THE RELATIVE EFFECTS OF EXERCISE AND CALORIC RESTRICTION IN CONTROLLING BLOOD CHOLESTEROL IN RATS

Thesis for the degree of Ph. D.

Michigan State University

Perry Brooke Johnson

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Major professor

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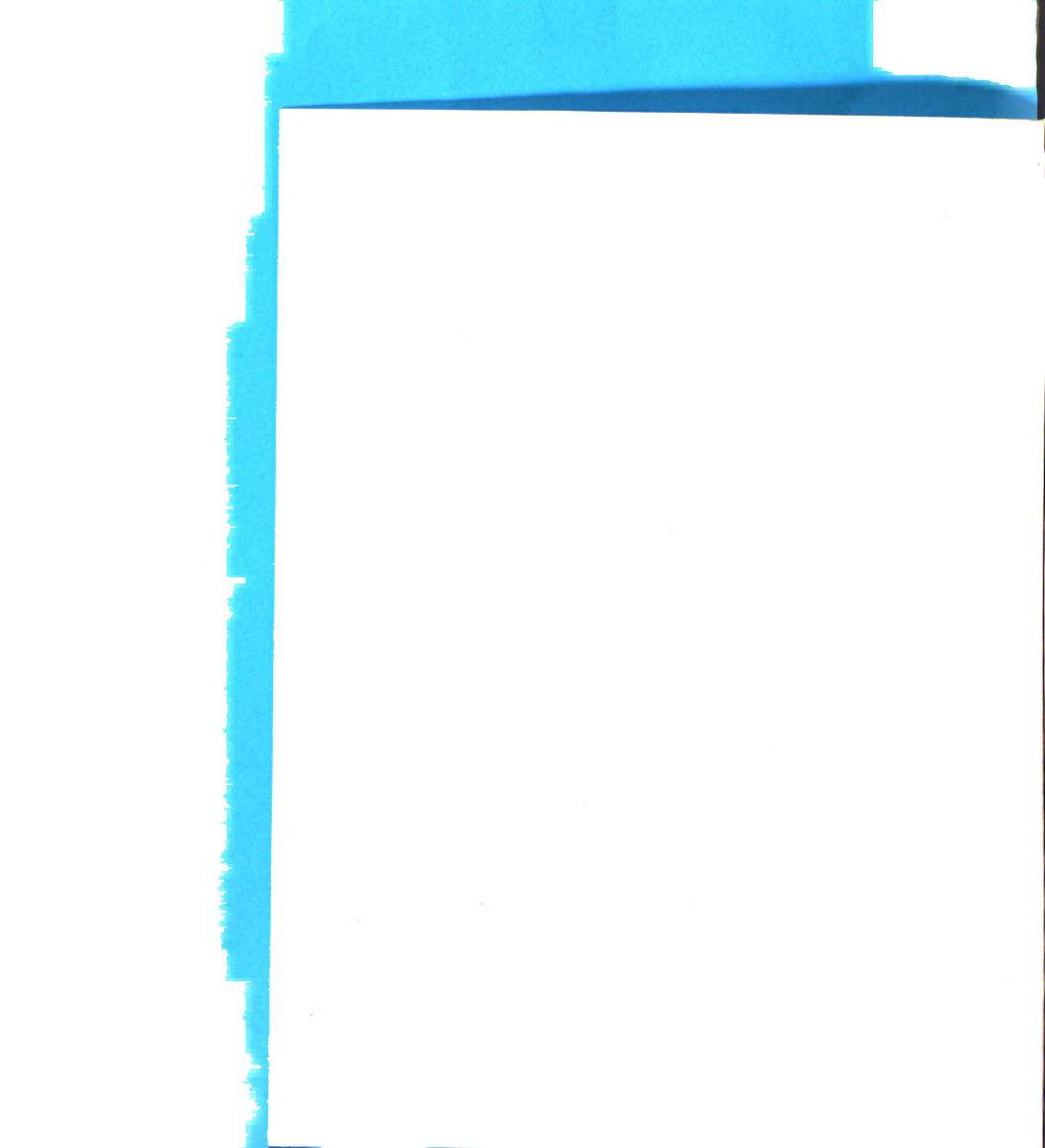

THE RELATIVE EFFECTS OF EXERCISE AND CALORIC RESTRICTION IN CONTROLLING BLOOD CHOLESTEROL IN RATS

by

PERRY BROOKE JOHNSON

AN ABSTRACT

Submitted to the School for Advanced Graduate Studies of
Michigan State University of Agriculture and
Applied Science in partial fulfillment of
the requirements for the degree of

DOCTOR OF PHILOSOPHY

Department of Health, Physical Education and Recreation

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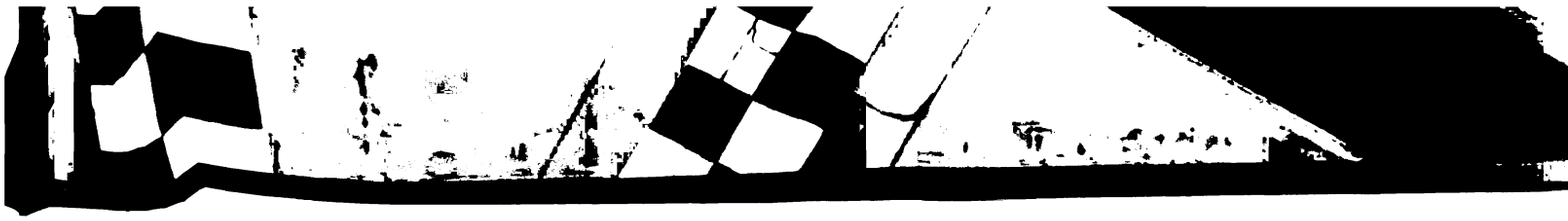
Approved:

Henry J. Montoye



Perry Brooke Johnson

ABSTRACT

With the relationship between excess serum cholesterol and atherosclerotic disease well established,[1,2] there is a need for investigating all possibilities for controlling serum cholesterol concentrations. There is evidence that serum cholesterol concentrations rise or fall paralleling weight gain or loss.[3,4,5] Recent work indicates that exercise decreases cholesterol level. Is exercise *per se* effective in controlling serum cholesterol, or is it effective only if weight is lost? Or is weight control by diet as effective as exercise? The purpose of the present investigation was to answer these questions.

In an effort to evaluate the relative effects of exercise and caloric restriction in controlling total blood cholesterol level, blood samples were taken from eighty-eight mature male albino rats for serum cholesterol assay. The animals were divided into four groups equated on the basis of body weight. One group was sacrificed immediately and the other three maintained for a fifteen-week experimental period. One group was sedentary and fed *ad libitum*, one was exercised regularly and fed *ad libitum* while the third group was sedentary, but maintained at a mean body weight comparable to that of the exercise group by careful diet restriction.

In addition, the experiment was designed to test whether differences in body weight and body fat could account for any differences in blood cholesterol.

Control and final total blood cholesterol concentration and final specific gravity, to be used as an estimate of body fat, were determined. The under-water weighing method was used to determine specific gravity. Control and final body weights also were recorded.

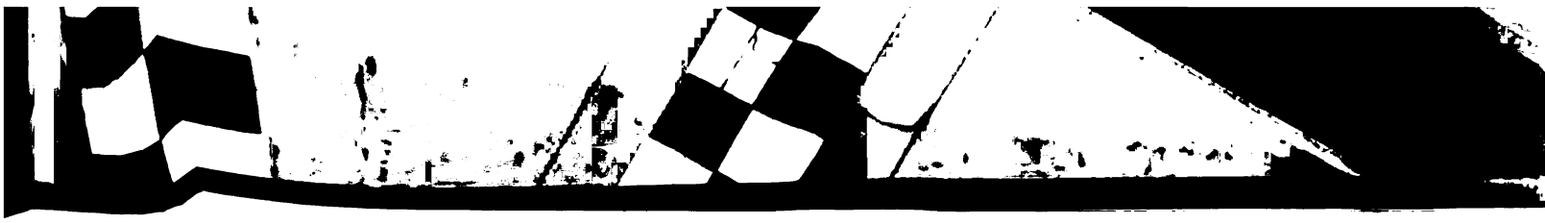
SUMMARY OF MEAN VALUES

Variable	Groups		
	Sedentary	Diet	Exercise
Final Weight	475.6 g	422.2 g	421.2 g
Spec. Gravity	1.0323	1.0256	1.0363
Final Chol. in mg%	96.41	95.32	73.10
Chol. Change	+30.59	+25.64	+13.85
Wt. Change	+148.2 g	+95.5 g	+92.0 g

The data were analyzed statistically, employing analysis of variance, analysis of covariance, students "t", and product-moment correlation coefficients.

It was concluded that caloric restriction was not as effective in controlling total blood cholesterol as was regular exercise. The mean cholesterol difference between the diet group and exercise group was significant even after removing the effects of the control cholesterol level.

The significant positive correlation between control and final cholesterol concentrations strengthens the theory that there is some predetermined, probably inherent factor which controls cholesterol concentration. However, this



relationship did not hold true within the exercise group. It is possible, then, that exercise is a factor which can alter this seemingly predetermined cholesterol concentration.

Although the sedentary group was significantly heavier than the diet and exercise groups, body weight was not significantly correlated with final cholesterol concentration. Therefore it cannot be concluded that high blood cholesterol and high body weight are associated.

Although the nonexercise animals were significantly less dense than the exercise animals, specific gravity was not significantly correlated with cholesterol concentration. Hence, it cannot be concluded that high blood cholesterols are associated with high percentage of body fat.

There was no significant relationship between cholesterol change and weight change, which is not surprising in view of the lack of relationship between final weight and cholesterol concentration.

The results seem to support the hypothesis that over and above its effect on weight and obesity, there is some effect of exercise which helps control blood cholesterol concentration.

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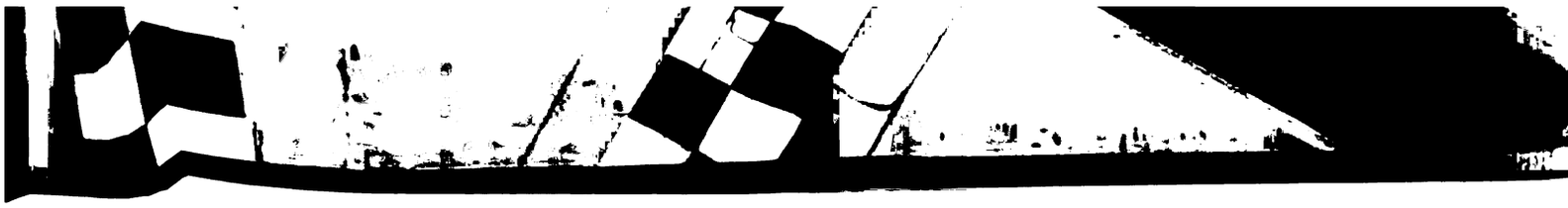
1. J. Groen and R. M. van der Heide, Atherosclerosis and Coronary Thrombosis, Trans. by J. Winsser, Rotterdam, WYT, 1956, 12-14.

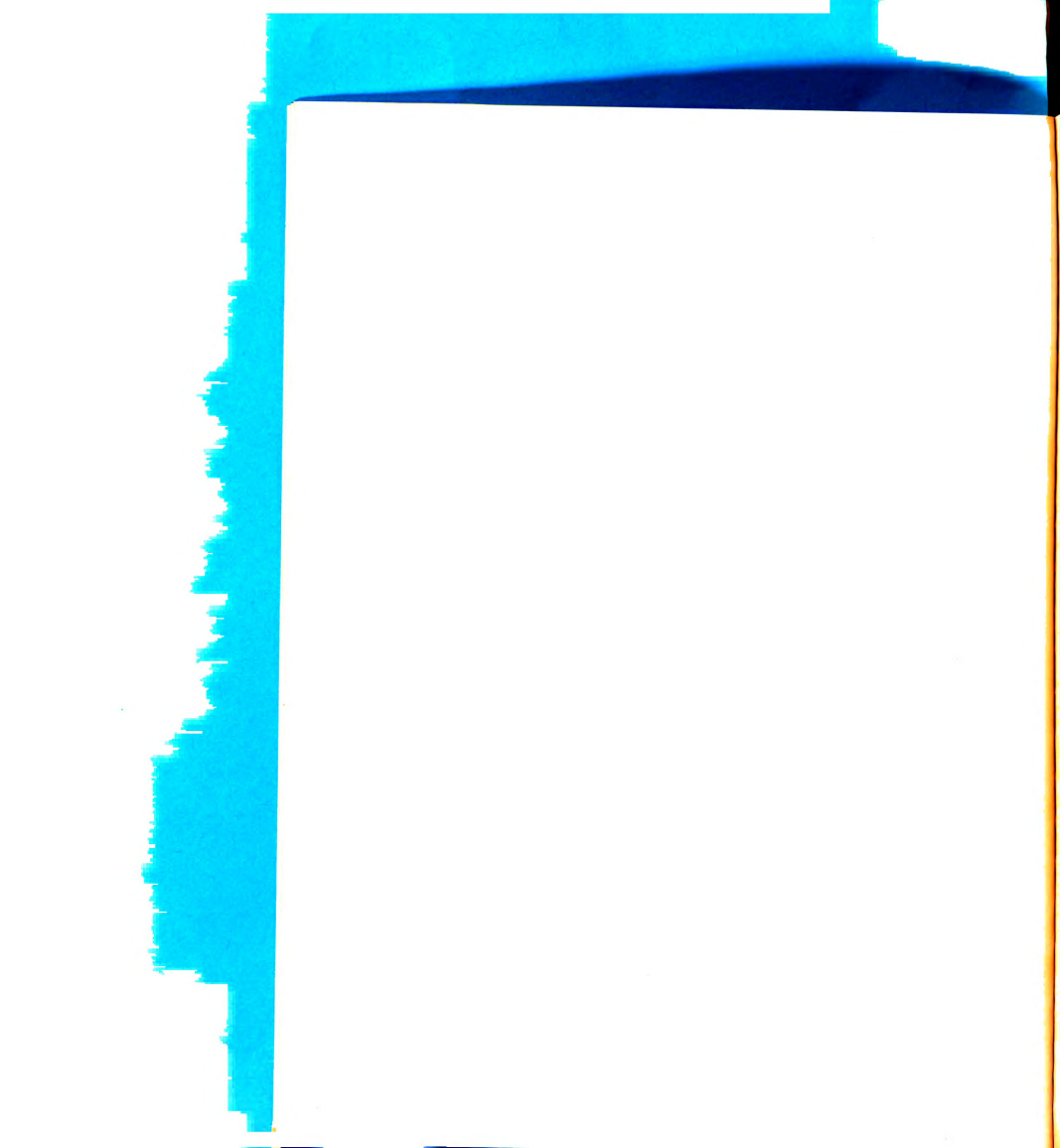
2. J. W. Gofman, et al., (Technical Group), E. C. Andrus, et al., (Comm. on Lipoprotiens, etc.), "Evaluation of Serum Lipoprotein and Cholesterol Measurements as Predictors of Clinical Complications of Atherosclerosis," Circulation, 14 (1956), 730.

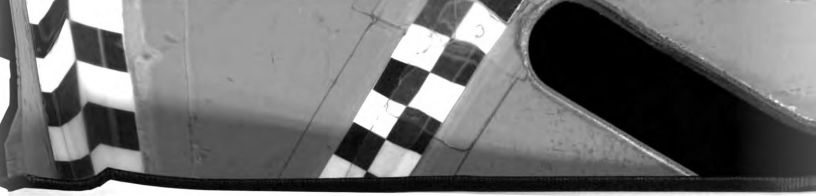


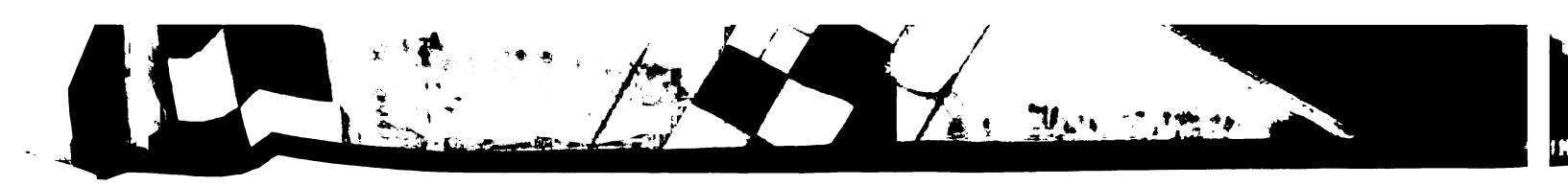
3. G. V. Mann, et al., "Exercise in the Disposition of Dietary Calories," New England Journal of Medicine, 253 (1955), 355.

4. J. T. Anderson, F. Grande and A. Keys, "Serum Cholesterol Concentration of Men in Semi-Starvation and in Refeeding," Federation Proceedings, 14 (1955), 426.

5. J. T. Anderson, A. Lawler and A. Keys, "Weight Gain from Simple Overeating. II. Serum Lipids and Blood Volume," Journal of Clinical Investigation, 36 (1957), 87.

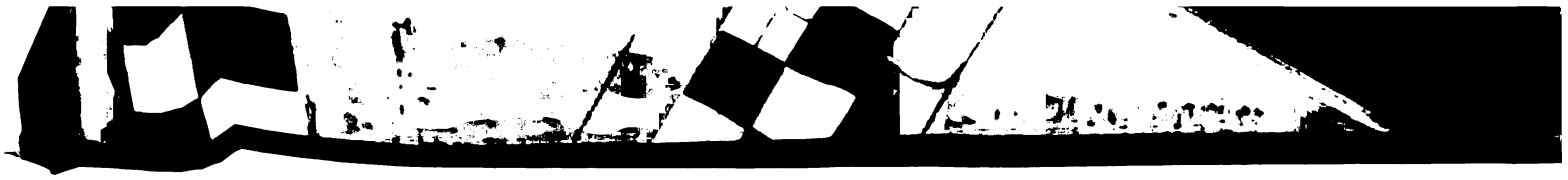






To the memory of my father...

PERRY BROOKE JOHNSON, JR.



ACKNOWLEDGEMENTS

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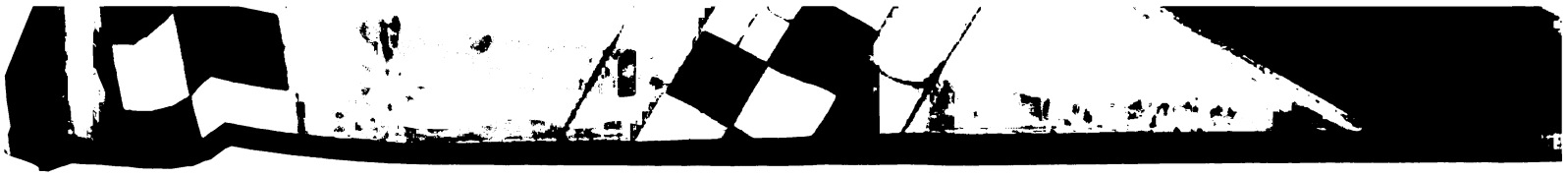
Grateful appreciation is also expressed to Dr. Evelyn Jones, for her time and patience in supervising the actual cholesterol assay, and Wynn Updyke, for his contribution to the long and tedious hours required during the experimental period.



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CHAPTER I

INTRODUCTION

In recent years there has been a growing interest in the relationship between fat metabolism and circulatory disorders. Considerable evidence tends to support the hypothesis that some abnormality in lipid metabolism is closely linked to atherosclerosis. Cholesterol is one of the lipids which has been extensively investigated in this connection.

Although there is little direct evidence that a high blood serum concentration of cholesterol actually causes atherosclerosis or related circulatory disorders, there are indications that high serum cholesterol concentrations are related to the atherosclerotic diseases. Groen and van der Heide,[1] in their comprehensive review of the total problem, cite the following factors which point to the possible importance of cholesterol: (1) a high percentage of cholesterol and its esters in foci of atherosclerosis, (2) experimental atherosclerosis produced in animals by high cholesterol feedings, and (3) geographical differences--in countries where atherosclerosis is rare, average blood

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[1] J. Groen and R. M. van der Heide, Atherosclerosis and Coronory Thrombosis, Health Organization. T. N. O., Publisher WYT, Rotterdam, 1956, trans. Johan Winsser, 12-14.

cholesterol level is lower. In summarizing the relationship between cholesterolemia and ischemic heart diseases, the authors say:[2]

> . . . we think it very acceptable, if not proven, that an increased blood cholesterol level promotes the development of atherosclerosis and arteriosclerotic heart diseases. The indications are especially clear when groups are compared with a very low and a very high blood-cholesterol level.

Most of the evidence in favor of the importance of cholesterol is indirect. However, a recent investigation by Gofman and co-workers indicates a more direct relationship between high cholesterol concentration and early onset of atherosclerotic changes.[3] They found high serum cholesterol effective in predicting these degenerative changes.

It is possible, then, that an excess of serum cholesterol contributes to the degenerative changes seen in atherosclerosis. On the other hand, there may be no causative effect, the two changes merely going hand-in-hand. In either case, with the relationship well established there is a need for investigating all possibilities for controlling and lowering serum cholesterol concentrations.

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[2] Ibid., 26.

[3] J. W. Gofman, et al., (Technical Group), E. C. Andrus, et al., (Comm. on Lipoprotiens, etc.), "Evaluation of Serum Lipoprotein and Cholesterol Measurements as Predictors of Clinical Complications of Atherosclerosis," Circulation, 14 (1956), 730.

In an effort to determine what variables affect the serum cholesterol concentration, much work has been done in the general area of diet and nutrition. There have been fewer studies concerning the possible role of physical exercise, but recent investigations seem to indicate that exercise may contribute considerably to the maintenance of normal serum cholesterol concentrations.[4,5,6,7] Mann[8] believes that "exercise is likely to have a biochemical relation to lipid metabolism."

I. The Problem

There is evidence which indicates that serum cholesterol concentrations rise along with an increase in

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[4]A. L. Myasnikov, "Influence of Some Factors on Development of Experimental Cholesterol Atherosclerosis," Circulation, 17 (1958), 110.

[5]H. Y. C. Wong, "Hypocholesterolization Effect of Exercise on Cholesterol-Fed Cockerels," Federation Proceedings, 16 (1957), 138.

[6]H. Montoye, et al., Unpublished data, Michigan State University, Department of Health, Physical Education & Recreation, 1959.

[7]I. H. Page, "Atherosclerosis--A Commentary," Federation Proceedings, 18 (1959), 50.

[8]G. V. Mann, "Diet, Exercise and Coronary Disease," Illinois Medical Journal, 116 (1959), 20.

body weight and decrease parallel to weight loss.[9,10,11] Recent work indicates that exercise is effective in decreasing serum cholesterol concentration, but only indirectly through decreasing body weight.[12] This evidence brings out an important question: is exercise *per se* effective in controlling serum cholesterol, or is it effective only if weight is lost or maintained? Or, is it possible that weight control by caloric restriction alone is as effective as exercise?

Statement of the Problem

It was the purpose of this study to investigate the relative effects of physical exercise and weight control on blood serum cholesterol concentrations in mature rats. The experiment was designed to test for differences in body weight, total body fat, and blood serum cholesterol concentration among three groups of rats (exercise, sedentary and

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[9] G. V. Mann, *et al.*, "Exercise in the Disposition of Dietary Calories," *New England Journal of Medicine*, 253 (1955), 355.

[10] J. T. Anderson, F. Grande and A. Keys, "Serum Cholesterol Concentration of Men in Semi-Starvation and in Refeeding," *Federation Proceedings*, 14 (1955), 426.

[11] J. T. Anderson, A. Lawler and A. Keys, "Weight Gain from Simple overeating. II. Serum Lipids and Blood Volume," *Journal of Clinical Investigation*, 36 (1957), 87.

[12] H. J. Montoye, *et al.*, "The Effects of Exercise on Blood Cholesterol in Middle-Aged Men," *American Journal of Clinical Nutrition*, 7 (1959), 144.

diet). In addition, an initial control group, sacrificed at the beginning of the experiment, was available for comparison with these three groups.

II. Limitations

Due to the ease in controlling such important factors as amount and intensity of exercise and diet restriction, rats were chosen for this investigation. It must be remembered that animal observations cannot be transferred to human interpretation. However, until evidence to the contrary is presented, phenomena observed in these mammals are extremely valuable in basic investigations related to this problem.

CHAPTER II

REVIEW OF RELATED LITERATURE

I. Exercise and Blood Cholesterol Concentration

Mann,[1] reporting the results of a study of three subjects, states that young men consuming high fat diets were able to double their caloric intake without increasing the level of their serum lipids or cholesterol so long as the surplus of energy was expended as heat and muscular energy. On the other hand, restricting exercise and allowing fat deposition doubled serum cholesterol concentration. The serum concentrations were returned to normal by food restriction and weight reduction.

Montoye and co-workers[2] investigated the effects of supervised exercise on total blood serum cholesterol in 31 sedentary middle-aged men. No effect was observed in mean serum cholesterol of subjects with "normal" initial serum levels, but three "high" level subjects showed large decreases compared to two controls (no exercise) with high initial levels, although one control also decreased. Changes in total serum cholesterol generally accompanied changes in body weight, regardless of whether the weight

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[1] G. V. Mann, et al., "Exercise in the Disposition of Dietary Calories," New England Journal of Medicine, 253 (1955), 355.

[2] H. J. Montoye, et al., "The Effects of Exercise on Blood Cholesterol in Middle-Aged Men," American Journal of Clinical Nutrition, 7 (1959), 144.

change was brought about by exercise. They concluded that exercise was effective in decreasing total serum cholesterol, but that the effect appeared to be indirect throught weight reduction.

Comparing ten sedentary subjects with seven active subjects, all middle-aged, Chailley-Bert and co-workers[3] found the active group had considerably lower blood serum cholesterol concentrations. In addition, three sedentary middle-aged workers (2 females and one male) with reasonably high blood cholesterol concentrations were placed on a conditioning program of daily 5 km. walks, plus cycling. Blood cholesterol was appreciably lowered in all three subjects following the exercise program. In one case the program lasted six months, in the others, four and two months.

Mann, Nicol and Stare[4] reported differences in cholesterol concentrations among Nigerian subjects in three separate areas of the country. Since total caloric and total fat content of the diet was similiar and since there was a difference in muscular exercise, they believed that the physical work patterns may have had an effect in controlling serum cholesterol concentrations.

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[3]Chailley-Bert, P. Labignette and Fabre-Chevalier, "Contribution à l'etude des variations du cholesterol sanguin au cours des activite's physiques," Presse Med., 63 (1955), 415.

[4]G. V. Mann, B. M. Nicol and F. J. Stare, "The Beta-Lipoprotein and Cholesterol Concentrations in Sera of Nigerions," British Medical Journal, 2 (1955), 1008.

In investigating the Bantu, Keys and co-workers[5] accounted for large variations in serum cholesterol not by physical activity differences, but by lower fat diets consumed by the heavy manual labor group. They concluded that the habitual diet, and especially its fat content, has much more influence on serum cholesterol concentration than physical activity per se.

The effects of daily treadmill walking in nine university students were studied by Taylor, Anderson and Keys.[6] The exercise consisted of two hours at 3.5 miles per hour, 10 per cent grade and equalled 1280 calories work. The diet was increased by 900 calories and the proportion of fat held constant. There was no change in mean body weight and no significant change in blood serum cholesterol concentrations. The authors conclude that this supports their hypothesis that serum cholesterol is related to the proportion of total calories derived from fat, not to exercise. The same authors in a similiar study of nine university students on a 1300 calorie daily treadmill walking program, again reported no serum cholesterol changes and no weight changes.[7] They concluded that in calorie balance, serum

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[5] A. Keys, et al., "Physical Activity and the Diet in Populations Differing in Serum Cholesterol," Journal of Clinical Investigation, 35 (1956), 1179.

[6] H. J. Taylor, J. T. Anderson and A. Keys, "Physical Activity, Serum Cholesterol and Other Lipids in Man," Proceedings of the Society for Experimental Biology and Medicine, 95 (1957), 383.

[7] H. J. Taylor, J. T. Anderson and A. Keys, "Effect on Serum Lipids of 1300 Calories Daily Walking," Federation Proceedings, 16 (1957), 128.

cholesterol concentration is independent of caloric intake, absolute fat intake and physical activity level when the kind of fat and percentage fat calories are constant.

Anderson, Lawler and Keys[8] studied twenty physically healthy schizophrenic men, increasing their caloric intakes without changing physical activity. Average total serum cholesterol concentrations rose 20 per cent during the first five weeks, then stayed relatively the same for fifteen weeks even though weight gain continued. They concluded that the rise in serum cholesterol tended to be proportional to rate of weight gain.

Brown and co-workers[9] used rabbits to determine the effects of exercise in preventing coronary atherosclerosis, admitting that there was probably little application to humans due to the differences in cholesterol metabolism. In rabbits on diets of 0.1 per cent and 0.5 per cent cholesterol, there was a lowering of total serum cholesterol after just four weeks of exercise. At the end of the comparatively short experiments (6, 8 and 12 weeks) exercise had no effect on the development of atheromata in the aorta and coronary arteries. Exercise was also ineffective in speeding disappearance from the vessels once the atheromata were developed. It should be pointed out, however, that these

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[8]Anderson, Lawler and Keys, op. cit., p. 81.

[9]C. E. Brown, et al., "Observations on Blood Vessels and Exercise," Journal of Gerontology, 11 (1956), 296.

rabbits were exercised only twenty minutes daily, which seems to be far less than a rabbit would normally exercise.

Rabbits were also used in an experiment by Myasnikov.[10] These rabbits, however, were exercised to exhaustion daily for six months. There was a marked decrease in the serum cholesterol concentration of the twenty-five exercise rabbits and also some reduction in the development of atherosclerotic changes.

Wong[11] reported a significant reduction in serum cholesterol concentrations of cockerels fed cholesterol and exercised daily for seven weeks as compared to a cholesterol fed, no exercise group. The author also noted a significant reduction in formation of atheromatous plaques in the abdominal aortas of the exercised birds.

Results of a recent study by Montoye and co-workers[12] indicate that exercise controls serum cholesterol concentration. Seven rats swum an hour daily for twelve weeks had a mean serum cholesterol level of 74.72 mg. per cent as compared to their seven paired littermates' mean level of 93.08 mg. per cent. The difference was significant at the

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[10] A. L. Myasnikov, "Influence of Some Factors on Development of Experimental Cholesterol Atherosclerosis," Circulation, 17 (1958), 110.

[11] H. Y. C. Wong, "Hypocholesterolization effect of Exercise on Cholesterol-Fed Cockerels," Federation Proceedings, 16 (1957), 138.

[12] H. J. Montoye, et al., Unpublished data, Michigan State University, Department of Health, Physical Education and Recreation, 1959.

5 per cent level of confidence. The correlation coefficient between carcass specific gravity and serum cholesterol concentration of 33 rats was -0.42, significant at the 5 per cent level. This indicates that serum cholesterol concentration is higher in fatter animals, as a low specific gravity is indicative of a high percentage of body fat.

Thomas and Garn,[13] testing 159 male medical students, found no significant relationship between serum cholesterol and body weight. They also correlated serum cholesterol with body weight/chest breadth and with lower thoracic fat. Again there were no significant relationships.

II. Specific Gravity as an Estimate of Body Fat

The low density of fat as compared to other body components logically indicates that there should be an inverse relationship between specific gravity and fat content of the carcass. Liuzzo and co-workers[14] reported a -0.99 correlation between carcass specific gravity and direct chemical analysis of total body fat in rats. Da Costa and Clayton[15] also reported a significant correlation, -0.63, in

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[13] C. B. Thomas and S. M. Garn, "Degree of Obesity and Serum Cholesterol Level," Science, 131 (1960), 42.

[14] A. Liuzzo, E. P. Reineke and A. M. Pearson, "Determination of Specific Gravity by Air Displacement," Journal of Animal Science, 17 (1958), 579.

[15] E. Da Costa and R. Clayton, "Studies of Dietary Restriction and Rehabilitation. II. Interrelationships Among Fat, Water Content and Specific Gravity of the Total Carcass of the Albino Rat," Journal of Nutrition, 41 (1950), 603.

the albino rat. They concluded that the regression lines obtained make indirect determination of fat possible when specific gravity has been determined. Their work also indicates that regardless of chronic pathological conditions affecting the body fat, this relationship still holds true. Similar studies with cattle[16] and guinea pigs[17] have also shown this inverse relationship.

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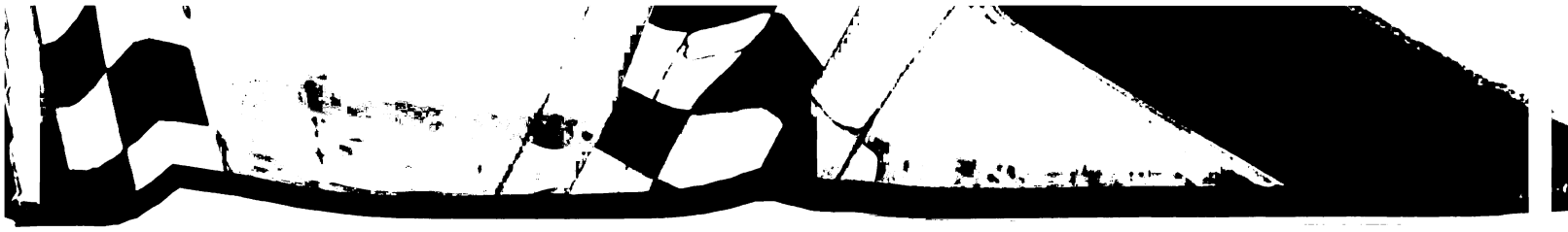
[16]H. F. Kraybill, H. L. Bitter and O. G. Hankins, "Body Composition of Cattle. II. Determination of Fat and Water Content from Measurement of Body Specific Gravity," Journal of Applied Physiology, 4 (1952), 579.

[17]E. N. Rathbun and N. Pace, "Studies on Body Composition. II. The Determination of Total Body Fat by Means of the Body Specific Gravity," Journal of Biological Chemistry, 158 (1945), 667.



CHAPTER III

PROCEDURE

General Experimental Design

In order to test for the relative effects of exercise and weight control in controlling serum cholesterol, eighty-eight mature male albino rats (Carworth) were divided into four groups as follows:

Group A = An initial control group, sacrificed at the beginning of the experiment.

Group S = A control group, sedentary (confined to small individual cages) fed ad libitum, maintained as such for fifteen weeks.

Group E = The experimental group, confined to the small cages, fed ad libitum, and subjected to fifteen weeks of vigorous exercise.

Group D = A second experimental group, sedentary (confined to cages), no exercise, and feeding restricted carefully so that body weight remained comparable to exercise animals during the fifteen-week period.

Body weights for the two experimental groups (E and D) and control group (S) were recorded bi-weekly and 18 ± 3

hours prior to sacrifice. Blood samples were taken at the beginning of the fifteen-week experimental period and immediately prior to sacrifice to be analyzed for total serum cholesterol. Carcass specific gravities, determined by under-water weighing, were used as an estimate of total body fat.

Methodology

Under light ether anesthesia, a control blood sample of 4 to 5 ml was withdrawn from the orbital sinus[1] of each of the 88 rats (refer to Figure 1, page 15). At the time of sampling, the animals' body weights ranged from 264.0 to 379.2 grams, with a mean weight of 326.8 grams. The samples were allowed sufficient time to clot, after which they were centrifuged for fifteen minutes at medium speed. The serum samples were transferred to vials which were held in frozen storage for subsequent cholesterol analysis.

After a recovery period of four days, the animals were divided into four groups equated on the basis of the body weights recorded at the time the control blood samples were taken. One group of twenty (referred to as Group A) was sacrificed immediately. The other three groups, two consisting of twenty-three rats each and the third, twenty-two (extras in case of deaths) were maintained throughout the fifteen-week experimental period.

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[1] S. H. Stone, "Method for Obtaining Venous Blood from the Orbital Sinus of the Rat or Mouse," Science, 119 (1954), 100.

Blood Sampling Technique

FIGURE 1

Each animal in Group A was weighed. In order to reduce the amount of food and waste material in the gastro-intestinal tract, food was withheld for 18 ± 3 hours prior to the time of sacrifice. This was deemed essential to standardized and accurate carcass specific gravity determinations. Following this fast, the animals were killed by injecting 1 ml of 3 per cent pentobarbital sodium. While this injection was taking effect, the electric clippers were used to remove as much of the animal's hair as possible. This, again was for the purpose of obtaining more accurate carcass specific gravity measures, as the hair tends to entrap air and thereby increase the bouyancy of the carcass.

The heart, thymus, liver, kidney, adrenals, testes and spleen were removed to furnish the data for a separate study of the effects of exercise on organ weights. The carcass was weighed in air and then washed carefully in detergent ("Blue Cheer") to remove any skin oil which might affect under-water weight. The carcass was then weighted with a sinker of known weight and weighed under water. The temperature of the water was recorded to make corrections in the calculation of specific gravity. The carcass specific gravity was calculated as follows:

$$\frac{\text{Wt. of dry carcass (in air)}}{\text{Wt. of dry carcass - Wt. of carcass under water}} \times \text{Temperature Correction Factor}$$

The remaining three groups were kept in individual wire mesh cages, 18 x 16.5 x 24 cm, where the room temperature was maintained at 21 - 23°C. There was no humidity

control. Cages were rotated weekly, from top to bottom of the cage rack.

The twenty-three sedentary animals (Group S, the control group) were confined to the cages for the entire fifteen week period. They were fed ad libitum. All groups were fed a stock diet[2] which supplied all of the known essential nutrients for the rat. (For composition of diet, see Appendix B.)

The twenty-three experimental exercise animals (Group E) were also confined to the cages and fed ad libitum, but they were subjected to fifteen weeks of exercise. The exercise progressed from an easy five minute swim with no weights attached, to sixty minutes swimming twice daily, with 3.5 per cent of the animal's body weight attached to its tail. The weight was attached as close to the rat's body as possible by means of a strip of adhesive tape. The entire exercise program was as follows:

First Four Weeks	- Progressed from 5' daily to 60' daily
Fifth Week	- Swam 60' daily
Sixth & Seventh Weeks	- Added 2% of body weight, progressed from 10' daily to 60' daily
Eighth Week	- 2%, 60' twice daily

[2] Wayne Laboratory Diets, "Allied Mills, Inc., Laboratory Diets Division, Chicago, Illinois.

Ninth Week	- Change to 3 1/2%, 60' twice daily
	- Tried 5%, 2 animals drowned
Final Six Weeks	- Changed back to 3 1/2%, continued 60' twice daily

The animals swam in a separate room in approximately 50 cm of water maintained at 35-37°C. They were swum simultaneously, half in each of two tanks, 60 x 60 cm. Room temperature in the swimming room was maintained at 31-32°C. during swimming. After swimming, the animals were dried and returned to their respective cages.

A second experimental group, Group D, consisting of twenty-two animals, was maintained in the restrictive cages for the entire fifteen-week period, just as the control group, with one exception. By means of food restriction an attempt was made to maintain the mean weight for this group as low as that of the exercise group. This was done by pairing each rat in Group D with a Group E rat of like weight at the beginning of the experimental period. Weights were recorded every two weeks, the pairs compared and the food allowance for each Group D animal for the next two weeks based on whether it was heavier or lighter than its mate. Individual differences and unpredictable weight gain patterns made this a difficult task. Although it was impossible to maintain each pair at the same weight throughout, the Group D mean weight was controlled quite nicely so that it was not significantly different from the exercise group.

A sample of the records kept might best illustrate the rather tedious method of weight control utilized. (See Appendix A.)

In the final four weeks, weekly comparisons were made in order to bring the mean weights of the two groups as close together as possible. The minimum ration for the Group D rats was 3.8 cm of Lab-Blox (6 grams). A check of the minimum daily essential nutrients[3] for rats and the content of the diet indicated that even at this ration, the daily essentials were present in sufficient quantity.

One rat in Group S became sick and died, reducing the number in this group to twenty-two. Three exercise rats drowned during the course of the experiment, one in the early weeks before weights were added and two later in the experiment when 5 per cent was added in an attempt to increase the intensity of the exercise. (See page 18, exercise program.)

At the end of the fifteen-week experimental period body weights were recorded and food withheld for 18 $\pm$ 3 hours. Following this overnight fast, the animals were lightly anesthetized, using ether, and 4-5 ml blood samples again withdrawn from the orbital sinus. The blood samples were prepared exactly as the control samples, and the serum frozen and stored for later cholesterol analysis. The

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[3] J. Q. Griffith and E. J. Farris, The Rat in Laboratory Investigation, J. B. Lipoincott Co., Philadelphia, 1942, 97-98.

animals were immediately sacrificed and specific gravities determined following the identical procedure described for Group A (See page 16).

Total serum cholesterol concentrations were determined, using the method described by Schoenheimer and Sperry,[4] as modified by Sperry[5] and Foldes and Wilson.[6] (See Appendix C for detailed outline of method.) The serum samples were coded by another person to eliminate any possibility of bias on the part of the author in carrying out the cholesterol assay. The assay was carried out in duplicate, except in a few cases where sufficient serum was not available. In cases where the second determination did not agree with the first, a third determination was run in order to clear up the discrepancy.

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[4] R. Schoenheimer and W. M. Sperry, "A Micro-Determination for the Determination of Free and Combined Cholesterol," Journal of Biological Chemistry, 106 (1934), 745.

[5] W. M. Sperry, "The Determination of Cholesterol," Journal of Biological Chemistry, 118 (1937) 377.

[6] F. F. Foldes and B. C. Wilson, "Determination of Cholesterol-Adaptation of Schoenheimer and Sperry's Method to Photoelectric Instruments," Analytical Chemistry, 22 (1950), 1210.

CHAPTER IV

RESULTS AND DISCUSSION

Results

The data were analyzed statistically. Analysis of variance and analysis of covariance[1] were both utilized with a partitioning of the between means sum of squares for comparisons.[2] Students "t" test was used to supplement analysis of variance wherever necessary. The product moment correlation coefficient[3] was employed where applicable.

Group means are listed in Table I, page 22, and presented graphically in Figure 2 on page 29.

Cholesterol

The "t" test indicates that there was no significant difference between the final mean cholesterol concentrations of Group S (96.41 mg%) and Group D (95.32 mg%). When the diet group--Group D--was compared to Group E (73.10 mg%) by analysis of covariance, the difference in mean cholesterols was significant ($F_2 = 10.40$).

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[1] W. J. Dixon and F. J. Massey, Jr., Introduction to Statistical Analysis, McGraw-Hill Book Co., Inc., New York, 1957, 2nd Edition, 211-215.

[2] C. H. Goulden, Methods of Statistical Analysis, John Wiley and Sons, Inc., New York, 1952, 2nd Edition, 87-88 and 326-327.

[3] A. L. Edwards, Statistical Analysis, Rinehart and Co., Inc., New York, 1950, 90-91.

TABLE I

SUMMARY OF MEAN VALUES

Variable	Groups				Significance of differences
	A	Sed.	Diet	Ex.	
Final Wt.(gms)	331.0	475.6	422.2	421.2	D vs. E(ns) S vs. D-E**
Spec. Gravity	1.0565	1.0323	1.0256	1.0363	S vs. D(ns) E vs. S(ns) E vs. S-D*
Cont. Chol. (mg%)	66.55	65.82	69.95	59.25	A vs. S vs. D vs. E(ns)
Final Chol. (mg%)	. . .	96.41	95.32	73.10	S vs. D(ns) E vs. D**
Chol. Change	. . .	+30.59	+25.64	+13.85	. . . . .
Wt. Change	. . .	+148.2	+95.5	+92.0	. . . . .

* Sig. at .05 level ** Sig. at .01 level

(ns) not significant

Although the control mean cholesterol concentrations were not significantly different (F = 1.96(ns)), the significant positive relationship between control and final cholesterol levels (+0.41) indicates that the final concentration was in part determined by the control. Analysis of covariance (see page 24) indicates that the difference between the Group E and Group D cholesterol concentrations is still significant after removing the effects of the control level. The reduced, but significant "F" ratio (F_2 = 5.88) indicates that there was some significant effect, over and above the control level effect which contributed to the difference in final cholesterol concentrations.

When all three Groups (S, D & E) were pooled together, there was a significant positive correlation between control cholesterol and final cholesterol concentration. Within the

TABLE II

CORRELATION COEFFICIENTS
CONTROL CHOLESTEROL VS. FINAL CHOLESTEROL

S,D,E (Pooled)	+0.41**
Sedentary	+0.40
Diet	+0.41
Exercise	+0.09

**Significant at .01 level

nonexercise groups, the Group S correlation was +0.40 and the Group D, +0.41. Within Group E, however, the correlation was only +0.09. (None of the group correlations were significant, due to the smaller N.)

TABLE III

ANALYSIS OF COVARIANCE
Y= FINAL CHOLESTEROL X= CONTROL CHOLESTEROL(COVARIATE)

Source	SS_x	SP_{xy}	SS_y	df	MS_y	"F_1"
Between Groups						
S vs. D vs. E	1334.14	2653.54	7138.10	2	3569.00	7.17**
Partitioned Comparisons						
S vs. D & E	. . .	. . .	1966.56	1	1966.56	3.95(ns)
D vs. E	. . .	. . .	5171.54	1	5171.54	10.40**
Error	12885.66	6875.46	30335.90	61	497.31	. . .
Total	14219.80	9529.00	37474.00	63	. . .	. . .

Source	SS_x	SP_{xy}	SS_y	$SS_{y'}$	df	MS	"F_2"
S vs. D&E	133.70	161.93	1966.56	1831.03	1	1831.03	4.12*
Error	12885.66	6875.46	30335.90	26667.43	60	444.46	. . .
Total	13019.36	7037.39	32302.46	28498.46	61	. . .	. . .
D vs. E	1200.44	2491.61	5171.54	2611.00	1	2611.00	5.88*
Error	12885.66	6875.46	30335.90	26667.43	60	444.46	. . .
Total	14086.10	9367.17	35507.44	29278.43	61	. . .	. . .

$SS_{y'}$ = adjusted sum of squares
"F_1" = F ratio unadjusted for control cholesterol
"F_2" = F ratio adjusted for control cholesterol
* Sig. at .05 level ** Sig. at .01 level

Note: "t" for S vs. D = o.15(ns)

Body Weight

The final mean body weights were treated by analysis of variance. The final mean weight of all three groups (S,D & E) was significantly greater than that of Group A.

TABLE IV

ANALYSIS OF VARIANCE
FINAL BODY WEIGHT

Source	SS	df	MS	"F"
Between Groups				
A vs. S vs. D vs. E	223460	3	74487	42.47***
Partitioned Comparisons				
A vs. S, D, E	181515	1	181515	103.49***
D vs. E	13	1	13	.01(ns)
S vs. D & E	41932	1	41932	23.91***
Error	140286	80	1754	. . . . .
Total	363746	83	. . .	. . . . .

***Sig. at .005 level

The effort to keep the Group D mean weight in line with the Group E mean was successful (See APPENDIX E for growth chart). The Group D mean weight of 422.2 grams was not significantly different from the Group E mean of 421.2 grams (F=0.01). Group S (475.6 grams) was significantly heavier when compared to E and D.

Final body weights were correlated with final cholesterol concentrations. The overall correlation (S, D & E pooled) was not significant, nor were the correlations within each group.

TABLE V

CORRELATION COEFFICIENTS
FINAL BODY WEIGHT VS. FINAL CHOLESTEROL

S,D,E (pooled)	+ 0.23
Sedentary	+ 0.36
Diet	+ 0.17
Exercise	- 0.32

When cholesterol change was correlated with weight change, neither the pooled nor the individual group correlations were significant.

TABLE VI

CORRELATION COEFFICIENTS
CHOLESTEROL CHANGE VS. WEIGHT CHANGE

S,D,E (pooled)	+ 0.16
Sedentary	+ 0.18
Diet	+ 0.12
Exercise	- 0.35

Fat, as Estimated by Carcass Specific Gravity

The mean carcass specific gravities are listed in Table I. These specific gravities are of the same general

magnitude as those reported by Montoye and co-workers[4] (exercise rats = 1.0429 and sedentary rats = 1.0356). They are lower than those obtained by Montoye and co-workers[5] in a later study (exercise rats = 1.0761 and sedentary rats = 1.0640). This apparent inconsistency can be explained by the fact that in both the present study and the first-mentioned study, the animals at sacrifice were approximately ten weeks older than in the later study which reported higher values. There is evidence that in this period of time, a considerable increase in percentage body fat takes place.[6,7]

The analysis of variance for specific gravities indicates that the nonexercise animals (Group S= 1.0323 and Group D= 1.0256) were not significantly different in body density. They were significantly less dense (fatter) than Group E, which had a mean specific gravity of 1.0363. When analyzed by the "t" test, Group E was not significantly

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[4]H. J. Montoye, et al., "Effects of Exercise on Endurance and Organ Growth in Rats," Accepted for publication by Research Quarterly.

[5]H. J. Montoye, et al., Unpublished data, Michigan State University, Department of Health, Physical Education and Recreation, 1959.

[6]M. C. Conrad and A. T. Miller, "Age Changes in Body Size, Body Composition and Basal Metabolism," American Journal of Physiology, 186 (1956), 209.

[7]M. W. Marshall, et al., "Effect of Dietary Fats and Carbohydrates on Digestibility of Nitrogen and Energy Supply, and on Growth, Body Composition and Serum Cholesterol of Rats," Journal of Nutrition, 69 (1959), 371.

denser than Group S. All three groups (S, D & E) were significantly fatter (less dense) than Group A (1.0565), the initial control group sacrificed at the beginning of the experiment. This is in agreement with previous work which indicates that the percentage of body fat increases with age.[8,9]

TABLE VII

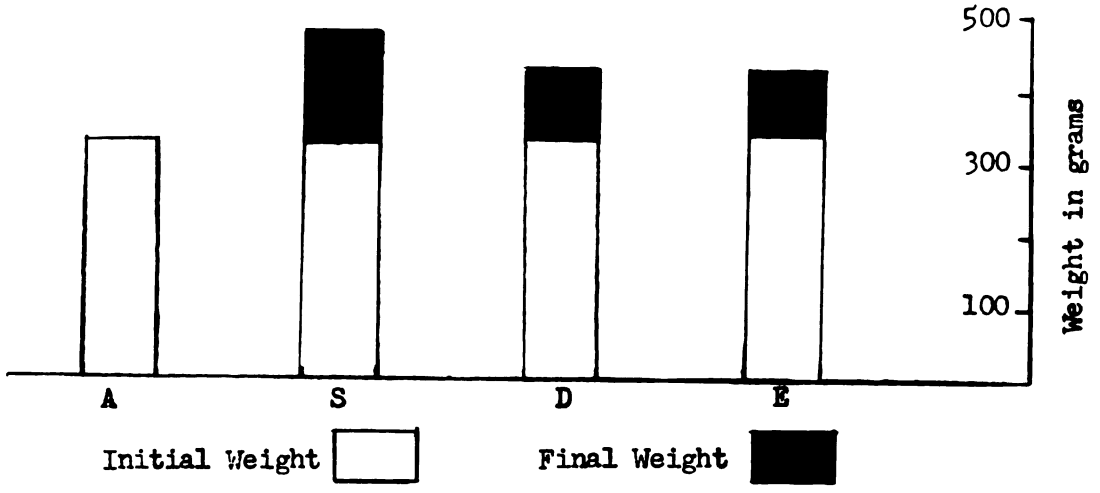
ANALYSIS OF VARIANCE
CARCASS SPECIFIC GRAVITY

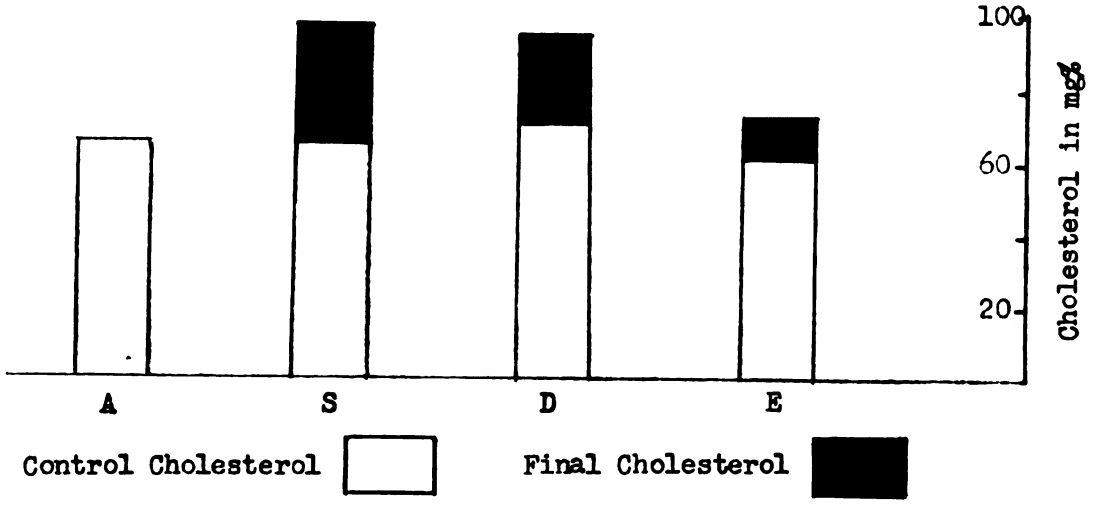
Source	SS	df	MS	"F"
Between Groups				
A vs. S vs. D vs. E	10943.26	3	3647.75	26.86**
Partitioned Comparisons				
S vs. D	484.54	1	484.54	3.57(ns)
E vs. S & D	732.20	1	732.20	5.39*
A vs. E, S, D	9727.26	1	9727.26	71.62**
Error	10865.75	80	135.82	. . . .
Total	21809.75	83	. . . .	. . . .

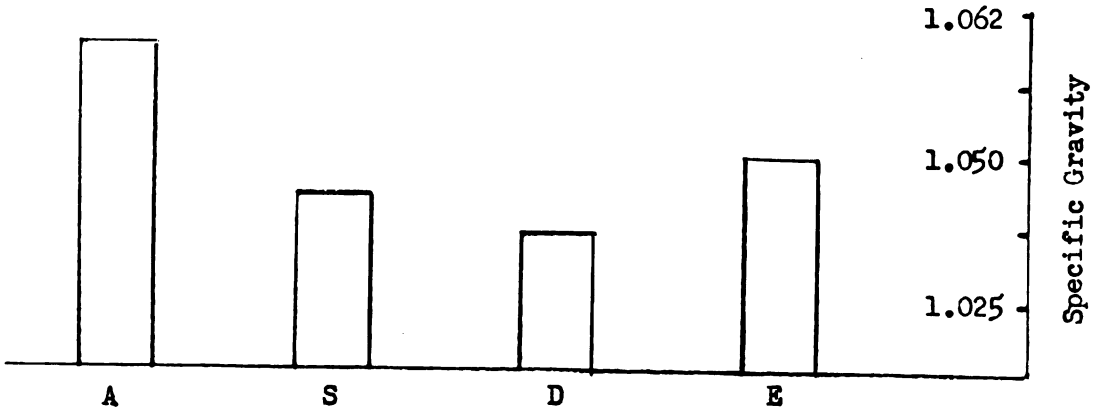
*Sig. at .025 level **Sig. at .005 level
Note: "t" for E vs. S = 1.15(ns)

[8] Ibid.

[9] Marshall, et al., op. cit.







Carcass Specific Gravity

COMPARISON OF MEAN VALUES

FIGURE 2

Carcass specific gravity was correlated with final cholesterol to test the possibility that cholesterol concentration is associated with percentage body fat. None of the correlations were significant.

TABLE VIII

CORRELATION COEFFICIENTS
CARCASS SPECIFIC GRAVITY VS. FINAL CHOLESTEROL

S,D,E (Pooled)	0.00
Sedentary	+ 0.10
Diet	+ 0.26
Exercise	+ 0.07

Discussion of Results

Cholesterol

The basic question involved in the present investigation, i.e., whether diet would be as effective as regular exercise in controlling serum cholesterol concentration, can be answered with reasonable certainty. The diet group, in spite of having a final mean weight equal to the exercise group, had a significantly higher cholesterol concentration (95.23 mg% as compared to 73.10 mg%). This difference was still significant even after removing the contributing effect of the control cholesterol levels.

It is also important to take note of the fact that the diet group, in spite of being significantly lighter than the sedentary group, did not have a significantly lower mean

cholesterol concentration. This tends to support the evidence which indicates that cholesterol levels increase with age.[10,11,12,13]

It is not surprising that the exercise group's mean cholesterol concentration (73.10 mg%) was significantly lower than the sedentary group (96.41 mg%). This is in agreement with the work done by Montoye and co-workers,[14] where the reported means for exercise and sedentary groups were 74.72 mg% and 93.08 mg%.

It is interesting to note that, after 15 weeks of three very different types of living among the three groups, there was still an overall significant positive relationship between the control cholesterol concentration and the final concentration. This lends support to the theory that cholesterol concentration is somehow predetermined and is

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[10] A. Keys, et al., "The Trend of Serum Cholesterol Levels With Age," Lancet, 263 (1952), 209.

[11] L. A. Lewis, et al., Serum Lipid Levels in Normal Persons," Circulation, 16 (1957), 236, 244.

[12] W. M. Sperry and M. Webb, "The Effect of Increasing Age on Serum Cholesterol Concentration," Journal of Biological Chemistry, 187 (1950), 110.

[13] Marshall, et al., op. cit.

[14] H. J. Montoye, et al., Unpublished data, Michigan State University, Department of Health, Physical Education and Recreation, 1959.

probably even inherited.[15,16] The very low, insignificant relationship within the exercise group may be an indication that regular exercise is effective in altering this pattern of seemingly predetermined blood cholesterol concentration.

Body Weight

Although the exercise and diet groups were equal in mean weight, and were significantly lighter than the sedentary group, there was no significant relationship between body weight and cholesterol concentration to explain the obtained differences in mean cholesterol concentration. This is in agreement with the evidence reported by Thomas and Garn[17] and Lewis and co-workers.[18]

The lack of a significant relationship between cholesterol change and weight change is seemingly contradictory to the work done by Montoye and co-workers[19] and

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[15] W. M. Sperry, "The Concentration of Total Cholesterol in the Blood Serum," Journal of Biological Chemistry, 117 (1937), 395.

[16] J. Groen, et al., "The Influence of Nutrition, Individuality and Some Other Factors, including Various Forms of Stress, on the Serum Cholesterol; An Experiment of Nine-Month's Duration in 60 Normal Human Volunteers," Voeding, 13 (1952), 556.

[17] C. B. Thomas and S. M. Garn, "Degree of Obesity and Serum Cholesterol Level," Science, 131 (1960), 42.

[18] Lewis, et al., op. cit.

[19] H. J. Montoye, et al., "The Effects of Exercise on Blood Cholesterol in Middle-Aged Men," American Journal of Clinical Nutrition," 7 (1959), 144.

others.[20,21,22] However, it should be remembered that the conditions of the present investigation and the conditions of the above mentioned studies were not actually comparable. The studies referred to involved mature, full-grown humans, capable of increasing or decreasing weight by means of caloric inbalance. The present animal investigation is complicated by one very important difference. Whereas these animals were also mature, the only way weight loss could have taken place would have been through some form of semi-starvation. This would have further complicated the issue. So, where the human studies are involved in weight reduction and weight gain as related to cholesterol reduction or increase, the present animal study could only be involved in controlling weight gain and controlling cholesterol increase. It should also be pointed out that the weight control involved here was gradual, taking place over a period of 15 weeks, and not a case of rapid weight loss as is often the case in the human studies. We would not expect, therefore, to obtain similar results from two such different situations.

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[20] G. V. Mann, et al., "Exercise in the Disposition of Dietary Calories," New England Journal of Medicine, 253 (1955), 355.

[21] J. T. Anderson, F. Grande and A. Keys, "Serum Cholesterol Concentration of Men in Semi-Starvation and in Refeeding," Federation Proceedings, 14 (1955), 426.

[22] J. T. Anderson, A. Lawler and A. Keys, "Weight Gain from Simple Overeating. II. Serum Lipids and Blood Volume," Journal of Clinical Investigation, 36 (1957), 87.

Due to the insignificant relationship it is concluded that control of weight gain did not account for differences in cholesterol level. The fact that the exercise group was significantly lighter than the sedentary group cannot be said to account for the fact that their mean cholesterol concentration also was significantly lower.

Fat, as Estimated by Carcass Specific Gravity

One would expect to find exercise animals leaner (more dense) than nonexercise animals. Such was the case in the present study when the exercise group was compared to the sedentary groups (S and D). The diet and sedentary groups, in spite of a significant difference in weight, were not significantly different in body density. This is an obvious contradiction to the logical assumption that sedentary animals fed ad libitum should be fatter because they presumably eat more and gain more weight. However, there is no assurance that differences in weight must be fat, for several reasons. First, it is impossible to know the amount eaten by the sedentary animals, as they were fed ad libitum. It is not entirely impossible that they ate no more than the diet animals. It is generally recognized that rats, unlike man, are more obedient to their homeostatic "built-in hunger control." And, for this reason, being inactive does not in itself cause obesity.[23] Second, although every precaution

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[23] A. C. Guyton, Textbook of Medical Physiology, W. B. Saunders Co., Philadelphia, 1958, 837.

was taken to insure that the diet rats' minimum daily rations contained all the ingredients necessary for normal growth, the constant caloric restriction may have had some effect on skeletal and, or, muscle development. Third, hunger being a form of stress, the poorly understood hormonal phenomena involved in stress may have had some effect here. Increased fat deposition in the diet group may have taken place in response to stress. Theoretically, this might occur through an increased pituitary adrenocortotrophic secretion and its resulting effect of increased glucocorticoid secretion by the adrenal cortex.[24]

Even though the exercise group was more dense (1.0363) than the sedentary group (1.0323), the lack of a statistically significant difference here was unexpected. Of course, the acceptance of the null hypothesis makes this result inconclusive. It cannot be said with reasonable certainty that there was a difference, but neither can it be concluded for certain that no difference exists. If there truly was no difference, the one plausible explanation is that the vigorous forced exercise was a stress and may have retarded skeletal and, or muscle development in the exercise rats to some extent. This, then, would mean that the big difference in body weight was not all due to excess fat.

In spite of the significant specific gravity difference between the exercise group and the nonexercise

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[24] Ibid., p. 902-903.

animals, no significant correlation between specific gravity and cholesterol concentration was found. This agrees with the work of Thomas and Garn,[25] Gofman and Jones,[26] and Keys,[27] whose indirect fat measures in humans were not significantly correlated with blood cholesterol level. One case of disagreement, however, is with the results of Montoye and co-workers.[28] They reported a significant correlation of -0.42. This is a difficult contradiction to explain, unless the previously discussed diet, caloric restriction and stress factors somehow affected the results.

At any rate, it cannot be concluded that differences in body fat account for the obtained significant differences in mean cholesterol levels.

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[25] Thomas and Garn, op.cit.

[26] J. W. Gofman and H. B. Jones, "Obesity, Fat Metabolism and Cardiovascular Disease," Circulation, 5 (1952), 515.

[27] A. Keys, et al., "Studies on Serum Cholesterol and Other Characteristics of Clinically Healthy Men in Naples," Archives of Internal Medicine, 93 (1954), 332.

[28] H. J. Montoye, et al., Unpublished data, Michigan State University, Department of Health, Physical Education and Recreation, 1959.

CHAPTER V

SUMMARY AND CONCLUSIONS

Summary

In an effort to evaluate the relative effects of regular exercise and diet in controlling total blood cholesterol level, blood samples were taken from eighty-eight mature Carworth male albino rats for serum cholesterol assay. The animals were then divided into four groups equated on the basis of body weights. One group, an initial control group (Group A) was sacrificed immediately. The other three were maintained for a fifteen-week experimental period. One group (Group S) was sedentary and fed ad libitum. One experimental group (Group E) was exercised regularly and fed ad libitum. A second experimental group (Group D) was sedentary, but maintained at a mean body weight comparable to that of Group E by careful diet restriction.

In addition to determining whether weight control by caloric restriction is as effective as exercise in controlling blood cholesterol, the experiment was designed to test whether differences in body weight and body fat could account for any differences in blood cholesterol.

Control and final total blood cholesterol concentrations and final carcass specific gravities, using the under-water weighing method, were determined. Control and final body weights also were recorded.

The data were analyzed statistically, employing analysis of variance, analysis of covariance, students "t", and product-moment correlation coefficients.

After considering the results and their statistical inferences, the conclusions in the following section were taken from the more general discussion of results.

Conclusions

1. Caloric restriction was not as effective in controlling total blood cholesterol as was regular exercise. The mean cholesterol difference between the diet group and exercise group was significant even after removing the effects of the control cholesterol concentrations.

2. The significant positive correlation between control and final cholesterol concentrations strengthens the theory that there is some predetermined, probably inherent factor which controls cholesterol concentration. However, this relationship did not hold true within the exercise group. It is possible, then, that exercise is a factor which can alter this seemingly predetermined cholesterol concentration.

3. Although the sedentary group was significantly heavier than the diet and exercise groups, body weight was not significantly correlated with final cholesterol concentration. Therefore it cannot be concluded that high blood cholesterol and high body weight are associated.

4. Although the nonexercise animals were significantly less dense than the exercise animals, specific

gravity was not significantly correlated with cholesterol concentration. Hence, it cannot be concluded that high blood cholesterols are associated with high percentage of body fat.

5. There was no significant relationship between cholesterol change and weight change, which is not surprising in view of the lack of relationship between weight and cholesterol concentration.

6. The results seem to support the hypothesis that over and above its effect on weight and obesity, there is some effect of exercise which helps control blood cholesterol concentration. This lends credence to Mann's[1] contention that "exercise is likely to have a biochemical relation to lipid metabolism."

Recommendations for Further Study

The evidence indicates that exercise has some direct effect on blood cholesterol. The next step, if a repeat of the present study confirms these results, is to investigate the exact means by which this effect is exerted. In any subsequent work, it is recommended that a careful check of food intake be maintained to add to the control of the study. It would also be extremely valuable to make periodic checks to assure normal and equal growth (skeletal and muscular) in each group.

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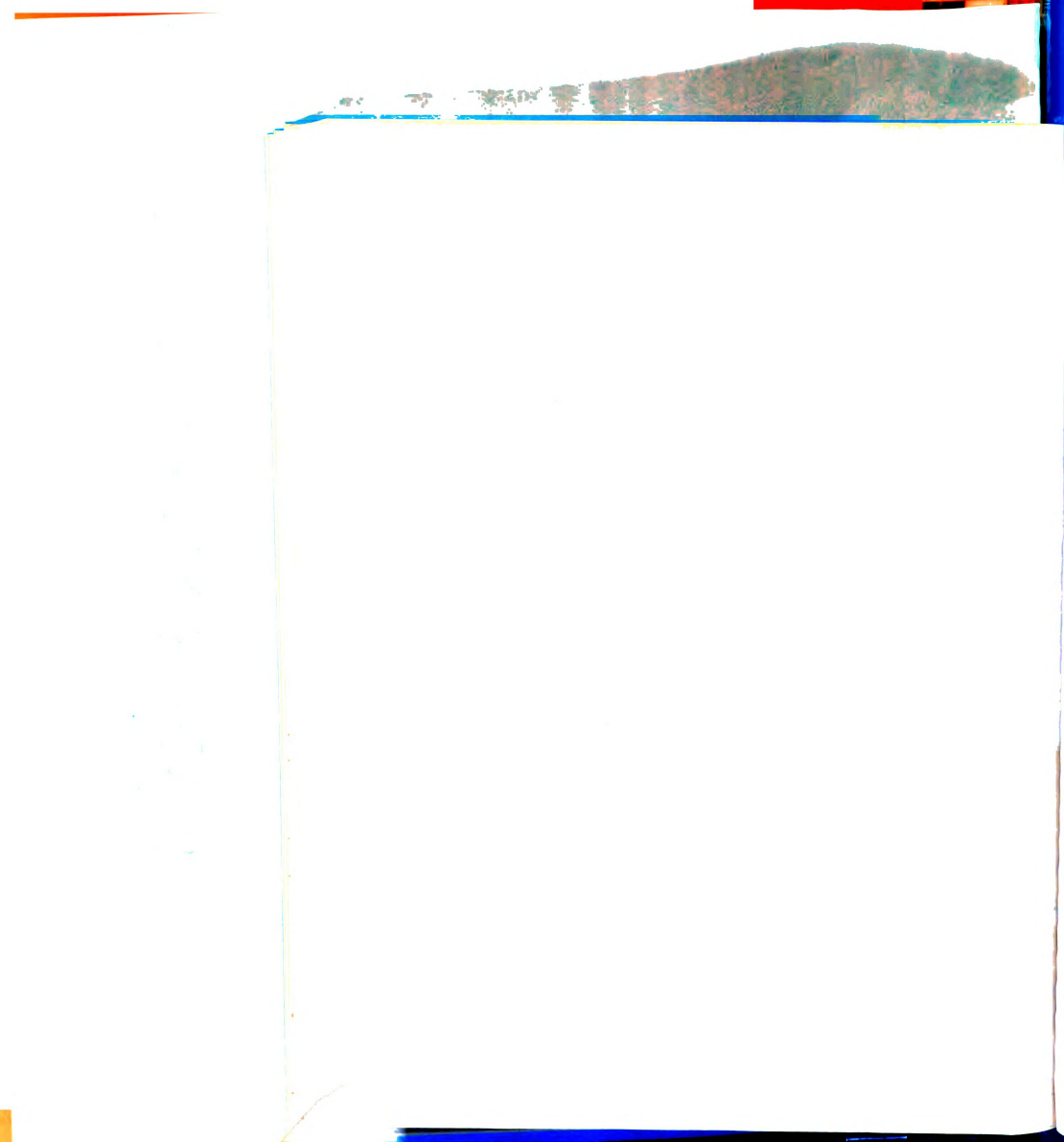
[1]C. V. Mann, et al., "Exercise in the Disposition of Dietary Calories," New England Journal of Medicine, 253 (1955), 349.



The next step might be to use animals which have reached a weight gain "plateau." Groups could then be equated on the basis of control cholesterol concentrations and one group sacrificed to give an estimate of control specific gravity. The experiment could then be concerned with weight reduction and cholesterol decrease and more closely parallel work with humans.

There is a need for investigating in more detail the specific gravity results. These results, if confirmed, mean revising our present beliefs concerning diet, exercise and body fat. There are several interesting possible explanations, as mentioned in the discussion. However, the first logical step is to confirm or refute these results. As part of the overall project, the carcasses were placed in frozen storage for this purpose. If the direct carcass fat assessments, when completed, confirm the results of the present study, we can proceed in further investigating this new concept.

The need for investigating the effects of strenuous exercise on growth has also been pointed up. It is important to know the point beyond which exercise becomes too strenuous to permit normal skeletal and muscular growth. This is important in ways other than its relationship to blood cholesterol, and could be very conveniently studied with rats.

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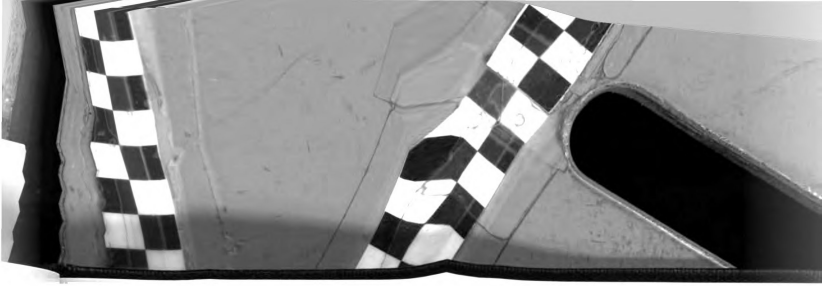
APPENDIX



APPENDIX A

SAMPLE OF DIET GROUP RATION CALCULATION

Rat #	Difference[1]	Last Ration[2]	Loss or Gain[3]	New Ration
14	+12	2	- 5	2
64	-22	AL	+ 7	AL
15	+10	4	+15	2.5
80	- 6	2.5	+ 1	3.5
53	+12	3.5	+14	2.5
89	-33	AL	+ 7	AL
13	+ 6	2.5	+ 5	2
99	+15	3.5	-11	3.5
4	-44	AL	- 4	AL
49	+ 7	3.5	-13	4
41	+12	4.5	+32	3
5	+ 4	3.5	+ 3	3.5
26	0	AL	- 4	AL
75	+ 4	2.5	+12	2
72	- 4	2	-13	3.5
1	+12	4	+21	2
76	+ 4	3.5	+19	2.5
47	-15	5	+ 3	AL
68	- 8	4	+ 8	4
86	+14	3.5	+15	2.5
46	+ 6	4	+24	2
9	+10	1.5	-13	1.5

[1]Difference= weight difference from Group E paired mate.

[2]Last ration= daily ration last week. Figures are in inches of Lab-Blox, one inch= approximately four grams of food.

[3]Loss or gain= weight lost or gained since last weighing.

The new ration was based on the last daily ration, the weight lost or gained on that ration, and the weight difference from the animal's paired mate.

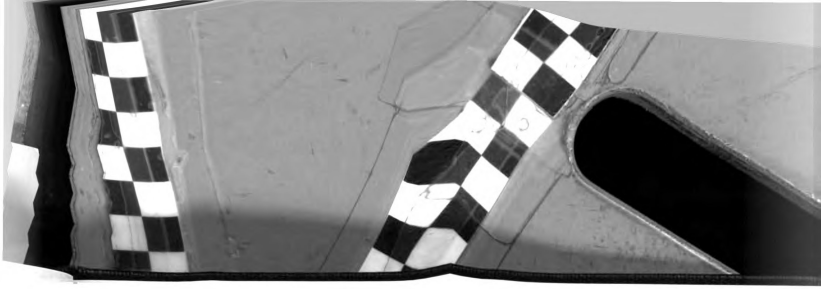
AL= ad libitum

APPENDIX B

COMPOSITION OF DIET[1]

Protein (min)	%	24.00
Fat (min)	%	4.00
Fiber (min)	%	4.50
Ash	%	8.60
Calcium	%	1.30
Phosphorous	%	1.00
Sodium	%	0.30
Potassium	%	1.00
Chlorine	%	0.60
Magnesium	%	0.20
Iron	PPM	340.00
Copper	PPM	11.00
Maganese	PPM	170.00
Zinc	PPM	30.00
Cobalt	PPM	0.60
Iodine	PPM	10.00
Thiamine	PPM	6.50
Riboflavin	PPM	6.50
Niacin	PPM	60.00
Calcium Pantothenate	PPM	18.00
Choline	PPM	1850.00
Vitamine E	PPM	35.00
Carotene	PPM	2.00
Vitamine A	USP units per lb.	5600
Vitamine D	USP units per lb.	2000

[1]Wayne Lab-Blox (mice and rats) Allied Mills, Inc.



APPENDIX C

TOTAL CHOLESTEROL DETERMINATION

Reagents

Absolute alchol - Acetone Soln. (1:1)
50% Potassium Hydroxide (5 grams - 10cc water)
Phenolphthalein
0.5% digitonin soln. (500 mg - 100 cc 50% alchol)
Heat to dissolve
Absolute anhydrous ether
Acetone - absolute anhydrous ether (1:1)
Glacial acetic acid
Con. sulfuric acid
Acetic anhydride

Extraction

1. Place 3 cc absolute alchol-acetone (1:1) into a 5ml. vol. flask.
2. Add 0.4 cc serum to form Ppt.
3. Bring to boil on steam bath.
4. Cool. Make to volume with abs. alchol-acetone mixture.
5. Filter through fat-free filter paper.

Hydrolysis and Precipitation

1. Pipette a 1 ml. sample of extract to determine total cholesterol. (Triplicates) Place in 5 cc centrifuge tube.
2. Add a drop of 50% potassium hydroxide and stir. Hold on water bath (37 to 40°C.) for 45 minutes.
3. Cool. Add 1 drop of phenolphthalein and titrate with 10% acetic acid in alcohol. (1 drop excess acid). Stir.
4. Add 2 cc of 0.5% alcholic digitonin to each tube. Stir.
5. Flocculate, 60°C (one hour). Stir. Remove rods carefully.
6. Cover and let stand overnight.

Washing the Precipitate

1. Remove stirring rod and place on rack so that the same rod will return to the same tube each time stirring is required.
2. Centrifuge 15 minutes at a centrifuge setting of 35.
3. Carefully pour off liquid portion.
4. Wash with acetone-ether (1:1) (absolute ether) solution, stirring and then centrifuge for 10 minutes, and remove liquid by pouring.

5. Repeat washing 2 times with absolute anhydrous ether.
6. Dry in sand bath at 110-115°C. till no trace of ether is detected (one hour).

Development of Color

1. Pipette 1 cc. glacial acetic acid into centrifuge tube to dissolve the precipitate. Stir with the same stirring rod used throughout.
2. Cool to room temperature.
3. Add 2 ml. freshly prepared cold (use ice bath) acetic anhydride and sulfuric acid (20:1) solution. Stir. Read 27 or 30 min. Later in Beckman Spectrophotometer at 620 mu.

Standard Curve

Prepare a series of standards 0.01 to 0.15 mg. cholesterol per ml. of glacial acetic acid and treat in the same manner beginning with the development of color process.

Plot optical density us. mg. chol/cc acetic acid.

Calculation

From the reading on the standard curve multiplied by a factor of 1250 you will get the mg. of cholesterol per 100 cc of blood.

$$\frac{5 \text{ ml. (total vol. of extract)}}{.4 \text{ ml. (Vol. serum)}} \times \frac{100}{1} = 1250 \text{ (factor)}$$

1250 X reading on curve = mg. cholesterol per 100 cc. of blood.

APPENDIX D

RAW DATA- GROUP A

N = 22

Rat #	Final Wt.[1]	Carcass Wt.(Dry)[1]	Carcass Wt.(UW)[2]	Carcass Sp.Gr.[3]	Final Chol.[4]
2	330	279.6	14.331	51	72
3	343	293.8	12.100	41	79
8	318	269.2	12.251	45	48
18	302	254.3	13.891	55	46
21	285	237.1	11.452	48	83
31	300	253.3	16.729	69	50
33	337	290.5	17.096	61	72
42	296	251.1	14.280	58	76
43	300	247.5	15.948	67	66
48	360	301.5	20.480	71	70
51	325	273.6	15.981	60	87
57	324	272.4	18.499	71	66
60	346	295.7	19.354	68	59
65	323	277.7	15.378	57	73
70	309	263.1	16.310	64	51
71	342	295.4	19.370	70	56
79	382	322.8	19.090	61	59
82	355	303.8	8.895	28	46
90	373	312.8	11.386	36	78
95	370	306.7	14.510	49	94

[1] Weight in grams

[2] Carcass weight under water

[3] 1.0 is omitted; i.e., 1.044 is specific gravity for rat #6

[4] Serum cholesterol in mg%

RAW DATA - GROUP S

N = 22

Rat #	Init. Wt.[1]	Final Wt.[1]	Carcass Wt.(Dry)[1]	Carcass Wt.(UW)[2]	Carcass Sp.Gr.[3]	Control Chol.[4]	Final Chol.[4]
6	353	598	519.5	22.15	44	58	118
7	304	467	405.3	11.88	29	90	93
10	342	520	448.0	19.32	44	68	92
11	316	425	363.2	16.22	46	55	107
12	331	447	396.9	14.43	37	65	78
22	333	494	440.0	10.43	23	112	165
23	370	554	480.7	13.31	28	49	115
28	290	446	385.2	14.70	39	70	150
30	342	482	413.4	21.46	53	42	84
32	297	460	397.0	15.37	39	54	123
36	323	462	396.9	15.58	40	80	88
44	337	539	469.3	10.74	22	75	97
59	306	434	392.5	6.62	16	77	66
56	346	494	434.5	11.80	27	65	102
58	295	451	394.8	11.32	28	56	68
61	331	466	409.2	15.72	39	62	73
62	269	375	325.7	6.92	21	58	73
63	312	451	393.5	13.88	36	82	85
66	365	595	524.6	13.95	26	79	108
73	327	460	417.2	18.02	44	53	72
74	334	467	409.6	-2.87	-08*	54	79
87	379	375	325.0	11.90	37	44	85

[1]Weight in grams

[2]Carcass weight under water

[3]1.0 is omitted; i.e., 1.044 is specific gravity for #6

*Rat #74 specific gravity = 0.992

[4]Serum cholesterol in mg%

RAW DATA - GROUP D

N = 22

Rat #	Init. Wt.[1]	Final Wt.[1]	Carcass Wt.(Dry)[1]	Carcass Wt.(UW)[2]	Carcass Sp.Gr.[3]	Control Chol.[4]	Final Chol.[4]
14	309	366	315.0	6.19	19	64	83
64	312	400	348.6	3.07	08	80	103
15	332	433	380.1	16.31	44	65	105
80	364	462	400.5	9.35	23	43	102
53	327	438	384.2	6.58	16	77	58
89	362	465	411.5	12.33	30	79	91
13	338	421	367.0	8.41	23	57	79
99	340	425	371.9	12.64	34	55	56
4	335	432	372.5	15.29	42	72	129
49	295	391	343.1	8.77	25	72	109
41	344	422	364.4	9.09	25	93	143
5	290	425	363.6	7.88	21	68	93
26	306	407	365.8	9.47	26	53	107
75	318	398	346.0	8.75	25	84	81
72	349	495	428.9	15.17	36	83	108
1	327	439	379.0	12.43	33	70	110
76	376	462	406.4	5.32	12	68	83
47	346	442	388.9	12.96	34	63	95
68	331	435	382.8	10.59	28	122	119
86	305	371	324.7	-2.37	-08*	49	86
46	281	353	298.6	11.55	39	56	86
9	300	406	345.8	9.96	29	66	71

[1]Weight in grams

[2]Carcass weight under water

[3]1.0 is omitted; i.e., 1.019 is specific gravity for #14

*Rat #86 specific gravity = 0.992

[4]Serum Cholesterol is mg%

RAW DATA - GROUP E

N = 20

Rat #	Init. Wt.[1]	Final Wt.[1]	Carcass Wt.(Dry)[1]	Carcass Wt.(UW)[2]	Carcass Sp.Gr.[3]	Control Chol.[4]	Final Chol.[4]
16	307	364	309.8	12.72	42	53	85
17	304	429	363.4	17.90	51	64	51
19	327	402	353.6	14.46	42	66	46
24	333	438	369.8	14.58	40	66	64
25	340	427	373.5	15.12	41	63	49
27	341	484	423.1	9.99	23	65	59
29	329	430	372.8	5.47	14	45	44
34	337	415	356.4	12.07	34	55	50
37	365	476	414.0	18.02	45	62	65
38	298	377	323.3	13.50	43	56	69
39	333	416	358.5	9.62	28	66	73
54	294	392	337.9	11.37	34	57	99
67	346	491	432.3	17.37	41	67	83
77	338	428	374.9	13.96	38	44	78
78	379	452	396.2	10.33	26	47	88
84	354	458	401.7	12.05	30	47	75
81	333	436	373.6	14.98	41	63	91
83	311	365	313.8	13.22	43	67	90
85	295	353	298.2	9.26	31	62	95
40	320	390	339.4	12.77	38	70	108

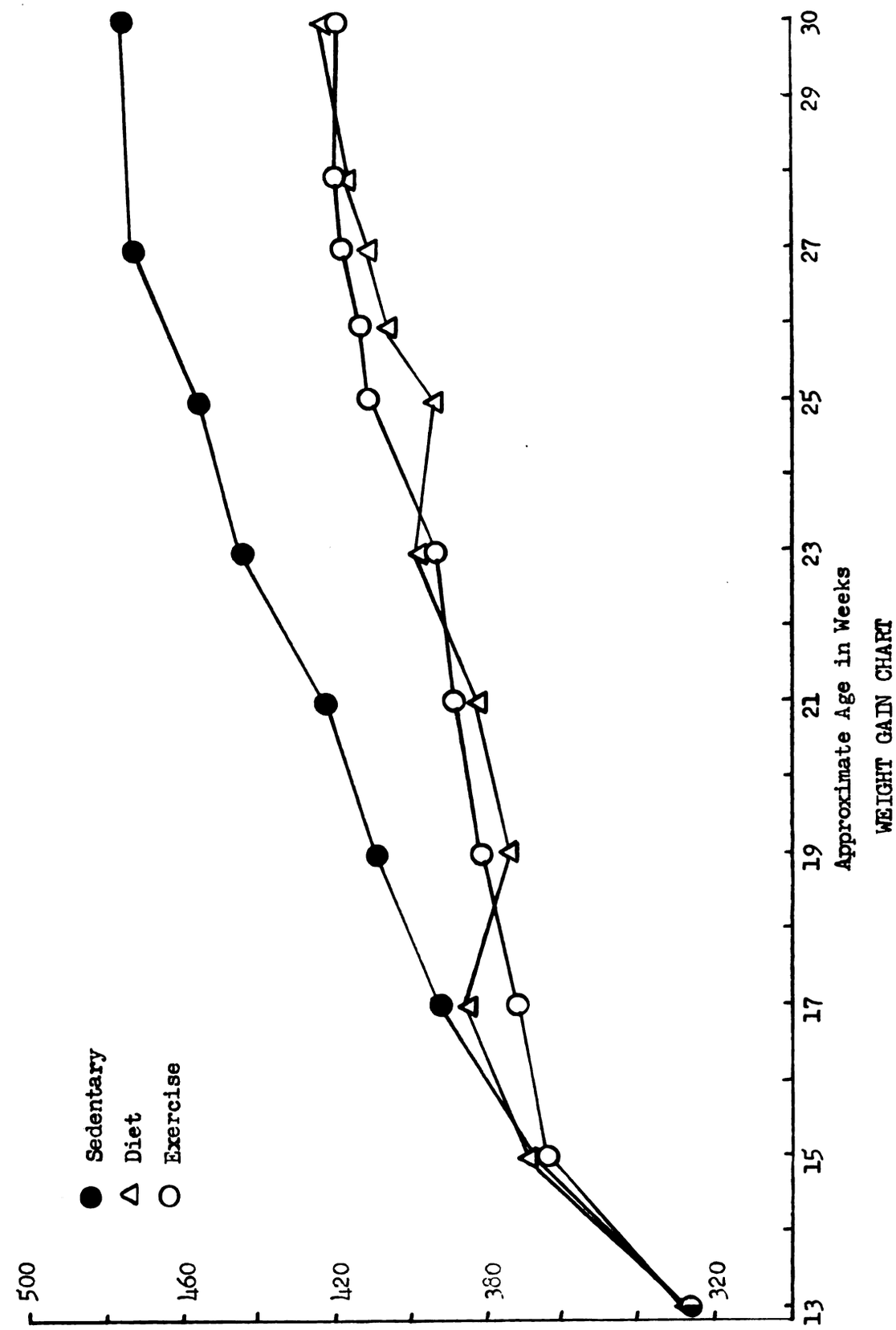
[1]Weight in grams

[2]Carcass weight under water

[3]1.0 is omitted; i.e., 1.042 is specific gravity for #16

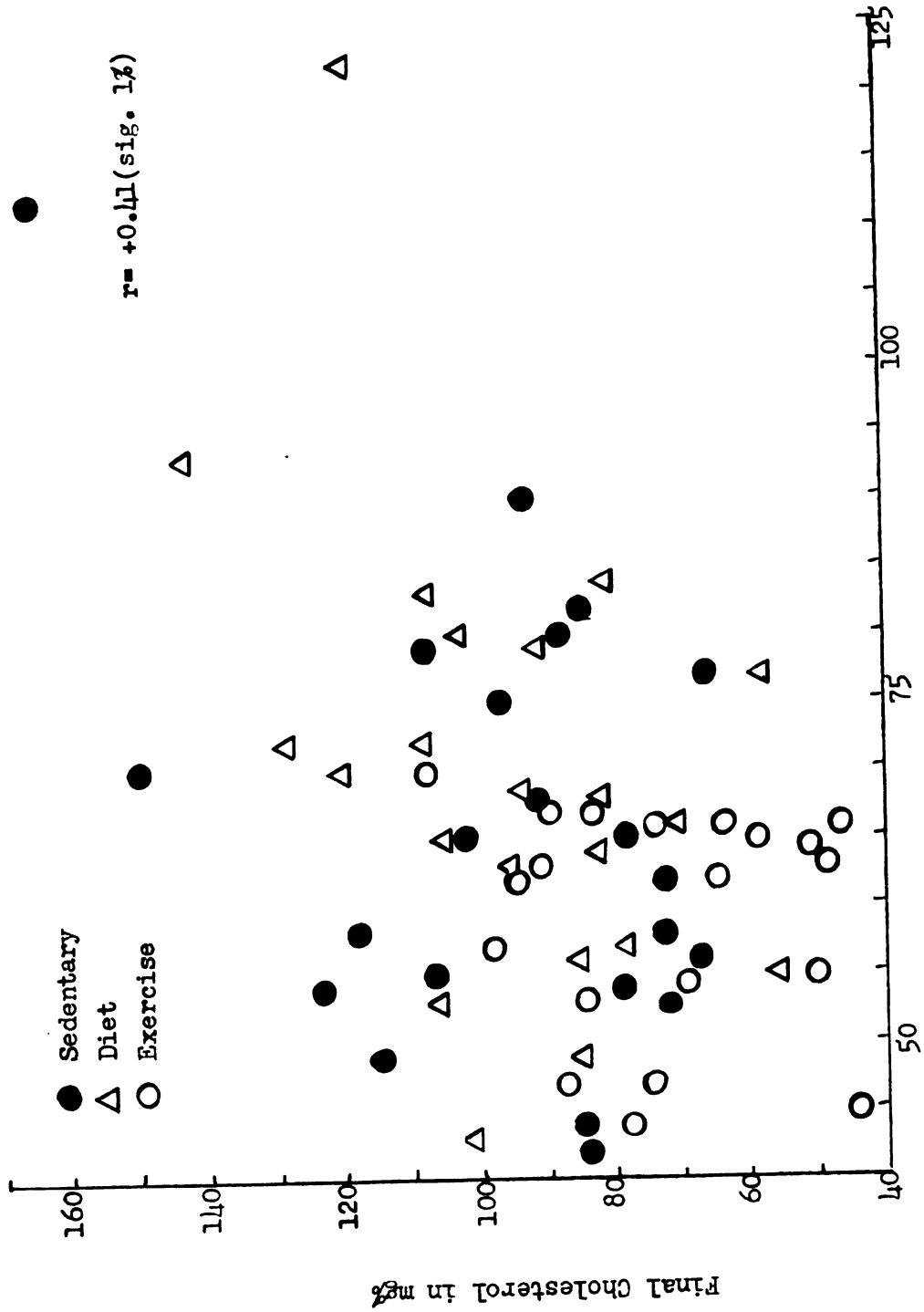
[4]Serum cholesterol in mg%

APPENDIX E

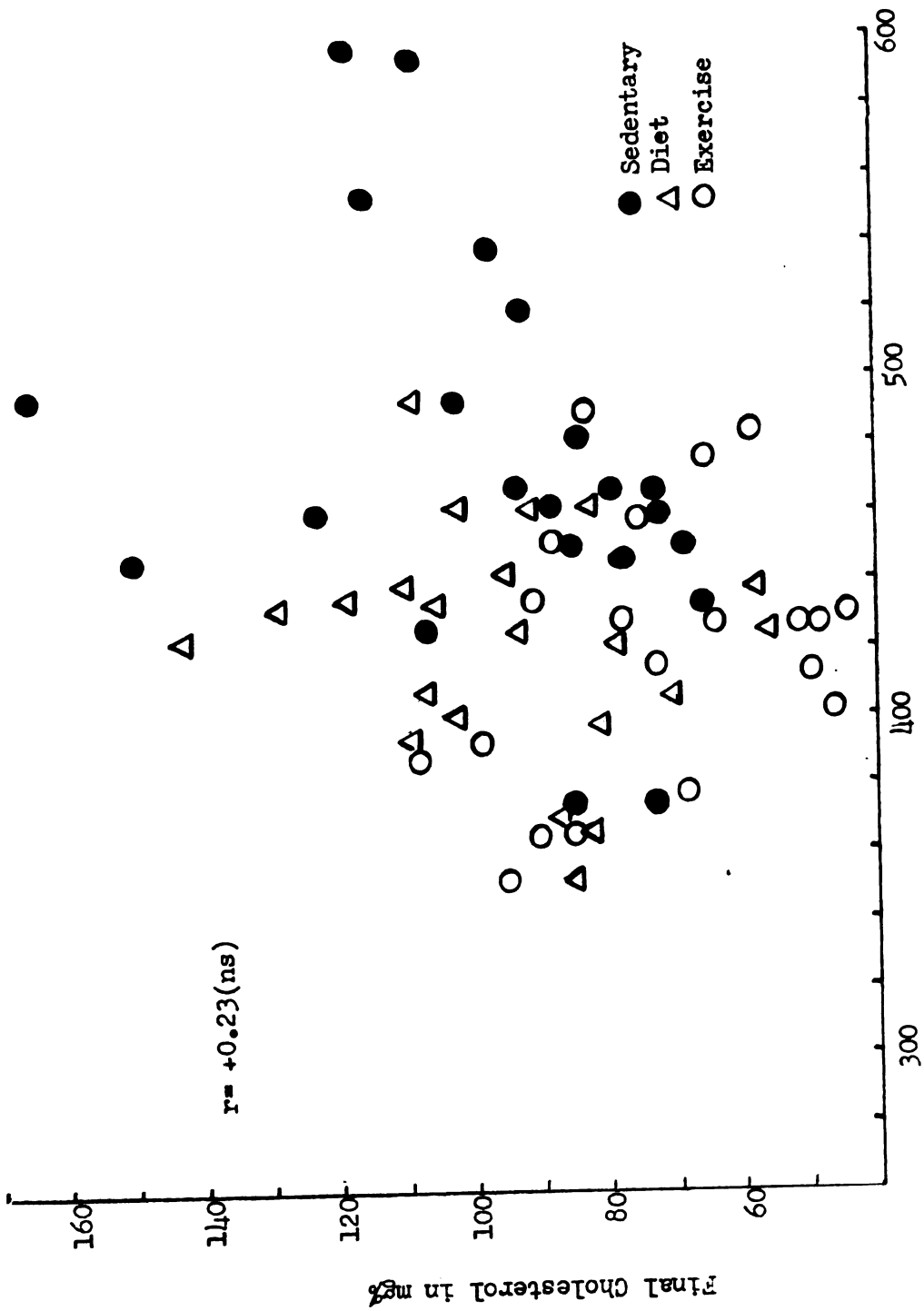


WEIGHT GAIN CHART

APPENDIX F

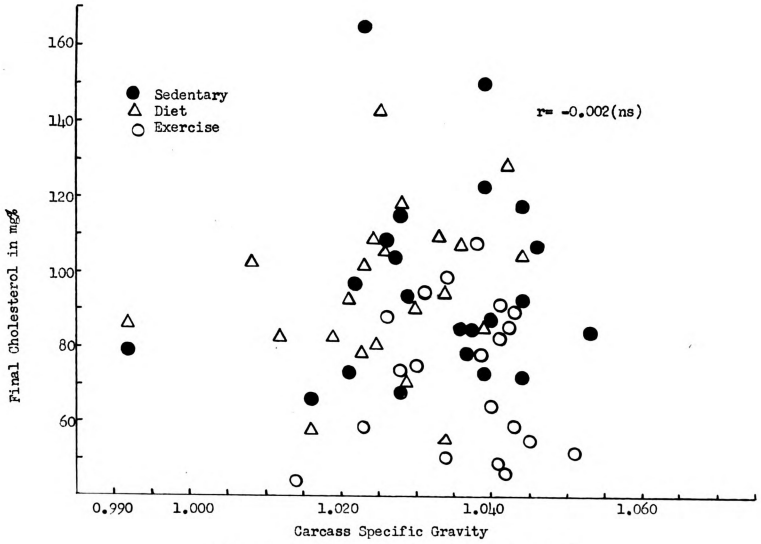


CONTROL CHOLESTEROL VS. FINAL CHOLESTEROL



FINAL BODY WEIGHT VS. FINAL CHOLESTEROL

WEIGHT CHANGE VS. CHOLESTEROL CHANGE



CARCASS SPECIFIC GRAVITY VS. FINAL CHOLESTEROL