods of fasting up to six days. The blood cholesterol decreased within a few hours after feeding. This subsequent lowering of blood cholesterol occurred regardless of the type of food eaten, whether fat, carbohydrate, protein or a mixed diet. Bose (1946) found that low cholesterol values occurred in starvation. In cases of starvation, Man and Gildes (1936) found that the cholesterol concentration in the blood seemed to vary directly with the state of nutrition.

Diet. The effect of diet on the concentrations of blood cholesterol over extended periods of time was first reported by Luden (1917) who from self observation found that a prolonged vegetable diet resulted in a decreased value for cholesterol and that an exclusive meat diet increased the concentration. Gardner and Gainsborough (1927) reported that blood cholesterol varied with the amount of cholesterol in the diet. On the other hand, Turner and Steiner (1939) concluded that no relationship existed between the type of diet and cholesterol concentrations in the blood. Neither maintenance on a high or low fat diet nor addition of cholesterol to the food influenced the blood cholesterol in their study.

Okey and Stewart (1933) determined cholesterol in the whole blood of four normal women who were kept on diet which contained varying amounts of cholesterol for approximately one month. Values reported for blood cholesterol were slightly higher when the diet contained 3.1 grams of cholesterol per day than when a control diet was used

which contained 0.77 grams. Values were higher when egg yolks were fed as a source of cholesterol than when cholesterol was mixed with butter, but these differences were slight and were not considered to be significant. Corwin (1938) reported that long term feeding on a high fat diet produced only slight hypercholesteremia in dogs but when the high fat diet was supplemented with lecithin there was a marked elevation of free cholesterol and combined cholesterol. Cholesterol administered in the solid state had no effect on the blood levels.

The specific effect of lecithin on the concentration of blood cholesterol was investigated by Steiner and Domanski (1941). Eight subjects were given 14 grams of lecithin and eight grams of cholesterol for five weeks. The average increase in the cholesterol concentration in the serum was about 101 milligrams per 100 cubic centimeters of blood at the end of this period.

These studies indicate that ingested cholesterol when given alone or when mixed with fat, apparently has little or no influence on the concentration of cholesterol in the blood, but when cholesterol is mixed with lecithin, an appreciable rise in the concentration of cholesterol in the blood may occur, probably through finer emulsification and more efficient absorption.

Keys, Mickelsen, Miller and Chapman (1950) studied the relationship between the cholesterol content of the diet and the concentration of cholesterol in the blood. Blood cholesterol was found to be independent of the intake over a wide range; however, when the diet was free of cholesterol, blood cholesterol was lowered at a rate

related to the previous concentration of cholesterol in the blood. A severe reduction of cholesterol in the diet was not easily achieved since cholesterol occurs only in foods of animal origin and is relatively stable. However, Keys found that a cholesterol free diet readily lowered the blood cholesterol in normal men and in men with extreme hypercholesteremia.

Obesity. The influence of the amount and the character of the blood lipids on the development of atherosclerosis has been studied extensively; however, the results which have been reported have been highly controversial. Diagnostic symptons of atherosclerosis do not appear until the advanced stages of the disease; therefore, the determination of its presence and extent in humans is difficult. Considerable evidence which has been obtained in experimental studies with rabbits has indicated that hypercholesteremia may be a factor in the development of atherosclerosis (Page, 1941). Investigations have been directed toward the possibility that this also may be true of the development of atherosclerosis in humans (Weinhouse 1943, Mann 1951). Further, the possibility that obesity is a predisposing f ctor toward atherosclerosis and that hypercholesteremia may be a factor in this relationship has been studied. Rony and Levy (1929) measured the blood cholesterol of 15 normal and 21 obese adults preceding the following a test meal of 500 milliliters of 20 per cent cream. There was no apparent change in the concentration of blood cholesterol. Conversely, Blotner in 1935 administered a test meal which was high in fat to 21 obese individuals and found that there was a marked

increase in blood cholesterol; this increase in blood cholesterol was not observed when the same test meal was given to 13 normal adults. The nature of the test meal was not described in the report. The results of Rony and Levy were confirmed by Oppenheimer and Bruger (1943) who repeated the experimental conditions of the previous study with seven normal and 13 obese individuals and found no increase in blood cholesterol following the test meal. These studies have indicated recognition of the problem relating to cholesterol metabolism in obesity. There is need for further investigation of the problem.

Methods on the Determination of Cholesterol.

Historical Development. Chemical methods for the determination of cholesterol date back to the early part of this century. Windaus in 1909 published a gravimetric procedure and Grigaut in 1910 published a gravimetric method for cholesterol determinations. Windaus found that cholesterol formed stoichiometric coordination compounds with digitonin and other saponins. On the basis of this observation Windaus precipitated cholesterol by digitonin in alcohol and determined it gravimetrically. Grigaut applied the Liebermann-Burchard reaction (1885) to an ether extract of cholesterol and measured the intensity of color produced by the reaction. In the Liebermann-Burchard reaction, acetic anhydride and concentrated sulfuric acid were added to a dilute solution of cholesterol in chloroform. The color which resulted was first blue, then green, and finally after long standing, a yellow brown.

For twenty years Windaus' gravimetric method was considered the standard procedure for the estimation of cholesterol. The method is considered excellent but it is laborious and time consuming.

The method was not applicable to routine analyses of small quantities of blood and tissue available for study as it was not possible to weigh accurately one or two milligram quantities of cholesterol digitonide with ordinary analytical balances.

Grigaut's method required five to ten cubic centimeters of blood and required three to five hours to extract the cholesterol. Autenrieth and Funk (1913) modified Grigaut's method and reduced the quantity of blood needed to two cubic centimeters and introduced the use of strong alkali to extract the cholesterol. The extraction time was thus reduced to 25 to 35 minutes. The use of strong alkali introduced a new problem. A brown tint developed which was believed to interfere with the colorimeter reading.

From 1916 to 1928, Bloor published a series of articles which presented modifications and improvements of the colorimetric procedure. He found that the cholesterol in blood behaved differently from pure standard cholesterol. Blood cholesterol reacted more readily and faded f ster than pure cholesterol. Bloor questioned whether the blood cholesterol was wholly or in part different from the pure cholesterol, or whether the difference resulted from changes in the rate of the reaction caused by impurities in the blood extracts. Later (1922), Bloor reported that pure cholesterol was not affected by alkaline saponification, and it was probable that some substance other than cholesterol was present in the blood which was sensitive

to alkali and gave a brown tint. In 1928, Bloor published another paper in which he reported the determination of total fatty acids and cholesterol in an alcohol-ether extract obtained by saponification and extraction; in 1929, Bloor published complete details for the oxidative determination of phospholipids, fatty acids, and cholesterol from the same extract.

Bloor had undoubtedly carried out the most extensive ground work for the colorimetric determination of cholesterol. In the same period of time, other laboratories also were concerned with improvements of methods for cholesterol determinations. Various modifications of the colorimetric procedure involved two processes, i. e., the isolation of cholesterol by extraction and the development of color in the extract.

Colorimetric determination of Cholesterol: Extraction. The extraction process may follow one of two steps: (1) the wet method which Bloor used extensively, and (2) the dry method of extraction. In wet extraction, blood is treated with alcohol-ether and filtered to remove the precipitated protein. The solvent is evaporated and the lipid material is taken up by chloroform. This method is preferred when more than one phase of the various lipid fractions of blood are studied. With the dry method, only cholesterol can be analyzed in the extract. The blood is dried on some substance as filter paper and then extracted with chloroform in a continuous extraction process. A comparison of the methods for wet extraction and dry extraction of cholesterol for blood which have been reported in the literature indicated that equa ly reliable results have been

obtained by the two methods. Only the total cholesterol content of the blood can be determined when the procedure of the dry extraction is used. The wet extraction method has the advantage that both total and free cholesterol may be determined from the same extract and that special continuous extraction apparatus is not needed.

The wet extraction method was used by Autenrieth and Funk (1913) and Grigaut (1910). Both investigators used ether for extraction. When used alone, ether requires a long extraction period; however, when ether is used with alcohol or when alcohol and acetone are used for extraction, only a few minutes are required to obtain complete extraction. Bloor, in his experiments (1916, 1917, 1928) used ether and alcohol in the ratio of three to one. Alcohol precipitates the protein and mixes with the water in the blood, and ether extracts the lipoid substances more completely. Schoenheimer and Sperry (1934) used a mixture of hot alcohol-acetone (1:1) for extraction of cholesterol. Sobel and Mayer (1945) found that cold extraction with alcohol and acetone (1:1) was as effective as hot extraction.

A method for dry extraction of cholesterol was first introduced by Myers and Wardell (1918). Blood was adsorbed on plaster of Paris and dried before extraction with chloroform. After adsorption, blood was in a finely divided state on the plaster of Paris. This condition facilitated extraction. The calcium salt also retained substances which added to the color development by Bloor's procedure. Results of this method agreed favorably with the method of Windaus (1909). Lieboff (1924) used paper discs to adsorb blood and reported that drying at high temperatures impaired the test slightly and gave lower

results. Lieboff reported that drying the blood on filter paper was not necessary. Ling (1928) reported the use of fat-free filter paper for the adsorption of blood and Abrahamson (1938) substituted absorbent cotton for filter paper and obtained similar results.

Colorimetric Determination of Cholesterol: Development of Color. Most methods using chloroform as the initial solvent used the Liebermann-Burchard color reaction. The blue-green color produced is measured colorimetrically or photometrically. This reaction in spite of its apparent simplicity, has proved difficult to control. The intensity and the shade of the color have been shown to be influenced by small differences in the concentrations of the reagents, by the presence of traces of water or other impurities, time and temperature (Hoffman 1941).

Schoenheimer and Sperry (1934) precipitated cholesterol with digitonin before application of the Liebermann-Burchard reaction to the digitonide dissolved in acetic acid. It was believed that isolation of cholesterol as the digitonide avoided errors which were introduced into other colorimetric methods by interfering chromogenic substances present in fatty extracts. Digitonin has an absorption curve similar to that of cholesterol. However, Schoenheimer and Sperry found that digitonin did not have an absorption peak in the range of 610 to 620 millimicrons where the color development by cholesterol was at its maximum. After precipitation of cholesterol digitonide, Schoenheimer and Sperry dissolved the precipitate with glacial acetic acid and then added separately acetic anhydride and sulfuric acid. The mixture was allowed to stand for fifteen

minutes at 25° C. and the intensity of the color was measured spectrophotometrically at a wave length of 610-620 millimicrons. Schoenheimer and Sperry were able to achieve by this method a degree of accuracy formerly obtained in the macrogravimetric method of Windaus; more-over, this method required only 0.2 cubic centimeters of blood where as Windaus employed 15 to 20 cubic centimeters of blood.

Sperry and Brand (1943) proposed a number of improvements of the earlier method of Schoenheimer and Sperry. Sperry and Brand suggested mixing the acetic anhydride and sulfuric acid beforehand so that the evolution of heat from the reaction did not affect the rate of color development. The color reaches a maximum in about fifteen minutes and then fades, so the temperature and time of reading must be selected for maximum color intensity.

The most recent modification of the method of Schoenheimer and Sperry was published by Foldes and Wilson (1950). The reagents were the same as in the earlier method with the exception that a solution of 0.5 per cent alcoholic digitonin was used instead of a solution of 0.4 per cent aqueous digitonin. The authors claimed that the use of the alcoholic digitonin resulted in a fine, evenly dispersed precipitate which was easier to handle than the coarse, sticky precipitate obtained when aqueous digitonin was used.

Other Methods for the Determination of Cholesterol

Most of the methods reported in the literature for the determination of cholesterol have applied the Liebermann-Burchard reaction for color formation; however, other reactions also have been employed

although the methods have not been as widely used.

Okey (1930) developed a micro method for the estimation of cholesterol in which the digitonide was oxidized with silver chromate-sulfuric acid and the excess dichromate was titrated with sodium thiosulfate. This method has given excellent results; however it is more time consuming than the colorimetric procedure of Schoenheimer and Sperry.

Bernoulli (1932) employed acetic chloride and anhydrous zinc chloride for color development in the determination of cholesterol. This reaction, called the Chugaen test, was considered to result in the formation of a compound which had a more stable color than that formed by the Liebermann-Burchard reaction. Bernoulli claimed that one part of cholesterol in 80,000 parts of solution could be detected by this procedure. This method has the disadvantage that fatty acid esters of cholesterol and ergosterol and phytosterol will give the same reaction as cholesterol with the test.

A color reaction which employed salicylaldehyde in a solution of chloroform, sulfuric acid and water was applied by Ohysama (1938) for the determination of cholesterol. This reaction resulted in a violet-colored substance which formed in the chloroform layer.

EXPERIMENTAL PROCEDURE

EXPERIMENTAL PROCEDURE

A series of determinations were made of the total and free cholesterol in blood serum of ten overweight college women who were subjects on a weight-control metabolism study conducted by the Foods and Nutrition Department at Michigan State College. Records of body weights and food intakes were available for inspection.

Subjects.

The subjects were overweight college women who ranged from 18 to 23 years of age. In September 1950, a preliminary study on weight reduction was begun. Subjects A.L., I.P., and V.S. participated in the study from September to December. After the Christmas vacation these women returned to the diet table and were continued on a weight reduction study with seven additional subjects who began the experimental study in January. The caloric intake for subjects A.L., I.P., and V.S. on their usual self-selected diet was determined initially in September. In January, these subjects were placed immediately on the weight reduction diet. However, the other subjects were permitted to eat without restriction from a mixed diet of foods typical of this area. All servings of food were weighed to determined the customary food intake of the subjects. Following this, all subjects were given the weight reduction diet. The calories of the diet were supplied chiefly by protein and fat. Subjects V.S. and I.P. reached expected weight before the period of observation was concluded. At this time,

the diets of these subjects were adjusted to maintain the desired weight. Since subjects S.S. and A.L. did not show appreciable weight loss on the reduction diet, their food intakes were reduced by ten and twenty per cent of the basic reduction diet, respectively.

Determinations of serum cholesterol were made at intervals of approximately two weeks from January, 1951 through March 1951. Thus values for serum cholesterol were obtained for seven subjects (A.W., M.J., D.VA., E.H., A.S., S.S., and F.G.) before weight reduction and at two week intervals during the period of weight reduction. Serum cholesterol values were not obtained for subjects A.L., V.S., and I.P. before weight reduction. Analysis of serum cholesterol were made during weight maintenance for subjects V.S. and I.P.

A group of eleven women students of average body weight volunteered to become control subjects. A series of three blood samples were taken at about one week intervals and serum cholesterol was determined. A record of a typical day's food intake was obtained from each subject and the caloric intake was calculated by the method of Donelson and Leichsenring (1945).

Sampling Techniques.

Samples of blood were taken from the subjects in the fasting state at approximately two weeks intervals. The blood was drawn from the fingertip from a small incision made by a lancet or a Bard-Parker blade. Approximately 1.0 cubic centimeter of blood was taken for each sample. After the blood clotted, the samples were centrifuged to separate the blood cells from the serum. Serum was used for cholesterol determinations in all cases.

During the period of study, cholesterol values were determined also on the serum of venous blood samples taken from the subjects. The samples were taken at least two hours after a meal. The blood was treated in the same manner as the fingertip sample and the serum was held in frozen storage until analysis. A comparison was made of the cholesterol of the venous blood serum and the cholesterol content of the serum of fingertip (capillary) blood which was analyzed within a week of the time that the venous samples were taken.

Chemical Procedure.

The concentration of total and free cholesterol in blood serum was determined by the method of Schoenheimer and Sperry (1934) with modifications according to Sperry and Brand (1943), Sobel and Mayer (1945) and Foldes and Wilson (1950). Three cubic centimeters of alcohol-acetone (1:1) solution were added to 0.2 cubic centimeters of serum in a five cubic centimeter volumetric flask. The solution was brought to boiling and held at boiling temperature for 30 seconds. The solution was cooled and made to volume. The protein precipitate was separated from the extract by filtration through fat-free filter paper.

An aliquot of one cubic centimeter of extract was used for the determination of total cholesterol. One drop of ten per cent potassium hydroxide was added to the extract to hydrolize the cholesterol esters. The solution was allowed to stand for 30 minutes and then titrated with ten per cent acetic acid in alcohol, using phenolphthalein as an indicator.

An aliquot of two cubic centimeters of the extract was taken for the determination of free cholesterol. With the exception of the

hydrolysis of cholesterol esters, the determinations of total and free cholesterol were carried out in the same manner. Both total and free cholesterol were precipitated with a 50 per cent alcoholic digitonin solution and allowed to stand overnight. The precipitate was separated by centrifuging and then washed with an acetone-ether solution and with anhydrous ether. The ether was removed by evaporation.

The digitonin precipitate was dissolved in glacial acetic acid and a solution of acetic anhydride-sulfuric acid (20:1) was added. The solution was allowed to stand for 27 minutes (no longer than 30 minutes) and the absorption of light was measured in the Beckman spectrophotometer at a wavelength of 620 millimicrons. Determinations of serum cholesterol were made in duplicate; however in some instances sufficient blood was not obtained to permit duplicate analyses.

A series of solutions of 0.01 to 0.10 milligram of pure cholesterol per one cubic centimeter of acetic acid were prepared and treated according to the above procedure. The absorption of light was measured and the values were plotted on semi-log paper against concentration of cholesterol. The graph was used as a reference curve for the calculation of the amounts of total and free cholesterol in blood serum.

In a preliminary study of this method, recoveries of pure cholesterol added to blood serum ranged from 94 to 98 per cent.

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

The subjects were overweight women who were apparently healthy in other respects. Routine physical examinations made by the student health service were negative. However, subject F.G. had received one grain of thyroid preparation since childhood and continued thyroid therapy during the study. The desired weights of the subjects were predicted from height and body stature on the basis of anthropometric measurements.[1] The subjects were moderately active college women as judged by their ability to carry a full time college program successfully. The age, height and predicted weights of the subjects are given in Table 2.

The age of the subjects ranged from 18 to 23 years; the subjects were from 8.0 to 51.2 per cent overweight at the beginning of the study. Initial weights are given for subjects V.S., A.L., and I.P. both at the beginning of the preliminary study and at the beginning of the period of observation. Estimations of the average loss per week for A.L., V.S., and I.P. were based on the period of preliminary study and the experimental period; thus the total reduction period was 24 weeks for subject A.L. and 19 weeks for subjects V.S. and I.P. The average loss of weight for all subjects was 0.67 kilogram per week. The rate of weight loss was satisfactory for all of the subjects except for A.L. who averaged only 0.20 kilogram per week. Two

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[1] The anthropometric measurements and predictions of desired weights were made by Dr. Margaret A. Ohlson.

Table 2

THE AGE, HEIGHT AND BODY WEIGHT OF TEN OVERWEIGHT WOMEN ON A WEIGHT REDUCTION DIET

Subject	Age	Height	Body Weight					Percent Overweight	
			Desired	Initial	Final	Total weight loss	Average wt. loss per week	Initial	Final
	years	cm.	kg.	kg.	kg.	kg.	kg.		
A.W.	18	167.6	61.4	79.6	70.2	9.4	0.94	29.7	14.4
M.J.	19	168.9	63.6	83.4	76.0	7.4	0.74	31.1	19.4
D.VA.	18	165.1	61.4	84.6	76.4	8.2	0.83	37.9	24.5
E.H.	18	161.3	59.1	89.3	80.3	9.0	0.90	51.2	35.9
A.S.	22	176.5	72.7	78.5	73.9	4.6	0.46	8.0	1.6
S.S.	22	156.2	54.5	59.1	56.0	3.1	0.70	8.5	2.7
F.G.	18	170.2	63.6	83.5	75.9	7.6	0.77	31.3	19.3
V.S.	23	166.4	61.4	73.4[1] 66.0[2]	61.0	12.4[1] 5.0[2]	0.65[4]	12.6[1]	-.66
A.L.	19	165.1	59.1	83.5[1] 81.3[2]	78.6	4.9[1] 2.7[2]	0.20[3]	41.1[1]	33.0
I.P.	22	154.9	54.5	60.3[1] 54.2[2]	51.4	8.9[1] 2.8[2]	0.47[4]	11.1[1]	-5.8

[1] beginning of preliminary study.
[2] beginning of study.
[3] 24 weeks.
[4] 18 weeks.

subjects, V.S. and I.P., achieved the desired weight loss which was 12.4 kilogram for V.S. and 8.9 kilogram for I.P. These subjects were then given diets planned to maintain body weight. At the end of the study, subjects A.S. and S.S. also had lost the desired amount of weight. The other subjects were from 14.4 to 35.9 per cent overweight at the end of the observational period.

The caloric intakes of the subjects on the unrestricted diet, the weight reduction diet, and the weight maintenance diet was given in Table 3. Since the women were apparently healthy individuals, it was assumed that the excess weight resulted from ingestion of calories greater than the output of energy. The caloric intake of the subjects during the period in which they ate without restriction averaged 1983 and ranged from 1789 to 2280 calories per day. It is probable that these intakes may be slightly below their previous customary intakes since weight losses from one to four pounds occurred during the two weeks on the self-selected diet. The caloric intakes for this period were below the recommendations of the Food and Nutrition Board of the National Research Council. This suggests that the recommended intake for calories for women may be high.

After a period of two weeks on the self-selected diet, the food intake of each subject was reduced to 1403 calories per day; these calories were supplied chiefly by protein and fat. The basic reduction diet supplied from 61.6 to 78.5 per cent of the caloric intakes of the subjects during the self-selected period. The basic reduction diet proved satisfactory for weight reduction for all of the subjects except for A.L. and S.S. for whom it was desirable to

Table 3

CALORIC INTAKE OF OVERWEIGHT SUBJECTS FOR EACH TWO WEEKS OF OBSERVATION

Subject	Average Caloric Intake					
	Self-selection	2 weeks	4 weeks	6 weeks	8 weeks	10 weeks
	cal./24 hrs.	cal./24 hrs.	cal./24 hrs.	cal./24 hrs.	cal./24 hrs.	cal./24 hrs
A.W.	1995	1403	1403	1403	1403	1403
A.L.	1789	1403	1403	1403	1123	1123
M.J.	1829	1403	1403	1403	1403	1403
D.VA.	1831	1403	1403	1403	1403	1403
E.H.	2140	1403	1403	1403	1403	1403
A.S.	1837	1403	1403	1403	1403	1403
V.S.	2235[1]	1403	1403	1513	1513	1513
I.P.	1998[1]	1403	1403	1709	1709	1709
S.S.	1897[1]	1403	1403	1403	1262	1262
F.G.	2280	1403	1403	1403	1403	1403

[1] Determined at beginning of preliminary study.

increase the rate of weight reduction. After six weeks on the reduction diet, A.L. and S.S. were given diets which supplied twenty and ten per cent less respectively of the foods of the basic reduction diet. Only two subjects, V.S. and I.P. attained adequate loss of weight so that they could be observed on diets planned to maintain body weight. Since I.P. was of small body build, the caloric intake was increased only to 1513 calories per day after weight reduction. This increase apparently was not adequate since she continued to lose weight. Subject V.S. was given 1709 calories per day for weight maintenance. This intake appeared to maintain body weight for the subject during the period of observation.

Serum Cholesterol during Weight Reduction.

The total cholesterol concentration in the blood serum of individual overweight subjects at two week intervals is shown in Table 4. The total serum cholesterol during the period on self-selected diets ranged from 141.9 to 227.5 milligram per 100 cubic centimeters and averaged 175.4 milligram per 100 cubic centimeters serum. Since V.S., A.L., and I.P. were on weight reduction diets at the beginning of the study, no values for the total serum cholesterol on the self-selected diets were available for these subjects.

The average values for total serum cholesterol were 167.9, 172.4, 172.3, 188.2 and 186.9 milligrams per 100 cubic centimeters for each two week interval, respectively. A graph showing the average changes in the total serum cholesterol and body weight for the subjects for each two week period is given in Figure 1. Changes in body weight and

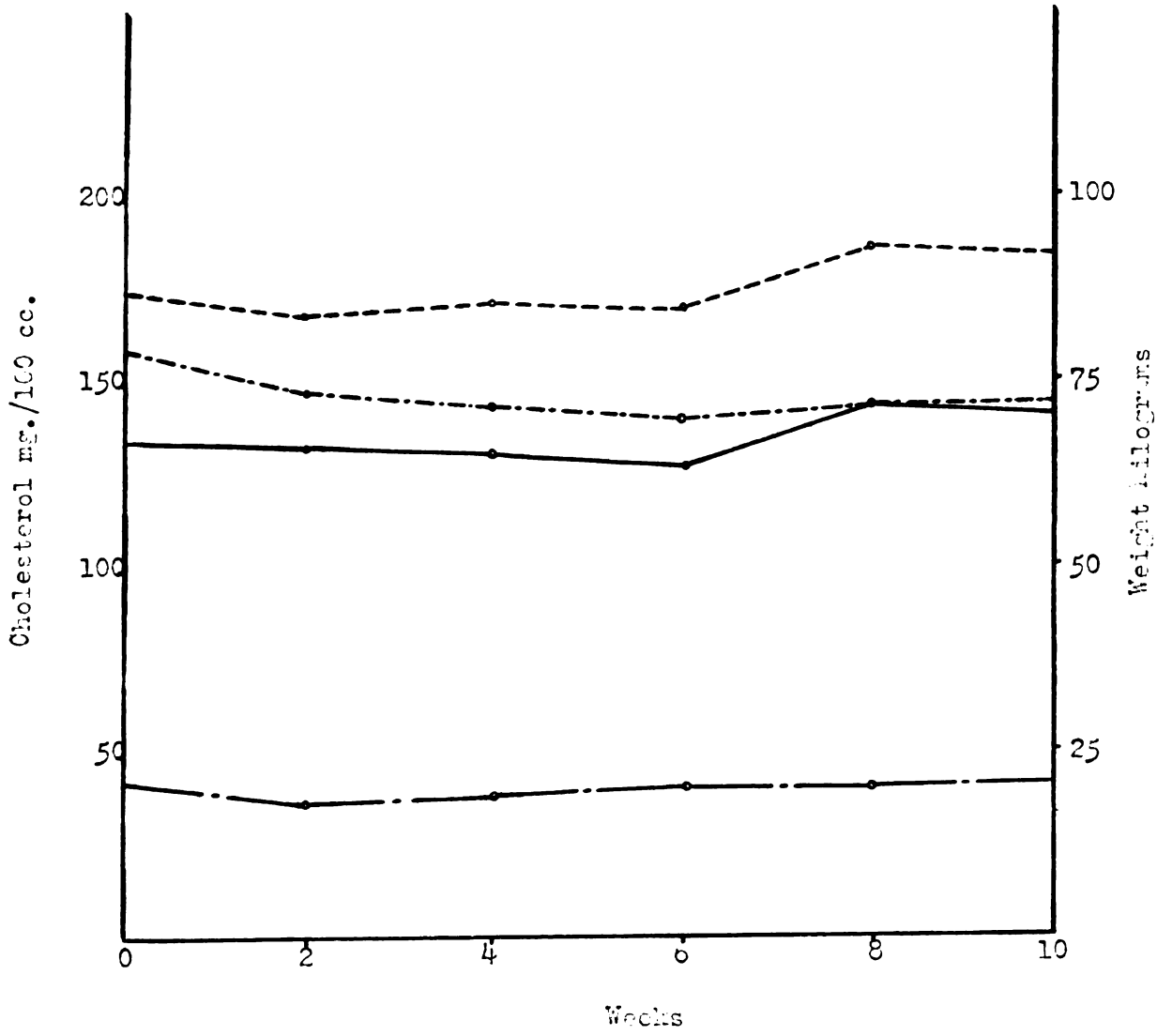
Table 4

TOTAL CHOLESTEROL OF BLOOD SERUM OF OVERWEIGHT WOMEN ON A SELF-SELECTED DIET, A WEIGHT REDUCTION DIET AND A DIET PLANNED FOR MAINTENANCE OF DESIRED WEIGHT

Subject	Serum Cholesterol on Self-Selected Diet	Serum Cholesterol on Weight Reduction Diet					Serum Cholesterol on Maintenance Diet		
		2 weeks	4 weeks	6 weeks	8 weeks	10 weeks	2 weeks	4 weeks	6 weeks
	mg./100 cc.	mg./100 cc.	mg./100 cc.	mg./100 cc.	mg./100 cc.	mg./100 cc.	mg./100cc.	mg./100 cc.	mg./100 cc.
A.W.	175.0	165.0	177.5	170.0	183.8	196.3			
M.J.	163.8	163.8	182.5	193.8	196.3	215.0			
D.VA.	141.9	163.8	162.5	147.5	155.0	147.5			
E.H.	145.0	138.8	138.8	138.8	143.8	146.3			
A.S.	227.5	248.8	245.0	226.3	261.3	252.5			
S.S.	179.4	152.5	183.8	197.5	180.0*	180.0*			
F.G.	195.0	177.5	185.0	205.0	207.5	183.8			
V.S.		147.5	141.3	133.8			127.5	141.9	146.9
A.L.		185.0	156.3	166.3	177.5	173.8			
I.P.		136.3	151.3	143.8			148.8	148.8	
MEAN	175.4	167.9	172.4	172.3	188.2	186.9	138.2	145.4	146.9

Figure I

GRAPH SHOWING THE AVERAGE CHANGES IN BODY WEIGHT AND IN THE TOTAL, FREE AND CHOLESTEROL ESTER OF BLOOD SERUM OF OVERWEIGHT WOMEN



in serum cholesterol for the individual subjects are given in Figure II. There was an initial decrease of total serum cholesterol for four of the subjects in the two week period following the introduction of the basic reduction diet. Following this, there was a tendency toward an increase in total serum cholesterol. This pattern was characteristic of four subjects, A.L., E.H., S.S., F.G.; an increase in total serum cholesterol during the experimental period also occurred for subjects M.J., D.VA., A.S. and I.P. However, the influence of time on total serum cholesterol values was determined statistically by analysis of variance (Table 5), and it was found that the changes in cholesterol for the successive periods were not statistically significant. Differences between individuals were highly significant.

The concentration of free cholesterol in the blood serum of the experimental subjects is given in Table 6. These values also are shown graphically in Figure II and the average of values for free cholesterol in the serum for the entire group is shown in Figure I. The concentration of free cholesterol averaged 41.2 milligram per 100 cubic centimeters of serum during the period of the self-selected diet and the values for the entire group ranged from 29.4 to 55.0 milligrams per 100 cubic centimeters for the group. Variations in concentration of free cholesterol in the serum followed the same pattern as for total cholesterol in that a decrease in free cholesterol values occurred in the two week period after the subjects were given the reduction diet. An analysis of variance showed that changes in the concentration of free cholesterol during the period on weight reduction were not statistically significant (Table 7).

Figure II

GRAPH SHOWING THE CHANGES IN BODY WEIGHT AND IN TOTAL, COMBINED AND FREE CHOLESTEROL OF BLOOD SERUM OF OVERWEIGHT WOMEN ON A WEIGHT REDUCTION DIET

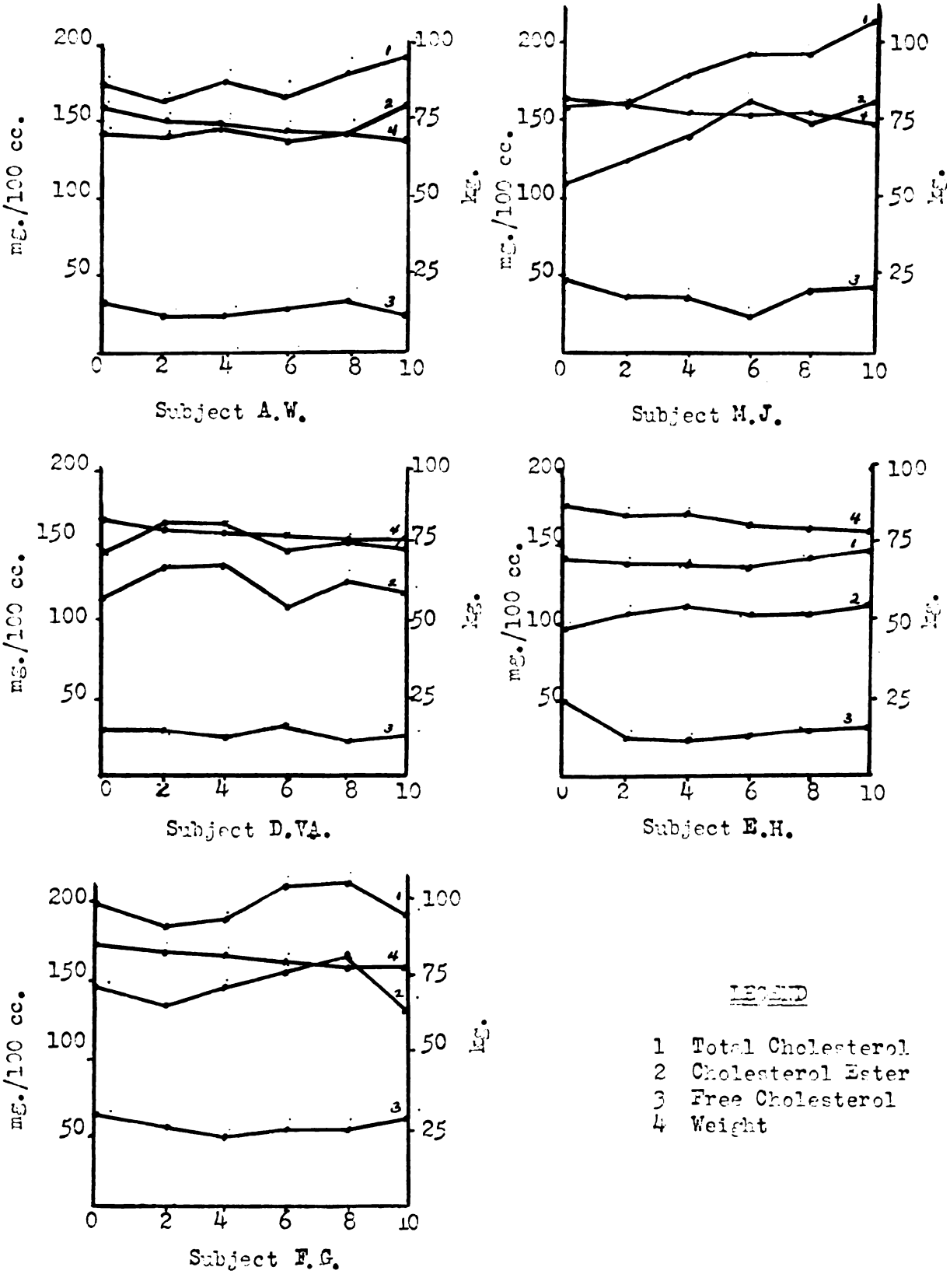


Figure II (continued)

GRAPH SHOWING THE CHANGES IN BODY WEIGHT AND IN TOTAL, COMBINED AND FREE CHOLESTEROL OF BLOOD SERUM OF OVERWEIGHT WOMEN ON A WEIGHT REDUCTION DIET

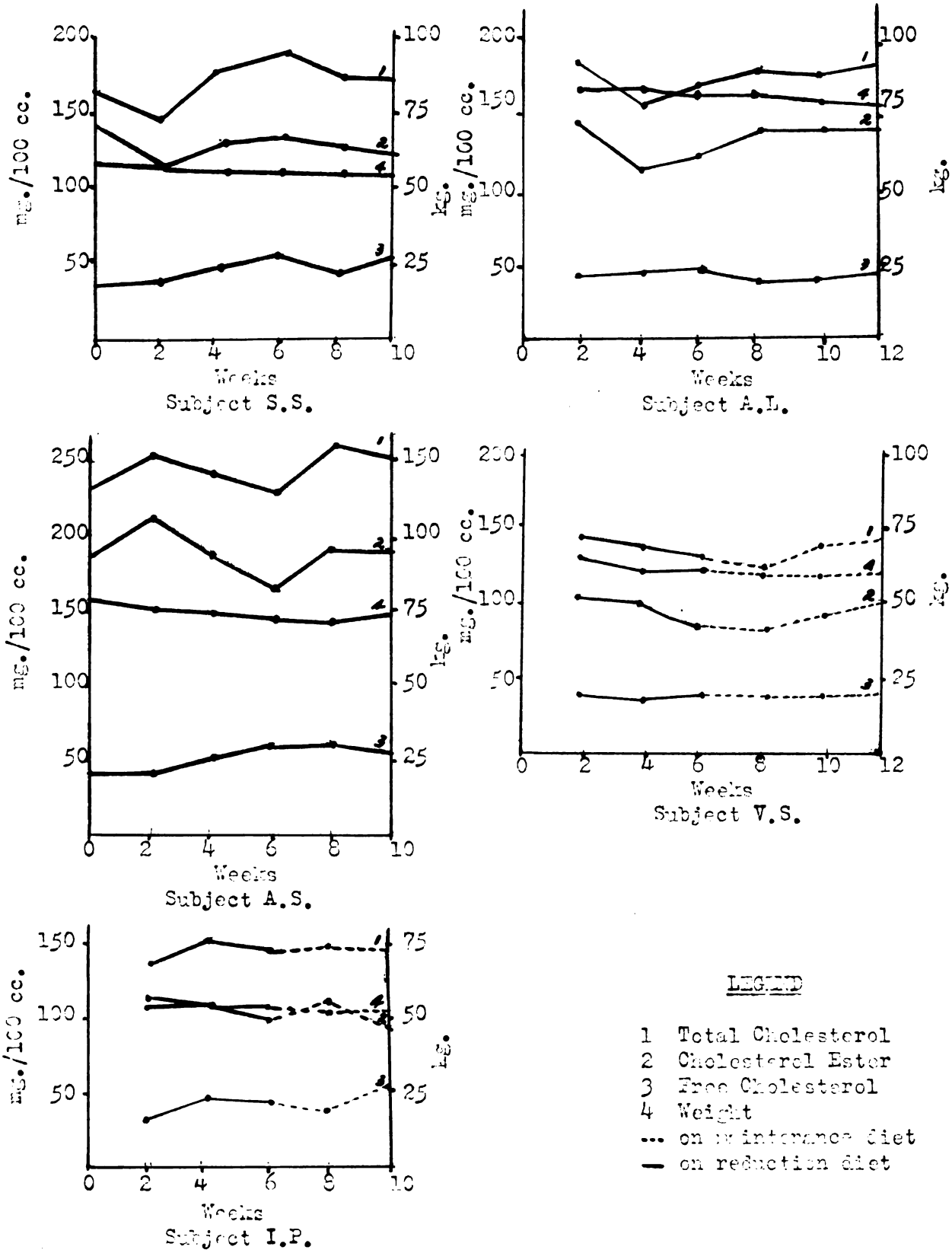


Table 5

ANALYSIS OF VARIANCE OF TOTAL CHOLESTEROL OF SEVEN SUBJECTS ON A WEIGHT REDUCTION DIET FOR TEN WEEKS

Source	Degress of Freedom	Variance	F Values	Predicted F Values	
				5%	1%
Total	41				
Subjects	6	6375.9	45.4	2.42	3.47
Time	5	320.7	2.3	2.53	3.70
Error	30	140.4			

Table 6

FREE CHOLESTEROL OF BLOOD SERUM OF OVERWEIGHT WOMEN ON A SELF-SELECTED DIET, A WEIGHT REDUCTION DIET AND A DIET PLANNED FOR MAINTENANCE OF DESIRED WEIGHT

Subject	Serum Cholesterol on Self-Selected Diet	Serum Cholesterol on Weight Reduction Diet					Serum Cholesterol on Maintenance Diet		
		2 weeks	4 weeks	6 weeks	8 weeks	10 weeks	2 weeks	4 weeks	6 weeks
	mg./100 cc.	mg./100 cc.	mg./100 cc.	mg./100 cc.	mg./100 cc.	mg./100 cc.	mg./100 cc.	mg./100 cc.	mg./100 cc.
A.W.	32.5	27.5	28.1	34.4	38.1	28.8			
M.J.	51.5	38.1	40.0	25.0	45.0	47.5			
D.VA.	29.1	26.3	22.5	35.0	21.3	28.8			
E.H.	45.6	29.4	25.6	33.1	35.6	35.0			
A.S.	40.6	40.0	54.4	62.5	64.4	58.4			
S.S.	34.4	37.5	51.9	56.9	48.1	55.3			
F.C.	55.0	48.8	42.5	50.0	48.8	55.6			
V.S.		40.6	39.4	45.6			40.6	43.8	41.3
A.L.		41.3	46.3	47.5	39.4	39.1			
I.P.		30.6	47.5	43.8			40.0	51.3	
MEAN	41.2	36.0	39.8	43.4	42.6	43.6	40.3	47.6	41.3

Table 7

ANALYSIS OF VARIANCE OF FREE CHOLESTEROL OF SEVEN SUBJECTS ON A WEIGHT REDUCTION DIET FOR TEN WEEKS

Source	Degrees of Freedom	Variance	F Values	Predicted F Values	
				5%	1%
Total	41				
Subjects	6	604.1	14.0	2.42	3.47
Time	5	79.2	1.5	2.53	3.70
Error	30	53.0			

The ratios of free to total cholesterol for the individual subjects are given in Table 8. The ratio of free to total cholesterol varied from 18.6 to 31.5 per cent during the self-selected diet period with an average of 23.9 per cent. The average ratios of free to total cholesterol for each two week interval during weight reduction were 21.8, 23.2, 25.4, 22.4, and 23.3 per cent. The averages for the entire group showed the constancy of ratio of free to total cholesterol which was reported by Sperry (1936). However variations in the ratio of free to total cholesterol for the individual subjects were much greater during the successive periods. The ratio of free to total cholesterol varied from 12.9 to 33.3 per cent during weight reduction for subject M.J. and from 16.1 to 27.6 per cent for subject A.S.

Values for the concentration of cholesterol ester in the blood serum were obtained from the difference of the total and free cholesterol values for each period and are shown in Table 9. The cholesterol ester of blood serum during the self-selected diet period ranged from 99.4 to 183.9 milligrams per 100 cubic centimeters and averaged 134.1 milligrams per 100 cubic centimeters. The average cholesterol ester concentrations for each two week interval during weight reduction were 131.9, 132.6, 128.9, 145.6 and 143.3 milligrams per 100 cubic centimeters of serum respectively. The relative constancy of the values for total cholesterol for the individual subjects during weight reduction may be seen in Figure II. Analysis of variance also showed that these changes were not significant (Table 10).

Table 8

RATIO OF FREE TO TOTAL CHOLESTEROL OF BLOOD SERUM OF OVERWEIGHT WOMEN ON A SELF-SELECTED DIET, A WEIGHT REDUCTION DIET AND A DIET PLANNED FOR MAINTENANCE OF DESIRED WEIGHT

Subject	Ratio of Free to total Cholesterol on Self-Selected Diet	Ratio of Free to Total Cholesterol on Weight-Reduction Diet					Ratio of Free to Total Cholesterol on Maintenance Diet		
		2 weeks	4 weeks	6 weeks	8 weeks	10 weeks	2 weeks	4 weeks	6 weeks
	%	%	%	%	%	%	%	%	%
A.W.	18.6	16.7	15.8	20.2	20.8	14.6			
M.J.	31.4	23.3	21.9	12.9	22.9	22.1			
D.VA.	20.5	16.0	13.8	23.7	13.7	19.5			
E.H.	31.5	21.2	18.5	23.9	24.8	23.9			
A.S.	17.9	16.1	22.2	27.6	24.6	23.1			
S.S.	19.2	24.6	28.2	28.8	26.7	30.7			
F.G.	28.2	27.5	23.0	24.4	23.5	30.3			
V.S.		27.5	27.9	34.1			31.9	30.8	28.1
A.L.		22.3	29.6	28.6	22.2	22.5			
I.P.		22.5	31.4	30.4			26.9	34.5	
MEAN	23.9	21.8	23.2	25.4	22.4	23.3	29.4	32.7	28.1

Table 9

CHOLESTEROL ESTER OF BLOOD SERUM OF OVERWEIGHT WOMEN ON A SELF-SELECTED DIET, A WEIGHT REDUCTION DIET AND A DIET PLANNED FOR MAINTENANCE OF DESIRED WEIGHT

Subject	Serum Cholesterol on Self-Selected Diet	Serum Cholesterol on Weight Reduction Diet					Serum Cholesterol on Maintenance Diet		
		2 weeks	4 weeks	6 weeks	8 weeks	10 weeks	2 weeks	4 weeks	6 weeks
	mg./100 cc.	mg./100 cc.	mg./100 cc.	mg./100 cc.	mg./100 cc.	mg./100 cc.	mg./100 cc.	mg./100 cc.	mg./100 cc.
A.W.	142.5	137.5	149.4	135.6	145.6	167.5			
M.J.	112.3	125.6	142.5	168.8	151.3	167.5			
D.VA.	112.8	137.5	140.0	112.5	133.8	118.8			
E.H.	99.4	109.4	113.1	105.6	108.1	111.3			
A.S.	186.9	208.8	190.6	163.8	196.9	194.1			
S.S.	145.0	115.0	131.9	140.6	131.9	124.7			
F.G.	140.0	128.8	142.5	155.0	158.8	128.1			
V.S.		106.9	101.9	88.1			86.9	92.1	105.6
A.L.		143.8	110.0	118.8	138.1	134.7			
I.P.		105.6	103.8	100.0			108.8	97.5	
MEAN	134.1	131.9	132.6	128.9	145.6	143.3	97.9	94.8	105.6

Table 10

ANALYSIS OF VARIANCE OF CHOLESTEROL ESTER OF SEVEN SUBJECTS ON A WEIGHT REDUCTION DIET FOR TEN WEEKS

Source	Degrees of Freedom	Variance	F Values	Predicted F Values	
				5%	1%
Total	41				
Subjects	6	3904.7	19.8	2.42	3.47
Time	5	161.8	0.82	2.53	3.70
Error	30	197.1			

The ratio of cholesterol ester to total cholesterol in the serum was calculated for each subject and the values are given in Table 11. From 65.9 to 87.1 per cent of the total cholesterol was present in the blood serum as the cholesterol ester fraction. Average values for the entire group during the successive periods were remarkably constant. Variations for the individual subjects during weight reduction were from 5.4 per cent for subject E.H. to 11.5 per cent for subject A.S.

The values presented above indicate that for this group of overweight women there was little change in the total serum cholesterol or in the fractions of serum cholesterol during weight reduction. The decrease in total cholesterol and free cholesterol which occurred for some of the subjects in the two week period after the subjects were given a restricted diet suggested the possibility of a relationship between the caloric intake and the concentration of serum cholesterol. The gradual increase which occurred following the initial decrease may have represented an adjustment to the restricted caloric intake or the influence of a diet which supplied a relatively high ratio of the calories from animal fats. Since the changes in serum cholesterol for the entire group were not found to be statistically significant, it would seem that the gradual increase in serum cholesterol which was observed for four of the subjects may have represented an adjustment to the restricted caloric intake. The values for serum cholesterol obtained in this study would seem to indicate that the excess body weight for these subjects at the beginning of the study did not influence the cholesterol metabolism.

Table 11

RATIO OF COMBINED TO TOTAL CHOLESTEROL OF BLOOD SERUM OF OVERWEIGHT WOMEN ON A SELF-SELECTED DIET, A WEIGHT REDUCTION DIET AND A DIET PLANNED FOR MAINTENANCE OF DESIRED WEIGHT

Subject	Ratio of Combined Cholesterol on Self-Selected Diet	Ratio of Combined to total Cholesterol on Weight Reduction Diet					Ratio of Combined to Total Cholesterol on Maintenance Diet		
		2 weeks	4 weeks	6 weeks	8 weeks	10 weeks	2 weeks	4 weeks	6 weeks
	%	%	%	%	%	%	%	%	%
A.W.	81.4	83.3	84.2	79.8	76.3	85.4			
M.J.	66.6	76.8	78.1	87.1	77.1	77.9			
D.VA.	79.5	84.0	86.2	76.3	86.3	80.5			
E.H.	68.5	78.8	81.5	76.1	75.2	76.1			
A.S.	82.1	83.9	77.8	72.4	75.4	76.9			
S.S.	80.8	75.4	71.8	71.2	73.3	69.3			
F.G.	71.8	72.5	77.0	75.6	76.5	69.7			
V.S.		72.5	72.1	65.9			68.1	69.2	71.9
A.L.		77.7	70.4	71.4	77.8	77.5			
I.P.		77.5	68.6	69.6			73.1	65.5	
MEAN	76.1	78.2	76.8	74.5	77.2	76.7	70.6	67.4	71.9

Serum cholesterol values also were obtained for subjects V.S. and I.P. on diets which were planned to maintain the desired weights of these subjects. This portion of the study was limited to six weeks for subject V.S. and four weeks for subject I.P. For both subjects, the serum cholesterol values on maintenance diets were within the ranges of values for these subjects during weight reduction.

Comparison of Serum Cholesterol Values for Overweight Women and Women of Average Weight.

Eleven healthy women of average body weight were selected to act as controls for a comparison of serum cholesterol values of overweight women and women of average weight. The age, height, weight, caloric intake and cholesterol concentrations of free, total, and cholesterol ester of the control subjects are given in Table 12. Ages of the women in the control group varied from 19 to 28 years; this was slightly above the range of ages for the subjects on the reduction diet.

The caloric intakes of the women of average weight varied from 1499 to 2498 calories per day. The average intake was 1859 calories per day or about 140 calories less than the average intake of the experimental subjects on self-selected diets.

The total serum cholesterol values for the control subjects range from 115.0 to 192.5 milligrams per 100 cubic centimeters; the average was 153.6 milligrams. Both the range of values and the average total cholesterol concentration of the serum of the women of average weight were below the values for the experimental subjects. However the

Table 12

THE AGE, HEIGHT, WEIGHT AND SERUM CHOLESTEROL OF ELEVEN HEALTHY WOMEN OF AVERAGE BODY WEIGHT

Subject	Age	Height	Weight	Caloric Intake	Serum Cholesterol				
					Total	Free		Ester	
	yrs.	cm.	kg.	cal./24 hrs.	mg./100 cc.	mg./100 cc.	per cent	mg./100 cc.	per cent
A.W.*	22	160.0	57.7	1855	160.0	55.0	34.4	105.0	65.6
K.M.	25	163.8	58.6	1712	157.2	47.3	30.7	106.9	69.3
J.F.*	24	172.7	75.0	1929	171.3	33.2	19.4	138.0	80.6
N.S.	19	167.6	64.5	1815	128.8	44.4	34.5	84.4	65.5
M.H.	20	167.6	58.1	1499	120.8	39.0	32.2	81.9	67.8
M.BE.*	25	168.9	61.8	1998	191.3	56.0	29.3	135.2	70.7
M.M.	22	162.6	54.5	1642	166.3	43.3	26.1	122.9	73.9
N.K.	20	165.1	56.8	2076	115.0	32.3	28.1	82.7	71.9
J.E.*	20	177.8	71.8	2498	145.0	33.1	22.8	111.9	77.2
J.W.	20	168.9	62.7	1763	145.0	44.0	30.3	101.0	69.7
M.BA.	28	162.6	50.0	1660	192.5	50.4	26.2	142.1	73.8

* Married.

individual differences within both groups were great enough that there was not a significant difference between the total serum cholesterol values of the overweight women and the women of average body weight (t-test, Fisher, 1938).

The combined cholesterol was the fraction of the total cholesterol which was lower for the women of average weight than the experimental subjects. The range of values for cholesterol ester for the control subjects was from 81.9 to 142.1 milligrams per 100 cubic centimeters of serum as compared with a range of 99.4 to 186.9 milligrams of cholesterol ester per 100 cubic centimeters of serum for the experimental subjects; the average concentration of cholesterol ester was 110.2 milligrams per 100 cubic centimeters for the control subjects and 134.1 milligrams per 100 cubic centimeters for the overweight women. Again, the difference was not statistically significant according to the "t" test (Probability = 0.10).

The values for free cholesterol for the eleven women of average weight was similar to the values for the experimental subjects. The range of values was 33.1 to 56.0 milligrams per 100 cubic centimeters with an average of 43.5 milligrams for the control group and 29.1 to 55.0 milligrams with an average of 41.2 milligrams for the experimental group. The mean ratio of free to total cholesterol concentration of the women of average weight was 28.3 per cent; this was five per cent less than the average ratio for the experimental subjects.

The women of the two groups were similar both in the range of ages and of heights. However the similarities were not close enough to justify pairing the subjects on the basis of desired weight, height

and age. The tendency toward higher serum cholesterol values for the overweight women when compared with women of average body weight suggests the desirability of extending this study to include a larger number of subjects and by pairing subjects on the basis of body build.

Comparison of Serum Cholesterol of Capillary and Venous Blood.

A comparison of serum cholesterol for capillary and venous blood is presented in Table 13. The average values for total cholesterol and cholesterol ester concentration in venous blood serum were within one milligram of the average values for capillary blood serum, but the average of free cholesterol in venous blood serum was three milligrams less than that of capillary blood. The average ratio of cholesterol ester to total cholesterol in the venous blood was within one per cent of that for capillary blood.

On the basis of this study, it would seem that the cholesterol content of capillary blood serum does not differ from that of the serum of venous blood.

Table 13

COMPARISON OF SERUM CHOLESTEROL VALUES FOR CAPILLARY AND VENOUS BLOOD

Subject	Total Cholesterol		Cholesterol Ester		Free Cholesterol		Ratio of Cholesterol Ester to Total Cholesterol	
	Venous Blood	Capillary Blood	Venous Blood	Capillary Blood	Venous Blood	Capillary Blood	Venous Blood	Capillary Blood
	mg./100 cc.	mg./100 cc.	mg./100 cc.	mg./100 cc.	mg./100 cc.	mg./100 cc.	per cent	per cent
A.L.	170.6	173.8	122.5	134.7	48.1	39.1	78.8	77.5
M.J.	163.8	180.0	141.6	125.6	38.4	38.1	78.6	76.8
D.VA.	126.3	163.8	95.6	137.5	30.6	26.3	75.7	84.0
E.H.	149.1	141.3	108.7	109.4	32.5	39.7	77.0	73.4
A.S.	256.3	248.8	203.7	208.8	52.5	40.0	79.5	83.9
V.S.	128.1	141.3	91.9	101.9	36.3	39.4	71.7	72.1
S.S.	196.6	166.2	142.8	123.4	53.8	42.8	74.3	72.7
I.P.	175.6	148.8	137.5	97.5	38.1	51.3	78.3	65.5
F.G.	185.0	177.5	131.9	128.8	53.1	48.8	71.3	72.5
MEAN	172.4	171.3	130.7	129.7	36.7	40.61	76.1	75.4

SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

The total and free cholesterol concentrations of blood serum were determined for ten overweight college women at two week intervals for a period of twelve weeks. The subjects were given controlled weight reduction diets after their customary caloric intakes were determined.

The serum cholesterol during the self-selected diet period averaged 175.4 milligrams per 100 cubic centimeters. There was an initial decrease of total cholesterol for four of the subjects after the introduction of the basic reduction diet; this was followed by a trend toward an increase in total serum cholesterol. The other subjects also showed a gradual increase in total serum cholesterol. However in serum cholesterol during the successive periods were not statistically significant.

The free cholesterol concentration of blood serum averaged 41.2 milligram per 100 cubic centimeters of serum during the period of the self-selected diet. Variations in the concentration of free cholesterol in the serum followed the same pattern as that for total cholesterol. An analysis of variance showed that changes in the concentrations of free cholesterol during the period on weight reduction were not statistically significant. Ratios of free to total cholesterol averaged 21.8 per cent during the weight reduction period.

The concentration of cholesterol ester was obtained from the difference of total and free cholesterol values for each period. The cholesterol ester averaged 134.1 milligram per 100 cubic centimeters.

The average ratios of cholesterol ester to total cholesterol during successive periods were remarkably constant. Individual variations ranged from 5.4 per cent to 11.5 per cent.

The values obtained indicate that for this group of overweight women, there was little change in the total serum cholesterol and in fractions of serum cholesterol during weight reduction.

The subjects lost an average of 0.67 kilogram of body weight per week. The rate of weight reduction was satisfactory in all of the subjects but one. Two subjects achieved the desired weight loss and were then given diets planned to maintain body weights. The values for blood serum cholesterol during the maintenance diet period for these subjects were within the range of the values for these subjects during weight reduction.

A comparison of serum cholesterol values for overweight women and women with average weight indicated that the values for the average total cholesterol concentration of serum of the women of average body weight were below the values of the experimental subjects. However, the difference was not statistically significant. Values of free cholesterol for the control group and the experimental group were similar. The mean ratio of free to total cholesterol concentration was five per cent less for the experimental subjects than the control group.

A comparison of serum cholesterol for capillary and venous blood showed that the cholesterol content of the blood serum of capillary blood does not differ from that of the serum of venous blood.

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