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The effects of a nine-week physical fitness program on plasma lipid concentrations, clotting time, and lysis time of nine young men (18-22) were investigated. The weight of each subject was maintained by a controlled diet. Relatively constant levels of fat calories, saturated fatty acids, and cholesterol were maintained. Total lipids, total and free cholesterol, phospholipids, triglycerides, non-esterified fatty acids, clotting time, and lysis time of plasma were determined. The means of each parameter were statistically analysed.

The mean concentrations of total lipids, phospholipid, triglyceride and non-esterified fatty acids, and the plasma lysis time were comparable to those found in adults. Both the mean and the individual plasma clotting times were longer than reported adult values. No relationship was found between individual plasma lipid concentrations and plasma clotting time.

In comparing the mean values of samples taken at the beginning and end of the study, a trend toward lowered plasma lipids and increased lysis time was noted; although these changes were not significant. There was a progressive increase in plasma clotting time during the nine-week period. This increase was highly significant.

The Plasma Lipid and Coagulation Response of Young Men to a Physical Fitness Program

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INTRODUCTION

Cardiovascular disease (CVD) is one of the leading causes of death in the western world. Recognizing this fact, scientists have made a concerted effort to determine the causes of CVD and to establish preventive measures. Investigations show that both diet and physical activity have an effect on the incidence of CVD.

One approach to the problem has been directed toward reducing the plasma concentrations of cholesterol and other lipids which are usually elevated in patients with CVD. Elevated cholesterol levels have responded to dietary manipulations such as the reduction of total fat (Meyer et al., 1954), or cholesterol (Connor, Hodges, and Bleiler, 1961), and the substitution of polyunsaturated fats for saturated fats in the diet (Fetcher et al., 1967). In overweight persons, weight reduction alone has been effective in lowering plasma cholesterol concentrations (Walker et al., 1953).

Another approach has been directed toward establishing a favorable balance between coagulation and fibrinolysis. Although the physiological mechanisms responsible for CVD have not been clearly identified, abnormalities of the clotting process have been noted (Panchenko and Bazaz'yan, 1965; Chakrabarti et al, 1968).

Exercise has an immediate effect both on plasma lipid levels and on the clotting-lysis response. Furthermore, population studies have shown that men employed in sedentary occupations have a greater incidence of CVD than do those whose work requires physical activity (Morris et al., 1953). However, most studies on the long term effects of exercise have been done on older men.

The value of a regular exercise program for young men, and the role of physical activity in the prevention of CVD need to be investigated. The object of this study is to assess the effect of regular physical activity on the plasma lipids and coagulation equilibrium of young men whose dietary intake is held constant.

REVIEW OF LITERATURE

Plasma Lipids

The role of elevated plasma lipids, particularly cholesterol, as a causative factor in CVD has been investigated by many workers. Among ten risk factors that contribute to coronary heart disease are high serum cholesterol and high serum triglycerides (Hatch et al., 1966). Control patients in Hatch's study exhibited an average of 1.9 abnormal findings while patients who had experienced myocardial infarctions had an average of 4.9. Turpeinen (1969) observed that a diet that lowered the serum cholesterol was followed by a decreased incidence of new coronary events. Garrett, Pangle, and Mann (1966) listed hypercholesteremia, hypertension, and obesity as coronary risk factors.

Albrink (1963) was one of the first to suggest that elevated plasma triglycerides may be useful in predicting the risk of CVD. She reported that over 80% of the men having had coronary attacks had elevated serum triglycerides, as compared with 38% of the control group who were 40 to 69 years old and 5% of the controls who were 20 to 29 years old. Hatch et al. (1966) found that the sum of the cholesterol and the triglyceride was more characteristic of patients who had had myocardial infarction than was either cholesterol or triglyceride alone.

If a high level of cholesterol or triglyceride is a predisposing factor in CVD, then any measure that would lower plasma levels of

these lipids would be of value in lessening the risk of coronary event.

Normal Plasma Lipid Levels

The lipid components of plasma that are measured in this study are total lipids, phospholipids, triglycerides, non-esterified fatty acids (NEFA) and cholesterol. Table 1 presents mean values for plasma lipids of young men, taken from several authors, and also the compilation of adult normal values from Henry (1964). The total plasma lipids of normal fasting adults may range from 450 to 1000 mg/100 ml plasma. As may be seen in Table 1, the mean total lipid values for young men fall well within these limits. In Marumoto's (1970) group the average was 464 mg/100 ml, while Lindholm (1956) found a mean of 914 mg/100 ml.

Methods of triglyceride determination give differing results. Early researchers computed the triglycerides as difference between total lipids and other lipid components. When triglycerides are analysed by laboratory methods, the plasma levels are lower. Triglyceride concentrations presented in Table 1 were determined by chemical analysis. Mean values reported for young men fall within the range of adult values although the spread of individual concentrations observed by Marumoto (1970) was somewhat narrower than those for all adults. This narrow range of individual values within a single age group is even more apparent when one looks at the phospholipid concentrations (Table 1).

TABLE 1. Mean fasting plasma lipids of young men.

Reference	Age	Total Lipid	Phospholipid	Cholesterol			Triglyceride	NEFA
				Total	Free	Free/Total		
	Yr.	mg/100 ml	mg/100 ml	mg/100 ml		%	mg/100 ml	mEq/L
Lindholm (1956)	20-29	914	225	159	59	37		
Hallgren et al. (1960)	23-38	588	197	224	69	31	76	19[a]
Svanborg and Svennerholm (1961)	16-35	610	208	192	64	33	84	0.75
Marumoto (1970)	18-19	464 336-594[b]	166 118-194	138 99-163	49 36-60	36 30-40	73 41-119	0.72 0.31-1.18
Henry[c] (1964)	Adult	450-1000	125-300	110-356		22-30	29-134	0.45-0.90

[a] mg/100 ml
[b] Range of 19 subjects
[c] Compilation of values for normal adults

Although total cholesterol of young men is within the range listed for all adults, the ratio of free:total cholesterol appears to be higher in the young men. Total adult range is listed as 22 to 30% while that of the young men in Marumoto's study was 30 to 40%.

Thus, plasma lipids may be affected by factors such as age and sex, and probably by relative body weight or body build. Lopez-S, Krehl, and Hodges (1967) have reported mean cholesterol levels by age. They show a gradual overall rise from 14 years to 69 years, beginning at 155 mg/100 ml and ending at 230/100 ml plasma. Lindholm (1956) presented data to show that total lipids, total cholesterol, free cholesterol, and phospholipids increased significantly with age in females. In males these parameters increased until age 50 and then remained static. He could find no variation of total lipid, total cholesterol, free cholesterol or phospholipid due to relative body weight or body build.

Plasma Lipids in Relation to Diet

Changes in dietary fats can alter plasma lipid levels; cholesterol is particularly affected by the source of fat. Turpeinen (1969) demonstrated that when milk fat was replaced by soybean oil in the diet of male hospital patients, their serum cholesterol levels decreased. When milk fat was restored, the serum cholesterol levels rose to the previous levels. Ahrens et al. (1957) also demonstrated that the source of fat can influence serum lipids. Patients on a formula diet in which

all dietary fat was from corn oil had lower levels of total cholesterol, free cholesterol, phospholipids and triglycerides than when they ate ad libitum. When the source of fat in the formula diet was changed from corn oil to coconut oil, total cholesterol, free cholesterol, and phospholipids increased. When the fat source was reversed the levels of total cholesterol, free cholesterol, and phospholipids decreased.

The question of the effect of dietary cholesterol on serum cholesterol levels is not resolved. Keys et al. (1956) reported that the ingestion of cholesterol failed to alter the serum cholesterol significantly. However, Steiner, Howard, and Akgun (1962) observed that the addition of crystalline cholesterol (3 gm) to a formula diet resulted in progressively increasing serum cholesterol levels. Steiner concluded that if cholesterol is presented in a form that is readily absorbed from the gastointestinal tract, then serum cholesterol levels will be elevated. Connor, Hodges, and Bleiler (1961) found that egg yolk caused a significant increase in serum cholesterol. The amount of cholesterol from egg yolk that produced the increase was 475 to 1425 mg/per day.

The source of carbohydrate as well as the source of fat can influence lipid response in man. Antar and Ohlson (1965) studied the effect of simple sugars versus starch as a source of carbohydrate in the diets of young men and young women, aged 20 to 25 years. A high sugar diet increased the total lipids, phospholipids and non-phospholipids, with the greatest increase in non-phospholipids. With the high starch

diet there was a decrease in these lipid parameters. Macdonald (1965) showed that young men on a low fat, high carbohydrate diet had increased serum glyceride levels when the carbohydrate was sucrose but no increase when the carbohydrate was cornstarch and partially hydrolyzed starch.

Plasma Lipids in Relation to Exercise

The effect of regular exercise programs on plasma lipids is modified by the initial lipid level and also by the nutritional state of the individual. Montoye et al. (1959) reported that a three-month exercise program had no effect on the serum cholesterol levels of middle-aged men if the initial serum level was "normal". If the initial concentration was high there was a decrease in serum cholesterol after the three-month period of exercise. Johnson and Wong (1961) also were unable to produce changes in serum cholesterol in swimmers who participated in a controlled exercise program. They concluded that greater exercise would be necessary to produce changes because initial cholesterol levels were low.

When assessing the effect of exercise on plasma lipids, the relationship of caloric intake to output must be considered. For example, Taylor, Anderson, and Keys (1957) found no significant changes in serum cholesterol levels of young men if calories were increased to meet the energy needs of the exercise. But Carlson and Fröberg (1967) showed a decrease of serum cholesterol,

phospholipids, and triglyceride and an increase in non-esterified fatty acids (NEFA) after a ten-day period of exercise when the caloric intake was restricted. Average intake was 200 calories per day and all subjects lost weight. The men on this study ranged in age from 20 to 50.

Marumoto (1970) found no significant changes in total lipids, free or total cholesterol, triglycerides, or non-esterified fatty acids after an 11-week fitness program. However, there was a significant decrease in phospholipids. The diet on this study was not controlled but mean body weight was maintained. Examination of dietary records indicated that the average daily intake of calories increased from 2900 at the beginning of the study to 3100 at the end. Fat comprised 40% of the calories in both periods.

The effects of the type of exercise were investigated by Campbell (1965). Phasic and static activity were defined: phasic exercise involved a rapid interchange of arms and legs with a rapid flexing and relaxing of muscles (cross country running and tennis) while static exercise occurred when muscles were held in a prolonged state of contraction with a slower rate of relaxing (wrestling, weight training, and tumbling gymnastics). The results showed a decrease of serum cholesterol with phasic activity but little change with static activity.

Not all investigators agree that exercise lowers plasma lipids. Calvy et al. (1963, 1964) observed the serum cholesterol of young Marine recruits who were in basic training. The results showed no significant change over a 5 1/2 month period. The subjects' diets were unlimited, with an average intake of 4500-5200 calories, 45% of which were supplied by fat. Johnson et al. (1959) studied college swimmers. After several months of controlled exercise there were no changes in the serum cholesterol.

Blood Coagulation

Many factors are involved in the formation and lysis of the blood clot and numerous methods have been used to measure the speed of these processes. Consequently, it is not always possible to compare the clotting or lysis times obtained in one study with those reported in another. However, comparisons within any one study indicate that blood coagulability varies with disease and dietary intake, and is also affected by exercise.

The Clotting Response

The clotting time of plasma is a measure of the time required for the sample to coagulate with or without accelerating factors. A number of studies have shown that the clotting of plasma is accelerated by lipemia following the ingestion of a fatty meal (O'Brien, 1956; Fullerton, Davie, and Anastasopoulos, 1953). Further investigation

by McDonald and Fullerton (1958a) showed that both animal fats and vegetable fats induced the same increase in coagulability after the high fat (85 grams) meal. Then McDonald and Fullerton (1958b) went on to show that the effects of the high fat meal on coagulation could be abolished by moderate physical activity.

The immediate effects of exercise on the blood clotting system of young men have been explored by several investigators. Egeberg (1963) reported that short term strenuous exercise decreased the clotting time in young healthy male subjects. Iatridis and Ferguson (1963) also found a significant acceleration of clotting after strenuous exercise. These observations were confirmed by Burt, Blyth, and Rierson (1964). There is very little reported about the effects of regular physical activity on clotting time. However, Keys and Buzina (1956) observed that the blood of physically active men clotted less rapidly than did the blood of sedentary men. Marumoto (1970) found a significant increase in the mean clotting time of plasma of young men after an 11-week fitness program.

The relationship between the coagulation mechanism and plasma lipid levels has been discussed by Panchenko and Bazaz'yan (1965). This study showed that a higher clotting power and a lower anti-coagulating system is correlated with high levels of blood lipids, cholesterol and -lipoproteins and lowered levels of non-esterified fatty acids in patients with coronary insufficiency.

Fibrinolysis

In simplest terms, the fibrinolysis time is a measure of the speed at which a fibrin clot may be lysed by the plasma. Hougie and Ayers (1960) were able to find no significant effect of fat feeding on fibrinolysis time. But Billimoria et al. (1959) reported a significant inhibition of fibrinolysis two hours after the ingestion of a butter meal. Furthermore, they found that moderate exercise could offset the effects of a fatty meal. The enhanced fibrinolysis induced by exercise lasted only one to two hours. Ogston and Fullerton (1961) also noted that the increased fibrinolysis after exercise was only transient. Furthermore, exercise in untrained subjects had a delayed depressing effect on fibrinolytic activity. Sherry et al. (1959) also observed enhanced fibrinolytic activity in the blood immediately following exercise. In this study, the exercise was strenuous. The male subjects showed more fibrinolytic activity after exercise than did the female subjects. Burt, Blyth, and Rierson (1964) also found a shortened fibrinolysin time after young college students had exercised to exhaustion.

The relationship between fibrinolytic activity and coronary-artery disease was investigated by Chakrabarti et al. (1968). They found that, of the survivors of myocardial infarction, 52% had defective fibrinolysis as compared to 19% in the healthy controls. They suggested that defective fibrinolysis be controlled as a preventive measure in coronary heart disease.

Burt, Blyth, and Rierson (1964) directed their attention to the coagulation-fibrinolysis equilibrium rather than to the individual components of clotting or lysis. They concluded that even though the clotting time was shortened with exercise the increased fibrinolysis was great enough to offset it. The end result was that the exercise to exhaustion affected the coagulation-fibrinolysis equilibrium by favoring fibrinolysis.

If clotting is accelerated and fibrinolysis is depressed in CVD, and if exercise can reverse these trends, then young men may be able to lower the risk of CVD in later life by participating in a continuing program of regular physical activity.

EXPERIMENTAL PROCEDURE

Experimental Plan

Subjects

Nine young college men who were enrolled in the Fitness Appreciation course (MPE 134) were selected as subjects. The subjects were apparently healthy, not receiving drugs, and not participating in varsity athletic programs. An effort was made to select young men who were not currently active in sports. The age range was 18 to 22.

Dietary Plan

All meals for the nine-week period were prepared by and served in one residence hall dining room. The initial caloric requirement was estimated by considering the factors of age, sex, and activity. Individual estimates were based on body weight. During the study, calories were adjusted so that the subjects neither gained nor lost weight. Daily menus were written for each subject using foods selected from the residence hall menu and daily food intake was recorded.

Average weekly intake of calories, protein, fat, carbohydrate, calcium, iron, vitamin A, thiamin, riboflavin, niacin, and ascorbic acid was estimated from data in Agriculture Handbook #8 (Watt and Merrill, 1963) and Home and Garden Bulletin #72 (U.S. Department of Agriculture, 1971).

The purpose of the controlled diet was to keep a fairly constant dietary intake from week to week.

Physical Fitness Program

The Fitness Appreciation class (MPE 134) consists of jogging and swimming. Twice each week a student and his partner run a selected course. The courses vary in length from 2 to 12 miles. To successfully complete the class, a student must jog the 12-mile course in 90 minutes. Once a week the students participate in a planned group activity.

Blood Samples

Thirty millilitres of venous blood were drawn from fasting subjects at three times during the study. The first sample was taken at the start of the exercise program and five days after the diet began. The second sample was taken four weeks later, and the third sample was taken at the end of the nine-week period.

The blood was collected in 10-ml Vacutainers which had been treated with EDTA (ethylenediaminetetraacetic acid) and the samples were packed in ice. The hematocrit and the hemoglobin analysis were done immediately on whole blood. Plasma was separated by centrifugation at 2500 rpm for 20 minutes at 5° C. Plasma clotting time was determined and the remainder of the plasma was stored at -10° C.

Methods of Blood Analysis

Hematocrit

Hematocrit was determined by the Wintrobe method (Wintrobe, 1961). Results were expressed as volume of packed red cells per 100 ml. whole blood.

Hemoglobin

The cyanomethemoglobin method was used to determine hemoglobin (Oser, 1965). Hemoglobin was oxidized to methemoglobin, and methemoglobin was converted to cyanomethemoglobin. Absorbance of the latter was read on a Bausch and Lomb Spectronic 20 colorimeter at 540 mμ, using Acuglobin from Ortho Diagnostics as a standard. Results are reported as grams hemoglobin per 100 ml. blood.

Clotting Time

Plasma clotting time (PCT) was measured by the modified Howell procedure as described by Davidsohn and Wells (1962). The PCT is the measure of the time required for a fibrin clot to form in decalcified plasma after the addition of calcium chloride. Plasma clotting time is reported in seconds.

Euglobulin Lysis Time

Fibrinolytic activity was measured according to the method of Iatridis and Ferguson (1962). The euglobulin fraction of plasma is

precipitated from plasma at pH 5.3. A fibrin clot is formed from bovine fibrinogen and calcium-thrombin. The euglobulin lysis time is the time required to completely dissolve the fibrin clot by the euglobulin fraction of the plasma. Euglobulin lysis time is reported in minutes.

Non-esterified Fatty Acids (NEFA)

The non-esterified fatty acids were determined by the method of Dole (1956). The lipids from 2 ml plasma were extracted into heptane, and the heptane phase was washed with sulfuric acid (Trout, Estes, and Friedberg, 1960). Aliquots of the heptane phase were titrated with sodium hydroxide with nile blue as an indicator. Non-esterified fatty acids are expressed as mEq/L of plasma.

Total Lipid Extraction

The lipids were extracted from 2 ml of plasma into chloroform-methanol, 2:1, V/V, using the procedure of Smith (1965). The lipid extracts were transferred to tared 1 ml volumetric flasks, and dried under nitrogen and the flasks were reweighed until a constant weight was obtained. Total lipids were expressed as mg/100 ml plasma. The lipids were redissolved in chloroform, made up to 1 ml volume, and aliquots were taken for the determination of cholesterol, phospholipids and triglycerides.

Free and Total Cholesterol

One hundred microliters of lipid extract were diluted to 1 ml with chloroform. Triplicate aliquots of 50 µl of the diluted extract were used for total cholesterol measurement. Total cholesterol samples were saponified and neutralized before cholesterol was determined. Free cholesterol was measured on triplicate aliquots of 100 µl of diluted lipid extract. The microprocedure of Smith (1961) was used for cholesterol determination. Cholesterol was precipitated as the digitonide. The digitonide was purified and treated with Liebermann-Burchard color reagent. Absorbance was measured in a Beckman model D U Spectrophotometer at 635 mu. Cholesterol was reported as mg/100 ml plasma.

Phospholipids

The microprocedure of Lowry et al. (1954) as modified by Hawthorne, Smith, and Pescador (1963) was used to determine lipid phosphorus. Forty µl of lipid extract were brought to 1 ml with chloroform. Twenty µl aliquots of the diluted sample were used for phosphorus determination. Lipid phosphorus is oxidized to inorganic phosphate, phosphomolybdic acid is formed and reduced by ascorbic acid to the molybdenum blue complex. Absorbance was measured in a Beckman model D U Spectrophotometer at 820 mµ. The phospholipid concentration was obtained by multiplying the phosphorus concentration by 25.

Triglycerides

Determination of triglycerides was done on 200 µl of lipid extract. The procedure of Van Handel and Zilversmit (1957) as modified by Chiu (1969) was used. Phospholipids were removed by adsorption onto activated silicic acid. Then glycerol was released by saponification of the triglycerides with potassium hydroxide. Glycerol was oxidized to formaldehyde. Formaldehyde was treated with chromotropic acid and the absorbance of the solution was measured photometrically. Triglycerides were expressed as mg/100 ml plasma.

Statistical Treatment

Student's *t* test

To determine the effect of the exercise program on plasma lipid concentrations, clotting time and lysis time, student's *t* test was performed on the means. A two tailed test was used with the critical value of *t* taken at $t_{.05}$ and n-1 or 8 degrees of freedom. Statistical analysis was done comparing the means of January and February, February and March, and January and March.

RESULTS AND DISCUSSION

Dietary Intake

Throughout the nine weeks, the diets of the subjects were controlled to assure a relatively constant intake of fat calories, saturated fatty acids and cholesterol. The average daily nutrient intake computed for each subject is presented in Table 2; the average daily intake of the group, computed for each week, is shown in Table 3. The dietary fat provided 40.4% of the calories, ranging from 42.4% in the second week to 39.1% in the eighth week. The ratio of linoleic acid to saturated fatty acids averaged 0.19, ranging from 0.25 during the first week to 0.16 in the seventh week. The average daily cholesterol intake, estimated from available data, ranged from 622 mg in the second week to 734 mg in the last week. For the nine week period the average daily intake was approximately 710 mg.

The average nutrient intake of each subject met or exceeded the Recommended Dietary Allowances (National Research Council, 1968) for young men aged 18 to 22 years, with few exceptions. Subject GG, who did not drink milk, fell slightly short of the recommended 800 mg of calcium, despite the inclusion of liberal amounts of cheese and ice cream in his diet. Although TC consumed less than the recommended allowances of 1.4 mg thiamine and 18 mg niacin, his intake of these nutrients was adequate in relation to his maintenance caloric requirement.

TABLE 2. Average daily dietary intake of each subject.

Subject	Food Energy	Protein	Fat	Satu-rated	Oleic	Lin-oleic	Carbohy-drate	Cal-cium	Iron	Vita-min A	Thia-min	Ribo-flavin	Niacin	Ascorbic Acid
	(Cal)	(G)	(G)	(G)	(G)	(G)	(G)	(MG)	(MG)	(IU)	(MG)	(MG)	(MG)	(MG)
D B	3125	132	139	59.9	52.2	9.9	352	2183	13.71	8402	1.54	3.67	18.5	142
D C	3416	143	153	63.7	57.7	11.7	372	2194	16.04	8669	1.68	3.79	22.3	144
A D	2999	130	138	58.5	52.2	10.4	321	2116	13.71	8094	1.50	3.56	18.3	116
T C	2554	105	113	44.6	43.8	10.4	288	1314	13.43	7119	1.31	2.42	17.7	111
G G	2826	109	118	43.8	46.6	12.2	335	756	16.66	7304	1.39	1.78	22.2	129
M G	3382	144	155	65.1	58.5	11.9	366	2198	15.95	8711	1.68	3.78	21.7	144
S K	3093	134	143	61.2	54.0	10.4	329	2245	13.80	8249	1.53	3.74	18.4	117
BMcK	3218	136	144	60.1	54.5	11.4	357	2173	15.08	8445	1.64	3.69	19.9	141
K W	3412	142	156	65.8	58.9	11.9	373	2191	15.64	8537	1.66	3.75	21.2	133

TABLE 3. Average daily dietary intake of nine subjects, by week.

Week	Food Energy	Protein	Fat	Satu-rated	Oleic	Lin-oleic	Carbohy-drate	Cal-cium	Iron	Vita-min A	Thia-min	Ribo-flavin	Niacin	Ascorbic Acid
	(Cal.)	(G)	(G)	(G)	(G)	(G)	(G)	(MG)	(MG)	(IU)	(MG)	(MG)	(MG)	(MG)
1	3016	125	132	52.4	51.1	13.0	344	1653	15.61	8020	1.63	3.02	19.8	165
2	2912	116	137	57.3	50.4	11.5	310	1908	12.76	7285	1.37	3.20	16.4	101
3	3191	136	142	60.7	53.8	10.0	353	2074	14.61	7966	1.54	3.45	19.8	150
4	3235	136	146	60.6	55.9	11.9	358	1886	14.69	6403	1.47	3.35	20.8	117
5	3215	130	143	58.3	54.3	10.7	364	1987	14.93	7455	1.68	3.35	18.5	134
6	3178	134	145	59.9	54.3	10.9	312	1930	15.37	9067	1.63	3.44	20.7	159
7	3052	132	136	56.1	50.3	9.3	336	2011	15.02	7746	1.42	3.40	19.4	125
8	3153	136	137	56.6	51.8	11.6	357	1992	15.56	9967	1.58	3.51	21.3	118
9	3072	135	142	60.8	55.5	11.3	323	1931	15.46	9623	1.61	3.46	21.3	141
Grand Average	3114	131	140	58.1	53.2	11.1	344	1930	14.89	8170	1.55	3.35	20.0	131

The caloric intake of each subject was adjusted to maintain his initial body weight. During the nine weeks, the dietary average for all subjects was 3114 Kcal. There was a gradual increase in caloric requirements through the fifth week, after which there was a gradual decline. Marumoto (1970) estimated the caloric intake of her subjects from diet histories taken before and after an 11-week jogging program. She concluded that young men required an average of 200 additional Kcal per day to offset the energy expenditure due to the exercise. The decline in caloric needs after the fifth week of the present study may represent increased muscular efficiency as the subjects adapted to the training. Furthermore the diet for the first week of this study was planned in anticipation of increased caloric needs due to the exercise program.

The weights of the subjects, their hematocrits and their hemoglobin concentrations remained stable throughout the study, as may be seen in Table 4. At all three sampling periods, the hematocrits and hemoglobins were well within the normal range. These measurements were performed in order to monitor the general state of health of the subjects.

TABLE 4. Height, weight, hematocrit and hemoglobin values for nine subjects. Observations at the beginning, middle and end of a nine-week exercise program.

Subject	Height	Weight			Hematocrit			Hemoglobin		
		Jan.	Feb.	Mar.	Jan.	Feb.	Mar.	Jan.	Feb.	Mar.
	ft/in	lb.			Red cells % whole blood			g/100 ml		
D B	6'2"	185	184	186	50.6	49.8	50.0	16.74	17.57	16.54
D C	5'10"	165	165	163	46.3	47.0	47.3	16.24	16.49	15.89
T C	5'8"	126	128	125	47.8	47.0	46.5	16.16	15.99	15.37
A D	5'7"	160	160	159	45.0	48.0	46.5	16.44	17.02	16.04
G G	5'10"	155	157	155	47.3	47.5	46.8	16.34	16.94	15.89
M G	6'0"	185	185	184	43.3	46.5	48.8	15.54	15.11	16.31
S K	5'11"	160	156	161	46.3	47.5	49.8	15.76	16.77	16.22
B McK	6'2"	185	188	186	49.6	49.5	48.0	16.39	17.19	15.89
K W	6'0"	170	175	171	46.8	45.5	46.5	16.24	16.18	15.89

Plasma Lipids

The plasma lipid concentrations, clotting times, and lysis times are presented in Tables 5, 6, and 7. The mean values for triglycerides, non-esterified fatty acids (NEFA), and total lipids approximate those reported by other investigators (Table 1). The phospholipid and cholesterol concentrations fall within the ranges observed in normal adults (Henry, 1964), but they are considerably lower than the means reported for young men by Svenborg and Svennerholm (1961), Lindholm (1956) and Hallgren et al. (1960). However, Marumoto (1970) also noted low plasma cholesterol concentrations in her sample of 18-19 year old males. It is likely that the low cholesterol values found in the present study are also a function of age because the diet was far from hypocholesteremic; it contained liberal quantities of saturated fats and cholesterol. Several investigators have observed that plasma cholesterol concentrations increase with age (Lindholm, 1956; Lopez-S, Krehl, and Hodges, 1967).

At the end of the exercise program all of the plasma lipids had decreased (Tables 5, 6, and 7), but none of the decreases was statistically significant. When the means of the three sampling periods were compared, only the NEFA showed significant differences; at the February sampling, the mean plasma NEFA were significantly lower then they had been in January ($p \leq 0.05$).

TABLE 5. Plasma phospholipid, triglyceride and non-esterified fatty acids (NEFA). Observations made at the beginning, middle, and end of a nine week exercise program.

	Phospholipid mg/100 ml			Triglyceride mg/100 ml			N E F A m Eq/L		
Subject	Jan.	Feb.	Mar.	Jan.	Feb.	Mar.	Jan.	Feb.	Mar.
D B	121	117	128	66	63	86	0.60	0.38	0.50
D C	152	98	150	44	72	62	0.73	0.31	0.32
T C	231	218	292	103	136	109	0.47	0.44	0.33
A D	150	150	124	54	89	52	0.64	0.48	0.64
G G	194	164	128	56	136	46	0.98	0.35	1.34
M G	188	128	146	55	66	53	0.58	0.67	0.62
S K	136	100	125	37	36	27	0.92	0.44	0.52
B McK	179	169	147	60	61	80	0.62	0.63	0.32
K W	212	124	188	178	100	120	0.50	0.17	0.29
Mean	174	141	159	73	84	71	0.67	0.43*	0.54
St. Dev.	± 36	± 39	± 54	±44	±34	±31	± 0.17	±0.15	±0.33

*Significantly different from January (p - 0.05).

TABLE 6. Plasma concentrations of total and free cholesterol and their relationship. Observation at the beginning, middle and end of a nine-week exercise program.

CHOLESTEROL

Subject	January			February			March		
	Total	Free	Free/Total	Total	Free	Free/Total	Total	Free	Free/Total
	mg/100 ml		%	mg/100 ml		%	mg/100 ml		%
D B	90	28	31	96	24	24	88	27	31
D C	112	34	30	100	30	30	105	33	31
T C	185	62	34	233	62	27	190	69	36
A D	126	40	31	163	49	30	106	32	31
G G	140	46	33	147	41	28	107	36	34
M G	142	46	33	120	37	31	97	32	33
S K	90	28	31	120	39	32	93	26	28
B McK	152	50	33	184	48	26	108	34	31
K W	130	48	37	120	38	32	136	49	36
Mean	130	42	33	143	41	29	114	38*	32
Std. Dev.	±30	±11	± 2	± 44	±11	± 3	± 31	±13	± 3

*Significantly different from January and March (P - 0.05).

TABLE 7. Total plasma lipids, plasma clotting time, and lysis time.
Observations made at the beginning, middle, and end of a nine-week exercise program.

	Total Lipid mg/100 ml			Clotting Time Seconds			Lysis Time Minutes		
Subject	Jan.	Feb.	Mar.	Jan.	Feb.	Mar.	Jan.	Feb.	Mar.
D B	806	596	1064	181	148	204	150	225	45
D C	635	956	887	169	182	221	120	80	45
T C	894	1154	1056	250	286	240	210	150	180
A D	645	1020	498	199	227	263	110	225	80
G G	617	955	723	181	205	246	155	220	150
M G	659	594	532	189	236	240	175	230	165
S K	549	772	410	175	214	232	180	180	160
B McK	707	888	500	178	199	221	215	205	135
K W	1043	851	685	215	243	280	105	75	120
Mean	728	865	706	193	216	239*	158	177	120
Std. Dev.	±157	±187	±247	±25	± 39	± 17	±41	±62	±50

*Significantly different from January (P - 0.01).

The possibility that some of the subjects may not have been fasting at the time of the February sampling was examined and discarded because seven out of nine subjects showed lowered NEFA concentrations. Furthermore, blood sugars determined on these same samples were well within the fasting range. The free cholesterol, as a percentage of total cholesterol, was also significantly lower in the February samples. This would suggest that more degradation of cholesterol esters may have occured in the January and March samples. However, all plasmas were handled and stored in the same careful manner and the lipid analyses were performed at the same time.

The changes in weight, plasma lipids, clotting time and lysis time from January to March are presented in Table 8. The failure to observe significant changes in plasma lipid concentrations of young men in caloric balance confirms the observations of Taylor, Anderson, and Keys (1957). Changes due to exercise are less likely to be observed when the initial lipid concentrations are in the normal range (Montoye et al., 1959; Johnson and Wong, 1961). The results of this study do not agree with the observations of Marumoto (1970) that plasma phospholipids decreased as a result of exercise.

TABLE 8. Changes in weight, plasma lipids, clotting time and lysis time (January - March).

Subject	Weight	Total Lipid	Triglyceride	Phospho-lipid	NEFA	Cholesterol			Clotting Time	Lysis Time
						Total	Free	Free/Total		
	lb	mg/100 ml	mg/100 ml	mg/100 ml	mEq/L	mg/100 ml		%	sec	min
D B	1	258	20	7	-0.10	- 3	- 1	0	23	-105
D C	-2	252	18	- 2	-0.41	- 7	- 1	1	52	- 75
T C	-1	162	6	61	-0.14	5	7	2	-10	- 30
A D	-1	-147	- 2	-26	0	-20	- 6	0	64	- 30
G G	0	106	-10	-66	0.36	-33	-10	1	65	- 5
M G	-1	-127	- 2	-42	0.04	-45	-15	0	51	- 10
S K	1	-139	-10	- 11	-0.38	4	- 2	-3	57	- 20
B McK	1	-207	20	-32	-0.30	-43	-16	-2	43	- 80
K W	1	-358	-58	-24	-0.21	6	1	-1	65	15
Mean	- .11	- 22.2	- 2.0	-15	-0.127	-15.1	- 4.8	-0.2	45.6	- 37.8
Std. Dev.	1.17	221.1	24.2	35.8	0.241	20.8	7.6	1.6	24.7	39.9

Blood Coagulation

The plasma clotting times for all subjects are presented in Table 7. With one exception, the time required for a clot to form in recalcified plasma exceeded the normal range of 105-150 seconds reported by Davidsohn and Wells (1962). In the current study, clotting times ranged from 148-286 seconds. These findings support the conclusion of Marumoto (1970) that the plasma of young men clots more slowly than does that of older persons.

The mean clotting times increased progressively during the nine-week exercise program, from 193 seconds in January to 216 seconds in February and 239 seconds in March. Although the mean clotting time of the February sample was not significantly different from that of January or March, the overall change from January to March was highly significant ($p \leq 0.01$). This finding agrees with the observations of Keys and Buzina (1956) on middle-aged men; the blood of active men coagulated less rapidly than did the blood of sedentary men. It also confirms the finding of Marumoto (1970) that the plasma clotting time of young men increased significantly following an 11-week jogging program. In Marumoto's study, there was a significant increase in total lipids and a significant decrease in phospholipids following the exercise program. In the present study, all of the plasma lipids decreased but none of the changes was statistically significant. Thus, no relationship between plasma lipids and clotting times could be

demonstrated. Possibly the initial plasma lipids of these young men were too low to show any dramatic change.

The plasma lysis times for all subjects are also shown in Table 7. Most of these values are within the range of 100-500 minutes reported by Iatridis and Ferguson (1962) for normal subjects. The mean lysis time decreased from 158 minutes at the beginning of the study to 120 minutes at its conclusion but the change was not statistically significant. In February, the mean lysis time was greater than the mean of the January or the March samples; again there was no statistical significance. Enhanced fibrinolytic activity immediately after exercise has been reported by a number of investigators (Sherry et al., 1959; Ogston and Fullerton, 1961). Billimoria et al., (1959) reported that the acceleration of fibrinolysis persisted for only one to two hours. Ogston and Fullerton (1961) also noted the transient nature of the enhanced fibrinolysis following exercise. However, in untrained subjects, fibrinolytic activity was depressed on the morning after exercise. Possibly insufficient training may have accounted for the greater lysis time observed in February of the present study.

No consistent relationship between plasma lipid concentrations and fibrinolytic activity could be demonstrated. As in the case of the clotting times, the plasma lipids of these young subjects may have been too low to exert any effect. Decreased fibrinolysis does occur

during postprandial lipemia (Billimoria et al., 1959; Ogston and Fullerton, 1961).

It is apparent that a sustained moderate exercise program can depress plasma clotting and increase fibrinolysis, even in young men. The exercise program did not significantly affect the fasting plasma lipids, which were already low. However, it is possible that regular physical exercise may prevent the periodic hyperlipemia which accompanies the ingestion of meals.

SUMMARY

Nine apparently healthy young men were maintained on a controlled diet while they participated in a nine-week physical fitness program. The individuals were kept in caloric balance as monitored by body weight. Average caloric intake was 3114 Kcal with 40% of the calories as fat. The ratio of saturated fatty acids to linoleic acid and the amount of dietary cholesterol were kept relatively constant. Venous blood samples were drawn at the beginning, ,middle, and end of the program. Plasma samples were analysed to determine concentrations of total lipids, phospholipids, triglycerides, free and total cholesterol and non-esterified fatty acids (NEFA). Plasma clotting and lysis times were measured. The changes in these parameters were analysed statistically to determine the effect of the exercise program.

The mean concentrations of total lipids, phospholipids, triglycerides, free and total cholesterol and NEFA were in the range expected for healthy adults. However, phospholipids and free and total cholesterol were lower than the published values for young men. Clotting times were longer than reported values but lysis times were within the expected range.

Although mean plasma lipid concentrations decreased due to the exercise program, the decrease was not significant. Plasma

clotting time was significantly prolonged by exercise ($p \leq 0.01$) but lysis time showed no significant change.

It is concluded that a regular exercise program did not change plasma lipid concentrations or lysis time of young men who had initial values in the normal range and who were in caloric balance. However, the regular exercise program significantly prolonged the plasma clotting time.

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