THE EFFECT OF MILK CONSUMPTION AND EXERCISE ON THE SERUM CHOLESTEROL OF RAT AND MAN

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ABSTRACT

THE EFFECT OF MILK CONSUMPTION AND EXERCISE
ON THE SERUM CHOLESTEROL
OF RAT AND MAN

by Jeanne Sherburne

A study was conducted to investigate the effects of milk consumption and exercise on the serum cholesterol concentrations of rat and man. Forty-eight male rats were divided into three groups, one of which was sedentary. Two groups were exercised daily by swimming. Powdered whole milk was added to the diet of one of these groups in amounts to provide thirty per cent of the calories. At the end of twelve experimental weeks blood was secured from the animals by cardiac puncture and cholesterol determinations were made on the sera by a modification of the Schoenheimer-Sperry method. The sedentary rats had higher cholesterol concentrations and greater weights than the exercised rats.

The higher cholesterol concentrations appeared to be related to changes in weight and only indirectly to the absence of exercise. The inclusion of powdered whole milk had no effect upon serum cholesterol concentrations or body weights in the exercised rats.

Nine athletes were divided into two groups, with

and without milk, cheese and ice cream in the diet. They exercised three times weekly on the motor-driven treadmill for eight experimental weeks. Before the beginning of the experiment and also during the first experimental week, twenty-four hour recall diets were secured for seven days by a graduate dietitian. Nutrient intakes were evaluated. Before the beginning of the experiment and during the last week of the experiment blood samples were taken by venipuncture and cholesterol determinations were made on the sera by a modification of the Schoenheimer-Sperry method. Body weights remained constant for the two groups. No significant effects of exercise, with or without milk consumption, were noted on blood cholesterol concentrations. There was a decreased intake of calcium, riboflavin and fat and an increased intake of carbohydrate in the restricted diet group. Intakes of calcium and riboflavin were below the amounts recommended by the Food and Nutrition Board of the National Research Council.

According to the results of this study, it is not advantageous to attempt to alter blood cholesterol concentrations of humans by changes in milk intake. There appears to be an indirect advantage in the control of serum cholesterol concentrations through the regulation of exercise and diet for caloric equilibrium.

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By

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INTRODUCTION

Atherosclerosis is a common medical problem today. It is believed to be related to serum cholesterol concentrations. Because of the present interest in attempts to change blood cholesterol concentrations, there has been much research on this subject in recent years. Most of the work has been concerned with altering the amounts of fat or saturated fatty acids in the diet. Some research has involved changes in physical activity. The present investigation into the effects of milk consumption and exercise on serum cholesterol concentrations combines both dietary components and physical activity.

REVIEW OF LITERATURE

It is generally agreed that dietary cholesterol has little effect on serum cholesterol concentrations and that the fat content of the diet appears to be a determining factor. In an early experiment, Messinger ('50) demonstrated that the addition of powdered egg yolks to the diet of man caused a greater elevation in the blood serum cholesterol concentrations than did the inclusion of the same amount of pure cholesterol. The difference in response was attributed to the fat content of the egg yolk. Authorities are not in agreement on the exact causative factor. Some support the contention that it is the fat *per se*, and others maintain it is related to the degree of saturation in the fatty acids of the fat.

Animal Studies

Dietary Experiments

Portman and coworkers ('56) reported the effects of dietary cholesterol on the serum cholesterol concentration of three groups of Cebus monkeys placed on iso-caloric diets. These diets contained fixed amounts of protein and cholesterol with different amounts of corn oil and sucrose. After eight weeks of the diet, with corn oil comprising 10%, 32% and 42% of the calories, the serum cholesterol concentrations of the monkeys were greater in the medium and high fat groups than in the low fat group. When corn oil was lowered to 10% of the calories, the serum cholesterol concentration decreased; and when the corn oil was increased, the serum cholesterol concentration also increased. After changing the diets to hydrogenated cottonseed oil, these associates observed increases in serum cholesterol concentrations. However, the hydrogenated cottonseed oil did not have any more elevating effect on the serum cholesterol than did the plain corn oil, when dietary cholesterol was not added. Diets containing lard and added cholesterol raised serum lipids more than diets containing corn oil and added cholesterol. Hydrogenated cottonseed oil raised the serum cholesterol concentrations even higher than nonhydrogenated cottonseed oil.

Later Portman and Sinisterra ('57) showed that with Cebus monkeys dietary hypercholesterolemia resembled essential hypercholesterolemia in man. No tissue liposis occurred after several months of serum cholesterol concentrations of 600 mg% or greater. There was a delayed rate of esterification of injected radiocholesterol in dietary hypercholesterolemia in monkeys which was similar to endogenous radiocholesterol in xanthoma tuberosum in man. The disappearance curves of labeled exogenous cholesterol were similar both quantitatively and qualitatively to those observed in man. Portman and Sinisterra observed that the effects of different fats on cholesterol concentrations were not due to the difference in division of cholesterol between the serum and the other tissues. Monkeys on diets with lard and cholesterol developed serum cholesterol concentrations much higher than those on diets with corn oil and cholesterol, though the analyses of other tissues were similar. Differences in absorption and turnover of exogenous radiocholesterol depended on the fatty acid or fat used for administration. The tests showed that cholesterol esters of linoleic and oleic acids were well absorbed but cholesterol stearate was not, probably because stearic acid is poorly absorbed. The fats which caused lowered blood cholesterol concentrations were high in linoleic acid, and those which increased cholesterol concentrations were low in linoleic and high in oleic acid. The highest concentrations of serum cholesterol were obtained when 45% of the calories were fed as triolein.

Alfin-Slater and coworkers ('54) examined the effect of essential fatty acid deficiency on the plasma and liver cholesterol in the rat. Contrary to their expectations, the concentrations of the cholesterol esters did not decrease when the fatty acids of the diet were reduced.

These authors postulated that since oleic acid and other more saturated fatty acids formed cholesterol esters which were not metabolized rapidly, it was probable that the concentration of plasma cholesterol in the rat did not reflect the body stores.

After analysis of cholesterol esters in liver and plasma of rats receiving varying amounts of linoleic acid, Klein ('57) reported that linoleic acid was the major polyunsaturated constituent of the liver esters. In the plasma other fatty acids increased as the linoleic acid in the diet increased, and there were significant quantities of tetraenoic acid. The polyunsaturated fatty acid content of the plasma esters exceeded that of the liver esters by a factor as large as ten. The metabolism of plasma cholesterol differed from that of liver cholesterol, and the esters were not in simple equilibrium.

Klein ('58) later reported that rats fed corn oil or hydrogenated cottonseed oil showed increasing plasma and liver cholesterol concentrations as the linoleic acid of the diet was raised. Hence, Klein concluded that rat cholesterol concentrations were related directly to the polyunsaturated fatty acid content of the diet and not to the total fat.

By feeding thirteen different fats, with and without 1% of added cholesterol, Okey and associates ('59) studied the effects of unsaturated dietary fats upon the liver and serum lipids. The rats consuming butterfat developed higher cholesterol concentrations than those on all other rations except menhaden oil. Rats fed butter alone did not have any higher concentrations of liver and serum cholesterol than those consuming fats with a lower natural content of cholesterol. However, when cholesterol was added to the butter diet, the liver cholesterol concentrations were the lowest observed, except for the rats on coconut oil diets. After butter was centrifuged to remove the phospholipid and then was included in the diet, it produced elevated liver cholesterol concentrations in the rats which were fed cholesterol. The liver cholesterol concentrations tended to diminish when there was a decrease in iodine number of the food fats, though the serum cholesterol concentrations remained at a constant level. These results supported the conclusion that the composition of the fatty acid fraction of cholesterol esters was somewhat influenced by the amount of linoleate in the diet.

Tillman and coworkers ('60) tested the effects of diet upon clotting time and lysis of plasma in three groups of rats on diets containing propylthiouracil. These diets had previously produced myocardial infarctions in other animal experiments when butter was used. Rats consuming a normal diet remained healthy, with normal serum cholesterol concentrations. Rats on a corn oil diet ate only half as much as the normal rats and stopped growing.

The third group, with isocaloric amounts of butter, had higher plasma cholesterol concentrations and greater plasma turbidity than the rats in the corn oil group. Plasma cholesterol concentrations in the corn oil-fed rats were only slightly higher than in the normal group. Twenty per cent of the butter-fed rats developed myocardial infarctions. None of the rats consuming corn oil or the normal diet developed myocardial infarctions.

Exercise experiments

Wong and associates ('56) tested the effects of exercise on experimental atherosclerosis in chicks treated with androgens and found that exercising on the treadmill did not affect the blood cholesterol concentrations but did reduce the formation of atheromatous plaques in the abdominal aorta. However, later work (Wong et al., '57) with cockerels showed that exercise could overcome the effects of an atherogenic diet by lowering blood cholesterol concentrations as well as reducing the atheromatous plaques.

Brown and coworkers ('56) tested the effects of exercise in preventing coronary atherosclerosis in rabbits and showed that exercise caused a lowering of total serum cholesterol concentrations but did not alter free unesterified cholesterol concentrations. No essential differences in the atheromatous plaques in the blood vessels of these animals were observed. Apparently once atheromata were developed, exercise did not affect the rapidity of the deposit or the resorption of cholesterol from the vessels.

In a study of the effects of physical exercise on atherosclerosis, Myasnikov ('58) used 10 rabbits as controls and added cholesterol to their diets for six months. Twenty-five rabbits receiving cholesterol in the diet were exercised on the electric treadmill daily, until fatigued. Eight rabbits received no cholesterol in the diet but were exercised on the treadmill. The cholesterol concentrations were analyzed twice each month, and after six months the rabbits were sacrificed. The aorta, coronary arteries and myocardium of each were studied for atherosclerotic plaques. Exercise caused a definite decrease in blood cholesterol in those animals receiving cholesterol in the diet. The physical exercise, because of intensifying metabolism in the body, resulted in more intensive assimilation of alimentary cholesterol, thereby lowering the blood cholesterol concentration. The exercised rabbits on diets without added cholesterol showed no morphological changes except for hypertrophy of the myocardium.

Human Studies

Dietary Studies

Population Surveys. Kinsell and coworkers ('52) showed large amounts of vegetable fats in the diet caused a lowering of the concentration of serum cholesterol. At the same time Keys and associates ('52) reported serum cholesterol concentrations of populations on high fat diets advanced with age; while the citizens of Naples, who subsisted on low fat diets, did not have increases in serum cholesterol after age thirty. This was important because Gertler and coworkers ('50), in relating serum cholesterol concentrations to coronary artery disease, had found a steady significant rise in serum cholesterol concentrations with advancing age.

Bronte-Stewart and coworkers ('55) reported an inter-racial survey from South Africa, with a highly significant difference in the cholesterol concentrations when classified according to race. The greatest difference in the environmental factors was the diet. A later investigation (Bronte-Stewart _et al_., '56) showed a correlation between changes in cholesterol and changes in fat intake. Olive oil did not raise cholesterol concentrations, while butter, beef drippings and beef muscle did have an elevating effect.

Group Studies. By 1954 it had become obvious that increasing cholesterol intakes did not always show a correlating rise in serum cholesterol. Hardinge and Stare ('54) studied 86 vegetarians who included milk and eggs in their diet, 26 pure vegetarians, and 88 non-vegetarians. The average calories from fat were approximately 38 per cent.

The non-vegetarians had slightly higher intakes of animal fat and total fat calories than did the other two groups. The non-vegetarians had a greater intake of dietary cholesterol than the vegetarians who consumed milk and eggs, while the pure vegetarians had practically cholesterol-free diets. No significant difference was found between the serum cholesterol concentrations of the non-vegetarians and the vegetarians with added milk and eggs. This indicated a direct correlation between the blood cholesterol concentration and the intake of animal fat. The pure vegetarians, with liberal use of plant oils and no animal fat had the lowest cholesterol concentrations of the three groups. There was no correlation between the weights of the subjects and their cholesterol concentrations, though the mean weight of the pure vegetarians was twenty pounds less than the mean weight of the non-vegetarians. Perhaps this indicated that calorie utilization was less effective when the food source was plants rather than a combination of plant and animal foods.

Paterson and Derrick ('57), in a long range observation of the relationship of serum cholesterol concentrations to lipid deposits in coronary arteries, obtained blood samples from a series of 800 patients in a veterans hospital, twice in 1953 and yearly thereafter. The serum from each collection was kept frozen, and after the death of the patient the cholesterol concentrations of the serum and of the fresh coronary arterial tissue were compared. At the time of the second interim report in 1957 there had been 129 deaths, and the post mortem studies had been completed on 88 patients. No significant relationship was established between the cholesterol concentrations of the serum and the lipid deposits in arterial tissue. Paterson and Derrick proposed that a study should be carried over a long period of time, since it was possible that lipid deposits might occur in waves and might continue after the cause for them no longer existed.

In a study of the causes of coronary heart diseases, Dawber and coworkers ('57) found that there was an increased risk of arteriosclerotic heart disease in persons with serum cholesterol concentrations above 260 mg per cent. There was little relationship between obesity and cholesterol concentrations.

Laboratory Studies

Mayer and associates ('54) reported that there was a significant increase in cholesterol concentration as the proportion of dietary fat, regardless of the source of the fat, was increased; and that alterations in the amounts of dietary fat, whether animal or vegetable, produced parallel changes in plasma cholesterol.

Keys and coworkers ('55) found the average concentration of cholesterol in the sera of men whose diets were high in fat was 25% to 50% greater than the average when the diets were low in fat, and alterations in dietary fat produced significant changes in serum cholesterol concentrations within a few weeks.

Beveridge and associates ('55) reported changes in proportion of vegetable fat and animal fat in diets of five male subjects, aged 33 to 41. The first experimental diet, with 28.4% of the calories as vegetable fat, caused a serum cholesterol decrease of 33 mg per cent. When the calories from vegetable fat were increased to 58.5%, the serum cholesterol concentration decreased by 47 mg per cent. When isocaloric quantities of butter were consumed, the serum cholesterol concentration increased above the control diet by the fourth and seventh day, and then dropped on the eleventh day to the same initial level. Addition to the vegetable fat diet of an amount of cholesterol equal to that in the animal fat diet produced a decrease of 38 mg % in the serum cholesterol by the eleventh day. Consumption of a diet high in animal fat resulted in increased plasma lipids. These findings suggested that vegetable fat alone had no tendency to increase the plasma lipids, but, in conjunction with some unknown substance of animal origin which was present in a non-vegetable diet, it did produce a higher concentration of the lipids.

Later these associates (Beveridge _et al_., '56) reported that the change from a diet with 58.5% of the calories from corn oil to a diet containing isocaloric quantities of butterfat, lard, beef drippings and chicken fat caused increased plasma cholesterol. Butterfat exerted a more marked effect than the other fats. Changing from a mixed free choice diet to a formula diet did not alter plasma cholesterol concentration, even though the formula contained 60% of the calories as butterfat. When increasing amounts of butterfat were replaced by corn oil, however, there was a significantly progressive decrease in cholesterol concentration.

Beveridge ('57) later tested the effects of the various components of corn oil on the concentrations of plasma cholesterol. Alpha-tocopherol had no effect, but beta-sitosterol caused nearly as much decrease in serum cholesterol concentrations as did the original corn oil. The intimate mixing of beta-sitosterol with the rest of the diet probably permitted the formation of a mixed crystal complex, and therefore decreased the absorption of cholesterol. Beta-sitosterol was not effective when it was fed before or after the meal. Beveridge proposed that corn oil also formed the mixed crystal complex with cholesterol when fed as part of the diet.

Keys and coworkers ('57) reported that serum cholesterol concentrations changed in response to the kind of fat in the diet, with the largest part of the change occurring during the first week. Four oils were tested: corn oil (iodine number 120, linoleic acid content 45%); sunflower seed oil (iodine number 131, linoleic acid content 61%); sardine oil (iodine number 188, linoleic acid content 0-2%); and butterfat (iodine number 32, linoleic acid content 3%). The butterfat diet caused cholesterol concentrations which were 52 mg% higher than the corn oil diet, 35.8 mg% higher than the sunflower seed oil diet, and 39.8 mg% higher than the sardine oil diet. These findings did not support the theory that the serum cholesterol concentration in man is a simple inverse function of the essential fatty acid content or the degree of unsaturation, since the sardine oil was the most highly unsaturated and the sunflower seed oil had the highest linoleic acid content. All serum cholesterol concentrations in this experiment were significantly lower on corn oil than on the other oils. Keys and associates proposed that serum cholesterol concentrations were related to total fat rather than inversely to the essential fatty acids and that the observed effect of vegetable oil in lowering serum cholesterol concentrations was not due to the essential fatty acids.

Kinsell and Sinclair ('57) objected to these conclusions (Keys _et al._, '57) in that the actual quality and quantity of the fatty acids had not been determined and that the linoleic acid values were not established by laboratory analysis but were taken from the literature.

Sunflower seed (iodine number 131) was assumed to have a linoleic acid content of 61%, but variations of 19% to 72% have been known. Corn oil was assumed to have a linoleic acid content of 45%, but samples of U.S. corn oil have assayed at 16% to 68% linoleic acid. Therefore, Kinsell and Sinclair proposed that Keys' figure of 45% for the linoleic acid content of corn oil was too low; that iodine numbers are not adequate for determining unsaturation of oils in relation to their effects on serum cholesterol concentrations; and that the efficiency of absorption of the oils must be determined. These associates observed from their own data that vegetable oils which are fresh, readily absorbable, and under controlled conditions caused a decrease in serum cholesterol concentration which was approximately proportional to the iodine number.

Because of the controversy initiated by Kinsell and Sinclair ('57), Keys and associates ('57b) published additional data on the iodine values and percentage saturation of the fatty acids in the oils which they had used. Although they did not deny the role of the amount of saturation in the fatty acids and its effect on the serum cholesterol, they reasserted their thesis that saturated fats had a great effect in elevating the serum cholesterol concentrations, and that corn oil had a lowering effect which was additional to that which was due to its fatty acid content.

In another report, Keys *et al*. ('57c) stated that the saturated fatty acids had twice as much effect on the serum cholesterol concentrations as the polyethenoids which acted in the opposite direction. Oleic acid, a monoethenoid, had a small but uncertain effect on the raising of serum cholesterol concentrations. Keys and coworkers predicted, on the basis of previous laboratory work, that the removal of 1 oz of butter (57% saturated with 4% polyethenoid fatty acids) would produce a three times greater decrease in cholesterol concentration than the addition of 1 oz of sunflower seed oil (10% saturated with 62% linoleic acid content). Thus they expected the substitution of 1 oz of sunflower seed oil for 1 oz of butterfat to cause a decrease of 20 mg% in the serum cholesterol concentration, with three quarters of the change caused by the reduction in butterfat content of the diet. These associates predicted this cholesterol concentration decrease for a man in caloric equilibrium at 2600 calories, with cholesterol concentrations under 230 mg% when subsisting on an ordinary high fat diet. Greater reductions were expected in serum cholesterol concentrations for people who were hypercholesterolemic.

McCann and coworkers ('59) reported studies on the effects of vegetable oils on serum lipids in subjects with serum cholesterol concentrations above the mean for their ages. During the experimental period the subjects consumed approximately 80 ml of peanut oil formula in three doses daily, before each meal. The formula supplied 960 calories, 96 g fat, 24 g carbohydrate and 23 g linoleic acid. Two samples of blood were taken for control measurements at intervals of one week, and additional samples were collected weekly. In the second study safflower oil was used in amounts to provide 250 calories, 28 g fat and 20 g linoleic acid. The dietary fat from animal sources was decreased from 76% to 33%, and cholesterol intake from 707mg to 497 milligrams. The mean ratio of saturated to unsaturated fats was reduced by 33% while the essential fatty acid intake was increased 100 per cent. McCann and coworkers observed a sharp drop in serum cholesterol concentrations after the first week, followed by a rise during the second and third week. During the fourth week the concentrations decreased to a level equal to that at the end of the first week. Both peanut oil and safflower oil were effective in causing a slight lowering of serum cholesterol concentrations, and it was concluded that unsaturated fatty acids should be incorporated into the diet pattern for best results.

Schendel and Hansen ('58) studied 48 infants with kwashiorkor and measured serum cholesterol concentrations during an eleven month period in which skim milk formulas, with no added fat, were given to the infants. As the kwashiorkor improved, serum cholesterol concentrations rose.

At the beginning of the observation, mean total cholesterol was 93 mg%, compared with normal controls of 173 mg per cent. As the infants responded to the treatment, cholesterol concentrations were doubled, but there was no significant change in cholesterol concentrations in infants who did not show a clinical response. Sitosterol might have been responsible for the original low cholesterol levels, since the diet was almost totally vegetable. The synthesis of cholesterol may have been reduced, and diarrhea may have led to increased excretion of sterols. Schendel and Hansen concluded that increases in cholesterol concentration which correlated with clinical improvement reflected the renewal of esterification mechanisms in the liver.

Davis and associates ('60) gave formulas containing 21% fat, 64% carbohydrate and 15% protein to ten patients with coronary heart disease, while adjusting calories so that body weight remained constant. Cholesterol concentrations were measured during two weeks' intake of safflower oil formula and two weeks of equal quantities of safflower oil and coconut oil. Serum cholesterol concentrations were reduced as well with 20% fat as with higher amounts of unsaturated fat in formula feedings. These researchers concluded that wide variations of fat levels in formula feedings showed similar results, and that the optimal fat level was perhaps in the 20% to 40% range.

Exercise Studies

Morris and associates ('53) reported a study of the incidence of coronary heart disease in various occupations in England. The incidence of heart disease in bus drivers was greater than in bus conductors. These authors related this to the increased physical activity in the work of the conductors. A study of the heart disease incidence in postal workers showed that there was less disease in those with active jobs than in more sedentary work. Further observations showed that differences in incidence of death rate and history of coronary heart disease were due to differences in physical activity. These associates concluded that physical activity might act upon the coronary circulation in addition to creating the demands made upon it.

Mann and associates ('55) reported that differences in cholesterol concentrations of Nigerian subjects in three separate areas of the country could not be attributed to total caloric or total fat content of the diet, since these were similar. These authors believed that a difference in muscular exercise might have had an effect in reducing or controlling serum cholesterol concentrations in a manner similar to the effect of exercise on carbohydrate tolerance in diabetics. According to this theory, the lower concentrations of cholesterol were caused by muscular activity and energy expenditure, and a large muscle mass or large muscular expenditure was important in the prevention of hyperlipemia and perhaps in the prevention of atherosclerosis.

Keys ('56) concluded from his observations with the Bantu that differences in physical activity do not explain large variations in serum cholesterol concentrations. Men on heavy manual labor also consumed diets which were lower in fat than those of the other Bantu; hence, Keys attributed the lower cholesterol concentrations to the fat content of the diet rather than to the physical activity.

In an investigation of the serum lipids and atherosclerosis among Yemenite immigrants in Israel, Toor and coworkers ('57) reported that the recent immigrants had lower serum cholesterol concentrations and lower mortality due to atherosclerosis than those who had lived in Israel longer. The only differences in the populations were income and total food intake. Because the recent immigrants worked harder, with less food, they were in a state of caloric imbalance and therefore developed lower cholesterol concentrations. Four years later, Toor and associates ('60) investigated this same group of immigrants who had then been in Israel for five years. These immigrants were now living under better conditions, with a higher caloric intake and a higher percentage of calories from fat than in their previous diet. Their serum cholesterol concentrations were also higher than previously, and were caused by the increased calorie and fat intake, as well as the change in ratio of saturated and unsaturated fatty acids.

Exercise Experiments

Taylor and associates ('57) tested the effects of physical activity on serum cholesterol, by increasing physical activity by 1300 calories on the motor driven treadmill and adjusting food intake so that there was no loss in weight and no change in proportion of calories from fat. No changes were observed in serum cholesterol. Therefore it was concluded that serum cholesterol concentrations are related to the proportion of the total calories derived from fat, and that they are determined by the fat transport load from the intestine to the liver and from the liver to the fat depots. This fat transport load is influenced by the circulation rate, which is influenced by exercise. Though the fat intake was increased by 46 g daily, the increase in circulation prevented an increase in serum cholesterol concentrations.

In an earlier report from the same laboratories (Anderson et al., '55), physical activity was kept constant at 2 1/2 miles of walking daily, and energy balances were changed. After a period of semi-starvation, the serum total cholesterol was 88% of that of the controls, and at the end of a refeeding period of 5300 calories it was 132% of the controls maintained on a constant diet of 3200 calories.

This experiment was repeated. The cholesterol concentrations were 78% of the controls during semi-starvation and 112% of the controls after refeeding with 4000 calories.

Another report (Anderson *et al.*, '56) showed that in obese subjects maintained on 1200 calorie reducing diets, some with 58% of the calories as fat and some with 18% of the calories as fat, there was a decrease in serum cholesterol concentrations in all subjects, when exercise was kept constant. The reduction of cholesterol concentrations was greater in the low fat than in the high fat group when the weight loss was identical for both groups. These authors concluded that in caloric undernutrition and in low fat consumption, serum cholesterol concentrations are lowered by both factors working independently and simultaneously.

This experiment was repeated except that the subjects were overfed, (Anderson *et al.*, '57). During a 20-week period with an intake of 3400 calories, the men who gained the most weight had increased serum cholesterols by 40 mg% during the first 10 weeks, with no change in cholesterol during the last 10 weeks, though the weight gain continued. Anderson and coworkers concluded that a gain in body weight might be used to predict an increase in cholesterol during the beginning phase of period of weight increase. When fat intake was increased, there was an accompanying rise in the serum cholesterol concentrations,

even though the percentage of calories from fat was decreased. This probably occurred because cholesterol was part of the lipoprotein-fat transport system.

Mann and coworkers ('55) further investigated this subject to determine whether the amount of total caloric turnover controlled the serum lipoprotein concentrations and whether the disposition of calories from the diet controlled the serum cholesterol concentration. According to this hypothesis, an excess of food that was converted to body fat would cause high concentrations of serum cholesterol, and caloric balance or deficit would result in low concentrations. By varying the total caloric turnover and the disposition of the excessive calories, these associates concluded that young men on high fat diets were able to double their caloric supply without increasing the concentrations of the serum lipids, as long as the excess calories were used up in exercise. However, when exercise was restricted so that fat deposition occurred, there was an approximate doubling of serum cholesterol concentrations when the caloric intake was doubled. During a period of weight reduction serum cholesterol concentrations promptly returned to the original levels.

Chailley-Bert and coworkers ('55) found that cholesterol concentrations of active subjects were lower than those of sedentary subjects. Daily exercise in three sedentary hypercholesteremic subjects caused lowered total cholesterol concentrations.

Montoye and associates ('59) observed 16 male subjects in a supervised exercise program of swimming and calisthenics and 15 sedentary controls. No appreciable difference was found in the serum cholesterol concentrations within either group when initial and final values were compared. When the subjects of both groups were combined, changes in serum cholesterol concentrations usually accompanied changes in body weight, regardless of exercise. These authors concluded that the effects of exercise in decreasing serum cholesterol concentrations were indirect and were related to loss of weight.

The literature which was reviewed was primarily concerned with the hypercholesterolemic effect of butterfat and the hypocholesterolemic effects of exercise. In order to investigate these further, two studies were designed to test both the dietary and the exercise factors. These experiments, one with rats and one with humans, were planned to test the effects of milk consumption upon serum cholesterol concentrations while maintaining exercise at a high level.

EXPERIMENTAL METHOD

Animals[1]

Sixteen weanling litter-mate trios of male rats of the Sprague-Dawley strain were housed individually in cages in a room with no direct sunlight. The cages (5" x 5" x 12") were rotated daily to compensate for possible light and temperature deviations. This study was carried out over a six month period. For two months the rats consumed a stock diet (Appendix A). The animals were then divided into three experimental groups by alternating the ranked weights within each litter, and the animals were numbered. During a period of twelve weeks the following regimes were continued: Group A (animals 1-16) received a stock diet and were exercised; group B (animals 17-32) received a stock diet with 30% of the daily caloric intake supplied by powdered whole milk and were exercised; group C (animals 33-48) received a stock diet and were sedentary.

Each animal in the two exercise groups received equal amounts of conditioning. They swam in their respective

[1]This study was carried out under the direction of Dr. Henry J. Montoye, Department of Health, Physical Education and Recreation, Michigan State University, as part of MSU Research Project Number 941, 1957. Details are reported by Ackerman ('59).

groups twice daily, Monday through Friday, and once on Saturday for twelve weeks. During the first week the periods of swimming were short. During the second and third weeks the swimming periods were ten minutes in length, and metal weights equal to four per cent of the body weight were attached to the rats. Beginning with week four, no weights were attached to the animals and swimming times were increased to thirty minutes, twice daily. Thereafter the swimming times were increased by five minutes weekly, until a one hour period was attained. The water temperature was standardized at 36-37° Centigrade.

At the end of the twelfth experimental week the animals were anesthetized with sodium pentobarbital and blood was obtained by cardiac puncture. The blood samples were allowed to clot and then were centrifuged. The clear supernatant serum was pipetted off and was frozen.

Humans[1]

Seven university freshmen who were candidates for the track team and two graduate students who had been outstanding endurance runners were selected as subjects.

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[1]This study was carried out under the direction of Dr. Gale E. Mickles and Dr. Wayne Van Huss, Department of Health, Physical Education and Recreation, Michigan State University. This was part of a study of the effects of milk upon endurance performance which was sponsored by the National Dairy Council.

All of these subjects were in good physical condition for participation in an endurance study. They ranged in age from 19 to 24 years except for subject number 9 who was 32.

Training period

For three weeks prior to the beginning of the experiment, all subjects trained to exhaustion on the motor-driven treadmill, running twice each week at 10 mph up an 8.6% grade. Three times each week the subjects ran at varying speeds on the level treadmill, in order to determine the fastest rate at which all subjects could attain a steady state and continue for fifteen minutes. The steady state was assumed when the pulse rate leveled off and did not increase again during the fifteen-minute period. The maximal speed which could be attained by the most poorly conditioned subject was 6 mph.

Blood samples

During the week prior to the experiment, 10-ml samples of blood were taken from each subject three times by venipuncture. The blood samples were allowed to clot and were centrifuged. The clear supernatant serum was pipetted off and was frozen. During the last week of the experiment three samples of blood, taken from each subject by venipuncture, were treated in the same manner.

Grouping of subjects

Four subjects were assigned at random to each of two groups. Group I (subjects 1, 2, 3, and 4) was instructed to consume a self-selected diet, including at least three pints of milk daily and two pints of ice cream weekly. Group II (subjects 5, 6, 7 and 8) was instructed to eliminate milk, cheese and ice cream from the diet. No attempt was made to eliminate milk and cheese in cooked dishes. The ninth subject was arbitrarily assigned to group II. Subjects 1, 6 and 9 ate in their respective homes. The other six subjects ate in a university dining hall. It was decided that when milk, cheese or ice cream were served in the dining hall all subjects would accept the food, and those in group II would give their restricted foods to those in group I. This resulted in a higher intake of these foods for group I than would have been possible without special arrangements.

Dietary records

For one week before the start of the experiment, a graduate dietitian secured twenty-four hour recall diets (Appendix B) from each subject by daily interview. Dining hall menus were used to verify the specific foods served. During the first experimental week recall diets were again secured from each subject daily. Nutritional intakes of each subject were calculated, using the method of Leichsenring and Wilson ('51). Sizes of portions were estimated from observations of the dining hall procedures and serving utensils.

The nutritional intakes were compared with the recommendations of the Food and Nutrition Board of the National Research Council ('58) for boys 16-19. The food consumption of subject 9 was not included in the comparisons because no pre-experimental diet was available. The intakes for week one were compared with week two for each group by the Student "t" test for paired variates (Goulden, '52).

Experimental period

For eight experimental weeks each subject ran once weekly for fifteen minutes at 6 mph on the flat. Twice weekly, each ran to exhaustion at 10 mph up the 8.6% grade. During this time respiratory gases were collected and analyzed. Details of this study were reported in 1961 (Van Huss *et al.*, '61).

Chemical Method

Cholesterol determinations of the sera were made in triplicate by the Schoenheimer-Sperry method ('34), modified by Sperry ('37) and Foldes and Wilson ('50). Serum was added to an alcohol-acetone mixture, the protein was precipitated and filtered, and the cholesterol was precipitated as the digitonide. The sample was washed once with an acetone-ether mixture and twice with anhydrous ether. After drying, the digitonide was dissolved in glacial acetic acid and treated with Liebermann-Burchard reagent (acetic anhydride and sulfuric acid). A color reaction was produced which followed Beer's law. The cholesterol concentration was determined by a comparison with the color produced by a standard cholesterol solution with a Beckman Spectrophotometer, model DU.

RESULTS

Animals

A total of thirteen rat cholesterol analyses were unavailable due to drowning of rats during swimming, difficulty in securing blood by cardiac puncture, and laboratory losses during cholesterol determinations. The mean cholesterol concentrations and mean body weights of the two groups of rats on the stock diet are listed in Table 1. The mean cholesterol concentrations and mean body weights of the exercised rats are listed in Table 2. Individual data are recorded in Appendix C. Significant differences in total and free cholesterol concentrations and in body weights were observed when the exercise group on the stock diet and the sedentary group were compared by the paired "t" test (Goulden, '52). No significant differences were observed between the two exercise groups.

Humans

Cholesterol

The average serum cholesterol concentrations for the two groups of the athletes are shown in Table 3. Individual data are recorded in Appendix D. Some of the

Table 1. Mean serum cholesterol concentrations and mean body weights of rats on stock diet compared by paired "t" test

	Exercise group	Sedentary group	Number of litters	t
Total serum cholesterol	74.7 mg/100 ml	93.1 mg/100 ml	8	2.59*
Free serum cholesterol	20.9 mg/100 ml	25.1 mg/100 ml	8	2.88*
Final body weight	414.1 g	453.4 g	10	4.22**

* Significant at 0.05 level
**Significant at 0.01 level

Table 2. Mean serum cholesterol concentrations and mean body weights of exercised rats compared by paired "t" test

	Added milk group	Stock diet	No. of litters	t
Total serum cholesterol	84.9 mg/100 ml	74.8 mg/100 ml	9	1.63
Free serum cholesterol	23.4 mg/100 ml	20.9 mg/100 ml	9	1.21
Final body weight	417.7 g	415.1 g	10	0.26

Table 3. Comparison of effects of exercise, with and without milk consumption, on mean serum cholesterol concentrations of athletes

Serum cholesterol	Milk Diet		t	Restricted Diet		t
	Initial	Final		Initial	Final	
	mg/100 ml	mg/100 ml		mg/100 ml	mg/100 ml	
Total	180.1	189.3	.47	190.3	202.9	.72
Free	51.6	58.8	1.21	55.7	60.6	1.90

pre-experimental samples were lost during laboratory procedures. Two samples of sera were available for all subjects except number one. Three samples of sera were available for all subjects for the experimental period. No significant differences were shown in either the total or free cholesterol by the "t" test between initial and final values in each group (Table 3).

Body weight

The mean weights of the two groups of athletes at weekly intervals are shown in Figure 1. Individual weights are recorded in Appendix E. The analysis of variance for body weights is shown in Table 4.

Diet

The nutritional analysis of the recall diets is listed in Table 5, with individual data in Appendix F. The changes in dietary intake of the athletes were tested for significance by the "t" test, comparing the pre-experimental and the experimental weeks for each of the two groups. The changes in intake of fat, carbohydrate and riboflavin were significant at the 5% level. The change in intake of calcium was significant at the 1% level. Intakes of calories, protein and vitamin A were not significantly different.

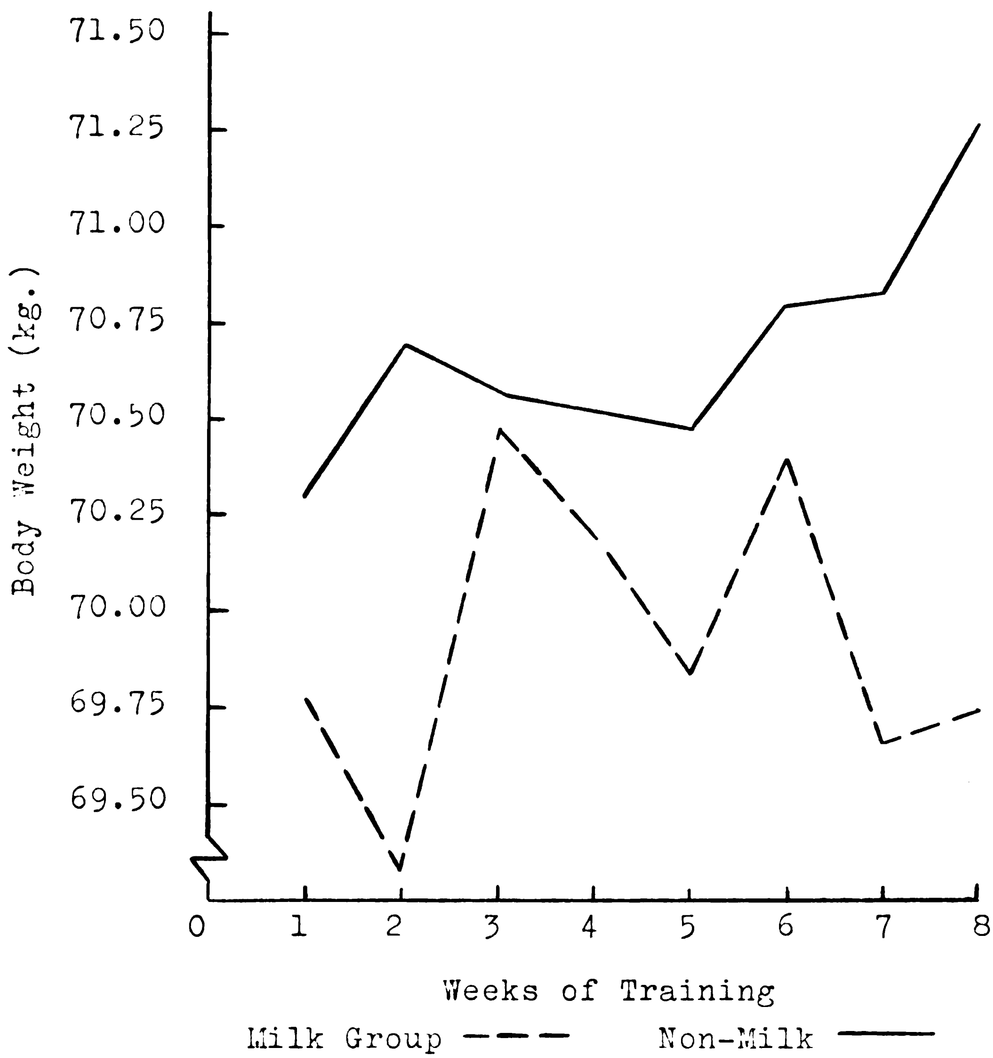

Figure 1. Weekly mean weights of athletes in milk and non-milk groups

Table 4. Analysis of variance for body weight

Sources of variance	Degrees of freedom	Sum of squares	Mean square	F-Value
Total	69*	907		
Groups	1	10	10.00	25.00**
Weeks	7	3	0.43	1.07
Individuals	7	870	124.29	310.73**
G x W interaction	7	5	0.71	1.78
Error	47	19	0.40	

* Two degrees of freedom were lost because two missing values were estimated.
** Significant at the 0.01 level.

Table 5. Nutritional intake. Comparison of week one with week two for each group of athletes

Nutrients	Recommended*** Dietary Allowances (Boys 16-19)	Unrestricted Diet			Restricted Diet		
		Week I	Week II	"t"	Week I	Week II	"t"
Calories	3600	3321	3544	2.06	3265	3340	1.26
Protein (g)	100	129	137	2.76	116	106	-1.44
Fat (g)	Not established	156	158	0.39	131	114	-3.75*
Carbohydrate (g)	Not established	383	401	1.11	398	474	4.56*
Calcium (g)	1.4	2.01	2.21	1.33	1.62	0.70	-6.01**
Vitamin A (IU)	5000	6317	7634	1.36	4542	4470	-0.06
Riboflavin (mg)	2.1	3.47	3.72	1.40	2.85	1.63	-6.67**

* Significant at the 0.05 level
** Significant at the 0.01 level
*** Food and Nutrition Board, National Research Council Recommended Allowances

A comparison was made of nutrients in the experimental diets and those recommended by the Food and Nutrition Board of the National Research Council ('58). Standards for boys 16-19 years were used because all subjects with dietary analyses were under 25 years of age. After milk restriction the calcium and riboflavin intakes fell below the recommended allowances (Table 5).

DISCUSSION

Animals

The mean total cholesterol concentration of the exercised rats on the stock diet was significantly different from that of the sedentary group. The rats in the sedentary group had the higher serum total and free cholesterol means and the greater mean weight. This would be expected with lesser amounts of exercise and unrestricted food. The above results are in accordance with the findings of Montoye and associates ('59) who reported that in middle-aged men changes in cholesterol concentrations were reflections of changes in weight. Although experimental results cannot be transferred from one species to another, it is not unreasonable to expect that higher cholesterol concentrations would be observed in the heavier rats as an indirect result of the lack of exercise. This relationship between changes in body weight and in serum cholesterol concentrations is also in agreement with the findings of Taylor and coworkers ('57) in studies with humans. If changes in serum cholesterol are related to the fat transport per unit of circulation, then lack of exercise with subsequent weight gain would cause higher amounts of fat per unit of circulation, as evidenced by raised serum cholesterol concentrations.

Therefore, the higher cholesterol concentrations in the sedentary rats may be related to the exercise and calorie imbalance.

The exercised rats receiving stock diet plus milk did not have significantly different serum total and free cholesterol concentrations from those on the stock diet without milk. Since exercise was similar in both groups, it can be concluded that the replacement of 30% of calories with powdered whole milk had no effect.

Humans

Cholesterol concentrations

The initial and final serum cholesterol concentrations were not significantly different, whether or not milk was included in the diet. This would be expected in accordance with the findings of Mann and associates ('55) who reported that serum cholesterol concentrations in young men did not change as long as excess calories were used up in exercise. The fat intake was constant in the unrestricted diet group. The percentage of calories from fat was decreased from 36% to 30% in the restricted diet group. The stability of the serum cholesterol concentrations is in agreement with the proposal of Davis and coworkers ('60) that changes in dietary fat levels in the range of 20%-40%

of the total calories do not affect serum cholesterol concentrations.

Body weight

The significant F value for individuals indicates that the subjects differed in body weight. The significant F value for groups shows that the two groups differed significantly in weight. This difference is due to the groups not being matched in body weight at the beginning of the experiment and is not due to the effect of milk. The insignificant F value for weeks indicates that there was no significant change as the experiment progressed due to factors other than milk restriction. The insignificant value for group-weeks interaction indicates that the change in weights for the two groups was not different as the experiment progressed, and that therefore milk restriction did not have any effect on body weight. Since body weight was constant, changes in serum cholesterol concentrations would not be expected (Montoye et al., '59, Taylor et al., '57).

Dietary Evaluation

The nutritional evaluation shows that when milk, cheese, and ice cream were eliminated from the diets, the calcium and riboflavin intakes were below the recommended allowances of the Food and Nutrition Board of the National

Research Council ('58) except for one subject. The intake for this subject was sufficient because he consumed an average of twelve servings of enriched cereal products daily, which was approximately one and one-half times the cereal intake of the other subjects on the restricted diet.

The calorie intake was maintained by the increased consumption of fruits, cereals, gravies, desserts, soft drinks and candies. The raised intake of carbohydrate foods, especially the desserts, soft drinks and candies, is not nutritionally desirable, since the calories are accompanied by very few other nutrients.

Since the elimination of milk, cheese and ice cream from the diet caused the dietary levels of calcium and riboflavin to fall below those recommended by the National Research Council ('58), attempts to lower blood cholesterol concentrations by this type of diet would appear to be undesirable. Since many college freshmen have not attained complete growth, it is imperative that their food supply adequate intakes of the essential nutrients. Moreover, the use of a non-milk diet at the college level would probably be repeated in the high school training of athletes, at an age when calcium is especially important for bone growth. The low intakes of calcium and riboflavin could have far-reaching effects because other young people have a tendency to copy the athletes' diet.

In both groups the caloric intake was below the recommended allowances for boys 16-19 years of age. This lower caloric consumption is probably adequate, since there was no weight loss among the athletes, even though they were maintaining a high level of exercise. It is probable that the recommendations for caloric intake are too high for present conditions.

The Vitamin A consumption was slightly below the recommended allowances in the restricted diet group during both weeks. The small deficit is not alarming. However, if a similar study were carried out in which butter and milk were eliminated from the diet, it is possible that the Vitamin A inadequacy might be critical.

CONCLUSION

An experiment was devised to test the effects of milk consumption and exercise on concentrations of blood cholesterol (free and total) in rat and man. The sedentary rats had higher cholesterol concentrations and greater weights than the exercise rats. The higher cholesterol concentrations appear to be related to the increase in weight and only indirectly to absence of exercise. No changes in the cholesterol concentrations or body weights of the exercised animals were observed when 30% of the calories were replaced by whole milk solids.

In two groups of trackmen, who were in good physical condition, exercise was maintained at a high level and milk consumption was varied. No changes in weight or in cholesterol concentrations were observed in either group. Exercise was a factor in maintaining constant weight and cholesterol concentrations. Elimination of milk, cheese and ice cream from the diet resulted in decreased dietary intakes of calcium, riboflavin and fat; and increased consumption of carbohydrate. On the restricted diet, intakes of calcium and riboflavin were below the amounts recommended by the Food and Nutrition Board of National Research Council.

According to the results of this study, it is not advantageous to attempt to alter blood cholesterol concentrations by changes in milk intake. There appears to be an indirect advantage in the control of serum cholesterol concentrations through the regulation of exercise and diet for caloric equilibrium.

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Appendix A. Composition of stock diet for rats

46.1 %	Ground Corn
20.0	Soybean Oil Meal (44% Protein)
10.0	Fish Meal
5.0	17% Dehydrated Alfalfa Meal
10.0	Dried skim milk
5.0	Sucrose
0.5	Trace minerals salt
3.0	Corn Oil
0.1	Merck B Vitamins
0.05	Vitamin A and D mix
0.25	Pfizer's Q and Vitamin B 12 Supplement

20 g/100 # Tocopherol Acetate

Appendix B. Twenty-Four Hour Diet Recall

Date ____________ Group __________

Name

EVENING SNACK	TYPE	AMOUNT

	SUPPER		DINNER	
	Type	Amount	Type	Amount
Meat				
Potato				
Vegetable				
Salad				
Condiments				
Bread				
Butter				
Dessert				
Beverage				
Milk				

AFTERNOON SNACK	TYPE	AMOUNT

MORNING SNACK	TYPE	AMOUNT

BREAKFAST	TYPE	AMOUNT
Fruit		
Cereal		
Eggs		
Bread		
Butter		
Condiments		
Beverage		
Milk		

Appendix C. Serum cholesterol concentrations of rats

	Exercise Stock Diet			Exercise Added Milk			Sedentary Stock Diet	
	Total cholesterol	Free cholesterol		Total cholesterol	Free cholesterol		Total cholesterol	Free cholesterol
	mg/ 100 ml	mg/ 100 ml		mg/ 100 ml	mg/ 100 ml		mg/ 100 ml	mg/ 100 ml
1.	97.5	13.8	17.	89.4	33.3	33.	76.3	22.5
2.	66.3	21.3	18.	87.9	27.4	34.	107.5	24.4
3.	----	----	19.	82.9	21.9	35.	----	----
4.	95.4	27.3	20.	85.9	20.8	36.	----	----
5.	----	----	21.	82.5	22.7	37.	78.3	23.9
6.	75.3	28.8	22.	----	----	38.	88.4	30.6
7.	59.7	22.0	23.	95.2	26.9	39.	82.5	30.5
8.	62.2	13.8	24.	95.0	21.3	40.	92.5	15.0
9.	75.0	24.4	25.	74.1	21.6	41.	----	----
10.	----	----	26.	76.3	19.2	42.	78.8	17.5
11.	----	----	27.	----	----	43.	68.8	21.3
12.	78.8	20.0	28.	----	----	44.	117.4	32.11
13.	59.4	17.4	29.	79.4	19.5	45.	----	----
14.	----	----	30.	----	----	46.	85.0	24.9
15.	81.3	22.0	31.	93.1	20.3	47.	89.7	21.6
16.	76.9	20.8	32.	64.1	20.0	48.	90.4	24.2

Appendix D. Serum cholesterol concentrations of athletes

Subject	Total cholesterol		Free cholesterol	
	Pre-experimental	Experimental	Pre-experimental	Experimental
	Unrestricted Diet			
	mg/100 ml	mg/100 ml	mg/100 ml	mg/100 ml
1	209.6	274.6	54.6	78.5
2	194.7	171.9	53.3	49.0
3	140.1	147.1	47.6	51.6
4	176.0	163.9	50.7	55.9
	Restricted Diet			
	mg/100 ml	mg/100 ml	mg/100 ml	mg/100 ml
5	229.9	184.8	64.5	64.0
6	183.8	212.1	59.2	60.1
7	149.0	189.2	59.2	50.0
8	231.1	221.9	68.8	70.6
9	157.8	206.5	46.6	58.2

Appendix E. Weights of subjects

Subject	Weight							
	Week							
	1	2	3	4	5	6	7	8
	kg	kg	kg	kg	kg	kg	kg	kg
1	72.67	72.39	73.09	72.56	71.71	72.35	72.15	71.42
2	67.57	67.35	67.52	68.48	67.39	67.56	67.78	68.95
3	71.25	70.73	72.95	71.59	71.73	73.15	70.99	71.76
4	67.54	66.86	68.34	68.25	68.47	68.56	67.30[1]	66.87
5	72.33	71.42	71.65	72.04	72.10	72.44	71.89	73.26
6	66.68	67.45	65.57	66.60	66.52	67.17	66.46	67.75
7	69.72	69.34	71.24	70.56	69.74	71.25	71.53	71.18
8	77.12	78.32	78.53	77.87	77.37	77.83	73.24	77.95
9	65.67	66.90	65.93	65.77	66.67	65.32	65.93	66.32[1]

[1]Calculated value

Appendix F. Nutritional evaluation of diets of athletes

Subject	Calories	Pro g	Fat g	CHO g	Ca g	Vit A IU	Riboflavin mg
MILK DIET							
Pre-experimental week							
1	2587	113	149	338	2.21	4761	3.60
2	3686	132	160	426	1.99	5280	3.48
3	3200	123	147	331	1.79	7546	3.24
4	3809	142	167	436	2.06	7631	3.58
Average	3321	129	156	383	2.01	6317	3.47
Experimental week							
1	3011	127	138	315	2.23	5333	3.68
2	4040	147	172	475	2.53	[illegible]577	4.20
3	3380	125	162	369	2.12	7549	3.49
4	3745	147	161	444	1.94	7678	3.50
Average	3544	137	158	401	2.21	7634	3.72
RESTRICTED DIET							
Pre-experimental week - no restriction							
5	3451	128	139	424	1.79	7217	3.15
6	2919	95	116	322	1.24	3250	2.15
7	3209	115	131	409	1.46	4337	2.97
8	3481	127	136	437	1.93	3312	3.14
Average	3265	116	131	398	1.62	4542	2.85
Experimental week - restriction of milk, cheese, ice cream							
5	3659	119	125	514	0.84	4571	1.87
6	3062	104	109	413	0.58	2956	1.43
7	3175	98	115	455	0.73	7251	1.68
8	3465	103	106	527	0.64	3103	1.54
Average	3340	106	114	474	0.70	4470	1.63