71-23,907

WATT, Edward William, 1937-
THE EFFECTS OF TRAINING AND DETRAINING ON THE DISTRIBUTION OF CHOLESTEROL, TRIGLYCERIDE, LIPOPROTEIN AND NITROGEN IN TISSUES OF ALBINO RATS.

The University of Michigan, Ph.D., 1970
Education, general

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THE EFFECTS OF TRAINING AND DETRAINING ON THE DISTRIBUTION OF CHOLESTEROL, TRIGLYCERIDE, LIPOPROTEIN AND NITROGEN IN TISSUES OF ALBINO RATS

by

Edward William Watt

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Education)
in The University of Michigan
1970

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ABSTRACT

THE EFFECTS OF TRAINING AND DETRAINING ON THE DISTRIBUTION OF CHOLESTEROL, TRIGLYCERIDE, LIPOPROTEIN AND NITROGEN IN TISSUES OF ALBINO RATS

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This study was conducted to test the hypothesis that moderate exercise training and detraining influence the concentration of cholesterol, triglyceride, and nitrogen in selected tissues and the distribution of lipoprotein in blood.

Forty-eight 90 day male Albino rats (Sprague-Dawley) were randomly assigned into 4 groups (group n = 12). Groups I and III were subjected to 8 weeks of progressive exercise training in motor driven running wheels. Detraining was effected in group III by discontinuing the exercise program for 8 weeks. Sedentary control groups (groups II and IV) were included for purposes of comparison. Rats were pair-caged in 8 1/4 x 11 x 8 inch cages randomly assigned to racks contained within a temperature and light controlled facility. They received water and a standard diet ad libitum throughout the experiment.

Body weight measures were made initially, at 8 weeks and at sacrifice. Resting heart rates were also measured at these times to assess training effectiveness (resting bradycardia). Animals were decapitated and blood samples drained immediately. The heart, right triceps, and an epididymal fat pad were quickly removed , cleaned, frozen on dry ice and lyophilized to obtain anhydrous tissue samples. Samples were assayed as follows:

blood and tissue cholesterol and triglyceride levels by autoanalyzer colorimetry, epididymal, as well as, duplicate analysis of heart and tricep cholesterol by Liebermann-Burchard colorimetry, total nitrogen of heart and triceps by Micro-Kjeldahl procedure, and serum lipoproteins by both paper and cellulose acetate electrophoresis.

Compared to controls, trained animals had lower mean body weights (458 vs 550 g) and resting heart rates (296 vs 340 beats/min). Significant increases in both body weight (487 vs 586 g) and heart rate (306 vs 331 beats/min) occurred with detraining. Training lowered blood cholesterol (105 vs 122 mg per 100 ml) and triglyceride (57 vs 106 mg per 100 ml) levels but had little or no effect on these lipid fractions in heart or skeletal muscle. Blood cholesterol and triglyceride levels remained significantly lower with detraining. Mean triglyceride levels in epididymal tissue were lower for both trained and detrained groups. The mean total nitrogen concentration of myocardial and skeletal muscle tissue was increased by training. Detraining resulted in a diminution of these elevated nitrogen levels but not to the low levels measured for sedentary controls. Serum lipoprotein electrophoresis results were inconsistent. Some resolution was observed in both experimental and control groups in the area defined as the pre-beta region of human serum.

The results of this experiment confirm previous reports that exercise training lowers blood cholesterol and triglyceride levels. Blood cholesterol and triglyceride levels remained low following detraining as substantiated through resting heart rate and tissue total nitrogen measures. Data from this study indicate that heart and skeletal tissue lipids may be independent of body weight, physical activity, and serum lipid levels.

ACKNOWLEDGMENTS

The author wishes to express his gratitude to Dr. Merle L. Foss for chairing the dissertation committee and for guidance during this doctoral program. Thanks are also extended to the other members of the committee for advice and helpful criticism offered.

The deepest appreciation is extended to Professor Walter D. Block for acceptance into his laboratory, for counsel, financial assistance, and without whom this thesis would not have been possible. Sincere gratitude is extended to Dr. Leigh S. Whitlock for preparing the data for computer analysis.

Special thanks are also extended to Mary Westhoff, Mara Markovs, and John Jarrett for their technical assistance and encouragement during this study. In addition, the help given by Mrs. Hinchcliff with the glassware was greatly appreciated.

Finally, the author wishes to thank Richard Lampman, doctoral student, for assistance with the dissection procedures.

DEDICATION

To Barbara Kay and John McKenzie

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CHAPTER I

INTRODUCTION

Numerous epidemiologic studies have indicated that individuals who maintain high serum blood cholesterol concentrations have a greater incidence of coronary heart disease than individuals with lower concentrations (35, 37, 56). In addition, clinical studies indicate that high blood concentrations of triglyceride also increase the risk of developing coronary heart disease (1). From these data it has been concluded that lowering of the concentration of blood lipids by dietary control could be important in reducing the lipid risk associated with this disease state (105, 106).

Further epidemiologic studies (80, 89, 102) have indicated that a lack of exercise seems to be associated with coronary heart disease. Studies have shown that cholesterol and triglyceride levels in the blood tend to be lower in persons engaged in regular physical activity than in sedentary persons. However, in the case of cholesterol, such observations are not unequivocally substantiated by experimental studies in man or in laboratory animals since some investigators have found a definite relationship while others have not (24, 60, 89).

Need for the Study

In spite of epidemiological studies indicating a lowering of cholesterol and triglyceride levels in blood with physical activity,

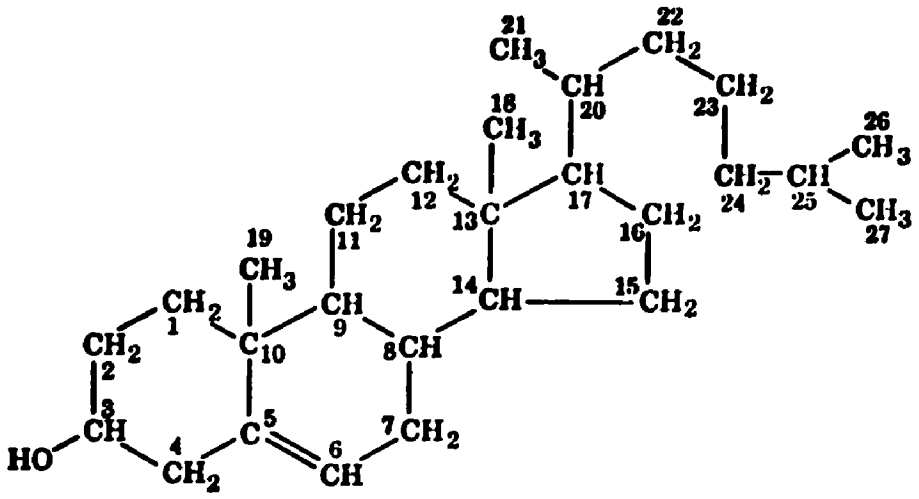
essentially very little experimental data exists showing comparable changes in lipid concentrations in various tissues. As a result, there is a great need for experimental study associating physical activity with concentrations of both cholesterol and triglyceride in blood and in tissues. Data of this type would strengthen the implication that physical exercise may be beneficial to man in terms of decreasing incidence of coronary heart disease because of influence in lowering the level of cholesterol and triglyceride in blood and tissues.

Purpose of the Study

The purpose of this study was to investigate the effects of a standardized exercise training program followed by a period of detraining on the content and interrelation of various lipid fractions in the blood, myocardial, skeletal, and epididymal tissues of albino rats. The protein content based on total nitrogen was also determined for the myocardial and skeletal tissues in order to possibly associate lipid changes with protein changes.

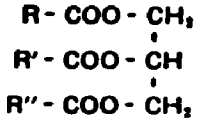
Definition of Terms

Four terms used frequently in this study were cholesterol, triglyceride, lipoprotein, and exercise training. Cholesterol is a perhydrocyclopentanophenanthrene derivative containing one double bond, one hydroxyl group and has the formula $C_{27}H_{45}OH$. It is the most abundant sterol found in mammals and is present in all animal tissues and blood in the free or esterified state. The structure of cholesterol is as follows:



Cholesterol

Triglycerides (commonly known as neutral fat) constitute the most abundant and widely distributed class of lipid in nature. They occur mainly as storage depots in plants and animals. Structurally they are fatty acid esters of the trihydroxyl alcohol glycerol. The general formula for a triglyceride is as follows:



Neutral fat

The R group representing three different fatty acids can also be the same, however, in nature they are different and usually palmitic acid, oleic acid, linolein acid, or arachidonic acid. They are usually C18 - 22 carbon acids and average about three double bonds per molecule of glyceride. A lipoprotein is a large macromolecule containing a portion of globulin type complex with lipid. This association solubilizes the lipid and is the major form in which lipids are transported in the body. Exercise training refers to an eight-week program of daily running in a motor-driven exercise wheel which involved progressive increases in the amount of work performed weekly (Appendix II).

Assumptions

Assumptions inherent in this study were that (1) the experimental animals maintained their same general state of health throughout the experiment; and (2) the animals ran in the motor driven exercise wheels with the same degree of "willingness" during each training session.

CHAPTER II

REVIEW OF THE LITERATURE

Effects of Exercise on Tissue Lipids

Experimental Studies

Evidence was produced in 1913 by Lafon (67) that lipids are removed from circulating blood by muscular tissue. Using the levator muscle of the upper lip of the horse, he found a lower concentration of lipids in venous blood than in arterial blood. This difference in arterio-venous concentration increased when the muscle contracted during chewing, suggesting a greater uptake of lipid by the contracting skeletal muscle than by the resting muscle.

Experimental work to quantitate the role of fat and carbohydrate in muscle metabolism was undertaken by March and Murlin (75) who investigated 17 young men during rest and exercise performed on cycle ergometers. The subjects were studied with high carbohydrate and high fat diets. From these investigations it was suggested that the respiratory exchange ratio could be employed to indicate the contribution of each substrate to the total metabolism during various states of energy expenditure and following various dietary intakes.

Since these experiments were performed using intact animals, the use of the respiratory exchange ratio to represent the metabolic process of individual working skeletal muscles was questioned by various investigators. Himwich and co-workers (48), Himwich and Castle (49),

and Himwich and Rose (50) attempted to resolve this question by a series of experiments in dogs in which they compared the respiratory quotients of isolated in situ gastrocnemius-plantaris muscles with the respiratory exchange ratios under various metabolic conditions. Their findings indicated that the muscle respiratory quotient and the respiratory exchange ratio were approximately equal at rest and during electrical stimulation. It was further shown that the muscle utilized carbohydrate and lipids for energy in the same proportion as the whole animal at rest.

Miller, Gold, and Spitzer (78) studied the uptake and incorporation of labeled ^{14}C palmitic and oleic acid by the thigh muscle of dog. During electrical stimulation, the rate of uptake of both of the fatty acids was increased. These findings were confirmed by Volk, Worthington, and Weinhouse(112) who reported that rat skeletal muscle could oxidize fatty acid in vitro. Their results were also substantiated by Neptune, et al. (87) for rat diaphram in vitro, and by Fritz, et al. (29) for rat skeletal muscle in vitro.

In view of the wide spread interest in the role of carbohydrate and fat in muscle contraction, Issekutz, Paul, and Miller (53) determined glucose and fatty acid metabolism in normal and pancreatectomized dogs during 4 hours of steady-state exercise on a motor driven treadmill. The rates of turnover and oxidation of plasma free fatty acids and of glucose were measured by infusing labeled palmitate or glucose into the dogs. Plasma free fatty acids represented the major energy supply while plasma glucose contributed very little during exercise. Essentially no differences were observed between normal and diabetic animals. Exercise

increased utilization of lipid as shown by a lower concentration of plasma free fatty acids, while carbohydrate utilization was decreased with a decreasing plasma level of insulin.

Barclay (3) investigated the utilization of free fatty acid and glycerides of in situ gastrocnemius-plantaris muscle of dogs at rest during electrical stimulation and during recovery. At rest, the mean value for free fatty acid release agreed reasonably well with the value of 1.54± 0.5 ueq/100 g per minute reported by Bass and Hudlicka (5) for similar preparations. Glycerides were taken up by the resting muscle preparations in the majority of experiments, but no consistent pattern for the uptake of glycerol was observed in the exercising and recovery preparations.

Experiments to determine the uptake and oxidation of individual free fatty acids in the human forearm during a 60 minute exercise period were performed by Hagenfeldt and Wahren (40). Muscular uptake of free fatty acid rose in parallel with the increase in concentration of fatty acids in the arterial blood. Of the fatty acids studied, the muscle showed a slight preference for linoleic and oleic acid as compared to palmitic acid. Respiratory quotient measurement of the forearm indicated a constant lipid oxidation during exercise with about 50% of this oxidation accounted for by the free fatty acids. Of the fatty acids oxidized 60% were converted to CO_2.

Young, et al. (117) studied fatty acid metabolism in man with a single injection of ^{14}C labeled palmitate. They found that approximately 37% of CO_2 was derived from free fatty acids at rest and approximately 50% during exercise. Contradictory evidence was presented by Masoro, et al. (76). Using ^{14}C labeled palmitate, they found no difference

between the levels of muscular phospholipids and triglycerides in monkey gastrocnemius and soleus muscle following 5 hours of stimulation. There was no evidence for an increased turnover rate. Only the cholesterol ester concentration in the exercising muscle tended to increase.

Basu, et al. (6) conducted a study with ten healthy students in the post-absorptive state during a 1 hour walk on a motor driven treadmill and observed a significant rise in plasma non-esterified fatty acid levels from 0.76 to 1.55 millimole/liter. It was suggested by these workers that lipid as a fuel for exercise was transported from adipose tissue as non-esterified fatty acid.

Experimental research into the relationship of serum cholesterol and exercise were conducted as early as 1921. Rakestraw (96) found an immediate slight reduction of 2-5 mg% in serum cholesterol in four subjects who participated in a single exercise period of either running up and down stairs or riding a bicycle ergometer. Chailley-Bert, Labignette, and Faber-Chevalier (23) compared the serum cholesterol levels of ten inactive subjects with seven active subjects of approximately the same age. The active group had lower mean serum cholesterol values (210 mg%). Mann, et al. (74) studied three students for 4 weeks and demonstrated that doubling their calorie intake with carbohydrate did not increase the serum cholesterol or lipoproteins as long as physical activity was increased correspondingly. When physical exercise was not increased during the period in which there was high caloric intake, the serum cholesterol levels increased in two of the three subjects. Mann concluded that physical activity can modify the

effects of diet on the serum cholesterol level. In a later experiment, the same investigator (74) reported no effect of exercise on serum cholesterol in nine young men who were in caloric balance.

Montoye (79) researched the relationship between weight, physical activity and serum cholesterol in 31 middle-age sedentary men. He observed no effect of activity among subjects who had initially low cholesterol levels ($\bar{X}$=189 mg%), while three subjects whose original values were high ($\bar{X}$=233 mg%) showed considerable decreases following the exercise program ($\bar{X}$=198 mg%). In three subjects in which there was a decrease in cholesterol, there was a parallel decrease in body weight. Montoye discussed this relationship further, hypothesizing that the effects of exercise may be indirect through a reduction of body fat.

The effect of 14 days of exercise training on blood lipid levels in 20 normal male subjects was studied by Fitzgerald, et al. (27). Determinations for total lipid were made before and after 8 minutes of vigorous exercise performed on a bicycle ergometer. The findings indicated that short bouts of strenous exercise significantly increased the total serum lipid levels. No changes were seen for the resting levels at the end of the training period. Campbell (15) determined the effects of one semester of physical activities on the serum cholesterol levels of 133 male college freshmen enrolled in cross-country running, weight training, gymnastics, wrestling, tennis, and golf. All subjects had normal serum cholesterol values at the beginning of the study. Those students enrolled in cross-country running and tennis activities demonstrated significant decreases in their serum cholesterol levels

(-19.8 - 7.5 mg%, respectively). Serum cholesterol values for the subjects enrolled in the more static activities remained unchanged.

In a study reported by Taylor, et al. (106) nine healthy university students were maintained on a high fat diet during four treatment periods. Period one consisted of 2 weeks of sedentary activity and a daily caloric intake of 3200 calories. Period two consisted of 1 week of increasing physical activity and a daily intake of 3200 calories. Period three comprised 3 weeks of daily walking for 2 hours on a motor driven treadmill and a daily intake of 4250 calories. The remaining period consisted of 1 week of sedentary living and a daily intake of 3100 calories. Their findings showed that serum cholesterol is independent of both caloric intake and physical activity when a subject is in caloric balance and the percentage of fat calories in the diet are held constant. Similar findings were reported by Hoffman, Nelson, and Goss (51) when they investigated 355 male officers, aged 40 - 55 years who were placed into two groups according to the amount of voluntary exercise performed. The 299 subjects who participated in the exercise program during the year of the study exhibited lower levels of cholesterol, βeta-lipoprotein and triglycerides.

Carlson and Pernow (21) studied six healthy males between 20 - 35 years of age. Exercise was performed on a bicycle ergometer with progressively increasing work loads. The plasma concentrations of non-esterified fatty acids in the arterial blood and in the femoral veins was determined at rest and work. During exercise the arterial level of non-esterified fatty acid decreased followed by an increase approximately 30 minutes later. Basu, et al. (6), Carlson, et al. (17), Carlson and Pernow (22), and Friedberg, et al. (28) all confirmed the initial

decreases in plasma free fatty acids during the early stages of work followed by increasing levels. They postulated that the initial lowering of plasma free fatty acids in the early stages of physical work was due to an efflux from the plasma to the working muscles. Carlson and Pernow stated that the increases observed were probably due to the increased mobilization of fatty acids from the adipose tissue as a result of increased sympathetic activity.

Carlson and Mossfeldt (20) studied the acute effects of prolonged heavy exercise on the concentration of plasma lipids and lipoproteins in skiers. The skiing time was approximately 8 - 9 hours. There was a significant fall in the concentration of plasma triglycerides and phospholipids in both groups studied. The most pronounced decrease of the plasma lipids was in the triglyceride fraction. No significant changes were seen for cholesterol.

Morgan, et al. (84) using a needle biopsy technique, studied the effect of exercise on the concentration of total phospholipid, cholesterol, and triglyceride in the trained and contralateral untrained vastus lateralis muscle of ten normal men. A weighted boot was fitted to one leg of each subject and the leg exercised to exhaustion daily for 4 - 6 weeks by performing a series of leg extensions. The phospholipid and triglyceride concentrations were higher in the trained muscle but the cholesterol was lower. The investigators concluded that apparent alterations in intracellar triglyceride stores may have been due to some contamination by adipose tissue incompletely removed in spite of their best efforts to do so.

A study to investigate the effects of exercise on plasma and tissue lipid levels (wet weight) of aging rats was performed by Carlson and

Fröberg (18). At the age of 20 months, the rats were subjected each day to 2 - 3 hours of running on a treadmill for five days a week over a three week period. Plasma lipid levels were high in the sedentary control rats and were significantly lower in the exercise group. Triglycerides were also lowered by approximately 55% and cholesterol and phospholipids by about 25% in the exercise groups. Liver triglyceride concentration decreased as a result of exercise, but the concentration of cholesterol and phospholipids remained unchanged. In red skeletal muscle the triglyceride concentration was lowered, while the phospholipid levels increased and cholesterol values were unaffected. No significant changes occurred in white skeletal and heart muscle.

No studies were located on the effects of exercise training followed by detraining on blood and tissue lipids.

Epidemiological Studies

Toor, et al. (110) and Brunner, et al. (11, 12) compared Yemenite Jews who had immigrated to Israel within the past 30 years with Israelites of European origin. The Yemenites were selected because they had lived on the Arabian Peninsula for some 2000 years in comparative isolation. These people had led a spartan life and were very active physically. The mortality from atherosclerosis was approximately four times greater among Yemenites who had immigrated to Israel in earlier years and were settled there, as compared to recent immigrants of the same age. The earlier immigrants were less active physically, had higher serum cholesterol levels and were heavier. Other Israelis of European origin were stated to have approximately the same high incidence of heart disease found in Great Britain, whereas the Yemenites revealed a much lower proportion of this disease. Mann, et al. (72) reported

similar differences when the cholesterols of rural and urban Central Americans were compared with an urban U.S. sample matched on sex and age. Initially, Mann concluded that the differences found in his investigations were based solely on percent calories from fat, but in a later paper (73) hypothesized that the differences could have been due to physical inactivity.

A study by Groen (37) attributed a vegetarian diet and a hardier life to the lower cholesterol values of Trappist monks when compared to a Benedictine order. The Trappist's cholesterol values were significantly lower than those of men of the same age in Cleveland (ages 18 - 40). They also found the average body weight and obesity greater among fathers than brethren of both orders (the brethren did all the heavy manual labor). This difference was attributed to exercise since there was little or no difference between fathers and brethren in caloric intake. There was little difference, however, in serum cholesterol values between fathers and brethren.

Gsell (38) and Gsell and Mayer (39) studied the population of Blatten, a mountain village in Switzerland characterized by a high dietary fat consumption (32 - 40%). When compared with a comparable town population, Blatten's inhabitants disclosed significantly lower cholesterol values. Other variables such as body size and weight, dietary factors, smoking habits, and racial differences did not explain this difference. The authors concluded that the heavy physical work of the village population, even up to ages 70 and 80, was the factor which kept the serum cholesterol values low. In Finland, where people consume a high fat diet, Karvonen (57) reported very little difference between

cholesterol levels of lumberjacks and townspeople. Karvonen emphasized physical activity as an important factor in keeping the cholesterol level relatively low.

Stamler (103) classified 784 men, ages 50 - 59, into various subgroups based on physical activity in occupation, and on sociological characteristics. Although he found a high incidence of coronary heart disease in all subgroups, significantly lower rates were observed in the foreign-born subjects compared to native born subjects. He also reported a mean cholesterol value of 239 mg%, with 20% of the men having levels of 275 mg% or greater. Blue collar workers, outdoor workers, and semi-skilled workers disclosed a lower incidence of coronary heart disease than indoor, white collar or executive personnel, although the differences were not significant.

Keys has been the leading proponent of the argument linking diet with serum cholesterol level. His studies in Italy (60, 61), Spain (64), the United States (58), and Japan (63) all point toward similar conclusions. People in those countries who consume a small amount of their calories in the form of animal fat have significantly lower serum cholesterol levels, and apparently have a lesser frequency of heart disease, than similar groups in the United States, where fat consumption is about 40% of the caloric intake. Keys, et al. (59) also reviewed data relative to the effects of physical activity on populations in Minnesota, Malmo, Bologna, Naples, the Island of Sardinia and three ethnic groups in Cape Province, South Africa. He concluded that differences in physical activity did not explain large differences in serum cholesterol when groups with different dietary habits were compared. Although he found that the Bantu manual laborers had lower serum cholesterol than other

men in the populations studied, he concluded it was because the Bantu consumed diets lower in fats. Keys did not negate some possibility of the effect of physical activity, but hypothesized that it contributed little toward explaining the differences in the cholesterol levels. In Finland, where cholesterol levels are generally higher than anywhere else in the world, Keys, et al. (62) studied 869 clinically healthy men ages 20 - 59. The subjects were divided by decades of age and classified according to occupational activity levels. Although Finnish people consume an extremely high percentage of animal fat in their diet, Keys reported that the more physically active men had lower mean serum cholesterol levels than the non-active men. The cholesterols of both active and non-active men increased through the decades, but the active men retained the lower mean in each group. This was true in East Finland, but not in West Finland. In this research, however, Keys concluded that the differences were determined by living habits and other environmental factors. However, it is interesting to note that at least in East Finland, physical activity appeared related to lower mean serum cholesterols, even though the Finnish people consume a diet rich in animal fats. Miller and colleagues (77) studied 189 Italian men of Neopolitan parentage who had lived in Boston all their lives, and reported their cholesterol levels similar to other Americans, but significantly higher than native Neopolitans. These investigators also found little correlation between degree of physical activity and serum cholesterol, and attributed the difference to diet.

Montoye (81) used a questionnaire-interview method to assess the amount and intensity of habitual physical activity of men age 20 - 64 in a total community of about 10,000 persons. The men were classified as

sedentary, moderately active or active on the basis of their occupational activity, leisure-time activity or a combination of both. Systolic and diastolic blood pressure, serum total cholesterol and glucose tolerance of men in the various activity groups were compared. When activity classification was based upon both occupational and leisure-time activities, the largest differences in the coronary heart disease risk factors appeared. The active men had significantly lower systolic and diastolic blood pressure and significantly lower serum total cholesterol. There were no significant differences in blood glucose.

CHAPTER III

METHODS

Sixty-two ninety day old male Albino rats of the Sprague-Dawley strain,[1] weighing between 229 and 321 grams were used in this study. The animals were identified by a numerical ear punch system and caged in pairs in stainless steel cages which measured 8 1/4 x 11 x 8 inches. They were housed in a temperature controlled room having a daily mean temperature of 23± 1.5°C. The cages were arranged in a randomized manner in order to minimize any possible effect of cage position on the experimental study. The room lighting was so regulated that the animals were exposed to 12 hours of light and 12 hours of darkness. All rats received water and were fed a standard diet[2] ad libitum throughout the experiment.

Selection of Animals for Experimental Groups

Sixty-two rats were preliminarily screened to determine their willingness and ability to run in a motor driven exercise wheel.[3] The wheel measured 43 x 4 1/4 inches. The screening tests consisted of four exercise sessions of five minutes duration at a speed of approximately 0.5250 mph.

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[1]Spartan Research Laboratories, Inc., Lansing, Michigan.

[2]Rockland Rat/Mouse Diet. Chesapeake Feed Company, Inc., Beltsville, Maryland.

[3]Wahman Manufacturing Company, Baltimore, Maryland.

(Appendix I). Forty-five rats were found satisfactory and were referred to as "runners", while 17 remaining rats were considered "non-runners".

The animals were divided into four groups of 12 rats each. These groups were designated as follows:

GROUP I	8 weeks exercise training
GROUP II	8 weeks sedentary controls for group I
GROUP III	8 weeks exercise followed by 8 weeks sedentary (detraining)
GROUP IV	16 weeks sedentary controls for group III

Selection of the animals for each group was based on the use of a table of random numbers. Groups I and III were randomly selected from the homogenous grouping of 45 animals previously determined as "runners". The animals not selected were pooled with the 17 "non-runners" making a total of 38 from which the non-exercise control groups II and IV were selected. The remaining 14 animals not assigned to the four experimental groups were used in establishing and evaluating the various experimental techniques used in the study.

Exercise Training and De-training Program

The exercise training program consisted of 67 running days. The speed and duration that the animals ran was based upon their progressive adaptation to the exercise work loads (Appendix II). Detraining was effected by eliminating the daily exercise training program for a comparable period of time (67 days).

Determination of Resting Heart Rates

Sixteen hours preceeding the determination of resting heart rates, pin electrodes were inserted subcutaneously into the dorsal skin directly above the left and right cutaneous maximus muscle of each of the 48 animals used in the study. It was assumed that this time period permitted the animals to adjust to the stress due to the insertion of the electrodes before any resting heart rate measurements were taken.

Resting heart rate measures were made on animal groups (n = 12) comprised of three rats from each of the four experimental groups. During the tests they were placed in individual cages measuring 7 1/4 x 9 1/2 x 7 inches. These cages were kept in an environmental chamber located within the exercise physiology laboratory. Electrical leads from a Sanborn Twin-Viso recorder[4] were attached to the pin electrodes of each rat with a pair of alligator clips. Wet and dry bulb temperatures and relative humidity data were recorded at the start and finish of each measurement period. During a subsequent two hour period, all temperature and humidity controls were shut down to reduce the noise level to a minimum. During the first hour of this period in which the animals were in the chamber, no resting heart rates were taken since this was considered an adjustment period. The following hour, six individual resting heart rates were recorded for each rat. These recordings were made at a time when the animals were not moving and were considered to be quiet and at rest. Each heart rate measurement was monitored for a 15 second period and a mean value based on six readings was calculated for each rat. At the end of the testing period, temperatures and humidity controls

[4]Sanborn Company, Cambridge, Massachusetts.

were activated to prepare the chamber for the next group. A test for significant differences between groups was performed using a one-way analysis of variance design (page 39).

Collection of Blood Samples

The animals were decapitated by guillotine and blood samples were obtained by draining the blood into 17 x 100 mm Falcon tubes which were placed into a refrigerator for 1 hour at 4°C. Each tube was rimmed with an applicator stick to facilitate the separation of the serum from the blood clot. The tubes were returned to the refrigerator for an additional hour until retraction was complete. The separated sera was decanted into 12 x 75 mm Falcon tubes and centrifuged at 2000 rpm for 10 minutes to ensure removal of all of the red blood cells and any fragments of the clot. The sera were transferred to clean 12 x 75 mm Falcon tubes and stored at 4°C.

Preparation of Tissues

Following decapitation and the securing of blood samples, tissues were quickly removed from the animal, washed in distilled water, rapidly frozen on dry ice, and stored in the frozen state at -20°C.

Each tissue was maintained in the frozen state by placing it on dry ice and manually cut into small fragments using a Bard Parker[5] surgical blade #22. The frozen fragments were placed in lyophilyzing flasks, shell frozen, attached to a Virtis-Super Freeze Dryer unit[6] and

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[5]Bard Parker Company, Inc., Rutherford, New Jersey, Danbury, Connecticut.

[6]Scientific Products Company, Pittsburg, Pennsylvania.

lyophilized for approximately 24 hours. The individual dried tissues were ground manually to a fine powder in separate porcelain mortar and pestles. The dry tissue powder was placed in Falcon tubes and stored in a vacuum dessicator at room temperature. The cold epididymal fat tissue was sliced into small pieces and placed in Falcon tubes and stored under vacuum at 4°C.

Cholesterol Determination in Tissues

Preparation of Tissue Extract

The following procedure was used for myocardial, skeletal and epididymal tissue.

A sample of tissue (0.1 to 0.2 gm) was placed in a 50 ml centrifuge tube and 5 ml of freshly made 33% alcoholic potassium hydroxide (KOH) was added. The tube was mechanically mixed and allowed to saponify overnight at room temperature. The following morning, 5 ml distilled water was added to the tube, the mixture shaken and allowed to stand for 5 minutes. Ten ml petroleum ether (boiling range 30 - 60°C) was added and the mixture mechanically mixed for 2 minutes. The sample was allowed to stand for 5 minutes until two clear layers separated. The supernatant petroleum ether layer was transferred to a 50 ml Erlenmeyer flask by means of a pipette. Two additional extractions with petroleum ether were made and added to the original extract. The total extract was then evaporated to dryness under reduced pressure in a 60°C water bath. Ten ml acetone:alcohol mixture (1:1 volume) was added to dissolve the residue. Two separate 4 ml aliquots of the extract were pipetted into separate 25 ml volumetric flasks and evaporated to dryness under reduced pressure in a 60°C water bath. After cooling to room temperature

the flasks were stoppered and analyzed for cholesterol by the manual method of Abell, et al. (2) Eight tissue samples were extracted at one time.

Cholesterol by the Manual Method (2)

Standard solutions of cholesterol were run in parallel in each series of tissue analysis. Duplicate 5 ml samples of the standard cholesterol solution (0.4 mg per ml) and 0.3 ml of 33% KOH solution were mixed in 25 ml glass-stoppered centrifuge tubes and incubated for 55 minutes at 37 - 40°C. Ten ml of petroleum ether and 5 ml of water were added and the tubes shaken vigorously for 1 minute. After centrifugation, 1, 2, and 3 ml respective aliquots of the petroleum ether layer were transferred into 25 ml volumetric flasks and evaporated to dryness. These standards were equivalent to 0.2, 0.4, and 0.6 mg of cholesterol.

The flasks containing the dry residues from the tissues previously prepared (See Preparation of Tissue Extract, page 21) and the standards were arranged so that a set of standards containing 0.2, 0.4, and 0.6 mg of cholesterol were analyzed at the beginning and at the end of each group of analyses. A clean empty Evelyn colorimeter tube was placed at the beginning of each set of analyses to serve as a blank. All the flasks were placed in a water bath at 25°C, a timer was started, and 6 ml of the modified Liebermann-Burchard reagent was added first to the blank flask and then at regular 30 second intervals to the standards and tissue samples in the flasks. Care was taken to wash down the entire surface of the flask with the reagent. The flasks were stoppered, shaken, and returned to the 25°C bath immediately following the addition of Liebermann-Burchard reagent.

The color was allowed to develope for 30 - 35 minutes in subdued light. The color intensity was measured in a Evelyn Photoelectric colorimeter[7] using a filter (620mu). The galvanometer readings obtained were converted to optical density (2 -log galvanometer reading).

Standard Curve

The optical density of the standards were plotted against the concentration of cholesterol in mg to yeild a standard curve (Fig. 1).

Reagents

1. Absolute ethyl alcohol (C_2H_5OH).
   U. S. Industrial Chemicals Company, New York.
2. Petroleum ether. B.P. 30 - 60°C.
   Merck & Company, Inc., Rahway, New Jersey.
3. Glacial acetic acid (CH_3COOH). Reagent grade.
   J. T. Baker Chemical Company, Phillipsburg, New Jersey.
4. Sulfuric acid (H_2SO_4). Reagent grade.
   Merck & Company, Inc., Rahway, New Jersey.
5. Acetic anhydride $(CH_3CO)_2O$. Reagent grade.
   Merck & Company, Inc., Rahway, New Jersey.
6. 33% Potassium hydroxide (KOH). Reagent grade.
   Dissolve ten grams KOH in 20 ml H_2O.

[7]Rubicon Company, Philadelphia, Pennsylvania.

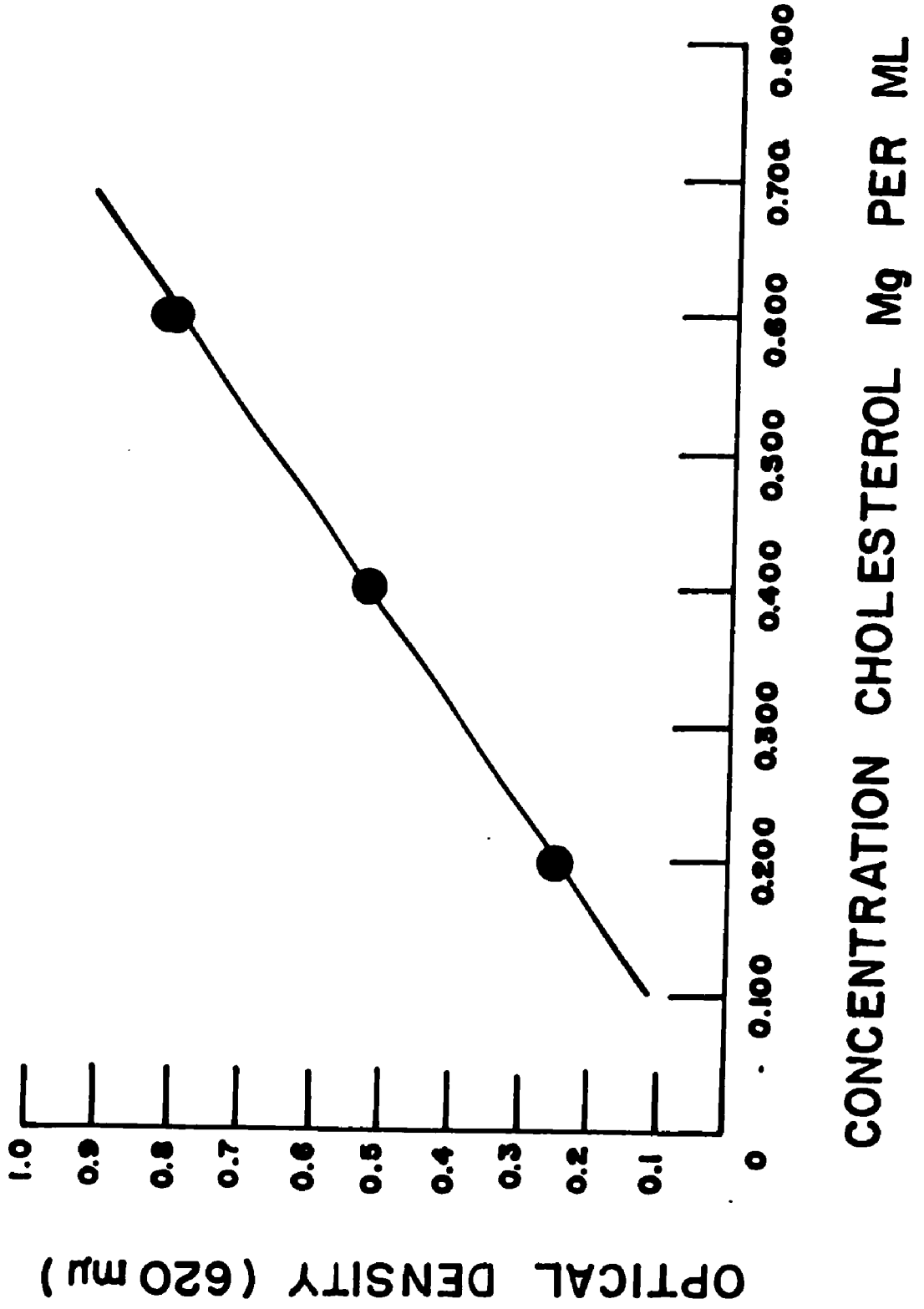


Figure I

RELATIONSHIP BETWEEN OPTICAL DENSITY (2 -LOG GALVANOMETER READING) AND CONCENTRATION OF A STANDARD CHOLESTEROL SOLUTION AS DETERMINED BY THE PROCEDURE OF ABELL, et al.

7. Alcoholic potassium hydroxide. Made immediately before using by adding 6 ml of 33% KOH to 94 ml of absolute ethyl alcohol (C_2H_5OH).
8. Standard Cholesterol solution,[8] 0.4 mg per ml, 100 mg of cholesterol was dissolved in absolute ethyl alcohol (C_2H_5OH) and the volume made to 250 ml.
9. Modified Liebermann-Burchard reagent. 20 volumes of the acetic anhydride $(CH_3CO)_2O$ was chilled to a temperature lower than 10°C in a glass-stoppered container, 1 volume of concentrated H_2SO_4 was added, and the well shaken mixture kept cold for 9 minutes. Ten volumes of glacial acetic acid (CH_3COOH) were added and the mixture allowed to warm to room temperature. The reagent should be used within 1 hour.

Cholesterol Determination in Blood and Tissues

Auto Analyzer Method (9)

A 0.5 ml sample of the serum was pipetted into a 15 ml centrifuge tube and 9.5 ml isopropanol was added. The mixture was thoroughly mixed and centrifuged at 2000 rpm. The dried residue obtained from a 4 ml aliquot of tissue extract (described under Preparation of Tissue, page 20) was dissolved in 2 ml of isopropanol and thoroughly mixed.

Aliquots (~2 ml) of isopropanol extracts from blood and tissue were poured into auto analyzer sample cups and placed into the sample plate of the Sampler II apparatus.[9] The extracts were aspirated from the

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[8]Eastman Chemical Products, Inc., Kingsport, Tennessee.

[9]Technicon Corporation, Tarrytown, New York.

sample cups by a stainless steel tube connected to polyethylene tubing and passed into Solvaflex tubing as shown in the flow diagram (Fig. 2).

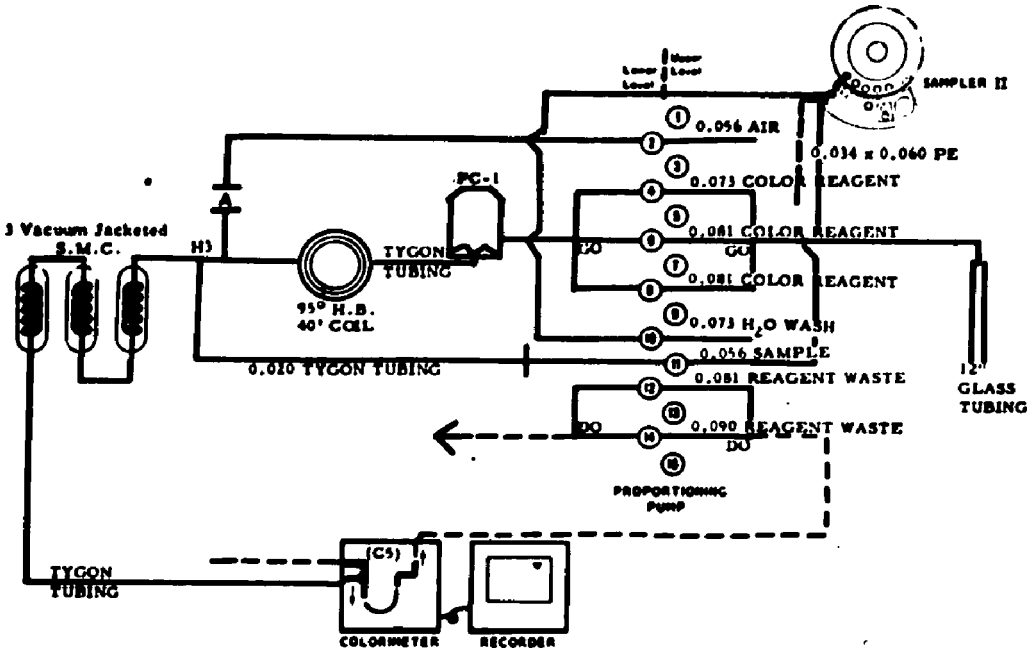


Figure 2

FLOW DIAGRAM SHOWING MANIFOLD ARRANGEMENT
FOR AUTOMATED CHOLESTEROL DETERMINATION

A 15 mm tubular flow-cell with a 550mu filter was used. The color concentration was registered on a continuous recording microammeter calibrated in percentage transmission. This was converted to optical density.

Standard Curve

The optical density is plotted against the various concentrations of cholesterol standard (Fig. 3).

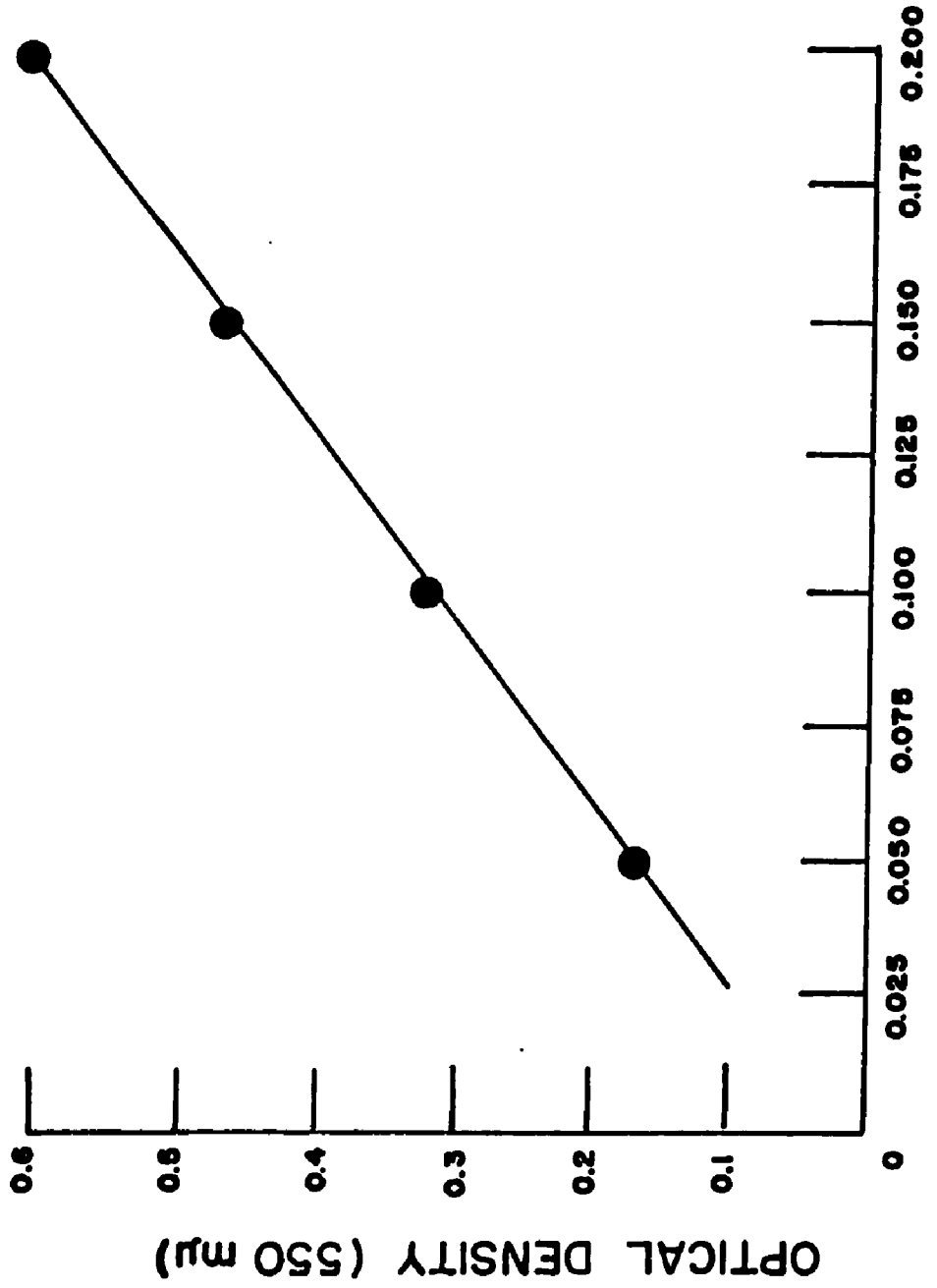


Figure 3

RELATIONSHIP BETWEEN OPTICAL DENSITY (2 -LOG PERCENT TRANSMISSION) AND CONCENTRATION OF A STANDARD CHOLESTEROL SOLUTION AS DETERMINED BY THE AUTOMATED PROCEDURE OF BLOCK, et al.

Reagents

1. Ferric choloride ($FeCl_3$) anhydrous. 825 mg $FeCl_3$ was dissolved in glacial acetic acid and made to a 2 liter volume. This was then mixed with 1 liter of concentrated H_2SO_4. The reagent was kept in a dark bottle.
2. Isopropanol, absolute, aldehyde free. Two liters of isopropanol, (Analytical Grade) was refluxed with 50 - 75 grams KOH pellets for 1 hour. The mixture was distilled and the first 50 ml of distillate discarded. This distilled isopropanol was stored in an air tight glass-stoppered bottle, with a minimum of air space above the liquid level.
3. Cholesterol Standard. A stock solution of cholesterol[10] (5 mg/ml) was made by dissolving 500 mg cholesterol in 100 ml of isopropanol.

Triglyceride Determination in Blood and Tissues

The triglyceride content of tissue and serum was analyzed according to the method of Block and Jarrett (8).

Tissue Extract

A sample (0.05 - 0.20 gm) of tissue was weighed on a Mettler analytical balance and placed in a 50 ml centrifuge tube. It was extracted by the procedure of Folch, et al. by adding 15 ml chloroform:methanol mixture (2:1 v/v) and mechanically mixed for 2 minutes and allowed to stand for 10 minutes. The suspension was then

[10] Technicon Corporation, Tarrytown, New York.

centrifuged at 2000 rpm for 10 minutes. The separated supernatant layer was decanted into a 50 ml Erlenmeyer flask. Extraction of the tissue was repeated first with 10 ml of extractant and followed by a 5 ml extraction to insure complete removal of all lipid. The extract was pooled and evaporated to dryness under reduced pressure in an atmosphere of nitrogen. The residue obtained was dissolved in 10 ml isopropanol and stored at 4°C until analyzed.

Analysis of Serum

A 0.5 ml sample of the serum was extracted with 9.5 ml isopropanol. Two grams of Zeolite was added to each sample and the mixture allowed to stand for 30 minutes. The sample was centrifuged at 2000 rpm for approximately 10 minutes. The supernate layer was decanted into a clean conical tube and 2 ml of the supernate layer was transferred into an autoanalyzer sample cup. The sample was saponified automatically, the glycerol converted to formaldehyde and condensed with 2,4 - pentanedione. The fluorescense that developes was determined in a Turner photofluorometer[11] model #111 equipped with a tubular flow cell, a Bausch and Lomb (405mu) interference primary filter and a Wratten #8 secondary filter (485mu). The fluorescense was recorded continuously on a microammeter. For details of the manifold used in this procedure see (Fig.4). Because free glycerol in the serum forms fluorescent substances with the reagents used, a non-saponificable material blank on all samples were determined in the following manner: the tubing above the jacketed H_3 fitting through which the sample, air and "Base" reagent flow, was disconnected (Fig. 4). This tubing was attached

[11]G. K. Turner Associates, Palo Alto, California.

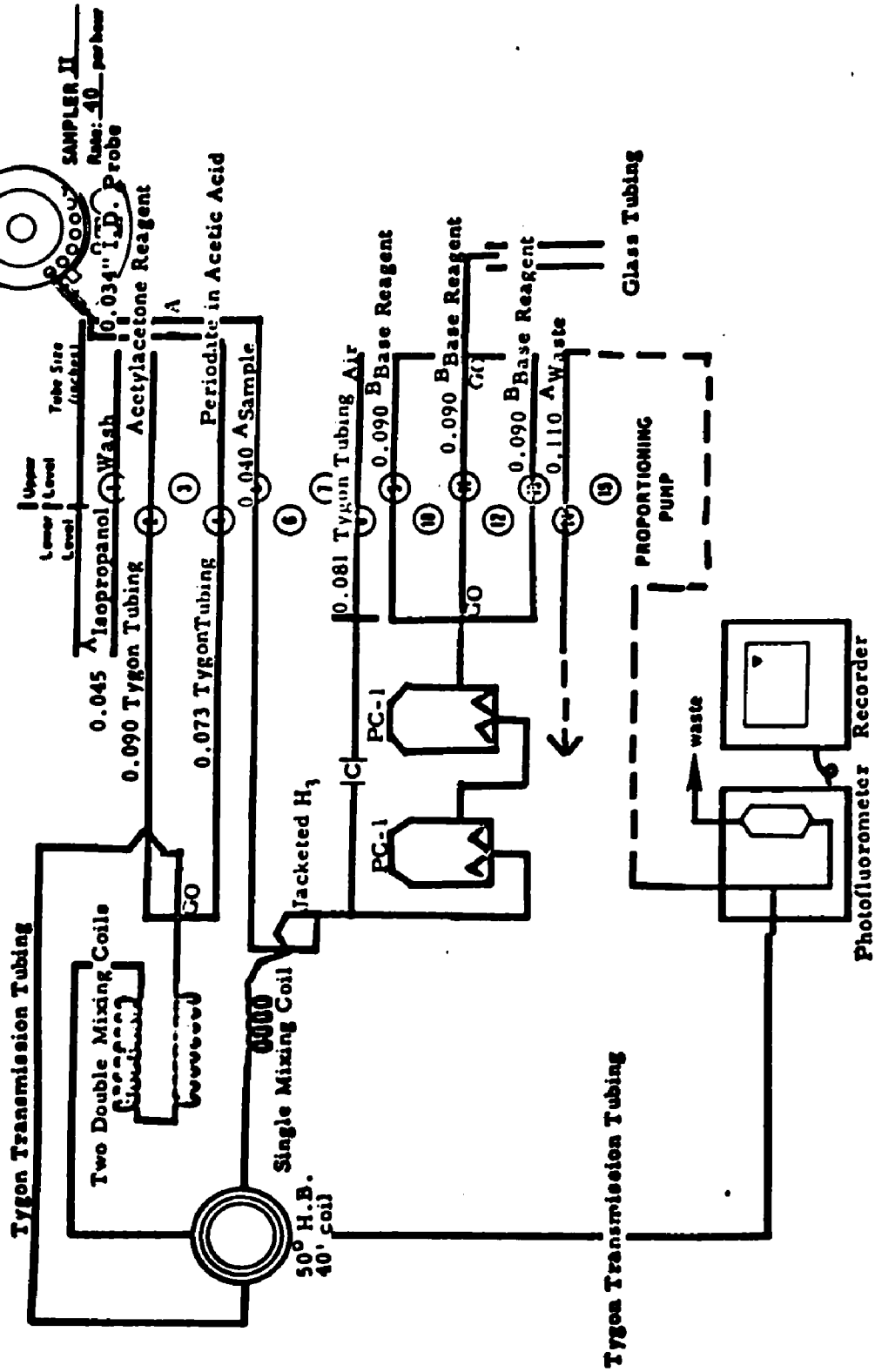


Figure 4

FLOW DIAGRAM OF MANIFOLD ARRANGEMENT USED IN THE DETERMINATION OF TRIGLYCERIDE BY THE AUTOMATED PROCEDURE OF BLOCK AND JARRETT.

(in place of the Tygon transmission tubing leading from the heating bath) to the GO fitting, which precedes the two double mixing coils. This modification of the manifold eliminated the initial heating and saponification steps and resulted in the determination of only the non-saponifiable material in serum that fluoreses under the experimental conditions used in the analytical procedure. These values were subtracted from the values obtained after saponification which were expressed as triglyceride concentration.

Standardization of Method

The standard curve was also established by subtracting the non-saponifiable material blank value from that obtained after saponification. The difference between the two values was plotted against the concentration of triolein (expressed in mg per 100 ml) to yeild a standard curve (Fig. 5).

Analysis of Tissue Extract

To each tissue residue dissolved in 10 ml isopropanol (described under the section of Tissue Extract, page 28) was added 2 grams of zeolite. The suspension was throughly mixed and allowed to stand for 30 minutes. The solution was centrifuged at 2000 rpm for approximately 10 minutes. The supernate fluid was decanted into a clean conical tube. Two ml was poured into a sample cup and analyzed as for the serum previously described (page 29).

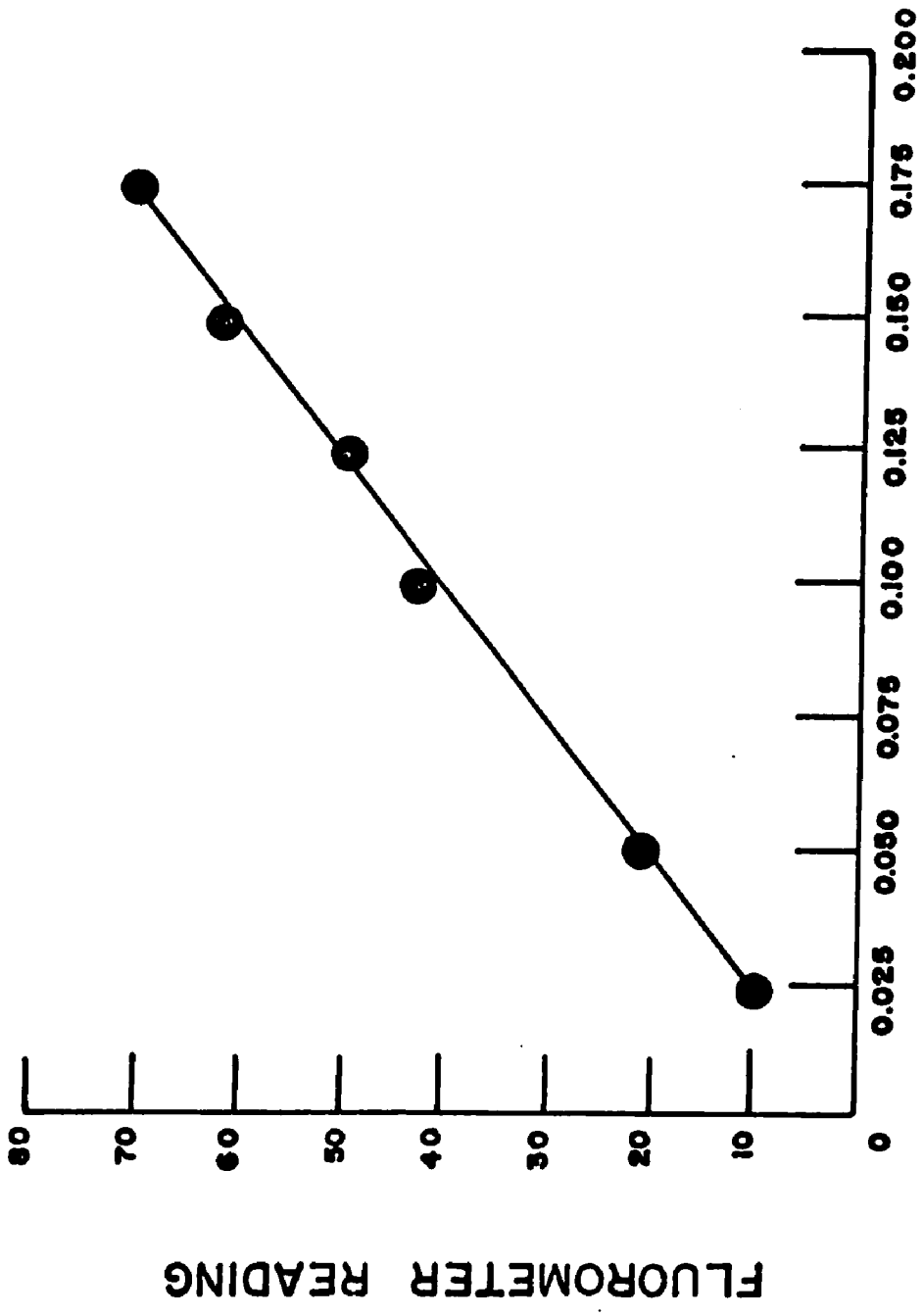


Figure 5

RELATIONSHIP BETWEEN FLUOROMETER READING AND CONCENTRATION OF A STANDARD TRIGLYCERIDE SOLUTION AS DETERMINED BY THE PROCEDURE OF BLOCK AND JARRETT.

Reagents

1. Isopropanol, absolute, aldehyde free. Two liters of isopropanol,[12] (Analytical Grade) was refluxed with 50 - 75 grams KOH pellets for 1 hour. The mixture was distilled and the first 50 ml of distillate discarded. The distilled isopropanol was stored in an air tight glass-stoppered bottle, with a minimum of air space above the liquid level. Aldehyde free isopropanol was used in all reagents requiring isopropanol.
2. Triolein Standard.

   A. Stock solution. The contents of a 500 mg vial of triolein[13] of 99+ percent purity was transferred to an 100 ml volumetric flask. Care was taken to wash the flask repeatedly with isopropanol. The solution was mixed well. The flask was brought to volume with isopropanol and stored at 4°C in an amber-colored glass bottle fitted with a Teflon lined screw cap.

   B. Working solutions. Various amounts of the stock solution were diluted with isopropanol to obtain triolein concentrations ranging from 50 - 250 mg/100 ml.
3. Zeolite mixture. 200 grams of Zeolite[14] were finely ground in a Waring Blendor and dried overnight at 110°C. To 20 grams of Lloyd Reagent[15] 4.70 gm anhydrous $CuSO_4$ and 20 gm of $Ca(OH)_2$

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[12] J. T. Baker Chemical Company, Phillipsburg, New Jersey.

[13] Applied Science Laboratories, Inc., State College, Pennsylvania.

[14] W. A. Taylor, Baltimore, Maryland.

[15] Hartman-Leddon Company, Philadelphia, Pennsylvania.

was added and throughly mixed. This mixture was stored in an amber colored glass-stoppered bottle at room temperature.

4. Base reagent. 5 grams of KOH pellets was dissolved in 250 ml of deionized H_2O in a 1 liter volumetric flask and made to volume with isopropanol. The reagent was made fresh daily.
5. Periodic Acid Reagent. 0.025 M $NaIO_4$ in 2M acetic acid. 120 ml concentrated CH_3COOH was added approximately 700 ml of deionized H_2O in a 1 liter volumetric flask and mixed. 5.35 grams $NaIO_4$[16] were added and mixed well until dissolved. The reagent was diluted to the 1 liter mark with deionized H_2O. The reagent was made fresh daily.
6. Acetylacetone Reagent. 154 grams $NH_4C_2H_3O_2$ was placed into a 1 liter volumetric flask. Deionized H_2O was added to the 1 liter mark and the contents throughly mixed. The pH of the 2M $NH_4C_2H_3O_2$ should be 6.0. The pH was adjusted as required by using either concentrated ammonium hydroxide or glacial acetic acid. 7.5 ml acetylacetone (2,3 - pentanedione) and 25 ml isopropanol was added to 1 liter of $NH_4C_2H_3O_2$ just before use. The reagent was made fresh daily and stored in an amber colored glass-stoppered bottle.

Total Nitrogen in Tissues

Micro-Kjeldahl Procedure (10)

A sample of tissue (10 to 20 mg) was weighed on a Mettler analytical balance and carefully introduced into a 15 ml micro-Kjeldahl flask which contained 1.3 grams of HgO,K_2SO_4 mixture. Two ml concentrated H_2SO_4

[16]Mallinckrodt Chemical Works, St. Louis, Missouri.

was added to the mixture and digested at a low temperature for 5 minutes. Two drops of 30% H_2O_2 (Superoxol)[17] was added and the mixture digested at a high temperature until the mixture was colorless indicating complete digestion of the tissue. This procedure usually required 1 1/2 to 2 hours. The digested mixture was allowed to cool and a small amount of deionized H_2O was added to dissolve the solids.

A 125 ml Erlenmeyer flask containing 5 ml 4% H_3BO_3 (Boric acid) and 4 drops of mixed indicator was placed under the delivery tube prior to the introduction of the sample into the distillation apparatus. The contents of each digestion flask was quantitatively transferred to the distillator boiling tube with a minimum amount of deionized H_2O. Eight ml of the $NaOH/NaS_2O_3 \cdot 5H_2O$ solution was pipetted into the rinsed distillator funnel and the stopcock was closed immediately. Distillation continued until approximately 20 ml of solution was distilled. Twenty-five ml of resin H_2O was then added to each sample and titrated with a standard solution of HCl, using a semi-micro burette. All samples were run in duplicate with an acceptable error of $\pm$ 2%. Two or three blanks were run intermittently with each set of samples.

Reagents

1. Sodium hydroxide sodium thiosulfate mixture ($NaOH/NaS_2O_3 \cdot 5H_2O$). 500 grams NaOH and 50 grams $NaS_2O_3 \cdot 5H_2O$ were placed in a 1 liter volumetric flask, dissolved in water, made to volume and thoroughly mixed.

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[17]Merck & Company, Inc., Rahway, New Jersey.

2. 4% Boric Acid (H_3BO_3). 40 grams H_3BO_3 were dissolved in H_2O in a 1 liter volumetric flask and made to volume.
3. Mercuric oxide potassium sulfate mixture (HgO/K_2SO_4). 420 grams K_2SO_4 and 14 grams HgO were thoroughly mixed by shaking and stored in a dark glass jar.
4. Mixed Indicator.

   A. Methyl Red. 0.04 grams of methyl red was placed in a 100 ml volumetric flask and dissolved in 7.44 ml of 0.02N NaOH and made to volume with deionized H_2O.

   B. Bromcresol Green. 0.04 grams of bromcresol green was placed in a 100 ml flask and dissolved in 2.88 ml 0.02N NaOH and made to volume.

   Two parts of methyl red and three parts bromcresol green were thoroughly mixed by shaking.

Determination of Lipoprotein in Blood Serum

The serum lipoproteins were analyzed in duplicate by electrophoresis on cellulose acetate membranes by the method of Ross and Brown (98) and on paper according to the method of Lees and Hatch (68). The electrophoresis of the serum samples were done within 48 hours after the blood was obtained.

Cellulose Acetate Electrophoresis

Electrophoresis was done in a Beckman microzone cell[18] using a Sephraphore III membrane (2.25 x 5.687 inches). A barbiturate supporting

[18]Spinco Division of Beckman Instruments, Inc., Palo Alto, California

media buffer, pH 8.6 and an ionic strength of 0.075 was used. Before being placed in the microzone cell, the membrane was immersed in the buffer 2 - 3 minutes in order for the membrane to become evenly saturated with the buffer. The membrane was removed, then blotted on filter paper and placed in the electrophoresis cell containing the pH 8.6 buffer. Serum samples were applied to the membrane by use of the Beckman sample applicator. Two applications of each sample were applied to the membrane. This was equivalent to approximately 0.5 microliters. Electrophoresis of the serum was done by applying a current of 250 volts at room temperature (22 ± 2°C) for 20 minutes. The membrane was removed from the cell at the end of this period and placed in a small covered tray containing a solution of "Oil Red O" for 36 hours at 37°C.

The membrane was then removed and placed in a small tray containing sufficient clearing solution to cover the membrane. After 1 minute, the membrane was removed and placed on a clean glass plate and dried in an over at 90°C for 30 - 40 minutes.

Reagents

1. Barbiturate Buffer. To 1 liter of water add 2.76 grams diethyl barbiturate pH 8.6, ionic strength 0.075.
2. "Oil Red O". To 600 ml ethanol add 0.4 grams of "Oil Red O"[19] and 400 ml deionized H_2O. This mixture was brought to a boil under a reflux condenser and allowed to cool overnight at 37°C. It was stored in a glass-stoppered bottle at 37°C.

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[19]Allied Chemical, Industrial Chemical Division, Morristown, New Jersey.

3. Clearing Solution. Five ml absolute methanol and 25 ml CH_3COOH were poured into a graduated cyclinder. To this mixture was added 70 ml resin H_2O.

Paper Electrophoresis

Electrophoresis was done at room temperature on Schleicher and Schuell 2043A paper strips in a Durrum hanging strip cell (Spinco Model R, Series D) for 16 hours at 110 volts with a current of 7/8 milliamp per strip. Barbiturate buffer[20] of ionic strength 0.075 and pH 8.6 was used in all experiments. Following electrophoresis the strips were dried in an oven at 120°C for 1/2 hour, then stained with "Oil Red O" at 35°C according to the method of Jencks and Durrum (54). The albumin-containing buffer was stored at 4°C between experiments, thereby enabling it to be used repeatedly.

Reagents

1. Barbiturate Buffer. Ionic strength 0.075, containing 0.001M EDTA (Ethylenediaminetetraacetic acid). To this buffer was added 10 grams/liter of Bovine Plasma Albumin, Fraction V.[21]
2. "Oil Red O". Prepared as for cellulose acetate electrophoresis, page 37.

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[20]Spinco Division of Beckman Instruments, Inc., Palo Alto, California.

[21]Armour Pharmaceutical Company, Chicago, Illinois.

Statistical Treatment

The means and standard deviations were determined for the data of all parameters measured and comparisons between groups were made by analysis of variance[22] as described in Snedecor (101).

Comparisons within groups for body weights and resting heart rates across time were made by the paired t test as described in Dixon and Massey (26) so that the variation between animals was removed. Significant differences were determined for the .05 and .01 levels using standard F and t tables.

Actual calculations were run on an IBM 360/67 computer.

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[22]Analysis of variance for one-way design - version of June 1, 1968. Written by Health Sciences Computing Facility, UCLA. Revised and adapted by Statistical Research Laboratory, University of Michigan.

CHAPTER IV

RESULTS

The results of these studies are presented in the following series of tables: body weight results (Table 1), resting heart rate results (Table 2), blood and tissue levels of cholesterol, triglyceride, and nitrogen (Table 3), and tissue lipid/nitrogen ratios (Table 4).

The means and standard deviations of all variables measured for each experimental and control group are shown. Statistically significant differences between groups at either the .05 or .01 level as determined through analysis of variance[23] are included. Significant differences ($P \leq .01$) within groups for the body weights and resting heart rates are also shown.[24]

Body Weights

Following 8 weeks of training, the mean ($\bar{X}$) body weight of group I was 91.3 grams less ($P \leq .01$) than the sedentary control group II. The $\bar{X}$ body weight of the exercise-detrained animals (group III) was also 36.4 grams lower than the sedentary control group IV, but this difference was not satistically significant at the .05 level. The animals in group III

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[23]Analysis of variance for one-way design - version of June 1, 1968. Written by Health Sciences Computing Facility, UCLA. Revised and adapted by Statistical Research Laboratory, University of Michigan.

[24]Paired t test as described in Dixon and Massey, Introduction to Mathematical Statistics. McGraw Hill, New York, 1957.

gained weight during 8 weeks of detraining with the result that their final sacrifice weight equaled the sedentary control group IV, (Table 1).

Table 1

MEANS AND STANDARD DEVIATIONS OF BODY WEIGHTS (g) OF EXPERIMENTAL ANIMALS

(n = 12 per group)

Measurement	Means & Standard Deviations	Group I 8 Weeks Training	Group II 8 Weeks Sedentary	Group III 8 Weeks Training & 8 Weeks Detraining	Group IV 16 Weeks Sedentary
Initial	$\bar{X}$	445.10	455.80	447.00	456.50
	$\pm S_D$	19.30	14.40	34.00	39.00
8 Weeks	$\bar{X}$	458.40**	549.70†	487.40	523.80†
	$\pm S_D$	37.90	36.30	44.40	72.20
16 Weeks	$\bar{X}$	-	-	586.20†	567.20
	$\pm S_D$	-	-	41.00	70.80

**Significant difference ($P<.01$) between groups

*Significant difference ($P<.05$) between groups

†Significant difference ($P<.01$) within groups

(For significant F ratios see Appendix)

Resting Heart Rates

After 8 weeks of exercise training, the $\bar{X}$ resting heart rates of groups I and III were 44 and 33 beats per minute slower ($P<.01$) than the corresponding $\bar{X}$ rates of the sedentary control groups II and IV. After 8 weeks of detraining, the $\bar{X}$ resting heart rate of group III was significantly elevated ($P<.01$) and there was no significant difference between the $\bar{X}$ rates of groups III and IV at 16 weeks (Table 2).

Serum Cholesterol and Triglyceride

Both serum cholesterol and triglyceride levels were significantly lower ($P<.01$) in group I after 8 weeks of training than in the sedentary control group II. Following 8 weeks of detraining, the serum cholesterol

and triglyceride levels of group III remained significantly lower ($P=.05$) than the sedentary control group IV (Table 3).

Serum cholesterol and triglyceride levels were plotted against body weight as shown in Figure 6. A linear relationship existed between these variables for groups I, II, and IV since higher levels were associated with heavier animals. A departure from this trend was observed in the detrained rats (Group III) since serum cholesterol and triglyceride levels remained lower although body weight increased.

Table 2

MEANS AND STANDARD DEVIATIONS OF RESTING HEART RATES (BEATS PER MINUTE) OF EXPERIMENTAL ANIMALS

(n = 12 per group)

Measurement	Means & Standard Deviations	Group I 8 Wk. Trg.	Group II 8 Wk. Sed.	Group III 8 Wk. Trg. 8 Wk. DeTrg.	Group IV 16 Wk. Sed.
Initial	$\bar{X}$	330.00	325.00	335.00	347.00
	$\pm S_D$	12.60	20.40	12.80	13.60
8 Weeks	$\bar{X}$	296.00**	340.00	306.00**	339.00
	$\pm S_D$	15.60†	23.20	14.40†	25.60
16 Weeks	$\bar{X}$	-	-	331.00	335.00
	$\pm S_D$	-	-	17.70†	21.20

**Significant difference ($P<.01$) between groups

*Significant difference ($P<.05$) between groups

†Significant difference ($P<.01$) within groups

(For significant F ratios see Appendix)

Tissue Cholesterol

There was an increase ($P<.05$) in the tricep cholesterol of group I (8 weeks training) when comparisons were made with group II (8 weeks sedentary) based on the automated procedure. When cholesterol was determined by the manual procedure, there was no difference between these same groups. Contrasting results were obtained for the detraining group III

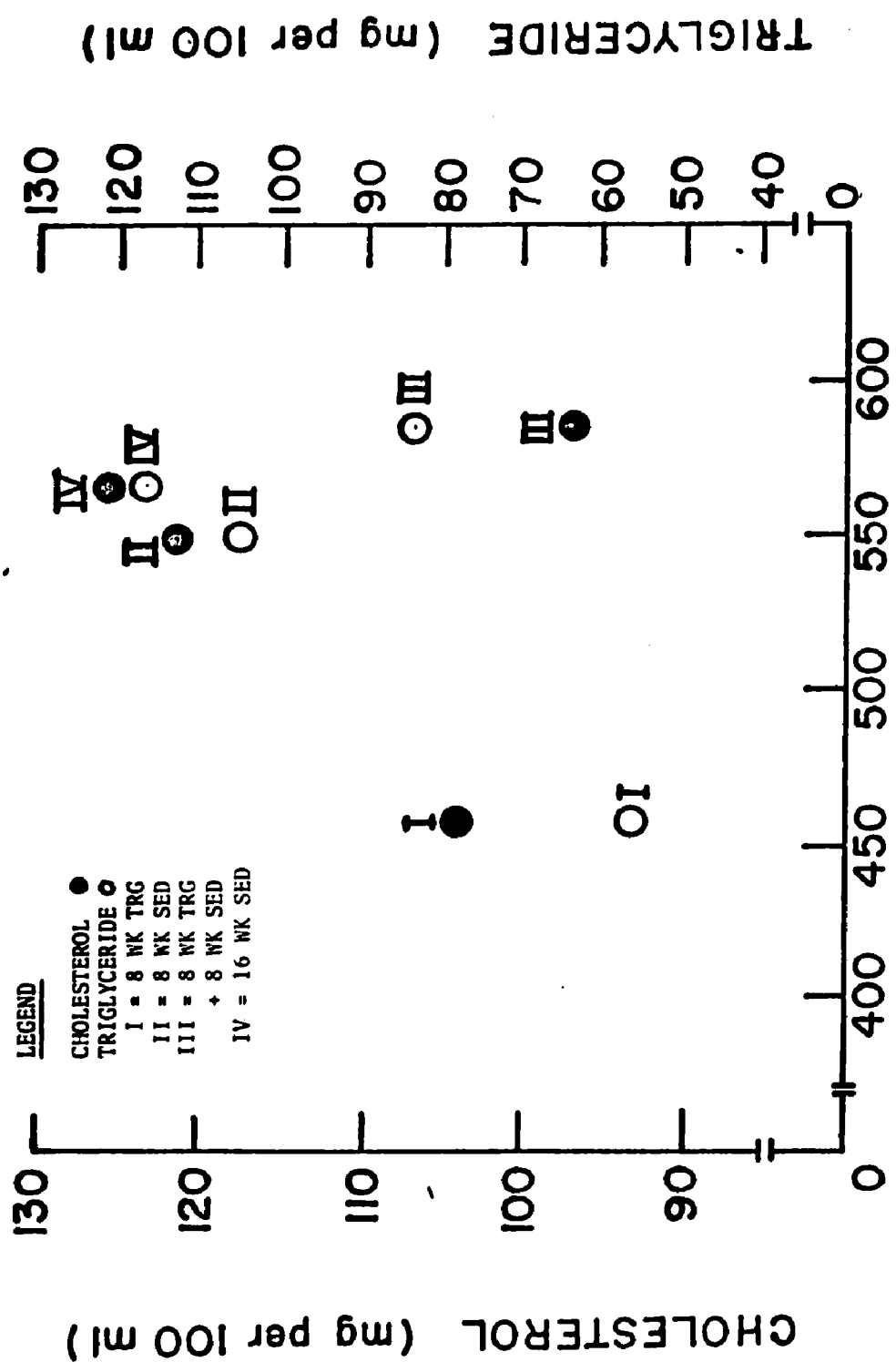


Figure 6

MEAN SERUM CHOLESTEROL AND TRIGLYCERIDE LEVELS AS RELATED TO BODY MEAN WEIGHT OF EXPERIMENTAL GROUPS

and sedentary control group IV as the triceps cholesterol was significantly lower in the detrained animals with the manual procedure. Additional contrast existed because these differences were not present when cholesterol was determined by the automated procedure (Table 3).

Tissue Triglyceride

The levels of triglyceride in epididymal tissue of the training groups I and III were lower ($P \leq .05$) than the levels in the corresponding sedentary control groups II and IV. There were no significant differences in heart and tricep triglyceride levels in any of the other experimental or control groups (Table 3).

Heart and Tricep Nitrogen

After training, the total nitrogen levels in heart and tricep muscle were greater ($P \leq .01$) in group I than in the sedentary control group II. Following 8 weeks of detraining, the total nitrogen levels of these muscles were also greater ($P \leq .01$) for group III as compared to the sedentary control group IV (Table 3).

Cholesterol and Triglyceride/Nitrogen Ratios

The triceps muscles of the detraining group III displayed a significant decrease compared to group IV in their $\bar{X}$ cholesterol to total nitrogen ratio based on either manual ($P \leq .01$) or automated procedures ($P \leq .05$). No significant differences in $\bar{X}$ cholesterol and triglyceride/nitrogen ratios were seen for any of the other tissues regardless of experimental groups (Table 4).

Table 3

MEANS AND STANDARD DEVIATIONS OF CHOLESTEROL: TRIGLYCERIDE AND NITROGEN IN THE BLOOD AND TISSUES OF EXPERIMENTAL ANIMALS

(n = 12 per group)

Variables (mg/g expressed as dry weight)		Means & Standard Deviations	Group I 8 Wk. Trg.	Group II 8 Wk. Sed.	Group III 8 Wk. Trg. 8 Wk. DeTrg.	Group IV 16 Wk. Sed.
CHOLESTEROL[a]						
Serum	mg/100 ml	$\bar{X}$	104.91**	121.66	97.25*	125.41
		$\pm S_D$	10.89	15.18	16.08	32.23
Heart	mg/g	$\bar{X}$	5.90	5.80	6.05	6.07
		$\pm S_D$	0.19	0.09	0.35	0.30
Tricep	mg/g	$\bar{X}$	3.01*	2.82	2.53	2.60
		$\pm S_D$	0.18	0.24	0.12	0.13
CHOLESTEROL[b]						
Heart	mg/g	$\bar{X}$	5.72	5.64	5.82	5.81
		$\pm S_D$	0.26	0.16	0.29	0.27
Tricep	mg/g	$\bar{X}$	2.61	2.53	2.11*	2.24
		$\pm S_D$	0.18	0.25	0.17	0.13
Epididymal	mg/g	$\bar{X}$	0.77	0.70	0.68	0.87
		$\pm S_D$	0.16	0.12	0.11	0.30
TRIGLYCERIDE						
Serum	mg/100 ml	$\bar{X}$	57.16**	105.58	83.83*	117.25
		$\pm S_D$	13.44	45.91	28.64	48.23
Heart	mg/g	$\bar{X}$	9.25	10.09	5.67	9.03
		$\pm S_D$	3.43	5.16	3.08	8.98
Tricep	mg/g	$\bar{X}$	6.49	7.75	9.92	12.59
		$\pm S_D$	2.88	3.25	6.07	5.83
Epididymal	mg/g	$\bar{X}$	968.08*	979.41	965.91*	976.50
		$\pm S_D$	13.64	10.32	11.96	12.48
TOTAL NITROGEN						
Heart	mg/g	$\bar{X}$	135.82**	131.78	130.76**	126.11
		$\pm S_D$	1.42	2.04	1.82	1.74
Tricep	mg/g	$\bar{X}$	139.71**	132.71	132.74**	129.09
		$\pm S_D$	2.16	1.99	2.37	0.77

*Significant difference ($P \leq .05$) between groups

**Significant difference ($P \leq .01$) between groups

[a]Automated procedure (Block et al.)

(For significant F ratios see Appendix)

[b]Manual procedure (Abell et al.)

Table 4

MEANS AND STANDARD DEVIATIONS OF LIPID/NITROGEN RATIOS IN TISSUES OF EXPERIMENTAL ANIMALS

(n = 12 per group)

Variables	Means & Standard Deviations	Group I 8 Wk. Trg.	Group II 8 Wk. Sed.	Group III 8 Wk. Trg. 8 Wk. DeTrg.	Group IV 16 Wk. Sed.
Man. Chol/Total N_2 Heart	$\bar{X}$	0.0421	0.0428	0.0445	0.0461
	$\pm S_D$	0.0020	0.0016	0.0025	0.0024
Auto. Chol/Total N_2 Heart	$\bar{X}$	0.0434	0.0440	0.0462	0.0481
	$\pm S_D$	0.0016	0.0011	0.0031	0.0028
Man. Chol/Total N_2 Tricep	$\bar{X}$	0.0186	0.0191	0.0159**	0.0174
	$\pm S_D$	0.0014	0.0021	0.0014	0.0011
Auto. Chol/Total N_2 Tricep	$\bar{X}$	0.0215	0.0212	0.0191*	0.0202
	$\pm S_D$	0.0015	0.0019	0.0010	0.0011
TG/Total N_2 Tricep	$\bar{X}$	0.0036	0.0038	0.0039	0.0035
	$\pm S_D$	0.0020	0.0020	0.0021	0.0025
TG/Total N_2 Heart	$\bar{X}$	0.0006	0.0007	0.0004	0.0007
	$\pm S_D$	0.0003	0.0004	0.0002	0.0007

*Significant difference ($P < .05$) between groups

**Significant difference ($P < .01$) between groups

(For significant F ratios see Appendix)

Electrophoresis of Serum Lipoproteins

Serum lipoproteins obtained from each of the experimental and control groups produced results which were quite variable. It was found that in some samples there were resolutions of beta and pre-beta bands, while in others there were very poor resolutions from which no beta or pre-beta resolutions could be determined.

From these results it was not possible to draw any definite conclusions regarding the electrophoretic patterns of the serum in the trained or non-trained animals.

CHAPTER V

DISCUSSION

The $\bar{X}$ body weights for all experimental groups were essentially the same at the beginning of the study. Following 8 weeks of exercise training, the body weights of groups I and III were lower than the corresponding sedentary control groups II and IV (Table 1). These decreases occurred with ad libitum feeding which suggests that the lower body weights resulted from an increase in energy expenditure. Similar differences in body weight have been reported for exercise experiments in which feeding was either ad libitum (43) or the food consumption of the exercised and control animals was equalized (32, 33, 109). In contrast, Parizkova and Koutecky (91) recently found that the body weight of trained and untrained rats was not significantly different after a treadmill training program that included 160 daily sessions of running. Gollnick and Taylor (34) have suggested that the lower body weights of exercise rats may be due to a decreased food intake since exercise may depress rather than stimulate their appetites. Thus in controlled feeding experiments, the exercised animals always limit the food consumption of the controls, and without such regulation the control animals gain much more weight than those that exercise. While Parizkova and Koutecky did not find any differences in gross body weight between exercised and sedentary rats, they did report that the trained animals had significantly less body fat than the sedentary controls.

Hanson, et al. (43) have reported similar differences in body composition between exercised and control rats fed high or low fat diets with ad libitum feeding.

No differences were observed in the initial resting heart rates in any of the experimental or control groups (Table 2). Following 8 weeks of forced running, groups I and III demonstrated a significant ($P<.01$) training bradycardia effect. The resting heart rates of the sedentary control animals (groups II and IV) remained essentially the same when compared to their initial resting rates (Table 2). Similar patterns have been shown in other animal studies (107, 109).

Explanations for the lower cholesterol levels observed in this study (Table 3) require a discussion of the pathways of cholesterol synthesis and degradation in the body. The major site for cholesterol synthesis in the body is the liver (14, 25). Accordingly, the relationship between endogenous cholesterol synthesis and plasma cholesterol levels resolves itself into the relationship of the rate of hepatic cholesterol synthesis and blood concentrations. Moderate changes in the rate of liver synthesis, however, do not necessarily induce similar changes in the plasma cholesterol content. Plasma cholesterol levels are also largely independent of dietary cholesterol (25, 65).

Degradation of cholesterol in the body was demonstrated in 1932 by Page (90). The main product of this degradation being cholic acid. In addition, since the sterol nucleus of cholic acid is not further metabolized by mammalian cells, the bile salts represent the main excretory products of the body's cholesterol pool (7). These findings were confirmed by Byers and Briggs (17) and other investigators (99, 100).

The processes concerned with the synthesis and the destruction of cholesterol appear to be chiefly responsible for the plasma concentration of this substance. Marked plasma changes, however, appear to be mediated by only one of these hepatic processes, namely the degradation of cholesterol (14).

All tissues capable of synthesizing cholesterol derive the steroid structure from acetyl-CoA. Other fates of acetyl-CoA are: resynthesis into body fat, oxidation in the Citric Acid Cycle, or conversion into ketone bodies in the liver. It would, therefore, seem that any condition that increases the utilization of acetyl-CoA for purposed other than cholesterol synthesis would tend to result in reduction of cholesterol synthesis. For example, an increase in fat mobilization stimulated by exercise would result in an increased amount of acetyl-CoA being utilized for fat synthesis in order to maintain essential body fat levels. Muscular exercise, by imposing an increased requirement for energy, would tend to divert increased amounts of acetyl-CoA into the Citric Acid Cycle. Finally, it has been shown (52) that increased fat mobilization can result in increased formation of ketone bodies from acetyl-CoA, and these ketone bodies may be utilized by the exercising muscles. Thus, there are three major metabolic pathways by which the supply of acetyl-CoA may be diverted away from cholesterol synthesis in the exercising animals.

When trained rats were subjected to 8 weeks of detraining by eliminating the daily forced exercise, they gained weight rapidly, exceeding the weights of their 16 week sedentary controls (group IV, Table 1). Their serum cholesterol levels, however, were lower ($P \leq .05$) than the control group IV (Table 3). This conflicts with the view of

Montoye, et al. (82) that increased blood cholesterol levels may be related to an increase in body weight. Their investigations on the effects of exercise on serum cholesterol, and liver lipids of rats showed that the lower serum cholesterol concentration in the exercised animals did not occur as a result of an increased accumulation of cholesterol in the liver. They did associate the lower cholesterol values measured with a leaner body mass in the exercised animals. A possible explanation for the lower blood cholesterol observed in the detrained animals (group III) may have been related to a lower body fat content which existed following 8 weeks of training.

A close association between cholesterol metabolism and the endocrine system has also been shown but the mechanisms are still somewhat obscure (25, 65). Studies in man (15, 16, 83) and animals (32, 89, 116) have associated lower serum cholesterol levels with an increased production of thyroid hormone. Support of this concept has come from recent experiments of Barnard, et al. (4) in which it was shown that training reduced the concentration of cholesterol in the livers of normal and hypophysectomized rats given thyroxine, but not in thyroidectomized or hypophysectomized rats not given throxine. Orma (89) has demonstrated an increased thyroid activity following exercise with accompanying lower serum cholesterol in cockrels. Investigations by Montoye, et al. (82) have shown a significant increase in the size of the adrenal glands as well as lower serum cholesterol levels in exercised rats. Supportive studies by Wong, et al. (116) also found adrenalhypertrophy with

accompanying lower serum cholesterol in exercised cockrels. Kyle, et al. (66) have demonstrated that injections of either ACTH or cortisone lowered serum cholesterol concentrations.

Gordon (35) has suggested that the cholesterol lowering effect of regular physical activity may be due to the presence of larger quantities of NADH in striate muscle following exercise which tends to suppress cholesterol synthesis. Supportive data regarding the concentrations of NADH has been presented by Holloszy (52) who found a two-fold increase in NADH in gastrocnemius muscle of trained rats run to exhaustion. Studies by Malinow, et al. (71) on rats indicate that the rate of cholesterol oxidation by muscle cells increased as a result of muscle contraction during treadmill running. That this increase in cholesterol oxidation may result in a drain on serum cholesterol pools was not suggested by these workers. Such an explanation seems plausible as an additional explanation for the lowered serum cholesterol levels observed in trained and detrained animals. The mechanism behind Malinow's findings are unknown.

It appears that no single factor is responsible for the lower serum cholesterol levels seen in exercise and exercise detrained animals. Specific mechanisms of cholesterol metabolism and control were not studied and explanations have therefore been based on the research cited. Since acetyl-CoA is a vital intermediate to the synthesis of cholesterol it appears most reasonable that training may have caused an increase in the utilization of acetyl-CoA for purposes other than cholesterol synthesis. This explanation does not exclude the possibility that training could have caused changes in normal endocrine function as

well as changes in cellular respiratory activity and increased cholesterol oxidation resulting in lower blood cholesterol levels as suggested by others.

The changes observed in the tricep cholesterol are conflicting and confusing as one method for the determination of cholesterol showed a difference, while another did not (Table 3). This lends support to the belief that there were no true differences in these tissues and that the differences observed were simply due to chance. Additional support to this statement may lie in the fact that no differences were seen in any of the remaining tissue cholesterol determinations for heart and tricep muscle (Table 3). These data suggest that tissue cholesterol concentrations may be independent of body weight, physical activity, and concentrations of cholesterol in the blood.

A review of the mechanism by which fat stores are laid down in animal tissue and subsequently mobilized in animal tissue is considered essential to the readers understanding of the triglyceride changes observed in this study.

Glucose is the major precursor of adipose tissue triglycerides (104). This substrate provides both the glycerol moiety of the triglycerides by way of α-glycerophosphate, and the fatty acid moiety by way of synthesis from acetyl-CoA. In addition amino acids can also form glucose or fatty acid after deamination. It appears that α-glycerophosphate is an obligatory acceptor onto which fatty acids are attached for storage as triglyceride in the adipose depots. Since there is, even in the steady-state, a continuing minimum rate of lipolysis in adipose tissue, there must be a continuing minimum rate of supply of α-glycerophosphate to permit the maintenance of depot fat during steady-state

conditions. White adipose tissue normally contains very limited amounts of glycogen and the pool of ∝-glycerophosphate is likewise small, (less than 0.2umoles/g). During normal circumstances, the ∝-glycerophosphate requirement must be met primarily by utilizing blood glucose. If this minimum requirement is not met and if lipolysis continues, free fatty acids will be released at a rate in excess of that at which they can be reesterified. This results in a net breakdown of triglyceride and release of free fatty acids to the liver causing an increase liver synthesis of triglyceride which in turn causes an increase in the plasma triglyceride levels. A net mobilization will occur if the steady-state of the animal is disrupted causing a disproportionate increase in the rate of breakdown of tissue triglycerides and/or a decrease in the rate of triglyceride synthesis.

Increased sympathetic activity has been proposed as the mechanism by which lipid mobilization is stimulated during exercise (19, 44, 45, 47). This proposal seems reasonable since the levels of catecholamines in blood increase during exercise (41, 42, 113). Evidence presented by Muir, et al. (86) has also shown that β-sympathetic blockage in man produces a 56% fall in blood free fatty acid during exercise and a 48% decrease in post-exercise concentration. These findings support the proposal that lipid mobilization is controlled by catecholamines levels through the sympathetic nervous system.

Free fatty acids mobilized from adipose tissue stores, enter the blood stream and combine with albumin. This free fatty acid albumin complex is then transported to the liver where the fatty acids are resynthesized back to triglycerides. The newly formed triglycerides then pass into the blood and attach to protein complexes to form

lipoproteins. It is this lipid-protein complex which enables triglyceride as well as cholesterol and other lipid fractions to be transported via the blood to the various tissues and organs of the body where lipase factors hydrolyze the triglyceride to free fatty acid and glycerol for utilization by the tissues.

The lower values for triglyceride seen in the adipose tissue and serum of the trained and detrained animals may be explained by the fact that these animals mobilized and utilized more fatty acid to meet the increased energy expenditure necessitated by the exercise training program, Since at least part of the endogenous plasma triglycerides are derived from the liver (46) decreased plasma levels of triglycerides could be caused by a decreased influx of triglycerides from the liver to the plasma. This might occur as a result of decreased liver triglyceride synthesis and hepatic uptake of plasma free fatty acid since they are important precursors to both liver and subsequently plasma triglyceride levels. It is also known that the hepatic blood flow is reduced during moderate and heavy exercise (114). This suggests that the uptake of free fatty acid in the liver may be decreased during exercise resulting in a decreased synthesis of liver triglyceride and lower plasma concentrations.

An increased efflux of fatty acid from plasma during exercise could be caused by an increased uptake and utilization of fatty acid in peripheral organs such as the working muscles. This explanation is reasonable from the functional viewpoint since exercise has been shown to increase the supply of fatty acid to working muscles in addition to increasing their uptake and utilization (6, 40). Such an increased utilization might be related to changes in blood flow and/or increased

amounts of lipoprotein lipase (lipase factor), the enzyme probably regulating the peripheral uptake of fatty acids (97). It has been reported by Nikkila, et al. (88) that exercise training increases the concentrations of lipoprotein lipase in myocardial tissue of rats. If increases in lipoprotein lipase also occur in skeletal muscle tissue with training, a possible mechanism for an increased efflux of plasma fatty acids to peripheral tissue exists. Increases in efflux might explain both the lower body weights and the lower resting serum triglyceride levels seen in the trained animals as well as the lower serum triglyceride measured for the detrained.

The lower triglyceride levels seen in the epididymal tissue of the trained and detrained animals suggest that these rats mobilized and utilized more fat from their adipose depots than the sedentary controls. In addition, this study has produced data on the intramuscular lipid stores of triglyceride and cholesterol. The results indicate that low levels of intermuscular triglyceride and cholesterol exist and that these levels are uneffected through exercise training. Masoro, et al. (76) also found low levels of triglyceride in gastrocnemius muscle of exercised and non-exercised pig-tailed monkeys. The finding that there were no significant differences in the levels of triglyceride or cholesterol in either the heart or tricep muscle for any of the experimental or control groups suggests that both variables are independent of body weight, physical activity, and blood levels.

The concentrations of total nitrogen in heart and tricep muscle of the trained and detrained rats groups I and III were greater ($P \leq .01$) than the sedentary control groups II and IV (Table 3). The observed differences may have been due to increases in both the contractile elements and respiratory enzymes of trained and detrained muscle tissues.

Support for the increased filament theory was presented by Penman (93) who found that an increase in the strength of human thigh muscle without hypertrophy was accompanied by an increased packing density of the contractile elements within the muscle cells. Evidence for other cellular changes induced by exercise training have also been shown by Holloszy (52) and Potter (95). In these studies, the exercise work capacity of the rats was also increased following exercise training. These increased work capacities were associated with increases in the levels of activity of mitochondrial respiratory enzymes in leg muscles, and in the capacity of the mitochondrial fraction of muscle to oxidize pyruvate. The increase in respiratory capacity appeared due to a net increase in respiratory enzymes as evidenced by increases in cytochrome C concentrations and total mitochondrial protein. Pattengale and Holloszy (92) have also found the concentrations of myoglobin to double in muscle in response to training. Fuge, et al. (30) and Wainio, et al. (115) studied the effects of protein deficiency in exercised rats and sedentary controls. The exercised animals demonstrated a two-fold increase in succinate dehydrogenase, cytochrome oxidase, succinate oxidase, and cytochrome C in gastrocnemius muscle. However, these animals demonstrated reduced work capacities when compared to exercised animals fed a standard laboratory diet. No attempt was made in this study to determine either contractile filament density or respiratory enzyme activity. However, on the basis of evidence in the literature, it appears quite likely that the increases seen in the total nitrogen of heart and muscle in the exercised and exercised detrained rats could be due to increases in both respiratory enzyme activity and greater filament density in the tissues.

The lower $\bar{X}$ cholesterol/nitrogen ratio calculated for the triceps muscle of detraining group III compared to sedentary group IV must be viewed with caution. This low ratio represents both a tissue cholesterol level that was inconsistantly altered (Table 3) and a highly significant increase in total nitrogen. This calculation tends to accentuate the significance of the lowered tissue lipids. There were no significant differences in any of the remaining lipid/nitrogen ratios. For purposes of comparison between the experimental and control animals, therefore, it appears that tissue lipid levels remained unchanged and that the decreases in lipid/nitrogen ratios mainly reflected increases in nitrogen concentrations.

The serum lipoproteins obtained from each of the experimental and control groups produced results which were quite variable. It was found that in some samples there were definite resolutions of beta and pre-beta bands, while in others there were very poor resolutions from which no beta or pre-beta resolutions could be determined.

From these results, it was not possible to draw any definite conclusions regarding the electrophoretic patterns of the serum in the trained or non-trained animals.

Summary

Albino rats were subjected to an 8 week exercise training program followed by an 8 week detraining program to determine possible changes in cholesterol, triglyceride, and lipoproteins in blood and in tissues. The protein content based on total nitrogen was also determined in the myocardial and tricep tissue in order to possibly associate lipid changes with protein changes.

Conclusions

1. Training lowered blood cholesterol and triglyceride concentrations, but had little or no effect on these lipid fractions in myocardial or skeletal muscle tissues.
2. There were decreases in the concentration of triglyceride in the epididymal tissue of the trained and detrained rats.
3. Training caused an increase in the total nitrogen concentration of myocardial and skeletal muscle. With detraining, these elevated nitrogen levels diminished but not to the low levels measured in sedentary controls.
4. The results of electrophoresis were inconsistent, and no definite conclusions could be determined from these findings. There were indications both in experimental and control rats of some resolution in an area identified as the pre-β region in human serum.
5. Body weight as well as resting heart rates were lowered by exercise training. Detraining caused a rapid increase in both body weights and resting heart rates.

APPENDICES

APPENDIX I

CALIBRATION OF SINGLE RAT EXERCISE WHEEL

Wheel circumference: 43.0"

Pulleys Drive:Driven Diam.(ins.)	Time for 10 revs $\bar{X}4$ (secs)	Ins/sec	Ft/sec	Ft/min	mph	Cm/sec	Meters/min	kph
2 : 5	72.0	5.972	.4977	29.862	.3393	15.169	9.101	.5461
2 : 4	59.2	7.264	.6053	36.318	.4127	18.451	11.071	.6643
2 : 3	42.5	10.118	.8432	50.592	.5749	25.670	15.402	.9241
2 : 2	29.5	14.576	1.2147	72.628	.8253	37.023	22.214	1.3328
3 : 5	47.4	9.072	.7706	46.204	.5250	23.043	13.826	.8296
3 : 4	39.1	10.997	.9164	54.638	.6208	27.932	16.759	1.0055
3 : 2	19.2	22.396	1.8663	111.978	1.2725	56.886	34.132	2.0479
4 : 5	34.0	12.647	1.0539	63.234	.7185	32.123	19.274	1.1564
4 : 3	20.1	21.393	1.7828	106.968	1.2155	54.338	32.603	1.9562
4 : 2	13.5	31.852	2.6543	159.258	1.8097	80.904	48.542	2.9125
5 : 4	23.1	18.615	1.5513	93.078	1.0577	47.282	28.369	1.7021
5 : 3	16.8	25.595	2.1329	127.974	1.4542	65.011	39.007	2.3404
5 : 2	11.0	39.091	3.2576	195.456	2.2210	99.291	59.575	3.5745

APPENDIX II

RAT EXERCISE TRAINING PROGRAM

Day	Time (min.)	Ratio	Speed (mph)	Miles ran
1	10	2:4	0.5250	0.0875
2	10	2:4	0.5250	0.0875
3	10	2:4	0.5250	0.0875
4	10	2:3	0.5749	0.0958
5	10	2:3	0.5749	0.0958
6	10	2:3	0.5749	0.0958
7	10	2:3	0.5749	0.0958
8	Pinned rats - 2:30 p.m. No Training			
9	Measured H.R. Commenced 6:45 a.m. - 6 p.m.			
10	No running - removed pins			
11*	20	2:4	0.5250	0.1750
12	20	2:3	0.5749	0.1916
13	30	2:3	0.5749	0.2874
14	30	2:3	0.5749	0.2874
15	30	2:3	0.5749	0.2874
16	30	2:3	0.5749	0.2874
17	Rest day - no running			
18	30	2:3	0.5749	0.2874
19	30	2:3	0.5749	0.2847
20	30	2:2	0.8253	0.4126
21	30	2:3	0.5749	0.2874
22	40	2:3	0.5749	0.3749
23	40	2:3	0.5749	0.3749
24	40	2:3	0.5749	0.3749
25	40	2:3	0.5749	0.3749
26	40	2:3	0.5749	0.3749
27	40	2:3	0.5749	0.3749
28	40	2:3	0.5749	0.3749
29	45	2:3	0.5749	0.4186
30	30	2:3	0.5749	0.2874
31	Rest day - no running			
32	30	2:3	0.5749	0.2874
33	45	2:3	0.5749	0.4186
34	45	2:3	0.5749	0.4186
35	45	2:3	0.5749	0.4186
36	Rest day - no running			
37	45	2:3	0.5749	0.4186
38	45	2:3	0.5749	0.4186
39	50	2:3	0.5749	0.4790
40	50	2:3	0.5749	0.4790
41	50	2:3	0.5749	0.4790

*Commence 8 week training

APPENDIX II continued

Day	Time (min.)	Ratio	Speed (mph)	Miles ran
42	50	2:3	0.5749	0.4790
43	50	2:3	0.5749	0.4790
44	Rest day - no running			
45	55	2:3	0.5749	0.5227
46	55	2:3	0.5749	0.5227
47	55	2:3	0.5749	0.5227
48	55	2:3	0.5749	0.5227
49	55	2:3	0.5749	0.5227
50	55	2:3	0.5749	0.5227
51	Rest day - no running			
52	30	2:3	0.5749	0.2874
53	60	2:3	0.5749	0.5749
54	60	2:3	0.5749	0.5749
55	60	4:4	0.8253	0.8253
56	30	4:4	0.8253	0.4126
	Animals displayed very sore feet at 4:4 speed, therefore, returned to 2:3 ratio.			
57	Rest day - no running			
58	Rest day - no running			
59	60	4:4	0.8253	0.8253
60	90	2:3	0.5749	0.8623
61	90	2:3	0.5749	0.8623
62	90	2:3	0.5749	0.8623
63	90	2:3	0.5749	0.8623
64	60	4:4	0.8253	0.8253
65	30	2:3	0.5749	0.2874
66	30	2:3	0.5749	0.2874
67	90	2:3	0.5749	0.8623
68	90	2:3	0.5749	0.8623
69	90	2:3	0.5749	0.8623
70	90	2:3	0.5749	0.8623
71	90	2:3	0.5749	0.8623
72	90	2:3	0.5749	0.8623
73	90	2:3	0.5749	0.8623
74	90	2:3	0.5749	0.8623
75	90	2:3	0.5749	0.8623
76	90	2:3	0.5749	0.8623
77	Pinned all rats - no running			
78	Determined resting heart rates - 6:45 a.m. - 4:00 p.m.			
79	90	2:3	0.5749	0.8623
80	Animals fasted prior to sacrifice (12)			
81	Remaining 12 animals fasted prior to sacrifice			
	GROUP III COMMENCE DETRAINING.			
			Total mileage run	32.5136

APPENDIX III

ANALYSIS OF VARIANCE: BODY WEIGHT

Group I and Group II (8 weeks)

	Sum of Squares	Degree of Freedom	Mean Square	F. Ratio
Between Groups	49959.375	1	49959.375	36.238**
Within Groups	30329.583	22	1378.617	
Total	80288.958	23		

Group III and Group IV (8 weeks)

Between Groups	7920.666	1	7920.666	2.202
Within Groups	79121.166	22	3596.416	
Total	87041.832	23		

Group III and Group IV (16 weeks)

Between Groups	2166.000	1	2166.000	0.646
Within Groups	73759.333	22	3352.696	
Total	75925.333	23		

**Significant ($P \leq .01$)

*Significant ($P \leq .05$)

APPENDIX IV

ANALYSIS OF VARIANCE: RESTING HEART RATES

Group I and Group II
(8 weeks)

	Sum of Squares	Degree of Freedom	Mean Square	F. Ratio
Between Groups	11382.663	1	11382.663	29.113**
Within Groups	8601.453	22	390.975	
Total	19984.116	23		

Group III and Group IV
(8 weeks)

Between Groups	6358.642	1	6358.642	14.772**
Within Groups	9469.592	22	430.436	
Total	15828.234	23		

**Significant ($P \leq .01$)

*Significant ($P \leq .05$)

APPENDIX V

PAIRED t TESTS OF BODY WEIGHTS

(n = 12 per group)

Groups	initial	8 Weeks	16 Weeks	
I $\bar{X}$	445.1	453.4	-	
±SD	19.3	38.0	-	
II $\bar{X}$	455.8	549.7	-	1
±SD	14.5	36.3	-	
III $\bar{X}$	447.0	487.4	586.2	2, 3
±SD	34.0	44.5	41.0	
IV $\bar{X}$	456.5	523.8	567.2	1, 3
±SD	39.0	72.2	70.9	

1) Significance at the .01 level between initial and 8 weeks.

2) Significance at the .01 level between 8 weeks and 16 weeks.

3) Significance at the .01 level between initial and 16 weeks.

PAIRED t TESTS OF RESTING HEART RATES
(Beats per minute)

(n = 12 per group)

Groups	Initial	8 Weeks	16 Weeks	
I $\bar{X}$	330.0	296.0	-	1
±SD	12.6	15.7	-	
II $\bar{X}$	325.0	340.0	-	
±SD	20.4	23.2	-	
III $\bar{X}$	335.0	306.0	331.0	1, 2
±SD	12.8	14.4	17.7	
IV $\bar{X}$	347.0	339.0	335.0	
±SD	13.6	25.6	21.2	

1) Significant at .01 level between initial and 8 weeks

2) Significant at .01 level between 8 weeks and 16 weeks.

APPENDIX VI

ANALYSIS OF VARIANCE: SERUM CHOLESTEROL

Group I and Group II

	Sum of Squares	Degree of Freedom	Mean Square	F. Ratio
Between Groups	1683.375	1	1683.375	9.640**
Within Groups	3841.583	22	174.617	
Total	5524.958	23		

Group III and Group IV

Between Groups	4760.166	1	4760.166	7.335*
Within Groups	14277.166	22	648.962	
Total	19037.332	23		

ANALYSIS OF VARIANCE: SERUM TRIGLYCERIDE

Group I and Group II

Between Groups	14065.041	1	14065.041	12.289**
Within Groups	25178.583	22	1144.481	
Total	39243.624	23		

Group III and Group IV

Between Groups	6700.041	1	6700.041	4.357*
Within Groups	34617.961	22	1573.541	
Total	41318.002	23		

**Significant ($P < .01$)

*Significant ($P < .05$)

APPENDIX VII

ANALYSIS OF VARIANCE: EPIDIDYMAL TISSUE TRIGLYCERIDE

Group I and Group II

	Sum of Squares	Degree of Freedom	Mean Square	F. Ratio
Between Groups	770.666	1	770.666	5.626*
Within Groups	3221.833	22	146.446	
Total	3992.499	23		

Group III and Group IV

Between Groups	672.041	1	672.041	4.494*
Within Groups	3289.916	22	149.541	
Total	3961.957	23		

ANALYSIS OF VARIANCE: TRICEP TISSUE CHOLESTEROL

(Automated Proccdure)

Group I and Group II

Between Groups	0.20906	1	0.20906	4.446*
Within Groups	1.03438	22	0.04701	
Total	1.24344	23		

Group III and Group IV

(Manual Procedure)

Between Groups	0.10800	1	0.10800	4.375*
Within Groups	0.54309	22	0.02468	
Total	0.65109	23		

**Significant ($P < .01$)

*Significant ($P < .05$)

APPENDIX VIII

ANALYSIS OF VARIANCE: TOTAL NITROGEN IN HEART TISSUE

Group I and Group II

	Sum of Squares	Degree of Freedom	Mean Square	F. Ratio
Between Groups	97.687	1	97.687	31.377**
Within Groups	68.492	22	3.113	
Total	166.179	23		

Group III and Group IV

Between Groups	129.502	1	129.502	40.579**
Within Groups	70.209	22	3.191	
Total	199.711	23		

**Significant (P< .01)

*Significant (P< .05)

APPENDIX IX

ANALYSIS OF VARIANCE: TOTAL NITROGEN IN TRICEP TISSUE

Group I and Group II

	Sum of Squares	Degree of Freedom	Mean Square	F. Ratio
Between Groups	294.770	1	294.770	68.325**
Within Groups	94.912	22	4.314	
Total	389.682	23		

Group III and Group IV

Between Groups	79.570	1	79.570	25.396**
Within Groups	68.929	22	3.133	
Total	148.499	23		

**Significant ($P < .01$)

*Significant ($P < .05$)

APPENDIX X

ANALYSIS OF VARIANCE: CHOLESTEROL/ NITROGEN RATIO IN TRICEP MUSCLE

(Manual Cholesterol Procedure)

Group III and Group IV

	Sum of Squares	Degree of Freedom	Mean Square	F. Ratio
Between Groups	0.000013	1	0.000013	8.377**
Within Groups	0.000035	22	0.000001	
Total	0.000048	23		

Group III and Group IV

(Automated Cholesterol Procedure)

Between Groups	0.000007	1	0.000007	6.511*
Within Groups	0.000023	22	0.000001	
Total	0.000030	23		

**Significant ($P < .01$)

*Significant ($P < .05$)

APPENDIX XI

CALCULATION FOR SERUM CHOLESTEROL

$$K^* = \frac{\text{Optical Density}}{\text{Mg Cholesterol of Standard}}$$

$$\bar{X}K = \frac{\sum K}{\text{Total Number of Standards}}$$

$$\text{Mg. Cholesterol per 100 ml blood} = \frac{\text{Optical Density of Sample}}{\bar{X}K}$$

*K corresponds to the slope of the line when concentration is plotted verses optical density.

APPENDIX XII

CALCULATION FOR SERUM TRIGLYCERIDE

$$K^* = \frac{\text{Fluorometer Reading of Standard}}{\text{Mg Triglyceride of Standard}}$$

$$\bar{X}K = \frac{\sum K}{\text{Total Number of Standards}}$$

$$\text{Mg. Triglyceride per 100 ml blood} = \frac{\text{Fluorometer of Sample}}{\bar{X}K}$$

*K corresponds to the slope of the line when concentration is plotted verses fluorometer reading.

APPENDIX XIII

CALCULATION FOR TISSUE CHOLESTEROL

$$K^* = \frac{\text{Optical Density of Standard}}{\text{Mg. Cholesterol of Standard}}$$

$$\bar{X}K = \frac{\sum K}{\text{Total Number of Standards}}$$

$$\text{Mg. Cholesterol} = \frac{\text{Optical Density of Sample}}{\bar{X}K}$$

$$\text{Mg. Cholesterol per gram Tissue} = \frac{\text{Mg. Cholesterol of Sample}}{\text{Weight of Tissue analyzed}} \times \text{Dilution Factor}$$

*K corresponds to the slope of the line when concentration is plotted verses optical density.

APPENDIX XIV

CALCULATION FOR TISSUE TRIGLYCERIDE

$$K^* = \frac{\text{Fluorometer Reading of Standard}}{\text{Mg. Triglyceride of Standard}}$$

$$\bar{X}K = \frac{\sum K}{\text{Total Number of Standards}}$$

$$\text{Mg. Triglyceride} = \frac{\text{Fluorometer Reading of Sample}}{\bar{X}K}$$

$$\text{Mg. Triglyceride per gram Tissue} = \frac{\text{Mg. Triglyceride of Sample}}{\text{Weight of Tissue analyzed}} \times \text{Dilution Factor}$$

*K Corresponds to the slope of the line when concentration is plotted verses fluorometer reading.

APPENDIX XV

CALCULATION FOR TOTAL NITROGEN OF TISSUES

$$\frac{14.0 \times \text{Normality of Acid} \times \text{ml. Acid Delivered} \times 100}{\text{Weight of Tissue Sample}} \times 10$$

= Mg Nitrogen per gram Tissue[1]

[1]Multiply by 6.25 to obtain estimate of protein content.

APPENDIX XVI

STANDARDIZATION OF ACID AND BASE[1]

Four grams of Potassium Acid Phthlate, 99.95% pure were weighed in a clean weighing bottle and dried in an oven at 110°C. for 2 hours. The bottle was removed and placed in a dessicator containing anhydrous $CaSO_4$ and allowed to cool to room temperature. Three samples (0.7 - 0.19 gm) of potassium acid phthalate were weighed and quantitatively transferred to individual 125 ml Erlenmeyer flasks. The solution was dissolved in 50 ml distilled water. A drop of phenolphtalein indicator was added and titrated with a standard NaOH solution.

Example

.1N base required.

4 gms of NaOH was dissolved in H_2O in a 1 liter volumetric flask and made to volume.

Standardization of NaOH

817.12 mg of KHP was titrated with 38.4 ml of NaOH solution.

Calculation

$$817.12 \text{ mg KHP} \times \frac{1 \text{ meq}}{204.22} \times \frac{1}{38.4} = 0.1023N$$

Standardization of HCl

Approximately 0.1N (8.3 ml HCl made to 1 liter with H_2O)

Three samples of 25 ml HCl was titrated with the standard NaOH (0.1023N).

[1]Pierce W. C. and E. L. Haenisch. Quantitative Analysis. John Wiley and Sons, Inc., New York, 1948.

APPENDIX XVI continued

Sample	NaOH used	HCl used
1	23.6 ml	25 ml
2	23.6 ml	25 ml
3	23.6 ml	25 ml
	$\bar{X}$ 23.6 ml	$\bar{X}$ 25 ml

Calculation

$$23.6 \times 0.1023 = 2.41428$$

$$2.41428 = 25x$$

$$x = .09657$$

$\therefore$ Normality = .09657

CONCENTRATION OF CHOLESTEROL IN RAT HEART

	Sample	Wt Tissue Analyzed (g)	O.D.	Mg Chol per Sample	Mg Chol per gm Tissue [2]
	.05		.1723	.0563	
	.10		.3050	.0996	
	.15		.4350	.1421	
	.20		.5690	.1859	
Group I	1	.018348	.3190	.1042	5.681
	2	.014912	.2882	.0941	6.316
	3	.022112	.3920	.1281	5.793
	4	.023436	.4320	.1411	6.023
	5	.021984	.3920	.1281	5.827
	6	.024214	.4260	.1392	5.749
	7	.020600	.3720	.1215	5.901
	8	.024470	.4560	.1490	6.089
	9	.019854	.3720	.1215	6.123
	10	.021808	.3920	.1281	5.874
	11	.021360	.3720	.1215	5.691
	12	.021044	.3770	.1232	5.854
Group II	13	.020720	.3690	.1205	5.819
	14	.020320	.3620	.1183	5.821
	15	.022650	.3950	.1290	5.699
	16	.020316	.3620	.1183	5.822
	17	.021096	.3870	.1264	5.994
	18	.019968	.3570	.1166	5.842
	19	.022070	.3850	.1258	5.700
	20	.021886	.3920	.1281	5.853
	21	.021756	.3850	.1258	5.783
	22	.024136	.4170	.1362	5.646
	23	.021336	.3740	.1222	5.728
	24	.020292	.3670	.1199	5.910

[1] By automated procedure: Block, et al.

[2] Expressed as dry weight tissue

CONCENTRATION OF CHOLESTEROL[1]
IN RAT HEART

	Sample	Wt Tissue Analyzed (g)	Corrected O.D.	Mg Chol. per Sample	Mg Chol per gm Tissue[2]
	.2		.2460	.1898	
	.4		.5230	.4035	
	.6		.7820	.6034	
Group I	1	.03669	.2460	.1898	5.173
	2	.02982	.2255	.1739	5.831
	3	.04422	.3370	.2600	5.879
	4	.04687	.3640	.2809	5.993
	5	.04397	.3250	.2508	5.703
	6	.04843	.3520	.2716	5.608
	7	.04120	.2950	.2276	5.524
	8	.04894	.3690	.2847	5.817
	9	.03971	.3190	.2461	6.197
	10	.04362	.3320	.2561	5.872
	11	.04272	.3030	.2338	5.472
	12	.04209	.3100	.2392	5.683
Group II	13	.04144	.3010	.2323	5.605
	14	.04064	.3010	.2323	5.716
	15	.04530	.3370	.2600	5.739
	16	.04063	.3010	.2323	5.717
	17	.04219	.3140	.2423	5.743
	18	.03993	.2903	.2240	5.609
	19	.04414	.3230	.2492	5.645
	20	.04377	.3210	.2477	5.659
	21	.04351	.3080	.2377	5.463
	22	.04827	.3720	.2870	5.945
	23	.04267	.2924	.2256	5.287
	24	.04058	.2950	.2276	5.608
	.2		.2537	.1958	
	.4		.5230	.4035	
	.6		.8170	.6304	

[1]By Abell, et al.

(K = 1.296)

[2]Expressed as dry weight

CONCENTRATION OF CHOLESTEROL[1]
IN RAT HEART

	Sample	Wt Tissue Analyzed (g)	O.D.	Mg Chol. per Sample	Mg Chol. per gm Tissue[2]
	.05		.1739	.0545	
	.10		.3190	.1000	
	.15		.4620	.1448	
	.20		.6020	.1887	
Group III	1	.02150	.4140	.1297	6.036
	2	.01594	.3050	.0956	5.998
	3	.01376	.2636	.0826	6.005
	4	.01743	.3320	.1040	5.970
	5	.02108	.3920	.1228	5.826
	6	.01666	.3190	.1000	6.002
	7	.01667	.3770	.1181	7.089
	8	.01789	.3190	.1000	5.589
	9	.01729	.3320	.1040	6.015
	10	.01760	.3420	.1087	6.180
	11	.01100	.2076	.0650	5.915
	12	.01572	.3010	.0943	5.998
Group IV	13	.01704	.3190	.1000	5.868
	14	.01718	.3570	.1119	6.510
	15	.00983	.1871	.0586	5.966
	16	.01191	.2480	.0777	6.521
	17	.01488	.2882	.0903	6.071
	18	.01852	.3620	.1134	6.123
	19	.01875	.3470	.1087	5.801
	20	.01098	.2147	.0673	6.129
	21	.01470	.2840	.0890	6.051
	22	.02106	.3920	.1226	5.816
	23	.01863	.3870	.1213	6.511
	24	.01626	.2882	.0903	5.552

[1]Automated procedure Block, et al. (K = 3.190)

[2]Expressed as dry weight

CONCENTRATION OF CHOLESTEROL[1]
IN RAT HEART

	Sample	Wt Tissue Analyzed (g)	Corrected O.D.	Mg Chol. per Sample	Mg Chol. per gm Tissue[2]
	.2		.2291	.1870	
	.4		.4850	.3959	
	.6		.7390	.6032	
Group III	1	.04300	.3190	.2604	6.054
	2	.03188	.2310	.1885	5.914
	3	.02752	.1888	.1541	5.600
	4	.03486	.2577	.2103	6.033
	5	.04217	.3080	.2514	5.962
	6	.03332	.2366	.1931	5.796
	7	.03334	.2636	.2151	6.454
	8	.03578	.2384	.1946	5.439
	9	.03459	.2441	.1992	5.760
	10	.03520	.2499	.2040	5.795
	11*	.02200	.1503	.1175	5.333
	12	.03145	.2236	.1825	5.803
Group IV	13	.03408	.2403	.1961	5.755
	14	.03437	.2616	.2135	6.211
	15*	.01966	.1382	.1080	5.493
	16*	.02383	.1855	.1450	6.086
	17	.02976	.2024	.1652	5.551
	18	.03705	.2861	.2335	6.303
	19	.03750	.2696	.2200	5.868
	20*	.02196	.1643	.1284	5.849
	21	.02941	.2059	.1680	5.715
	22	.02981	.2007	.1638	5.495
	23	.03726	.2696	.2200	5.905
	24	.03253	.2218	.1810	5.564
	.2		.2441	.1992	
	.4		.4980	.4065	
	.6		.7760	.6334	

[1]By Abell, et al. (K = 1.225)
[2]Expressed as dry weight (*K = 1.279)

CONCENTRATION OF CHOLESTEROL[1] IN RAT TRICEP

	Sample	Wt Tissue Analyzed(g)	O.D.	Mg Chol. per Sample	Mg Chol. per gm Tissue[2]
	.05		.1871	.0540	
	.10		.3470	.1002	
	.15		.5020	.1450	
	.20		.6580	.1900	
Group I	1	.02113	.2366	.0683	3.232
	2	.02373	.2819	.0814	3.431
	3	.02115	.2129	.0614	2.907
	4	.03778	.1707	.0493	1.304
	5	.02489	.2441	.0705	2.832
	6	.02489	.2676	.0772	3.105
	7	.02269	.2182	.0630	2.776
	8	.01972	.1990	.0574	2.913
	9	.02119	.2328	.0672	3.173
	10	.02116	.2164	.0625	2.952
	11	.01985	.2041	.0589	2.968
	12	.02379	.2422	.0699	2.939
Group II	13	.02650	.2616	.0755	2.850
	14	.02252	.2059	.0594	2.640
	15	.02392	.2273	.0656	2.744
	16	.02035	.2076	.0599	2.946
	17	.02179	.2255	.0651	2.987
	18	.01893	.1756	.0507	2.679
	19	.02036	.1922	.0555	2.725
	20	.02195	.1990	.0574	2.618
	21	.02919	.2950	.0852	2.919
	22	.02522	.3030	.0875	3.470
	23	.02144	.1939	.0560	2.610
	24	.02915	.2716	.0784	2.690

[1]Automated procedure Block, et al. (K = 3.462)

[2]Expressed as dry weight

CONCENTRATION OF CHOLESTEROL[1]
IN RAT TRICEPS

	Sample	Wt Tissue Analyzed (g)	Corrected O.D.	Mg Chol. per Sample	Mg Chol. per gm Tissue[2]
	.2		.2460	.1856	
	.4		.5160	.3894	
	.6		.8240	.6218	
Group I	1	.04227	.1612	.1216	2.878
	2	.04747	.1612	.1216	2.562
	3	.04230	.1397	.1054	2.492
	4	.03091	.1024	.0772	2.500
	5	.04818	.1596	.1204	2.500
	6	.05905	.1956	.1476	2.499
	7	.04398	.1457	.1099	2.500
	8	.04126	.1367	.1031	2.500
	9	.04238	.1707	.1288	3.039
	10	.04233	.1565	.1181	2.790
	11	.03972	.1352	.1020	2.568
	12	.04760	.1565	.1181	2.481
Group II	13	.05301	.1821	.1374	2.592
	14	.04505	.1235	.0931	2.068
	15	.04784	.1442	.1088	2.274
	16	.04070	.1457	.1099	2.701
	17	.04358	.1643	.1240	2.845
	18	.03786	.1221	.0921	2.433
	19	.04073	.1235	.0932	2.288
	20	.04390	.1412	.1065	2.427
	21	.05838	.2182	.1646	2.820
	22	.05044	.1939	.1463	2.901
	23	.04289	.1397	.1054	2.458
	24	.05932	.2024	.1527	2.619
	.2		.2596	.1959	
	.4		.5530	.4173	
	.6		.8240	.6218	

[1]By Abell, et al. (K = 1.325)

[2]Expressed as dry weight

CONCENTRATION OF CHOLESTEROL[1]
IN RAT TRICEPS

	Sample	Wt Tissue Analyzed (g)	O.D.	Mg Chol. per Sample	Mg Chol. per gm Tissue[2]
	.05		.1739	.0545	
	.10		.3190	.1000	
	.15		.4620	.1448	
	.20		.6020	.1887	
Group III	1	.02515	.2218	.0695	2.7631
	2	.02099	.1643	.0515	2.5621
	3	.01921	.1612	.0505	2.6304
	4	.01849	.1427	.0447	2.4191
	5	.01987	.1643	.0515	2.5905
	6	.01880	.1549	.0485	2.5824
	7	.01547	.1192	.0373	2.4134
	8	.01456	.1135	.0355	2.4429
	9	.02425	.1805	.0565	2.3331
	10	.01828	.1518	.0475	2.6028
	11	.02276	.1939	.0607	2.6704
	12	.01751	.1367	.0428	2.4471
Group IV	13	.01613	.1308	.0410	2.5418
	14	.02111	.1772	.0555	2.6309
	15	.01964	.1643	.0515	2.6221
	16	.01793	.1580	.0495	2.7603
	17	.01572	.1278	.0400	2.5467
	18	.02552	.1871	.0586	2.2981
	19	.01907	.1549	.0485	2.5458
	20	.01591	.1397	.0437	2.7506
	21	.02008	.1772	.0555	2.7645
	22	.01880	.1612	.0505	2.6863
	23	.01785	.1518	.0475	2.6640
	24	.01651	.1308	.0410	2.4818

[1]Automated procedure Block, et al. (K = 3.190)

[2]Expressed as dry weight

CONCENTRATION OF CHOLESTEROL[1]
IN RAT TRICEPS

	Sample	Wt Tissue Analyzed(g)	Corrected O.D.	Mg Chol. per Sample	Mg Chol. per gm Tissue[2]
	.2		.2422	.1854	
	.4		.5380	.4119	
	.6		.7890	.6041	
Group III	1	.05031	.1659	.1270	2.475
	2	.04199	.1079	.0826	1.967
	3	.03843	.1065	.0815	2.120
	4	.03698	.0996	.0762	2.060
	5	.03976	.1010	.0773	1.944
	6	.03760	.1037	.0794	2.111
	7	.03095	.0809	.0619	2.000
	8	.02913	.0718	.0549	1.884
	9	.04850	.1308	.1001	2.063
	10	.03656	.1093	.0863	2.286
	11	.04552	.1382	.1058	2.324
	12	.03502	.0969	.0741	2.115
Group IV	13	.03226	.0901	.0689	2.135
	14	.04222	.1322	.1012	2.396
	15	.03928	.1177	.0901	2.293
	16	.03587	.1079	.0826	2.302
	17	.03145	.0915	.0700	2.225
	18	.05104	.1337	.1023	2.004
	19	.03814	.1093	.0836	2.191
	20	.03184	.0982	.0751	2.358
	21	.04018	.1264	.0967	2.406
	22	.03762	.1192	.0912	2.424
	23	.03571	.0982	.0751	2.103
	24	.03304	.0915	.0700	2.118
	.2		.2616	.2003	
	.4		.5270	.4035	
	.6		.8030	.6148	

[1]By Abell, et al.

(K = 1.306)

[2]Expressed in dry weight

CONCENTRATION OF CHOLESTEROL[1] IN RAT EPIDIDYMAL TISSUE

	Sample	Wt Tissue Analyzed(g)	Corrected O.D.	Mg Chol. per Sample	Mg Chol. per gm Tissue[2]
	.2		.2518	.1947	
	.4		.5160	.3990	
	.6		.7890	.6102	
Group I	1	.31979	.3900	.3016	.9431
	2	.37351	.2518	.1947	.5213
	3	.39579	.4030	.3116	.7874
	4	.37804	.4140	.3201	.8469
	5	.39488	.4850	.3750	.9498
	6	.37639	.2903	.2245	.5964
	7	.39154	.5230	.4044	1.0330
	8	.37798	.2861	.2212	.6012
	9	.38886	.3320	.2567	.6602
	10	.37148	.3590	.2776	.7473
	11	.37398	.4470	.3457	.9243
	12	.36290	.3400	.2629	.7245
Group II	13	.38817	.3590	.2776	.7152
	14	.38841	.3770	.2915	.7506
	15	.39285	.3690	.2853	.7264
	16	.39635	.2422	.1873	.4725
	17	.37328	.3100	.2397	.6422
	18	.39720	.4120	.3186	.8021
	19	.38874	.3400	.2629	.6764
	20	.36327	.2840	.2196	.6046
	21	.37370	.2636	.2038	.5455
	22	.37020	.3590	.2776	.7499
	23	.38007	.4410	.3410	.8973
	24	.37342	.4290	.3317	.8884
	.2		.2480	.1918	
	.4		.5270	.4075	
	.6		.8030	.6210	

[1]By Abell, et al. (K = 1.293)

[2]Expressed as anhydrous weight

CONCENTRATION OF CHOLESTEROL[1]
IN RAT EPIDIDYMAL TISSUE

	Sample	Wt Tissue Analyzed(g)	Corrected O.D.	Mg Chol. per Sample	Mg Chol. per gm Tissue[2]
	.2		.2537	.1944	
	.4		.5230	.4007	
	.6		.7820	.5992	
Group III	1	.40691	.3300	.2528	.6212
	2	.41402	.3320	.2544	.6144
	3	.42508	.4030	.3088	.7264
	4	.29228	.2218	.1699	.5812
	5	.32295	.3030	.2321	.7186
	6	.37712	.2756	.2111	.5597
	7	.32637	.3800	.2912	.8922
	8	.32834	.3820	.2927	.8914
	9	.36395	.2840	.2176	.5978
	10	.38147	.2840	.2176	.5704
	11	.36224	.3420	.2620	.7232
	12	.35106	.3470	.2659	.7574
Group IV	13	.39217	.3280	.2513	.6407
	14	.34863	.5090	.3900	1.1186
	15	.32964	.3590	.2750	.8342
	16	.37398	.5120	.3923	1.0489
	17	.33372	.3800	.2911	.8722
	18	.33946	.3950	.3026	.8914
	19	.35932	.3010	.2306	.6417
	20	.34008	.5650	.4329	1.2728
	21	.37405	.4690	.3593	.9605
	22	.33765	.5120	.3923	1.1618
	23	.32781	.3850	.2950	.8999
	24	.33733	.4690	.3593	1.0651
	.2		.2577	.1974	
	.4		.5300	.4061	
	.6		.8030	.6153	

[1]By Abell, et al.

[2]Expressed as anhydrous weight (K = 1.305)

APPENDIX XX

CONCENTRATION OF SERUM CHOLESTEROL

Group I

Sample	Percent Trans	Optical Density	Mg per 100 ml
100	68.0	.1675	
200	49.3	.3030	
300	36.3	.4350	
400	26.3	.5730	
1	66.0	.1805	119
2	68.5	.1627	107
3	71.5	.1442	95
4	65.5	.1821	120
5	68.5	.1627	107
6	67.0	.1739	114
7	66.5	.1756	116
8	71.0	.1487	98
9	68.5	.1627	107
10	72.5	.1382	91
11	71.5	.1442	95
12	73.0	.1367	90

Group II

13	63.5	.1956	129
14	70.0	.1549	102
15	65.5	.1821	120
16	66.5	.1756	116
17	59.5	.2236	147
18	64.5	.1888	124
19	63.5	.1956	129
20	69.0	.1612	106
21	70.5	.1503	99
22	66.0	.1805	119
23	60.0	.2218	146
24	65.0	.1871	123

K = .00152

APPENDIX XX continued

CONCENTRATION OF SERUM CHOLESTEROL

Group III

Sample	Percent Trans	Optical Density	Mg per 100 ml
100	66.5	.1772	
200	46.5	.3320	
300	33.0	.4820	
400	23.5	.6290	
1	59.0	.2291	139
2	72.5	.1397	85
3	72.0	.1427	86
4	69.0	.1612	98
5	72.0	.1427	86
6	66.0	.1805	109
7	73.0	.1367	83
8	67.0	.1739	105
9	67.5	.1707	103
10	68.5	.1643	100
11	71.0	.1487	90
12	73.0	.1367	83

Group IV

13	62.0	.2076	126
14	47.5	.3230	196
15	57.0	.2441	148
16	63.5	.1973	120
17	67.0	.1739	105
18	56.0	.2518	153
19	57.0	.2441	148
20	71.5	.1457	88
21	65.5	.1838	111
22	74.5	.1278	77
23	66.0	.1805	109
24	62.5	.2041	124

K = .00165

APPENDIX XXI

CONCENTRATION OF SERUM TRIGLYCERIDE

Group I

Sample	Saponification	Blank	Mg per 100 ml
50	21.0	10.0	
100	31.0	10.0	
200	52.5	10.0	
250	57.5	10.0	
300	72.5	10.0	
350	83.5	10.0	
1	25.0	15.5	44
2	27.5	16.0	54
3	27.5	16.0	54
4	26.5	15.0	54
5	31.5	15.5	76
6	23.0	15.0	37
7	26.5	15.0	54
8	27.5	16.0	54
9	33.5	15.0	88
10	27.0	15.5	54
11	29.5	15.5	66
12	27.0	16.0	52

Group II

13	63.0	16.0	224
14	36.0	15.5	97
15	32.5	15.0	83
16	33.0	16.0	80
17	36.5	15.5	100
18	30.0	15.0	71
19	39.5	15.5	114
20	31.5	15.0	78
21	52.0	16.0	171
22	37.0	15.5	102
23	35.0	15.5	92
24	28.5	15.5	61

K = .212

APPENDIX XXI continued

CONCENTRATION OF SERUM TRIGLYCERIDE

Group III

Sample	Saponification	Blank	Mg per 100 ml
50	21.0	10.0	
100	30.0	10.0	
200	50.0	10.0	
250	58.0	10.0	
300	66.5	10.0	
350	76.5	10.0	
1	41.0	12.5	144
2	26.0	12.0	71
3	20.5	12.0	43
4	35.5	13.5	111
5	30.5	12.5	91
6	27.0	12.0	76
7	23.5	12.0	58
8	27.0	13.0	71
9	35.5	12.0	119
10	29.5	12.0	88
11	24.5	12.5	61
12	26.5	12.0	73

Group IV

13	38.5	12.5	131
14	37.5	13.5	121
15	24.5	12.0	63
16	26.5	12.5	71
17	52.5	12.0	205
18	30.5	12.5	91
19	52.0	14.0	192
20	37.5	13.0	124
21	20.0	12.0	40
22	38.5	13.0	129
23	34.0	12.0	111
24	39.0	13.5	129

K = .198

CONCENTRATION OF TRIGLYCERIDE* IN RAT HEART (DRY WEIGHT)

	Sample	Wt. Tissue Analyzed (g)	Fluor. Rdg. - Blank	Mg Triglyceride per Sample	Mg Triglyceride per gm Tissue
	.025		10.5	.025	
	.050		21.0	.050	
	.100		43.0	.103	
	.125		50.5	.121	
	.150		62.0	.149	
	.175		70.5	.169	
GROUP I	1	.010244	20.5	.049	9.646
	2	.012121	22.0	.053	8.748
	3	.014388	24.0	.057	8.038
	4	.009920	15.0	.036	7.286
	5	.011306	11.5	.027	4.900
	6	.010699	16.0	.038	7.602
	7	.010414	29.5	.071	13.656
	8	.012265	35.0	.084	13.476
	9	.011300	15.5	.037	6.608
	10	.009940	31.5	.075	15.272
	11	.011017	12.0	.028	5.246
	12	.011258	25.0	.060	10.698
GROUP II	13	.012800	19.0	.045	7.152
	14	.013784	26.5	.063	9.268
	15	.012376	24.0	.057	9.342
	16	.009088	12.0	.028	6.360
	17	.017226	38.5	.092	10.768
	18	.013179	26.0	.062	9.506
	19	.009904	9.5	.022	4.624
	20	.012797	29.0	.069	10.918
	21	.014529	72.0	.173	23.882
	22	.012459	22.0	.053	8.508
	23	.011147	35.0	.084	15.126
	24	.010713	12.5	.030	5.624

K = 415.0

*Dilution factor = 2

CONCENTRATION OF TRIGLYCERIDE* IN RAT HEART (DRY WEIGHT)

	Sample	Wt. Tissue Analyzed (g)	Fluor. Rdg. - Blank	Mg Triglyceride per Sample	Mg Triglyceride per gm Tissue
	.025		8.5	.026	
	.050		16.0	.049	
	.100		32.0	.098	
	.125		40.5	.124	
	.150		49.5	.152	
	.175		56.0	.172	
GROUP III	1	.007574	16.5	.050	6.693
	2	.005625	12.5	.038	6.818
	3	.008456	8.0	.024	2.904
	4	.007217	7.5	.023	3.189
	5	.006819	27.0	.082	12.158
	6	.007181	17.0	.052	7.271
	7	.006618	8.0	.024	3.711
	8	.006529	7.0	.021	3.290
	9	.006860	6.5	.019	3.909
	10	.006098	20.5	.062	10.321
	11	.006261	11.0	.033	5.396
	12	.008353	9.5	.029	3.493
GROUP IV	13	.003723	7.5	.023	6.190
	14	.003419	8.0	.024	7.184
	15	.004270	7.0	.021	5.032
	16	.005939	5.5	.016	3.127
	17	.004280	10.5	.032	7.532
	18	.005263	6.0	.018	3.501
	19	.003403	5.5	.016	4.967
	20	.004110	19.5	.059	14.569
	21	.004032	7.5	.023	5.714
	22	.004468	9.0	.027	6.183
	23	.003367	39.5	.121	35.997
	24	.004929	13.5	.041	8.409

K = 325.6

*No dilution required

CONCENTRATION OF TRIGLYCERIDE IN RAT TRICEP (DRY WEIGHT)

	Sample	Wt. Tissue Analyzed (g)	Fluor. Rdg. - Blank	Mg Triglyceride per Sample	Mg Triglyceride per gm Tissue
	.025		8.0	.023	
	.050		18.0	.051	
	.100		35.5	.102	
	.125		43.5	.125	
	.150		52.0	.149	
	.175		62.0	.178	
GROUP I	1	.01035	26.0	.074	7.232
	2	.01333	61.5	.177	13.284
	3	.01369	23.5	.067	4.942
	4	.01197	16.0	.046	3.847
	5	.01371	39.0	.112	8.190
	6	.01144	16.5	.047	4.152
	7	.01344	18.0	.051	4.855
	8	.01110	28.5	.082	7.392
	9	.01820	56.5	.162	8.938
	10	.01207	33.0	.095	7.871
	11	.01776	28.0	.080	4.539
	12	.01161	15.0	.043	3.720
GROUP II	13	.01322	43.0	.123	9.365
	14	.01627	23.5	.067	4.158
	15	.01589	42.5	.122	7.701
	16	.01687	46.5	.133	7.936
	17	.01693	51.0	.146	8.673
	18	.01634	24.5	.070	4.317
	19	.01528	32.0	.092	6.029
	20	.01541	64.0	.184	11.957
	21*	.01719	38.5	.121	14.125
	22	.01630	52.5	.151	9.273
	23	.01515	15.5	.044	2.945
	24	.01901	43.0	.123	6.512

K = 347.3

*K = 317.1

CONCENTRATION OF TRIGLYCERIDE
IN RAT TRICEPS (DRY WEIGHT)

	Sample	Wt. Tissue Analyzed (g)	Fluor. Rdg. - Blank	Mg Triglyceride per Sample	Mg Triglyceride per gm Tissue
	.025		9.5	.025	
	.050		19.5	.052	
	.100		37.5	.100	
	.125		47.0	.126	
	.150		35.5	.143	
	.175		62.5	.167	
GROUP III	1	.003516	8.5	.022	6.480
	2	.004105	18.5	.049	12.082
	3	.003356	21.0	.056	16.776
	4	.004009	8.5	.022	5.688
	5	.005500	39.0	.104	19.034
	6	.004960	10.5	.028	5.681
	7	.005347	9.0	.024	4.515
	8	.006099	12.5	.033	5.500
	9	.003369	4.0	.010	3.183
	10	.004126	31.5	.084	20.474
	11	.006604	31.5	.084	12.812
	12	.003934	10.0	.026	6.829
GROUP IV	13	.003723	20.5	.055	14.793
	14	.003419	22.0	.059	17.269
	15	.004270	21.0	.056	13.201
	16	.005939	58.5	.157	26.437
	17	.004280	19.5	.052	12.228
	18	.005263	15.0	.040	7.653
	19	.003403	8.5	.022	6.708
	20	.004110	5.5	.014	3.591
	21	.004032	13.5	.036	8.992
	22	.004468	22.5	.060	13.512
	23	.003367	16.5	.044	13.142
	24	.004929	25.0	.067	13.612

K = 372.5

CONCENTRATION OF TRIGLYCERIDE IN RAT EPIDIDYMAL TISSUE (ANHYDROUS WEIGHT)

	Sample	Wt. Tissue Analyzed (g)	Fluor. Rdg. - Blank	Mg Triglyceride per Sample	Mg Triglyceride per gm Tissue
	.025		7.5	.023	
	.050		16.0	.050	
	.100		32.0	.100	
	.125		40.5	.124	
	.150		49.0	.154	
	.175		56.0	.176	
GROUP I	1	.004387	26.5	.083	951
	2	.003394	21.0	.066	976
	3	.005023	31.0	.097	973
	4	.003173	19.5	.061	969
	5	.003354	21.0	.066	988
	6	.004974	31.0	.097	983
	7	.004473	27.0	.085	952
	8	.004238	26.5	.083	985
	9	.005393	32.5	.103	950
	10	.004281	26.0	.081	957
	11	.003781	23.0	.072	959
	12	.004320	26.5	.083	967
GROUP II	13	.003987	24.5	.077	968
	14	.003929	24.5	.077	982
	15	.003993	25.0	.078	987
	16	.004436	27.5	.086	976
	17	.004251	26.5	.083	983
	18	.004013	25.0	.078	982
	19	.004456	27.5	.086	972
	20	.005983	37.5	.118	988
	21	.005960	37.5	.118	992
	22	.004967	30.5	.096	967
	23	.005970	37.5	.118	990
	24	.003944	24.0	.075	960

K = 317.1

*Dilution factor = 50

CONCENTRATION OF TRIGLYCERIDE IN RAT EPIDIDYMAL TISSUE (ANHYDROUS WEIGHT)

	Sample	Wt. Tissue Analyzed (g)	Fluor. Rdg. - Blank	Mg Triglyceride per Sample	Mg Triglyceride per gm Tissue
	.025		9.0	.025	
	.050		18.0	.051	
	.100		34.0	.097	
	.125		43.5	.125	
	.150		50.5	.145	
	.175		60.0	.172	
GROUP III	1	.005197	36.0	.103	981
	2	.005232	35.5	.102	975
	3	.009440	63.5	.182	966
	4	.004326	29.5	.084	971
	5	.003479	23.5	.067	970
	6	.003597	24.5	.070	978
	7	.004583	31.0	.089	973
	8	.004223	28.5	.081	970
	9	.003421	22.5	.064	945
	10	.005646	37.5	.107	953
	11	.004576	30.5	.087	956
	12	.003414	22.5	.064	948
GROUP IV	13	.004560	31.0	.089	976
	14	.004023	27.0	.077	965
	15	.005686	39.0	.112	985
	16	.003901	26.5	.076	976
	17	.004764	32.5	.093	981
	18	.003475	23.5	.067	970
	19	.004433	30.5	.087	989
	20	.004026	27.0	.077	962
	21	.005897	40.5	.116	986
	22	.003197	22.0	.063	987
	23	.002651	17.5	.050	949
	24	.004365	30.0	.086	986

K = 347.9

HEART: TOTAL NITROGEN
(mg/gram dry weight)

Group 1

Sample*	Tissue weight analyzed (mg)	HCl (ml) delivered	Total nitrogen g/tissue	Mean value
1	11.53	1.16	136.01	136.09
1	10.92	1.10	136.18	
2	11.88	1.21	137.77	137.09
2	11.00	1.11	136.42	
3	10.72	1.11	139.98	139.04
3	10.67	1.09	138.11	
4	10.70	1.10	138.98	139.48
4	10.72	1.11	139.98	
5	11.99	1.20	135.31	135.08
5	11.93	1.19	134.85	
6	10.59	1.05	134.04	134.80
6	10.67	1.07	135.57	
7	11.70	1.18	136.35	136.80
7	11.82	1.20	137.25	
8	10.94	1.08	133.46	133.95
8	10.76	1.07	134.44	
9	11.55	1.15	134.61	134.84
9	11.61	1.16	135.08	
10	10.99	1.09	134.09	134.64
10	10.70	1.07	135.19	
11	12.00	1.20	135.19	135.08
11	12.02	1.20	134.97	
12	10.99	1.11	136.55	136.96
12	11.12	1.13	137.38	

$\bar{X}$ = 136.56

*Duplicate samples

HEART: TOTAL NITROGEN
(mg/gram dry weight)

Group II

Sample*	Tissue weight analyzed (mg)	HCl (ml) delivered	Total nitrogen g/tissue	Mean value
1	9.99	0.99	133.97	
1	10.13	1.00	133.46	133.71
2	10.81	1.07	133.82	
2	10.79	1.07	134.07	133.94
3	10.38	1.04	135.45	
3	10.43	1.03	133.51	134.48
4	9.66	0.94	131.55	
4	9.47	0.93	132.21	131.88
5	12.00	1.16	130.69	
5	11.94	1.15	130.21	130.45
6	8.81	0.85	130.44	
6	9.42	0.91	130.60	130.52
7	11.81	1.17	133.93	
7	12.02	1.19	133.84	133.88
8	11.95	1.15	130.01	
8	11.87	1.15	130.98	130.49
9	10.19	0.98	130.02	
9	10.21	0.99	130.09	130.55
10	14.20	1.41	134.24	
10	14.28	1.40	132.54	133.39
11	10.50	0.99	127.47	
11	15.56	1.48	128.59	128.03
12	14.99	1.44	129.87	
12	14.83	1.43	130.36	130.11

$\bar{X}$ = 131.78

*Duplicate samples

HEART: TOTAL NITROGEN
(mg/gram dry weight)

Group III

Sample*	Tissue weight analyzed (mg)	HCl (ml) delivered	Total nitrogen g/tissue	Mean value
1	12.16	1.19	132.31	
1	11.14	1.09	132.28	132.29
2	11.55	1.13	132.28	
2	11.24	1.11	133.51	132.89
3	16.76	1.64	132.30	
3	15.42	1.50	131.51	131.90
4	14.07	1.34	128.75	
4	13.08	1.27	130.23	129.49
5	12.87	1.22	128.16	
5	14.73	1.40	128.49	128.32
6	13.76	1.32	129.70	
6	14.22	1.35	128.35	129.02
7	13.32	1.26	127.89	
7	12.89	1.23	129.01	128.45
8	11.83	1.14	131.43	
8	12.47	1.22	132.27	131.85
9	10.16	1.00	133.07	
9	11.01	1.08	132.61	132.84
10	10.90	1.05	131.47	
10	10.52	1.03	132.37	131.92
11	12.85	1.25	131.51	
11	12.62	1.23	131.77	131.64
12	12.17	1.16	128.86	
12	12.12	1.15	128.28	128.57

$\bar{X}$ = 130.76

*Duplicate samples

HEART: TOTAL NITROGEN
(mg/gram dry weight)

Group IV

Sample*	Tissue weight analyzed (mg)	HCl (ml) delivered	Total nitrogen g/tissue	Mean value
1	12.36	1.19	130.16	
1	13.37	1.28	129.43	129.79
2	12.41	1.18	128.55	
2	14.02	1.32	127.29	127.92
3	12.21	1.15	127.33	
3	11.94	1.14	129.08	128.20
4	13.18	1.22	125.14	
4	14.73	1.37	125.74	125.44
5	12.34	1.13	123.80	
5	13.41	1.23	124.00	123.90
6	11.25	1.03	123.78	
6	11.63	1.07	124.38	124.08
7	12.08	1.11	124.22	
7	11.47	1.06	124.94	124.58
8	11.10	1.03	125.45	
8	12.01	1.12	126.07	125.76
9	12.85	1.19	125.20	
9	12.64	1.18	126.21	125.70
10	13.74	1.27	124.96	
10	14.03	1.31	126.23	125.59
11	14.30	1.34	126.68	
11	13.72	1.28	126.13	126.40
12	13.12	1.23	126.74	
12	14.11	1.31	125.52	126.13

$\bar{X}$ = 126.12

*Duplicate samples

TRICEP: TOTAL NITROGEN
(mg/gram dry weight)

Group I

Sample*	Tissue weight analyzed (mg)	HCl (ml) delivered	Total nitrogen g/tissue	Mean value
1	13.00	1.35	140.39	
1	18.00	1.87	140.45	140.42
2	11.64	1.18	137.05	
2	17.24	1.77	138.80	137.92
3	14.98	1.59	143.50	
3	15.61	1.67	144.63	144.06
4	15.94	1.63	138.25	
4	16.20	1.68	140.20	139.22
5	13.50	1.42	142.20	
5	14.91	1.56	141.45	141.82
6	14.40	1.49	139.89	
6	14.47	1.51	141.08	140.48
7	15.18	1.55	138.04	
7	15.02	1.53	137.71	137.87
8	16.10	1.67	140.23	
8	17.44	1.82	141.08	140.65
9	16.54	1.70	138.95	
9	15.44	1.57	137.47	138.21
10	15.61	1.57	135.97	
10	11.78	1.18	135.42	135.69
11	17.89	1.84	139.05	
11	20.61	2.14	140.38	139.71
12	17.79	1.84	139.83	
12	17.41	1.82	141.33	140.58

$\bar{X}$ = 139.71

* Duplicate samples

TRICEP: TOTAL NITROGEN
(mg/gram dry weight)

Group II

Sample*	Tissue weight analyzed (mg)	HCl (ml) delivered	Total nitrogen g/tissue	Mean value
1	16.28	1.78	131.21	131.45
1	15.50	1.51	131.70	
2	19.97	1.95	132.01	131.63
2	16.79	1.63	131.25	
3	13.00	1.25	129.99	129.30
3	14.82	1.41	128.62	
4	17.23	1.79	133.39	132.94
4	16.02	1.57	132.49	
5	15.63	1.56	134.93	134.09
5	16.03	1.58	133.25	
6	14.97	1.47	132.75	132.52
6	14.92	1.46	132.29	
7	14.86	1.47	133.74	134.42
7	16.81	1.68	135.11	
8	15.87	1.59	135.45	135.60
8	16.73	1.68	135.76	
9	15.56	1.50	130.33	130.21
9	14.03	1.35	130.09	
10	16.84	1.67	134.07	134.06
10	15.43	1.53	134.05	
11	15.48	1.55	135.37	135.08
11	17.25	1.72	134.80	
12	16.86	1.64	131.50	131.22
12	15.90	1.54	130.94	

$\bar{X}$ = 132.71

*Duplicate samples

TRICEP: TOTAL NITROGEN
(mg/gram dry weight)

Group III

Sample*	Tissue weight analyzed (mg)	HCl (ml) delivered	Total nitrogen g/tissue	Mean value
1	13.94	1.40	135.77	
1	15.07	1.52	136.36	136.06
2	14.91	1.43	129.66	
2	12.47	1.21	131.18	130.42
3	13.34	1.31	132.76	
3	15.15	1.47	131.18	131.97
4	15.35	1.52	133.87	
4	15.02	1.49	134.11	133.99
5	14.93	1.46	132.20	
5	16.21	1.59	132.61	132.40
6	19.60	1.98	136.57	
6	17.57	1.77	136.19	136.38
7	16.49	1.61	132.00	
7	15.42	1.51	132.39	132.19
8	18.00	1.77	132.94	
8	16.74	1.63	131.64	132.29
9	12.79	1.27	133.18	
9	14.53	1.44	133.98	133.58
10	13.32	1.34	134.99	
10	14.14	1.41	134.81	134.90
11	17.99	1.74	130.76	
11	16.78	1.61	129.71	130.23
12	17.80	1.68	127.60	
12	17.14	1.64	129.36	128.48

$\bar{X}$ = 132.74

*Duplicate samples

TRICEP: TOTAL NITROGEN
(mg/gram dry weight)

Group IV

Sample*	Tissue weight analyzed (mg)	HCl (ml) delivered	Total nitrogen g/tissue	Mean value
1	16.00	1.53	129.28	129.27
1	15.27	1.46	129.26	
2	17.93	1.70	128.18	128.13
2	16.36	1.55	128.09	
3	16.87	1.62	129.82	129.48
3	17.90	1.71	129.15	
4	18.60	1.76	127.92	128.91
4	20.71	1.99	129.91	
5	18.75	1.76	126.90	127.64
5	19.27	1.83	128.39	
6	17.16	1.63	128.42	128.95
6	16.08	1.54	129.48	
7	15.44	1.46	127.84	128.56
7	14.85	1.42	129.28	
8	16.85	1.61	129.18	129.26
8	15.47	1.48	129.34	
9	16.00	1.54	130.12	129.56
9	17.71	1.69	129.01	
10	18.60	1.77	128.65	128.87
10	15.29	1.46	129.09	
11	18.45	1.77	129.70	130.21
11	16.03	1.55	130.72	
12	18.90	1.81	129.47	130.35
12	14.01	1.36	131.24	

$\bar{X}$ = 129.09

*Duplicate samples

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