THE EFFECT OF EXERCISE AND CALORIC RESTRICTION ON LIVER CHOLESTEROL IN THE RAT

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Sister M. John Martin Martin, O. P.

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ABSTRACT

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By Sister M. John Martin Martin, O.P.

The high incidence of cardiovascular disease presents a serious problem in the United States. Epidemiological studies showed a statistical relationship between the incidence of coronary heart disease and atherosclerosis. The presence of large amounts of cholesterol in the blood and in the deposits in the intima of the arteries of atherosclerotic persons supports the hypothesis that altered cholesterol metabolism is involved. Cholesterol metabolism is influenced by many factors including the type and amount of deitary fat, caloric balance, age, sex and physical exercise. Since the liver is the principal site of cholesterol synthesis, it seems to be a better basis for evaluation of an individual's cholesterol status than serum cholesterol concentrations. The present investigation was designed to study the relative effects of exercise and caloric restriction on liver cholesterol in the rat.

Eighty-eight male rats (Carworth) were divided into four groups equated on the basis of body weight. The initial controls (Group A) were sacrificed at the begin-

ning of the experimental period. The remaining animals were maintained on a stock diet for fifteen weeks. The sedentary controls (Group S) were fed ad libitum; the exercise animals (Group E) were fed ad libitum and subjected to an exercise regimen; the diet-restricted animals (Group D) were sedentary and were fed restricted rations to maintain body weights comparable to those of the exercise animals.

The livers were macerated and the lipids were extracted with ethyl alcohol and ethyl ether. The liver lipids were determined gravimetrically after evaporation of the solvents from aliquots of the extracts. Free and total cholesterols were measured in suitably treated aliquots of the lipid extracts. The cholesterol was precipitated as the digitonide and analyzed colorimetrically.

Liver weights of Group A (mean, 9.92 gm.) were significantly less than the older animals of Groups S, D and E. The livers of the sedentary group (mean, 12.99 gm.) were larger than those of the other two groups. No difference in liver weights was observed between the exercise (mean, 11.82 gm.) and the diet-restricted animals (mean, 11.89 gm.). The correlation coefficient for the combined groups ($r = 0.630^{**}$) indicated a positive relationship

between body weight and liver weight.

The analysis of variance for liver lipids in mg./liver showed significant differences due to treatment. The means were 0.662 gm., 0.971, 0.885, and 0.789 for Groups A, S, D and E, respectively. The sedentary (7.50 per cent) and the diet-restricted rats (7.46 per cent) had significantly higher percentages of liver lipids than either the initial control (6.74 per cent) or the exercise animals (6.70 per cent). Liver lipids were correlated positively with body fat and liver weight.

No significant differences in total and free liver cholesterol in mg./liver were observed between the diet-restricted and exercise groups. The total and free cholesterol concentrations of the younger animals were less than those of the groups of older animals; the sedentary rats had higher liver cholesterol concentrations than any of the other animals.

No correlations between total serum cholesterol and total liver cholesterol were observed. Although exercise was more effective than caloric restriction in controlling serum cholesterol concentrations, both exercise and caloric restriction exerted a similar influence on liver cholesterol.

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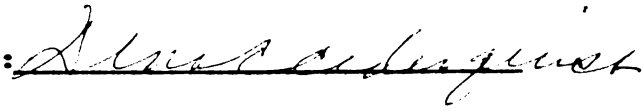
Approved by: 

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INTRODUCTION

The high incidence of cardiovascular disease in middle-aged persons thwarts man's quest for longevity. In the United States of America, the leading cause of death is coronary disease. Epidemiological studies showed a statistical relationship between the incidence of coronary heart disease and atherosclerosis. The presence of large amounts of cholesterol in the blood and in the deposits in the intima of the arteries of atherosclerotic persons supports the hypothesis that altered cholesterol metabolism is involved. Cholesterol metabolism is influenced by many factors including the type and amount of dietary fat, caloric balance, age, sex and physical exercise.

Although the physical activity of the average American has decreased over the last five decades and the incidence of coronary disease has increased, there is insufficient evidence to substantiate or refute a causal relationship. Caloric imbalance is closely related to the problem of inactivity. This is particularly true for aging individuals who tend to keep food intakes rather

constant even though they have decreasing calorie expenditures.

The present experiment was designed to study the relative effects of exercise and caloric restriction on liver lipids and cholesterol in the rat.

REVIEW OF LITERATURE

The hypothesis exercise plays an important role as a therapeutic and preventive agent in controlling serum and liver lipids and cholesterol concentrations is sustained by new evidence from both laboratory experiments and epidemiological studies. The association of decreased physical activity with positive caloric balance cannot be overlooked in the problem of atherosclerosis. Studies which are concerned with the effects of physical activity and caloric restriction on serum and liver lipids are reviewed.

Factors Related to Atherogenesis

Physical activity

In 1953 Morris and co-workers[1] studied coronary heart disease in a group of bus drivers and conductors in England. The incidence was higher and the severity was greater in drivers than in conductors. These findings led to a similar study of postal men who were physically active and civil servants engaged in sedentary occupations. The observations resembled those made for

transport workers; the incidence and severity of the disease was less in the postmen than in the sedentary groups. This hypothesis was supported also by an analysis of the Registrar-General's occupational mortality data.[2]

Mann and associates[3] observed differences in serum lipids of Guatemalans and North Americans. The rural Guatemalans were primarily manual laborers by occupation and had low-fat diets; the urban Guatemalans and North Americans were chiefly business or professional people and had high-fat diets. The rural Guatemalans had considerably lower serum cholesterol concentrations than either of the latter groups. The beta-lipoproteins were slightly lower in rural Guatemalan males than in North American males, but were frequently higher in rural Guatemalan females than in the comparable North American group. These investigators were of the opinion the fat content of the diet did not account for the differences in the serum lipid values since it did not permit an explanation of the dissociation of cholesterol and lipoprotein measurements.

Mann and co-workers[4] found the serum cholesterols of Nigerian men were considerably lower than those of Americans; but the relative concentrations of the beta-lipoprotein fractions were similar. The fat contents of the

diets were not considered significant factors. The authors hypothesized the low serum cholesterol of the Nigerian, like that of the Guatemalan, resulted from the muscular activity and energy expenditures of these groups.

To test this hypothesis in the laboratory, Mann et al.[5] studied three male undergraduates who were placed on a diet which contained twice as many calories but the same amount of fat as the normal diet. When increased physical activity burned the excess calories, the beta-lipoprotein, phospholipid, the total and free cholesterol concentrations remained unchanged; but as soon as exercise was restricted, positive caloric balance resulted. This was accompanied by weight gain and increased serum lipid values. The authors concluded the exercise was operative in preventing a positive caloric balance and the associated hyperlipemia.

Montoye and associates[6] reported the effects of exercise on blood cholesterol in middle-aged men. Thirty-one adult males were divided into a control group and an exercise group. The exercise group were under supervised exercise for three months, while the control group continued their routine activities. The subjects were categorized as "normal" or "high" depending on the initial serum cholesterol concentrations. No significant differ-

ences were observed between the initial and final serum cholesterol concentrations in the "normal" men. However, an appreciable decrease was noted in subjects with "high" initial serum cholesterol. When the subjects of both groups were combined, change in total serum cholesterol usually accompanied change in body weight even though the subject did not exercise. The investigators concluded exercise was indirectly effective in decreasing serum cholesterol by decreasing body weight.

In a contemporary epidemiological investigation of Yemenite immigrants, Toor and associates[7] observed lower serum cholesterol and total lipid concentrations in the recent immigrants than in the earlier migrants. The atherosclerosis mortality rate was four times higher for the individuals of the same age and sex in the latter than in the former group. The differences were due largely to dietary habits and occupational activity. Furthermore, the recent Yemenite immigrants worked harder and remained in a state of caloric imbalance. After nine or ten years in Israel[8] the recent Yemenite immigrants had improved their socio-economic status due to change in occupation. An increase of 43 per cent in their total caloric intake as well as a rise of 7 per cent in calories derived from fats was found. This transition was accompanied by ele-

vated serum cholesterol concentrations especially in men from 45 to 65 years old. It was assumed that the rise in serum cholesterol in the "semi-recent" Yemenites was due to positive caloric balance which resulted from overall higher caloric and fat intakes and the decreased activity.

Brown and co-workers[9] studied the effect of mechanically-induced exercise in cholesterol-fed rabbits. Exercise was effective in lowering total serum cholesterol concentrations. However, it was ineffective in inhibiting atheromatous deposits in the aorta and major arteries. Even though the serum cholesterol concentrations were lowered in the exercised rabbits, they were still sufficiently high to allow the development of advanced lesions. The duration of the cholesterol feeding, as well as the concentration of cholesterol in the supplement, determined the degree of involvement.

Conversely, Kobernick and associates[10] reported exercise was effective in lowering the incidence of atherosclerosis in rabbits fed high cholesterol diets. The animals were exercised daily in a manually operated drum. In an effort to correlate serum cholesterol concentrations with the severity of experimental atherosclerosis, these investigators[11] conducted further studies. All cholester-

ol-fed rabbits developed atherosclerosis, but the amount of aortic atherogenesis was distinctly less in exercised animals than in the sedentary rabbits. However, a comparison of average serum lipids, including esterified and unesterified cholesterol of exercised and sedentary animals showed no **significant** differences. Non-parallelism between severity of atherosclerosis and the concentrations of lipids was observed; an animal with the highest serum lipids had the least atherosclerosis; another with the lowest, an intermediate grade, and a third animal with intermediate serum lipid values, the most severe lesions. The authors concluded there was no correlation between the free or esterified cholesterol concentrations and either exercise or the severity of aortic atherosclerosis.

According to Myasnikov,[12] cholesterol-induced hypercholesteremia decreased markedly in rabbits exercised daily until fatigued on an electric treadmill. Atherosclerotic degeneration of the aorta and coronary arteries was somewhat reduced in the exercised animals. It appeared physical activity intensified metabolism in the body and thereby caused a more comprehensive assimilation of endogenous cholesterol and a consequent lowering of the serum cholesterol concentrations.

Brainard[13] studied the effect of prolonged exercise

on atherogenesis in the rabbits fed a cholesterol-rich diet *ad libitum*. The experimental animals were exercised in a revolving drum for eight hours daily. Exercise and rest were alternated at fifteen-minute intervals. Exercised rabbits exhibited aortic atheromatous plaques comparable to the non-exercised controls. At the end of the experiment, significantly higher serum cholesterol concentrations, lower mean liver cholesterol concentrations, and slightly higher mean body weights were observed in the active animals than in the sedentary ones. Possible explanations for dissimilarities between these findings and Kobernick's were considered. The latter limited the food intake of the sedentary and exercised animals producing exercised animals which were leaner and lighter than sedentary controls. The caloric balance effected by physical exertion was used to explain lower serum cholesterol in Kobernick's study.

Wong and co-workers[14] reported the inhibitory effect of exercise on atherogenosis in the abdominal aorta of young cholesterol-fed chicks. The birds were exercised on a treadmill twice daily during the eight-week experimental period. Exercise had no effect on the plasma cholesterol concentrations of androgen-treated birds on an atherogenic diet. In a later study, under

similar conditions, Wong and co-workers[15] observed marked increases in plasma cholesterol concentrations in non-exercised birds. Further investigation,[16] revealed similar results in cockerels fed an atherogenic diet and exercised twice daily for fifteen weeks.

Orma[17] compared cholesterol-fed cockerels confined to small cages with birds allowed to exercise freely. Exercise was ineffective in lowering serum cholesterol concentrations in cholesterol-fed cockerels. The incidence of atherosclerosis was higher and its severity more marked in the inactive cholesterol-fed cockerels than in the active cholesterol-fed birds. No differences were observed in either the serum lipid values or atherogenesis between the active and inactive controls fed a normal diet.

Warnock and associates[18] reported brisk walking retarded cholesterol-induced atherogenesis in cockerels fed ad libitum. Vascular and hepatic concentrations were significantly lower in exercised than in non-exercised controls. Food intakes and body weights were comparable in both groups.

Lewis and collaborators[19] investigated the effect of exercise on serum and liver lipids in rats fed high-fat diets. The exercised animals were forced to run in cages that alternately revolved for two minutes and stopped for

one minute. The exercise period was gradually increased to eight hours a day. Two studies were conducted with (1) simple, high-fat diets and (2) choline-containing high-fat diets (0.3, 1.0 or 3 per cent choline; 20 per cent protein as casein; 40 per cent butterfat by weight). The simple, high-fat experiment used 54 or 22 per cent coconut oil or soya oil. In one series of the simple high-fat diet a vitamin mixture containing no choline was used. Later, it was replaced with a vitamin mixture which contained choline. The controls were fed a commercial chow. The source and amount of fat as well as the vitamin supplement affected the serum and liver lipids. High-fat diets supplemented with the choline-containing vitamin mixture produced normal lipid concentrations in the serum and hepatic tissues. Under these conditions no significant differences attributable to exercise were observed in serum or liver lipids. This was also true in rats fed a chow diet.

Elevated serum and liver lipids were observed in rats on the simple, high-fat diets without choline. Five months of exercise were effective in lowering significantly the serum and liver cholesterol concentrations in the rats on the 54 per cent coconut oil diet containing the choline-free vitamin supplement. The mean liver

cholesterol concentrations were 8.6 and 4.6 (mg./gm.liver) in the non-exercised and exercised animals respectively. Both total serum lipid and cholesterol concentrations were significantly lower in the exercised rats than in the non-exercised animals. The same amount of exercise was effective in lowering significantly the liver cholesterol concentrations in the rats on the 54 per cent soya oil diet containing the choline-free vitamin supplement. Mean liver cholesterol concentrations were 15.2 and 9.3 (mg./gm.liver) in non-exercised and exercised animals respectively. No significant differences were found in serum lipid concentrations under these conditions. With either diet total liver lipids were unaltered by the exercise. Nine months of exercise was effective in lowering serum lipids in rats on the 40 per cent butterfat diets; the liver lipids were unaffected by exercise.

A recent study of Montoye and associates[20] examined the effect of exercise on rats fed a stock diet or a whole-milk supplemented stock diet. Animals on each diet were subjected to a daily swimming regimen for twelve weeks. Exercise was effective in lowering significantly both the total and free serum cholesterol in the rats on the stock-fed diet. Mean serum cholesterol concentrations

were: 74.72 mg. per cent total, 20.92 free, and 93.08 total, 25.10 free in the exercised and non-exercised groups respectively. The body weight of the exercised animals was less than the non-exercised at the end of the experiment. However, no correlation existed between total or free cholesterol with body weight.

Johnson[21] investigated the relative effects of exercise and caloric restriction on serum cholesterol. Caloric restriction was less effective than exercise in controlling serum cholesterol concentrations. Mean serum total cholesterol concentrations were: 73.10 mg. per cent, exercised group; 95.32, diet-restricted group, and 96.41, sedentary group. No correlation was found between body weight and serum cholesterol concentrations.

Caloric restrictions

In an early investigation, Entenman and associates[22] observed low serum cholesterol in dogs maintained on inadequate amounts of a normally balanced diet for twenty to thirty weeks. Generally, the ratio of free to total cholesterol rose perceptibly during the restriction. Striking changes in serum cholesterol were not noted during acute fasting which lasted as long as thirty days.

Firstbrook[23] fed young, growing rabbits a suspension

of cholesterol in water and restricted the diet so that the animals lost weight and failed to grow normally. Other things being equal, the atherosclerotic process was inhibited in animals which lost weight or gained relatively little.

McMillan and associates[24] measured the severity of atherosclerosis in rabbits fed various levels of cholesterol on normal and restricted food intakes. The caloric restriction limited the food intakes of the undernourished animals to approximately one-third that of the normal diet. Both young, growing rabbits and fully matured rabbits lost weight. In cases of extreme emaciation, additional food was allowed. Severe caloric restriction did not inhibit the development of aortic atherogenesis; however, undernutrition promoted hypercholesteremia in cholesterol-fed rabbits.

Goldner and co-workers[25] reported similar observations in atherogenic rabbits. When caloric intake was reduced to one-half or one-third of that of the ad libitum controls, the calorie-restricted animals showed significantly higher serum cholesterol concentrations. Caloric restriction seemed to enhance atherosclerosis in the rabbit maintained on high cholesterol intake.

Okey and Lyman[26] observed a marked decrease in food

intake and a lowered serum cholesterol in rats treated with an estrogenic hormone. This led to a second experiment which examined the effect of caloric restriction and an estrogenic hormone on serum and liver lipid concentrations. Intact controls, castrated controls and hormone-dosed castrates were fed either a 15 or 30 per cent protein diet containing cholesterol. Rats of the hormone-dosed castrate subgroup fed 30 per cent protein were used as pacemakers. Some of the rats (pair-fed) from each group were restricted to the mean amount of food consumed daily by the pacemakers; the remaining animals were fed ad libitum. After three weeks the rats were sacrificed. The hormone-dosed castrates ate about three-fourths as much food and attained about half the weight of the undosed. Pair-fed intact and castrate rats gained approximately twice as much as their hormone-dosed littermates with the same food intakes. A contrast between the serum and liver lipids was noted in the ad libitum and diet-restricted rats of the corresponding groups. In the diet-restricted rats, serum cholesterol was higher and liver cholesterol was lower than in rats fed ad libitum. The data demonstrated that both caloric restriction and estrogenic hormone treatment were capable of altering cholesterol transportation and storage. In

both cases the primary effect seemed to result in an increase in circulating cholesterol. With caloric restriction this increase was greater when the diet was high in protein. The authors questioned the reliability of serum cholesterol concentrations as an index of tissue cholesterol retention in view of the increase in serum cholesterol while liver cholesterol stores were depleted.

Okey et al.[27] studied further the effect of caloric restriction on cholesterol metabolism. Weanlings (Long-Evans strain) were fed adequate synthetic diets containing 15 per cent protein until the males reached 250 gm. and the females, 200 gm. Half of the animals were administered cholesterol supplements. At the beginning of the first experiment the rats were from 36 to 39 days old. Pre-experimental controls were sacrificed and the remaining animals were divided into ad libitum and restricted groups. The latter were limited to three-fourths of the daily food intake of the former. At the end of a three-week experimental period, the ad libitum controls and the diet-restricted animals were sacrificed. In all cases the liver size was decreased by dietary restriction. At the beginning of the period of dietary restriction, the 15 per cent protein diets (without cholesterol) produced a considerable accumulation of liver fat and cho-

lesterol. Males fed ad libitum without cholesterol continued to accumulate liver fat and cholesterol at a rate to be expected when the diet furnishes a minimum percentage of protein for good growth. The smaller amounts of absolute total liver fat and cholesterol stored by the restricted animals reflected, primarily, smaller liver sizes rather than lower percentages of lipid stored. The males on restricted diets had higher percentages, but lower amounts of liver lipid than did the young controls sacrificed at the beginning of the experiment.

For the male rats fed the 15 per cent protein diet with low cholesterol, mean liver lipid values were as follows: pre-experimental controls, 0.670 gm./liver or 4.2 per cent moist weight; ad libitum controls, 1.140 gm./liver or 7.8 per cent; and restricted group, 0.579 gm./liver or 6.3 per cent. The mean for absolute total liver cholesterol was highest in the rats fed ad libitum (45.0 mg./liver) and lowest in the restricted animals (30.4 mg./liver). The pre-experimental controls had an intermediate amount with 33.5 mg./liver. Although percentage differences were quite small the lowering of the amounts of liver cholesterol accomplished by dietary restriction approached significance.

The cholesterol-fed animals showed essentially the same degree of reduction of liver lipids and liver cholesterol concentrations with caloric restriction as did their littermates on the low-cholesterol diet. Mean serum cholesterols were usually slightly lower for the restricted groups than the ad libitum controls. Differences were not statistically significant.

In the second part of the study the same experimental design was used when the protein content was increased from 15 to 30 per cent at the beginning of the experimental period. In this study the animals were ten days older than the rats in the first study when they attained the desirable weight.

Caloric restriction decreased liver weights. Lower liver lipids were observed in the rats fed ad libitum without cholesterol. No significant differences were observed in liver cholesterol concentrations. The restricted males had moderate decreases in the mean liver lipid and cholesterol concentrations.

Serum cholesterol variations were insignificant in the animals fed no cholesterol. Dietary restrictions caused increases in serum cholesterol in cholesterol-fed animals. These investigators concluded the protein concentration in the diet, rather than caloric restriction

was the determining factor in maintaining the high level of circulating cholesterol.

In a recent study, Lee and Lucia[28] investigated the relationship between caloric requirements and weight maintenance in Long-Evans rats. The content of the lipid in the liver was examined as a possible factor in caloric-requirement adaptation. Male rats were fed a purified diet which contained 18 per cent casein and 8 per cent cottonseed oil. When the animals reached the weights of approximately 200 grams, dietary restrictions were initiated. The severity and duration of caloric restriction was varied within the nine groups: Group 1 (zero-time controls), sacrificed when the animals attained a weight of 200 gm.; Group 2, maintained at 200 gm. by caloric restriction for six weeks; Group 3, held at 200 gm. for fourteen weeks; Group 4, held at 200 gm. for twenty-six weeks; Group 5, held at 200 gm. for fourteen weeks and then fed ad libitum for six weeks; Group 6, held at 200 gm. for six weeks, then brought down to 130 gm. by more severe caloric restriction, sacrificed after five weeks at 130 gm.; Group 7, held at 200 gm. for six weeks; then brought down to 130 gm. and held for five weeks; then brought back to 200 gm. and held at this weight for four weeks; Group 8, held at 200 gm. for six

weeks, brought down to 130 gm. and held for five weeks, then brought back to 200 gm. and fed ad libitum for four weeks; Group 9, (ad libitum controls) fed ad libitum for twenty-six weeks.

Average body weights for rats restricted for forty-four days varied between 199 and 211 gm. with a mean of 205 gm. During this time the food consumption decreased 35 per cent. During the next 120 days of caloric restriction, (five animals of Group 4), body weight remained between 190 to 210 gm. with a mean of 200 gm.

Significant changes occurred in liver weights. At the end of one week, restricted animals had smaller livers than the ad libitum-fed rats ($p = 0.01$), but not significantly smaller than the initial controls. After eight weeks, restricted animals had significantly larger livers than the comparable control group or the initial controls.

No significant differences were observed in liver lipids or liver cholesterol within any of the groups. Mean liver total lipid values ranged from 3.50 to 4.18 per cent (wet weight of liver). Mean total liver cholesterol values, expressed as percentage of wet weight of liver, ranged from 0.16 to 0.24 per cent. The highest percentage was observed in the initial controls,

and the lowest in the rats fed ad libitum throughout the experiment.

Age

Okey and Lyman[29] studied the relationship of age, protein intake and liver lipid storage in rats. The mean liver cholesterol values for the group fed 10 per cent protein was about 0.4 per cent moist weight and 0.21 per cent for the animals on the 30 per cent protein diet. Although higher values were observed at the seventh week, during the ten-week experiment the changes, due to age, were minor. Previous studies on year-old control rats fed 12.5 to 17 per cent protein diets resulted in liver cholesterol values as high as 0.5 per cent. However, in older control rats on 20 per cent or more protein the liver cholesterol values were similar to those of the ten-week controls. Total liver lipids ranged from 7.3 to 11.8 per cent in groups fed 10 per cent protein; from 3.7 to 4.1 per cent for those fed 15 per cent protein, and from 3.3 to 4.0 per cent for those fed 30 per cent protein. Rats fed 17 and 28 per cent protein showed intermediate ranges of variation. No consistent variation with age was found. Correlation coefficients indicated no interdependence of liver cholesterol and food intake

in rats of the same age, sex and diet group.

Wilcox and Galloway[30] tested the effect of cottonseed oil and lard in male and female Sprague-Dawley rats. Diets containing two fats at three levels (5.0, 13.5 and 20.0 per cent) with and without cholesterol were fed for five and eight weeks. Total liver lipid and total cholesterol values were higher for the rats fed cottonseed oil than for the rats on the lard diet. Differences in weight gains were related to sex and to the number of weeks fed. Rats fed eight weeks had somewhat heavier livers than those fed five weeks. Increasing the experimental period from five to eight weeks resulted in higher liver values for total cholesterol, total lipids and phospholipids. These findings led to a further study of ten to sixteen-month old female rats that had been maintained on a stock diet or diets which supplied 20 per cent fat (butter, margarine, lard, soya oil or cottonseed oil). Mean total serum cholesterol values for all diets ranged from 111 to 163 mg./100 ml. Variations in liver cholesterol concentrations between fats were slight and the values obtained for the older animals were similar to the younger rats. On the stock diet the mean liver cholesterol concentrations were 2.2 mg./gm. of liver for the free, and 3.3 for the total. Sixty-seven per cent of the

cholesterol was present in the unesterified state. On the other diets, the free cholesterol ranged from 1.7 to 2.0 mg./gm. of liver; the total, from 2.3 to 2.7, and the free in total, from 65 to 82 per cent.

Methods of Isolation and Saponification of Liver Lipids

Various methods used in serum and tissue cholesterol analysis have been published. A modification of the Schoenheimer and Sperry[31] method has been established as accurate and reliable in quantitative analysis of free and total cholesterol. Therefore, lipid extraction procedures which utilized this method were investigated.

In the extraction process, lower aliphatic alcohols (methanol or ethanol) were commonly combined with another solvent such as ethyl ether or chloroform. For complete extraction the tissue was homogenized in the solvent, and tough tissues were ground in a suitable manner.

Dury and Treadwell[32] macerated liver samples in a mortar with sand and the lipids were taken up in 95 per cent ethyl alcohol. Extraction of liver lipids was accomplished by refluxing three times with 95 per cent alcohol and thrice with ether followed by distillation under reduced pressure of the combined extracts. The residue

was taken up in petroleum ether and washed with an equal volume of water; the petroleum extract was then dried over anhydrous sodium sulfate and made to a convenient volume. Suitable aliquots of the petroleum ether extracts were taken for lipid partition. The Schoenheimer and Sperry method was used for total and esterified cholesterol determinations.

According to the method of Fillios and Mann,[33] a known amount of liver was ground in anhydrous sodium sulfate and dried overnight at 65°C. After the dried tissue was extracted with redistilled chloroform in a Soxhlet extractor, the solvent was removed by evaporation and the residue was dissolved in redistilled petroleum ether. The petroleum ether extract was filtered into a volumetric flask and the filtrate made to volume with the solvent. Aliquots of this extract were used to determine various liver lipid components. Free and total cholesterols were determined according to a modification of the Schoenheimer and Sperry method.

In the method described by Okey and Lyman,[34] liver samples were homogenized with redistilled 95 per cent ethanol. The extraction was continued by one two-hour heating under the ethanol at 65°C. in a water bath. The extract was decanted through a filter and the liver ex-

traction was repeated for an additional hour with a second portion of ethanol. The liver extract was decanted, filtered and combined with the original extract. The residue was extracted with freshly distilled diethyl ether in a Soxhlet for twenty-four hours. The extracts were combined and made to volume. Aliquot portions were saponified by heating on a steam bath with fresh, normal sodium ethylate according to the method of Bloor.[35] The acidified residue was extracted with petroleum ether. A second portion was measured into a centrifuge tube and the solvent evaporated. The residue was taken up with acetone-alcohol, and the total cholesterol assay was completed by the Sperry and Webb[36] method. Free cholesterols were determined by evaporating an aliquot of the original alcohol-ether extract and completing the determinations according to Sperry and Webb.

Lee and Lucia[28] used the same solvents and similar procedures as Okey et al.[34] The chief differences were found in the times required for each extraction and in the procedure for the ether extraction. Weighed portions of the liver were ground in 95 per cent ethyl alcohol. The suspension was heated to 60°C. for thirty minutes and the alcohol was decanted. The tissue was re-extracted with hot alcohol and at room temperature with ethyl ether; each

extraction lasted one-half hour. The final ether extract was filtered and the combined extracts were brought to standard volume. Total lipid was measured by a modification of the method of Bloor. Total cholesterol was determined by a modification of the method of Sperry and Webb.[36]

EXPERIMENTAL PROCEDURE

Animals[1]

Eighty-eight young adult male albino rats (Carworth strain) were divided into four groups designated as Group A, twenty pre-experimental or initial control animals; Group S, twenty-three sedentary controls; Group D, twenty-two diet-restricted rats, and Group E, twenty-three exercise animals. The four groups were equated on the basis of body weight which averaged 331.0 gm. for Group A; 327.4 for Group S; 326.7 for Group D, and 329.2 for Group E.

Blood samples were taken before the animals were divided into their respective groups. After an 18 ± 3 hour fast, rats were anesthetized lightly with ether. Four to five ml. of blood were withdrawn from the orbital sinus. After the samples were allowed to clot, they were

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[1]Johnson[21] raised the animals and conducted this section of the experimental procedure under the direction of Dr. Henry J. Montoye and Dr. Wayne D. Van Huss of the Department of Health, Physical Education, and Recreation, Michigan State University, as part of the requirements for the degree of Doctor of Philosophy.

centrifuged for fifteen minutes at medium speed. The serum samples were transferred to vials and held in frozen storage for subsequent cholesterol analysis. The samples collected from the eighty-eight rats prior to the experimental period were used for initial control serum cholesterol analysis.

Following a fast of 18 - 3 hours the animals in Group A were sacrificed by a 1-ml. injection of 3 per cent sodium pentobarbital. The hair was shorn with an electric clipper and the specific gravity was determined. The livers and other organs were weighed and replaced in the carcasses which were packaged in individual plastic bags and held in a freezer at 0°C. At the time of the analyses of the carcasses, the livers were removed, repackaged in individual plastic bags and stored again in the freezer.

The remaining three groups (S, D, and E) were housed in individual mesh cages, 18 x 16.5 x 24 cm. in a room maintained at 21 to 23°C. Cages were rotated weekly from top to bottom. All of the rats were confined to their cages during the entire experimental period except the exercise rats which were removed for exercise six days a week.

In a separate room maintained at 31 to 32°C. the exercise animals were swum in groups of ten or eleven in a plastic-lined tank with the water maintained at 35 to 37°C. The animals were then dried and returned to their respective cages.

The fifteen-week exercise regimen was as follows: first four weeks, exercise increased progressively from five minutes daily to sixty minutes; fifth week, sixty minutes daily; sixth and seventh weeks, swimming progressed from ten minutes daily to sixty minutes daily, added weights equivalent to 2 per cent of body weight;[1] eighth week, sixty minutes twice daily, added weights 2 per cent; ninth week, sixty minutes twice daily, added weights 3½ per cent; during the ninth week, weights 5 per cent were tried and proved to be too strenuous (two animals drowned); and from the tenth week through the fifteenth week, the ninth week regimen was continued.

All the animals were fed a commercial stock diet.[2]

Groups S and E received ad libitum food for the entire experimental period. The ration for Group D was

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[1]Added weights were attached to the tail with adhesive tape as close to the animal's body as possible.

[2]The diet (Lab-Blox, Allied Mills, Inc., Chicago) contained: (in per cent) protein, 24.0; fat, 4.0; ash, 8.6; fiber, 4.5; calcium, 1.3; phosphorus, 1.0; sodium, 0.3;potassium, 1.0; chlorine, 0.6; magnesium, 0.2; The diet supplied (in parts per million): iron, 340.0; copper, 11.0; manganese, 170.0; zinc, 30.0; cobalt, 0.6; iodine, 10.0; thiamine, 6.5; riboflavin, 6.5; niacin, 60.0; calcium pantothenate, 18.0; choline, 1850.0; vitamin E, 35.0; carotene, 2.0; In addition, the diet provided (in International Units per pound): vitamin A, 5600, and vitamin D, 2000 units.

restricted in order to maintain body weights comparable to those of the exercise animals. At the beginning of the experimental period each animal in Group D was paired with an animal in Group E on the basis of body weight. Every two weeks, body weights of the matched animals were compared in order to determine the food allowance for each animal in Group D. During the last four weeks, weekly comparisons were made to equate the mean weights of the two groups as nearly as possible.

Throughout the experiment, one rat in Group S died; three exercise rats drowned. At the end of the fifteen-week experimental period, the mean body weight of the rats in Groups S, D and E were 475.6, 422.2 and 421.2 gm., respectively. After the food was withheld for 18 ± 3 hours, blood was taken. These samples were used for the final serum cholesterol determinations. The animals were treated in the same manner as Group A at the beginning of the experimental period.

Chemical Analyses

Liver lipid extraction

After a preliminary investigation of the method of Lee and Lucia,[28] and the procedures of Okey and Lyman,[34]

a modification of the former was adopted for the lipid extraction. Whole frozen livers were placed in a quart Mason jar and homogenized for one minute in a blendor[1] with 50 ml. 95 per cent redistilled ethyl alcohol. After an additional 100 ml. of the ethyl alcohol were added, the suspension was re-homogenized for two minutes. The jar was then placed in an upright position and the particles of liver on the cover, blades and sides were washed down with the solvent. The suspension was held in a hot water bath at 65°C. for thirty minutes with occasional shaking. After the alcohol was decanted and filtered into a 500-ml. volumetric flask, a second thirty-minute extraction with 150-ml. of hot ethyl alcohol was made. A final extraction was carried out at room temperature for thirty minutes with 100-ml. of ethyl ether. The ether extract was filtered into the flask containing the alcohol extracts and the combined extract was equilibrated overnight at 20°C. and then brought to volume with the alcohol-ether (3:1).

Liver lipid determination

Duplicate 50-ml. aliquots of the alcohol-ether extract were evaporated in a tared beaker on the Goldfisch extractor. The crude fat was dried in the oven for ten

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[1]Osterizer

minutes at 95°C., cooled in a desiccator and weighed. In the future, the crude fat will be referred to as liver lipid.

Free liver cholesterol assay

Free cholesterol analyses were carried out in duplicate; 1-ml. aliquots of the original alcohol-ether extract were evaporated to dryness on a hotplate under a hood. The residue was dissolved in acetone-alcohol (1:1), and the cholesterol was precipitated as the digitonide which was allowed to stand over two nights. After the precipitate was centrifuged, the supernatant was poured off and the precipitate was washed and recentrifuged once with acetone-ether and twice with anhydrous ether. The precipitate was dried at 110°C., dissolved in glacial acetic acid and cooled. A colorimetric determination, based on the color developed in the Liebermann-Burchard reaction (acetic anhydride and sulfuric acid), was made with a Beckman Spectrophotometer, Model DB. The cholesterol concentration was determined by a comparison with the color produced by a standard cholesterol solution.

Total liver cholesterol

Lipid saponification for the total cholesterol assay

was carried out in duplicate according to Bloor.[35] Ten-ml. aliquots of the lipid extract were pipetted into 125-ml. Erlenmeyer flasks and the saponification was accomplished with 10-ml. of sodium ethylate. The mixture was evaporated on the steam bath until a pasty residue remained. Five-ml. of diluted sulfuric acid (1:3) were added and the acid mixture was heated on the water bath for one minute at 50°C. Ten-ml. of petroleum ether were added to the hot mixture and the flask was rotated gently in the water bath for two or three minutes. The solvent was decanted from the watery residue into a 25-ml. volumetric flask fitted with a ground glass stopper. The heating and extraction were repeated with 5-ml. portions of petroleum ether until the acid water was clear and free from particles. After the petroleum ether extract was equilibrated for at least two hours at 20°C., the flask was brought to volume and the contents were well mixed. Two-ml. aliquots of the petroleum ether extract were used in the total cholesterol assay. After the sample was evaporated, the determination was completed in the same manner as the free cholesterol analysis.

Computations and Statistical Procedures

Since three livers were lost, missing values for total lipids and liver cholesterols were computed from the cor-

responding treatment means for total lipids (percentage) and for free and total cholesterols (mg./gm.). Absolute total and free cholesterol values were computed from the liver weight. Computed total lipids were obtained by multiplying the corresponding percentage of fat by the liver weight. In a few instances when saponification was judged incomplete, the total cholesterol was calculated on the basis of the mean per cent increase of the total over the free.

All data were statistically analyzed by the analysis of variance, (Snedecor[37]); and by the multiple range test (Duncan[38]) when appropriate. Correlation coefficients were made by an electronic computer (Mistic).

RESULTS AND DISCUSSION

Liver weights, total and free cholesterols in mg./liver and in mg./gm. of liver and total lipids in weight and per cent for individual rats are recorded in Tables I, II, III and IV for the initial control (Group A), sedentary (Group S), diet-restricted (Group D), and exercise (Group E) animals respectively. Mean values of the liver weights, total and free liver cholesterol, liver lipids and percentage of fat for each group are summarized in Table V.

Liver Weights

The analysis of variance showed significant differences in liver weights (Table VI). The significant range test indicated no significant differences between liver weights of the diet-restricted (mean, 11.89 gm.) and the exercise (mean, 11.82 gm.) groups. The livers of these animals were significantly larger than the initial control group (mean, 9.92 gm.) and smaller than the sedentary animals (mean, 12.99 gm.). Okey[27] reported smaller livers in young adolescent rats after three weeks of caloric re-

TABLE I

Individual and Mean Liver Weights and Liver Lipids of Control Animals (Group A)

Rat No.	Liver Weight (gm.)	Cholesterol (mg./liver) Total	Cholesterol (mg./liver) Free	Cholesterol (mg./gm.liver) Total	Cholesterol (mg./gm.liver) Free	Total Lipid (gm./liver) Absolute	Total Lipid % Wet Weight
2	10.30	37.8	37.1	3.67	3.60	0.88	8.51
3	9.93	30.9	21.9	3.11	2.20	0.71	7.12
8	11.93	35.5	31.1	2.97	2.61	0.64	5.39
18	12.48	28.9	27.4	2.32	2.19	0.63	5.06
21	9.46	24.2	20.3	2.56	2.15	0.49	5.22
31	10.07	31.9	27.6	3.17	2.74	0.60	5.95
33	9.04	28.1[1]	25.4	3.11[1]	2.81	0.74	8.17
42	8.86	30.1[1]	25.7	3.40[1]	2.90	0.61	6.86
43	8.24	26.2	24.8	3.19	3.00	0.64	7.80
48	10.11	37.3	33.9	3.69	3.35	0.73	7.20
51	9.44	31.9	28.5	3.38	3.02	0.71	7.56
57	8.41	27.0	24.9	3.21	2.96	0.65	7.71
60	8.58	31.9[1]	27.2	3.71[1]	3.17	0.60	6.98
65	8.92	27.0	22.2	3.03	2.49	0.54	6.03
70	9.79	35.7[1]	29.3	3.65[1]	2.99	0.57	5.85
71	9.23	33.8[1]	28.8	3.66[1]	3.12	0.57	6.21
79	10.68	39.2	27.0	3.68	2.53	0.77	7.17
82	9.67	40.6	34.2	4.19	3.54	0.81	8.38
90	13.58	40.7	29.6	3.00	2.18	0.80	5.89
95	9.70	30.4	26.7	3.13	2.75	0.55	5.66
Mean	9.92	32.5	27.7	3.29	2.82	0.662	6.74

[1]Computed values.

TABLE II

Individual and Mean Liver Weights and Liver Lipids of Sedentary Animals (Group S)

Rat No.	Liver Weight (gm.)	Cholesterol (mg./liver) Total	Cholesterol (mg./liver) Free	Cholesterol (mg./gm.liver) Total	Cholesterol (mg./gm.liver) Free	Total Lipid (gm./liver) Absolute	Total Lipid % Wet Weight
6	16.02	48.6	45.8	3.03	2.86	1.27	7.94
7	11.42	40.3	31.9	3.52	2.79	0.83	7.24
10	14.69	48.7	38.8	3.31	2.64	0.97	6.59
11	11.03	38.6	33.2	3.50	3.02	0.77	6.94
12	11.39	49.1	40.9	4.31	3.59	0.88	7.74
22	12.44	52.2	41.5	4.20	3.34	1.03	8.26
23	18.26	50.9[1]	42.3	2.79[1]	2.32	1.12	6.11
28	13.59	45.1	38.8	3.32	2.85	1.10	8.09
30	13.63	49.5	39.8	3.63	2.92	0.98	7.16
32	11.35	41.9[1]	34.8	3.69[1]	3.07	0.86	7.55
36	11.99	43.0	37.5	3.59	3.12	0.99	8.26
44	16.70	48.9	39.4	2.93	2.36	1.38	8.29
56	12.90	46.8	36.1	3.63	2.80	1.09	8.48
58	11.88	40.9	33.6	3.45	2.83	0.97	8.15
59	10.58	32.8	27.0	3.10	2.55	0.92	8.65
61	12.42	41.7[1]	34.6[1]	3.36[1]	2.79[1]	0.93[1]	7.50[1]
62	9.59	34.7	30.6	3.62	3.19	0.70	7.30
63	12.29	38.6	33.8	3.14	2.75	0.70	5.72
66	17.44	54.4	46.7	3.12	2.68	1.22	6.97
73	14.36	35.9	30.6	2.50	2.13	0.96	6.66
74	13.40	40.8	29.6	3.05	2.21	1.06	7.92
87	8.51	26.6[1]	22.1	3.12[1]	2.59	0.63	7.40
Mean	12.99	43.2	35.9	3.36	2.79	0.971	7.50

[1]Computed values.

TABLE III

Individual and Mean Liver Weights and Liver Lipids of Diet-restricted Animals (Group D)

Rat No.	Liver Weight (gm.)	Cholesterol (mg./liver) Total	Cholesterol (mg./liver) Free	Cholesterol (mg./gm.liver) Total	Cholesterol (mg./gm.liver) Free	Total Lipid (gm./liver) Absolute	Total Lipid % Wet Weight
1	11.64	32.8	31.9	2.82	2.74	0.72	6.19
4	11.63	36.2	26.3	3.12	2.26	0.85	7.34
5	11.69	39.8	33.5	3.40	2.87	0.82	7.00
9	10.56	43.1	29.0	4.08	2.75	0.69	6.50
13	13.09	33.4	31.7	2.56	2.42	0.86	6.58
14	10.21	27.2	24.8	2.66	2.43	0.68	6.66
15	13.54	42.7	38.8	3.15	2.86	0.86	6.36
26	12.18	32.2	27.4	2.64	2.25	0.95	7.83
41	10.99	34.3	32.5	3.12	2.96	0.81	7.36
46	9.81	29.9	27.0	3.05	2.75	0.80	8.15
47	11.19	47.1	33.3	4.21	2.98	0.90	8.06
49	10.94	29.1	27.2	2.66	2.49	0.89	8.10
53	13.41	41.1[1]	35.2[1]	3.07[1]	2.63[1]	1.00[1]	7.46[1]
64	10.84	36.8	27.2	3.39	2.50	1.01	9.33
68	13.71	33.4	27.4	2.44	2.00	1.15	8.41
72	15.09	48.9	41.3	3.24	2.74	1.08	7.18
75	11.25	33.2	29.5	2.95	2.62	0.89	7.93
76	13.02	40.0[1]	34.2[1]	3.07[1]	2.63[1]	0.97[1]	7.46[1]
80	12.68	40.2	35.4	3.17	2.79	0.96	7.54
86	9.28	30.5	27.4	3.29	2.95	0.79	8.50
89	12.63	35.0	33.0	2.77	2.62	0.94	7.43
99	12.28	32.5	31.6	2.65	2.58	0.84	6.84
Mean	11.89	36.3	31.2	3.07	2.63	0.885	7.46

[1]Computed values.

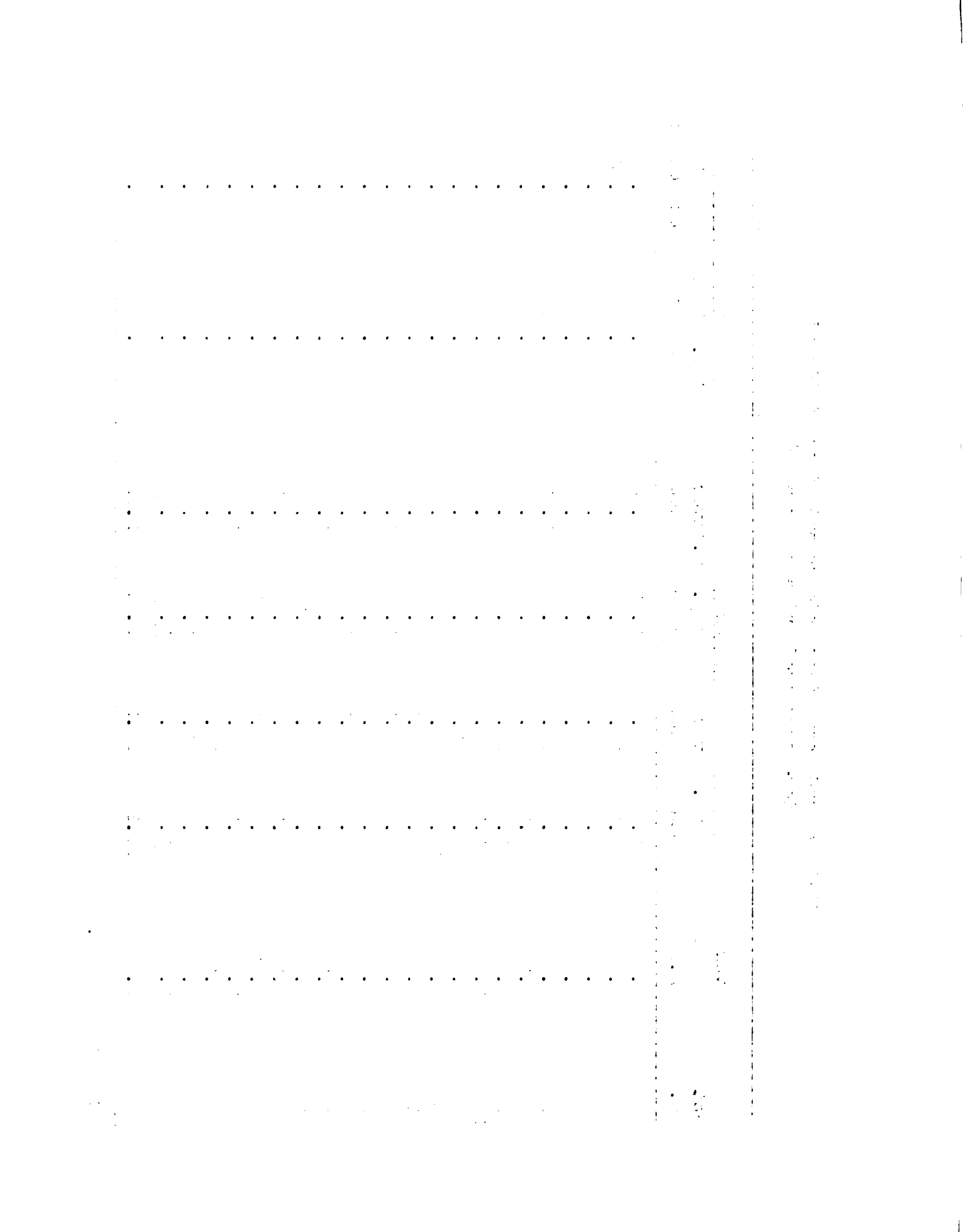

TABLE IV

Individual and Mean Liver Weights and Liver Lipids of Exercised Animals (Group E)

Rat No.	Liver Weight (gm.)	Cholesterol (mg./liver) Total	Cholesterol (mg./liver) Free	Cholesterol (mg./gm.liver) Total	Cholesterol (mg./gm.liver) Free	Total Lipid (gm./liver) Absolute	Total Lipid % Wet Weight
16	9.30	30.2	26.9	3.25	2.89	0.71	7.60
17	11.12	35.8	29.8	3.22	2.68	0.74	6.62
19	10.12	42.7	38.9	4.22	3.84	0.70	6.92
24	10.38	30.1	27.7	2.90	2.66	0.69	6.62
25	12.60	39.0[1]	34.1	3.09[1]	2.71	0.76	6.01
27	13.28	37.8	36.3	2.85	2.73	1.03	7.76
29	11.66	45.5	36.0	3.90	3.09	0.79	6.76
34	13.25	40.4	29.0	3.05	2.19	0.69	5.18
37	14.95	41.1	37.4	2.75	2.50	0.88	5.87
38	11.42	37.0	35.2	3.24	3.09	0.73	6.38
39	10.52	26.0	24.4	2.47	2.32	0.67	6.37
40	11.52	33.2	26.5	2.88	2.30	0.82	7.14
54	11.54	34.5[1]	30.2	2.99[1]	2.62	0.71	6.18
67	14.33	42.0	37.3	2.93	2.60	0.96	6.71
77	10.80	33.6	32.9	3.11	3.05	0.75	6.90
78	12.17	35.0	31.2	2.88	2.57	0.86	7.07
81	12.65	46.1	39.8	3.65	3.14	0.93	7.33
83	12.86	31.0	27.4	2.41	2.13	0.77	6.01
84	12.06	46.0	37.1	3.81	3.08	0.81	6.72
85	9.95	35.0	31.9	3.52	3.21	0.78	7.83
Mean	11.82	37.1	32.5	3.16	2.77	0.789	6.70

[1]Computed values.

TABLE V

Mean Values for Liver Weights and Liver Lipids of Control, Sedentary, Diet-restricted and Exercised Rats

Group[1]	No. of Animals	Liver Weights (gm.)	Cholesterol (mg./liver) Total	Cholesterol (mg./liver) Free	Cholesterol (mg./gm.liver) Total	Cholesterol (mg./gm.liver) Free	Total Lipid (gm./liver) Absolute	Total Lipid % Wet Weight
A	20	9.92	32.5	27.7	3.29	2.82	0.662	6.74
S	22	12.99	43.2	35.9	3.36	2.79	0.971	7.50
D	22	11.89	36.3	31.2	3.07	2.63	0.885	7.46
E	20	11.82	37.1	32.5	3.16	2.77	0.789	6.70

[1]Group A, initial control; Group S, sedentary; Group D, diet-restricted, and Group E, exercised animals.

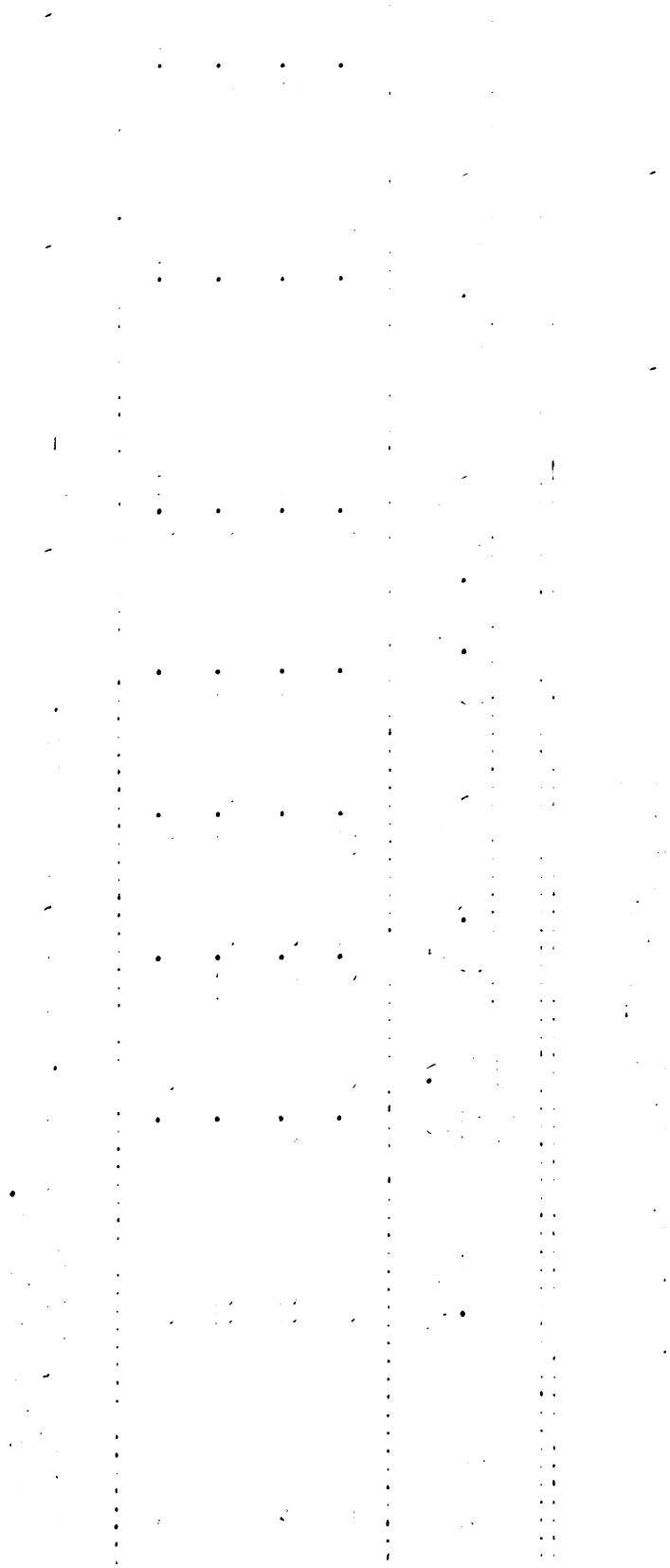

TABLE VI

Analysis of Variance and Multiple Range Test of Liver Weights in Control, Sedentary, Diet-restricted and Exercised Rats

LIVER WEIGHTS

Analysis of Variance

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	F
Total	83	349.0269		
Treatment	3	101.2964	33.7655	10.90**
Error	80	247.7305	3.0966	

Multiple Range Test[1]

a) Shortest Significant Ranges at 5% Probability Level

P:	(2)	(3)	(4)
R_p:	1.08	1.14	1.18

b) Results

Treatments:	A	E	D	S
Means:	9.92	11.82	11.89	12.99

**Significant at the 1% probability level.

[1]Explanation of Treatment Significance:

Any two means not underscored by the same line are significantly different.

Any two means underscored by the same line are not significantly different.

LIVER WEIGHTS

Analysis of Variance

striction than in the pre-experimental or ad libitum controls, and no significant differences between the pre-experimental and caloric restricted animals. In this study the diet-restricted rats had larger livers than the initial controls and smaller ones than the ad libitum sedentary animals. However, Lee and Lucia[28] found, at the end of one week, restricted rats had smaller livers than their ad libitum controls, but not significantly smaller than the pre-experimental controls. After eight weeks, restricted animals had significantly larger livers than either the comparable controls or the pre-experimental animals. Wilcox and Galloway[30] found rats which had been fed eight weeks had somewhat heavier livers than those fed five weeks. Differences may be due to the age of the animal, heredity of the rats, the degree of caloric restriction or dietary dissimilarities.

Correlation coefficients showed a positive relationship between body weights and liver weights (Fig. 1) for all groups combined ($r = 0.630$), for the older animals ($r = 0.830$) and within groups S, D and E ($r = 0.902$, 0.821 and 0.641, respectively. No correlation was found within Group A, the initial controls. This difference may be attributed to the ages of the animals.

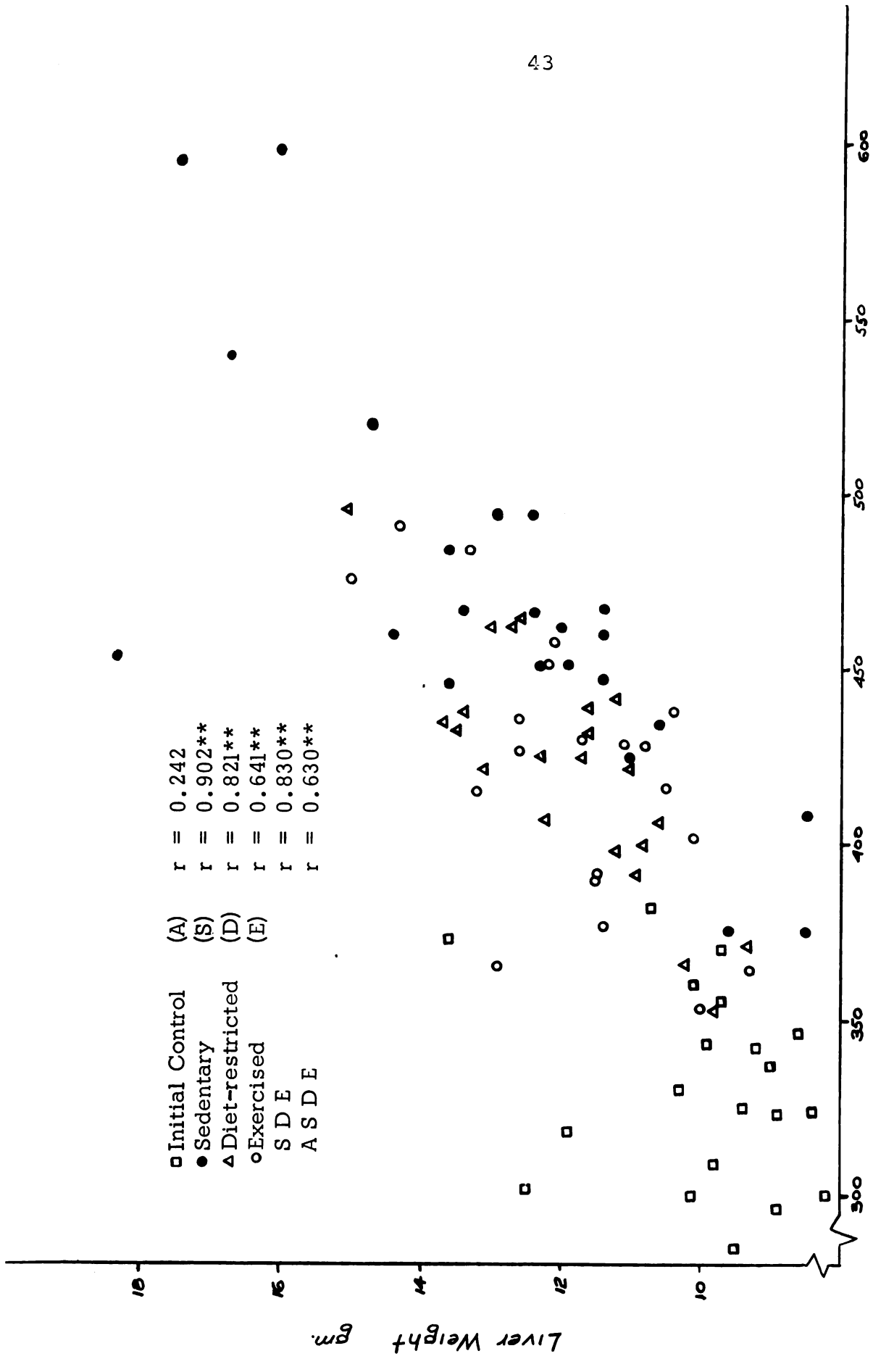

FIG. 1. Final body weight (gm.) plotted against liver weight (gm.).

Liver Lipids

Significant differences were observed in liver lipids (mg/liver) between all groups (Table VII). The mean liver lipid values were lowest in the initial control group (mean, 0.662 gm.) and highest in the sedentary controls (mean, 0.971 gm.). The diet-restricted (mean, 0.885 gm.) and exercise animals (mean, 0.789) were intermediate. The exercise group was significantly lower than the diet-restricted animals. Since the liver weights of the diet-restricted and exercise rats were not significantly different, the data indicated a greater accumulation of fat in the liver of the diet-restricted rats.

When the total lipids were expressed in per cent fat, no significant difference was found between the initial control and the exercise rats, nor between the diet-restricted and sedentary controls (Table VIII). However, the percentage of fat in the former groups (Group A, 6.74 and Group E, 6.70) was significantly lower than that of the latter groups (Group D, 7.46 and Group S, 7.50). The observed percentage of fat was in agreement with that reported by Okey *et al.*[27] in the *ad libitum* sedentary rats but not in the pre-experimental controls. This difference may have been due to variations in age or heredity.

TABLE VII

Analysis of Variance and Multiple Range Test of Absolute Total Lipid in Control, Sedentary, Diet-restricted and Exercised Rats

ABSOLUTE TOTAL LIPIDS

Analysis of Variance

Source of Variation	Degrees of Freedom[1]	Sum of Squares	Mean Squares	F
Total	80	2.5142		
Treatment	3	1.1002	0.3667	19.93**
Error	77	1.4140	0.0184	

Multiple Range Test[2]

a) Shortest Significant Ranges at 5% Probability Level

P:	(2)	(3)	(4)
R_p:	0.083	0.088	0.091

b) Results

Treatments:	A	E	D	S
Means:	0.66	0.79	0.88	0.97

[1]Three degrees of freedom lost due to missing values.

**Significant at the 1% probability level.

[2]For explanation of treatment significance see page 41.

TABLE VIII

Analysis of Variance and Multiple Range Test of Total Lipids Expressed in Percentage of Fat in Control, Sedentary, Diet-restricted and Exercised Rats

TOTAL LIPIDS
Percentage of Fat[1]

Analysis of Variance

Source of Variation	Degrees of Freedom[2]	Sum of Squares	Mean Squares	F
Total	80	68.6266		
Treatment	3	12.2143	4.0714	5.56**
Error	77	56.4123	0.7326	

Multiple Range Test[3]

a) Shortest Significant Ranges at 5% Probability Level

P:	(2)	(3)	(4)
R_p:	0.53	0.56	0.57

b) Results

Treatments:	E	A	D	S
Means:	6.70	6.74	7.46	7.50

[1]Per cent wet weight.

[2]Three degrees of freedom lost due to missing values.

**Significant at the 1% probability level.

[3]For explanation of treatment significance see page 41.

The mean liver lipid range, in the present study, was higher than that reported by Lee and Lucia[28] and within the range observed by Okey and Lyman.[29]

A positive correlation was observed between the amounts of body fat[1] and liver lipids (Fig. 2) for Groups A, S, D and E ($r = 0.804$) and for Groups S, D, and E ($r = 0.722$) and within all the groups except Group E. When the per cent body fat was plotted against the per cent liver lipids (Fig. 3) no correlations were observed within Groups A, D and E. A highly significant correlation was observed between liver lipids (gm./liver) and the liver weight in gm. (Fig. 4) for all groups combined ($r = 0.788$), for the older animals ($r = 0.766$) and within all groups except Group A. When the per cent liver lipids was correlated with the liver weight (Fig. 5) a negative relationship was found within Group A ($r = -0.454$). No other correlations were found.

Liver Cholesterol

Analysis of variance of liver cholesterol (mg./gm. liver) showed no significant differences between the groups (Table IX). However, significant differences were

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[1]Unpublished data.[39]

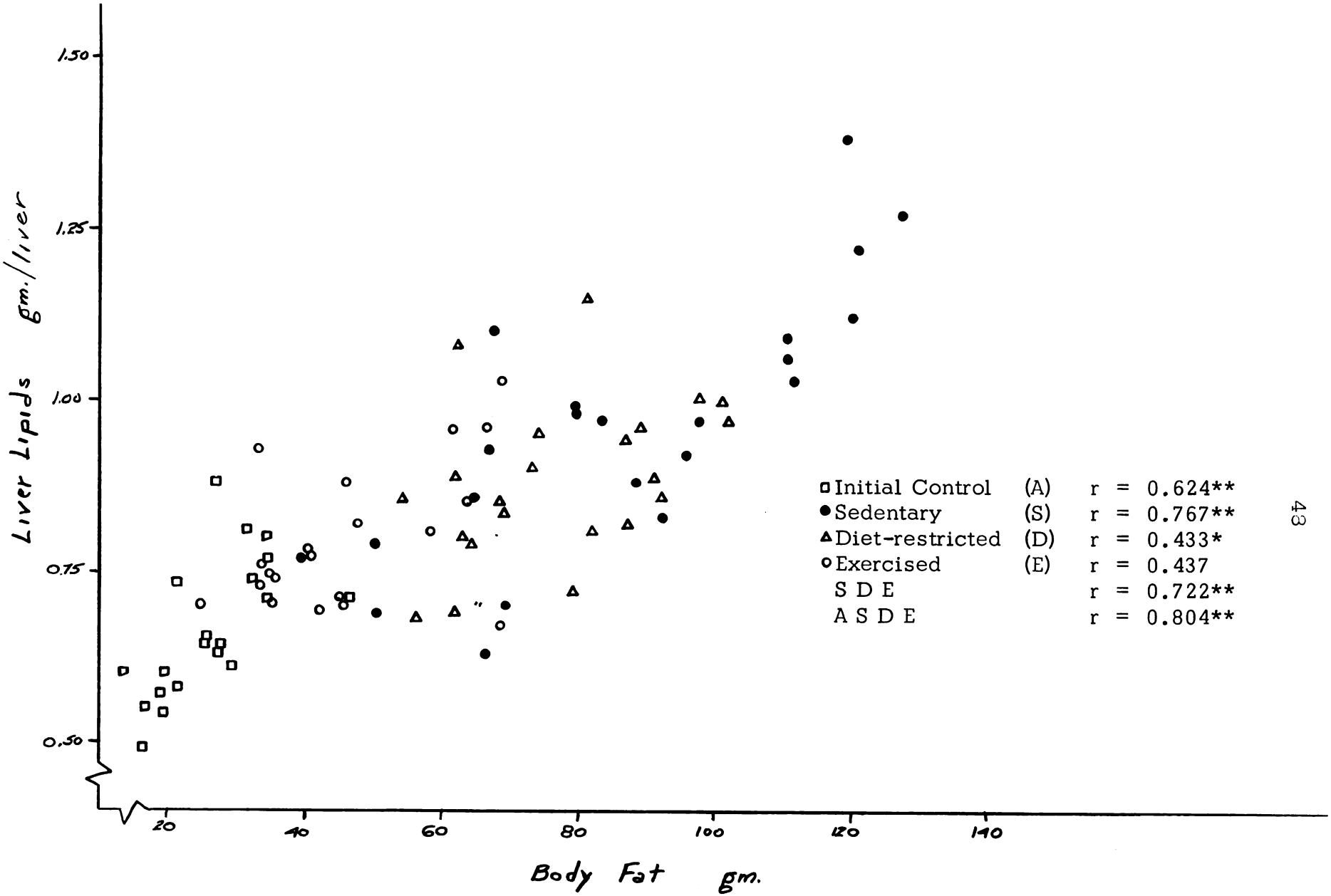

FIG. 2. Body fat (gm.) plotted against liver lipids (gm./liver).

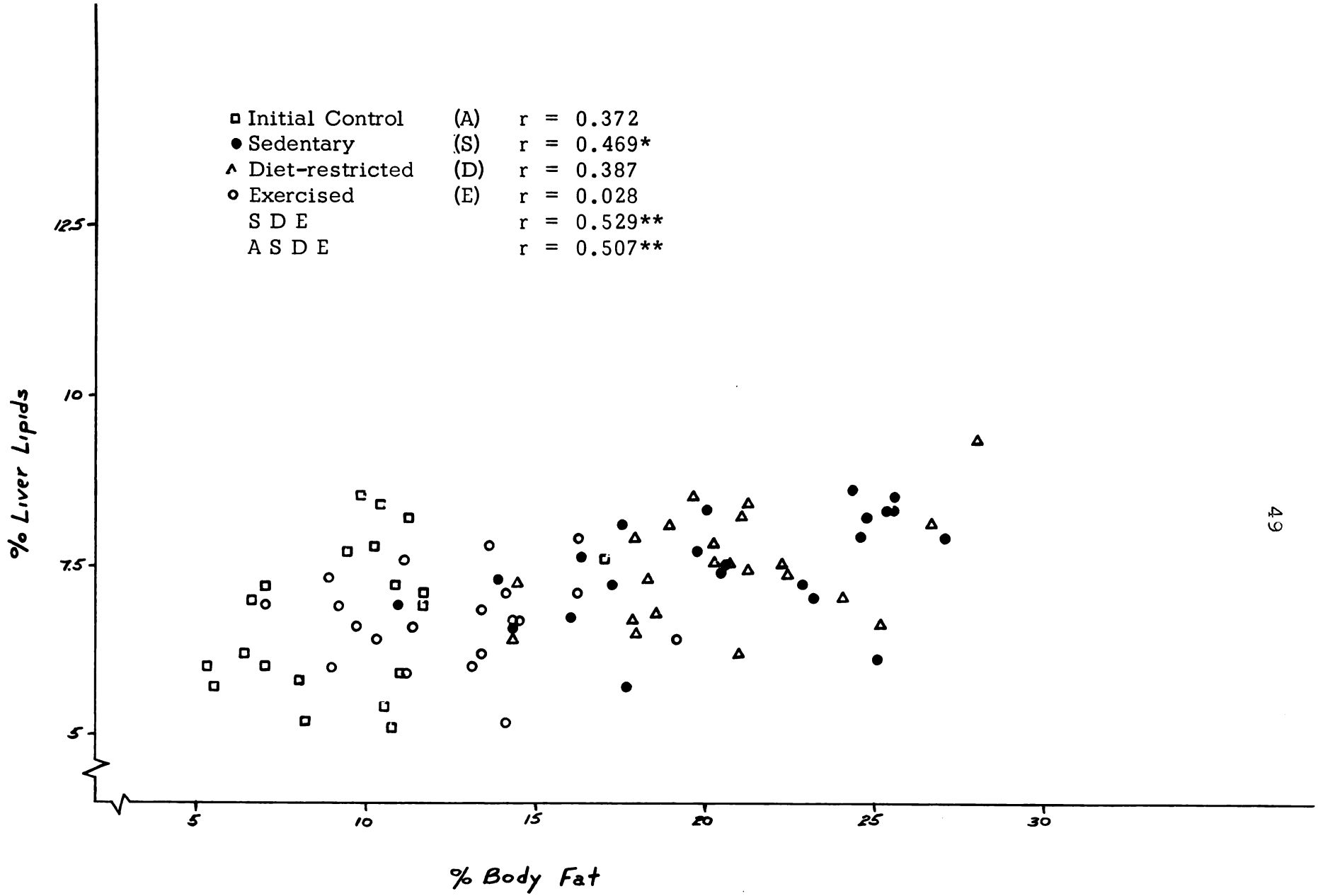

FIG. 3. Per cent body fat plotted against per cent liver lipids.

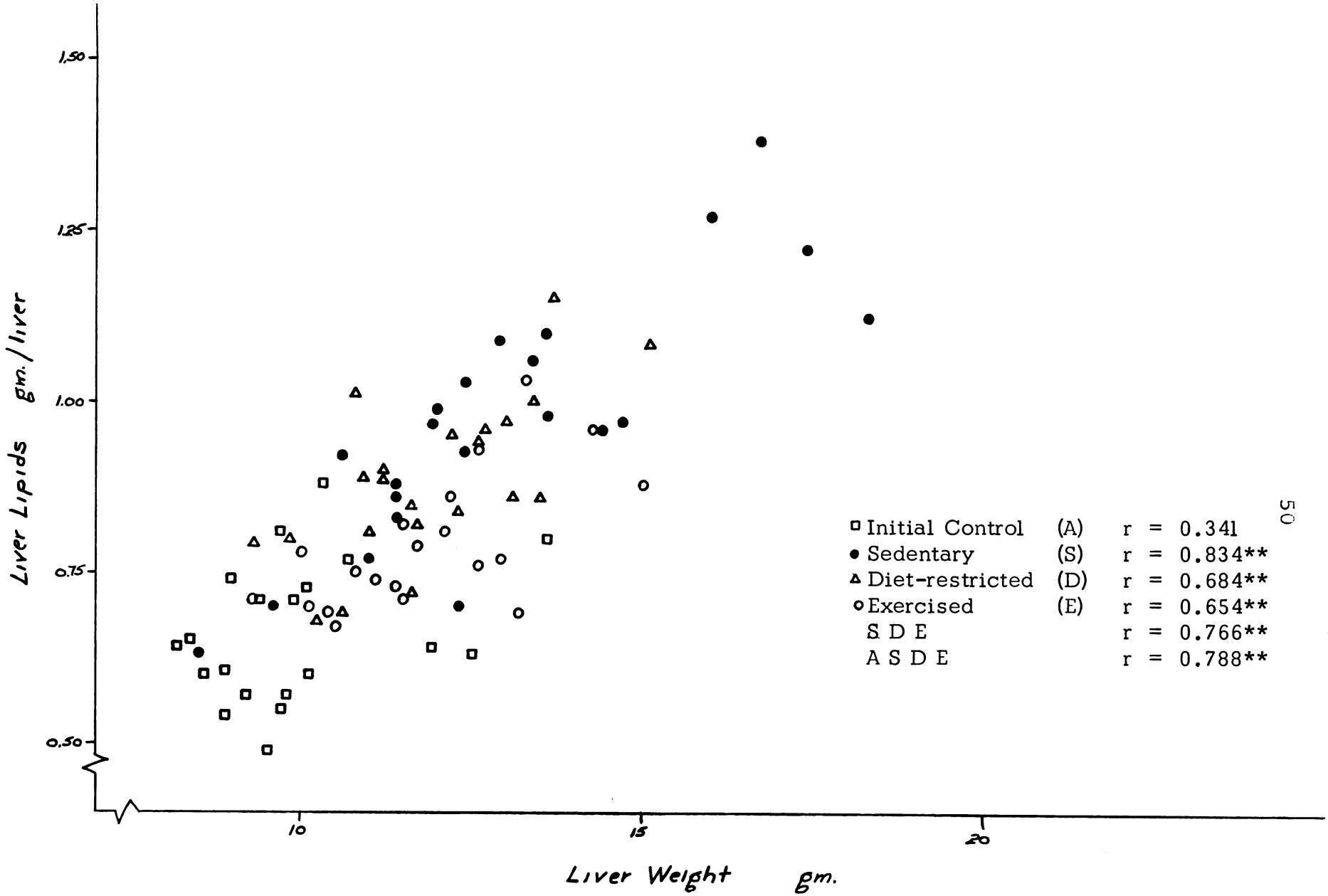

FIG. 4. Liver weight (gm.) plotted against liver lipids (gm./liver).

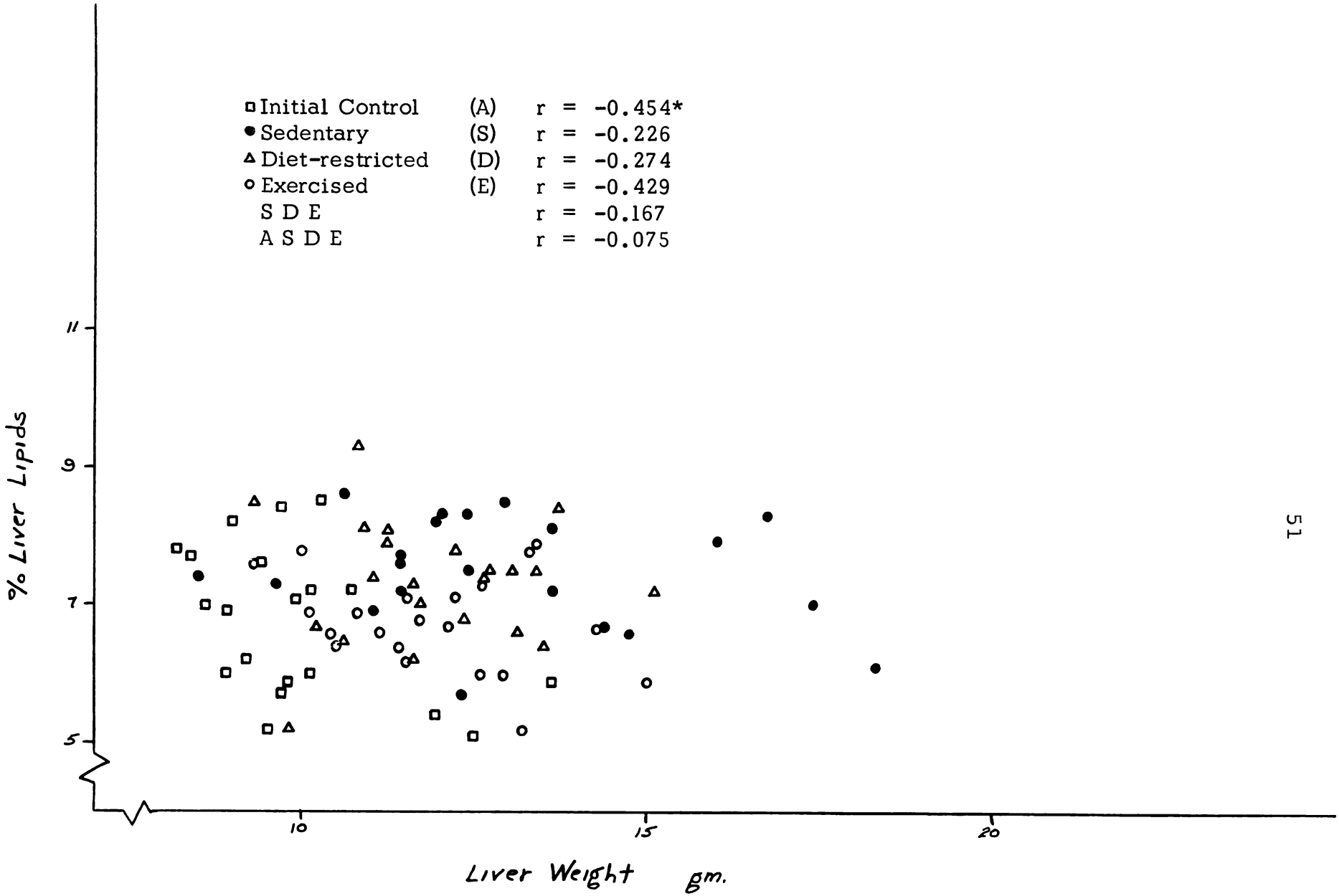

FIG. 5. Liver weight (gm.) plotted against per cent liver lipids.

TABLE IX

Analysis of Variance of Liver Cholesterol (mg./gm.liver) in Control, Sedentary, Diet-restricted and Exercised Rats

TOTAL CHOLESTEROL
(mg./gm.liver)

Source of Variation	Degrees of Freedom[1]	Sum of Squares	Mean Squares	F
Total	80	16.6826		
Treatment	3	1.1165	0.3722	1.84
Error	77	15.5661	0.2022	

FREE CHOLESTEROL
(mg./gm.liver)

Source of Variation	Degrees of Freedom[1]	Sum of Squares	Mean Squares	F
Total	80	11.3249		
Treatment	3	0.4557	0.1519	1.08
Error	77	10.8692	0.1412	

[1]Three degrees of freedom lost due to missing values.

observed in total and free cholesterol in mg./liver as indicated in Tables X and XI. The means of the total cholesterol (mg./liver) were 32.5, 37.1, 36.3 and 43.2 for the initial control, exercise, diet-restricted and sedentary groups, respectively. The value for the younger animals was lower than those for the older ones. Those for the exercise and the diet-restricted rats were the same, but they were lower than those for the sedentary animals. Okey et al.[29] reported rats up to one year of age showed liver cholesterol concentrations similar to those of the ten-week controls when the diet contained 20 per cent or more of protein. Wilcox and Galloway[30] found higher liver cholesterol concentrations in weanling rats which had advanced from five to eight weeks in age. However, the liver cholesterol concentrations of ten-to-sixteen-month-old females were similar to those for the younger rats. This was in agreement with the findings of Lee and Lucia[28] who recorded no significant differences in ad libitum-fed and calorie-restricted rats after twenty-six weeks. The mean values for the absolute total cholesterol reported by Okey et al.[27] were comparable to the current experimental group and the ad libitum-fed controls. However, their

TABLE X

Analysis of Variance and Multiple Range Test of Total Cholesterol (mg./liver) for Control, Sedentary Diet-restricted and Exercised Rats

TOTAL CHOLESTEROL
(mg./liver)

Analysis of Variance

Source of Variation	Degrees of Freedom[1]	Sum of Squares	Mean Squares	F
Total	80	4070.66		
Treatment	3	1251.19	417.06	11.39**
Error	77	2819.47	36.62	

Multiple Range Test[2]

a) Shortest Significant Ranges

P:	(2)	(3)	(4)
R_p:	3.72	3.92	4.05

b) Results

Treatments:	A	D	E	S
Means:	32.5	36.3	37.1	43.2

[1]Three degrees of freedom lost due to missing values.

**Significant at the 1% probability level.

[2]For explanation of treatment significance see page 41.

TABLE XI

Analysis of Variance and Multiple Range Test of Free Cholesterol (mg./liver) for Control, Sedentary, Diet-restricted and Exercised Rats

FREE CHOLESTEROL
(mg./liver)

Analysis of Variance

Source of Variation	Degrees of Freedom[1]	Sum of Squares	Mean Squares	F
Total	80	2610.63		
Treatment	3	724.09	241.36	9.85**
Error	77	1886.54	24.50	

Multiple Range Test[2]

a) Shortest Significant Ranges at 5% Probability Level

P:	(2)	(3)	(4)
R_p:	3.05	3.21	3.32

b) Results

Treatments:	A	D	E	S
Means:	27.7	31.2	32.5	35.9

[1]Three degrees of freedom lost due to missing values.

**Significant at the 1% probability level.

[2]For explanation of treatment significance see page 41.

diet-restricted animals had lower absolute total liver cholesterol than their pre-experimental controls. This does not agree with the findings of the present study.

A positive correlation (r = 0.476 to 0.738) of liver weight and total liver cholesterol in mg./liver (Fig. 6) seemed to indicate the amount of liver cholesterol was related to the size of the liver. However, it appeared the differences in absolute total cholesterol were due merely to larger livers, since the correlations of the total liver cholesterol in mg./gm. of liver and liver weight (Fig. 7) were negative for all the groups combined (r = -0.279) and for the older animals (r= -0.257).

When total liver cholesterol in mg./liver (Fig. 8) was plotted against liver lipids in gm./liver, a positive correlation was observed for the combined Groups A, S, D and E (r = 0.686), for the combined Groups S, D, and E (r = 0.603) and within all the groups (r = 0.448 to 0.721) except Group D. No positive correlation was observed in total liver cholesterol in mg./gm. of liver and liver lipids in gm./liver (Fig. 9). As shown in Fig. 10 free liver cholesterol in mg./liver was correlated with the liver weight (r = 0.684 to 0.738) except within Groups A and E. Similarly the free liver cholesterol in mg./liver

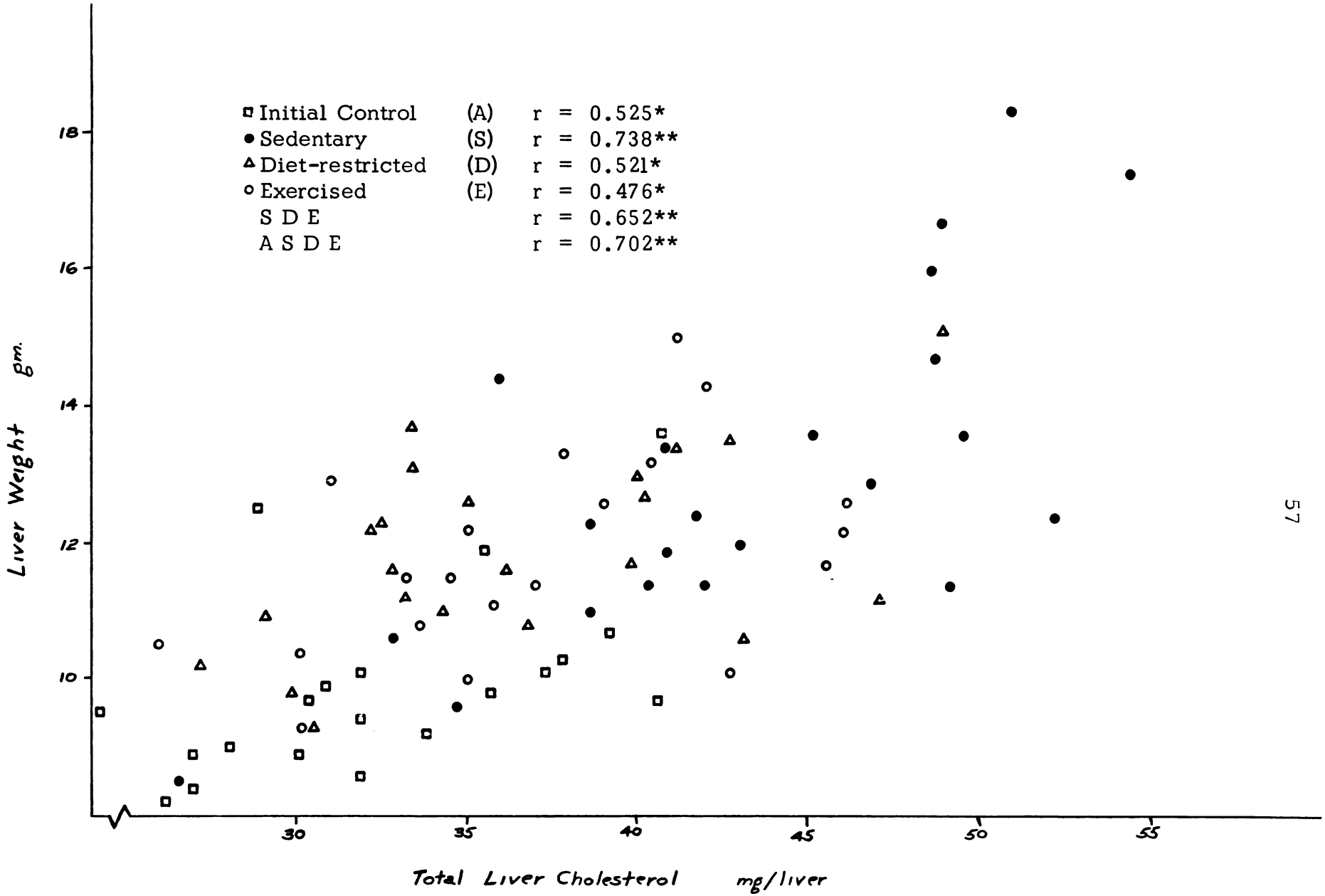

FIG. 6. Total liver cholesterol (mg./liver) plotted against liver weight (gm.).

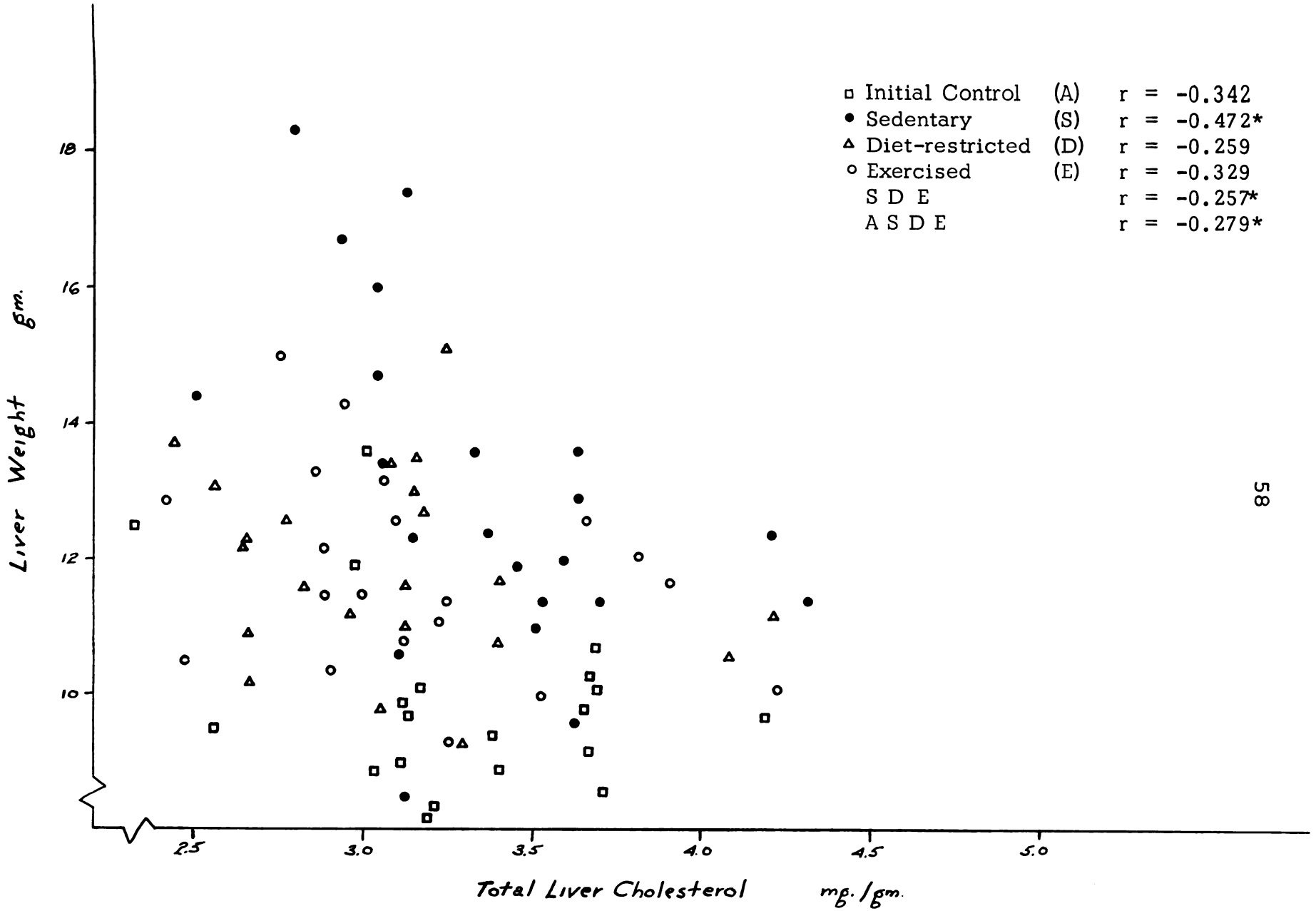

FIG. 7. Total liver cholesterol (mg./gm.) plotted against liver weight (gm.).

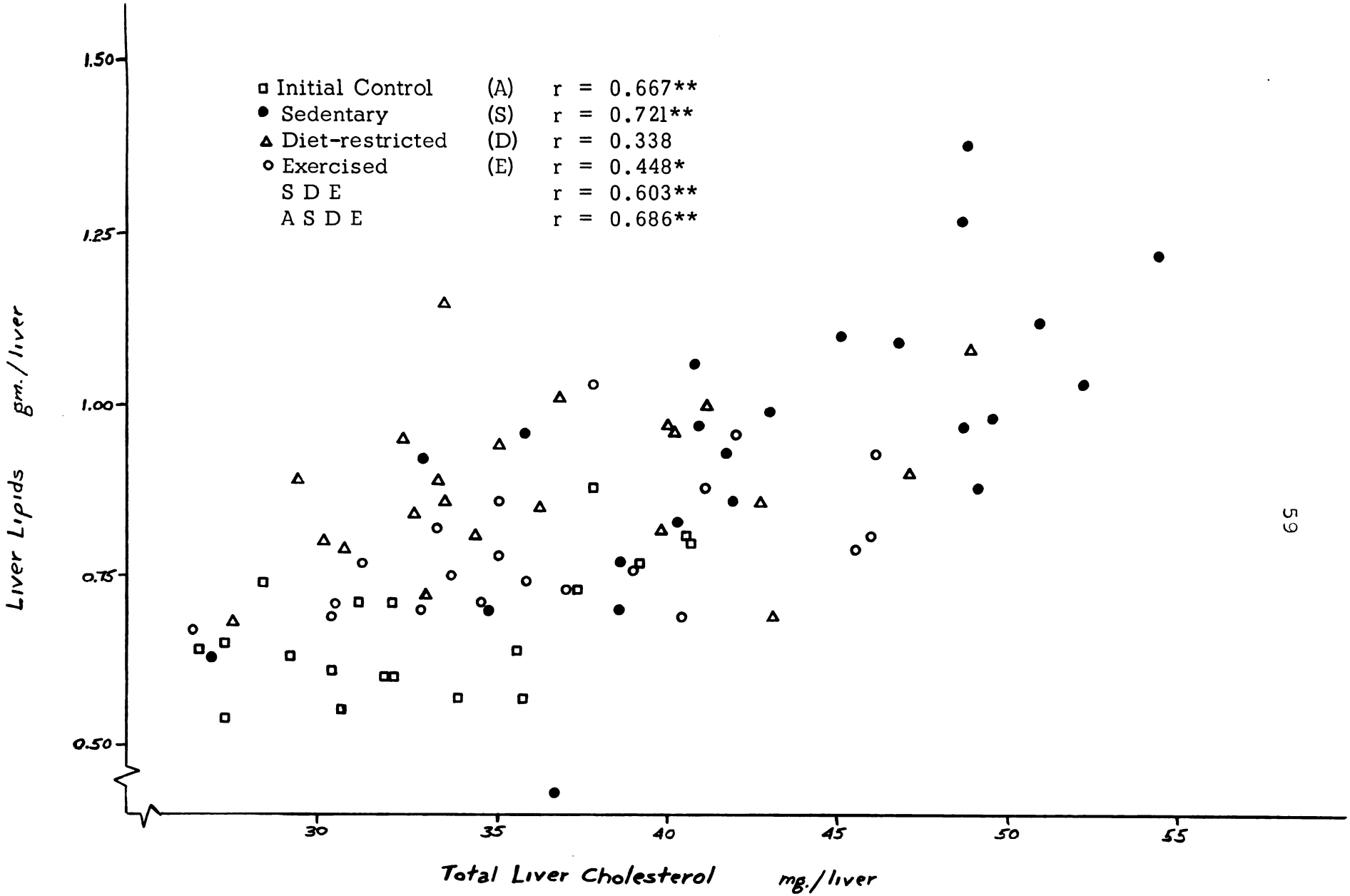

FIG. 8. Total liver cholesterol (mg./liver) plotted against liver lipids (gm./liver).

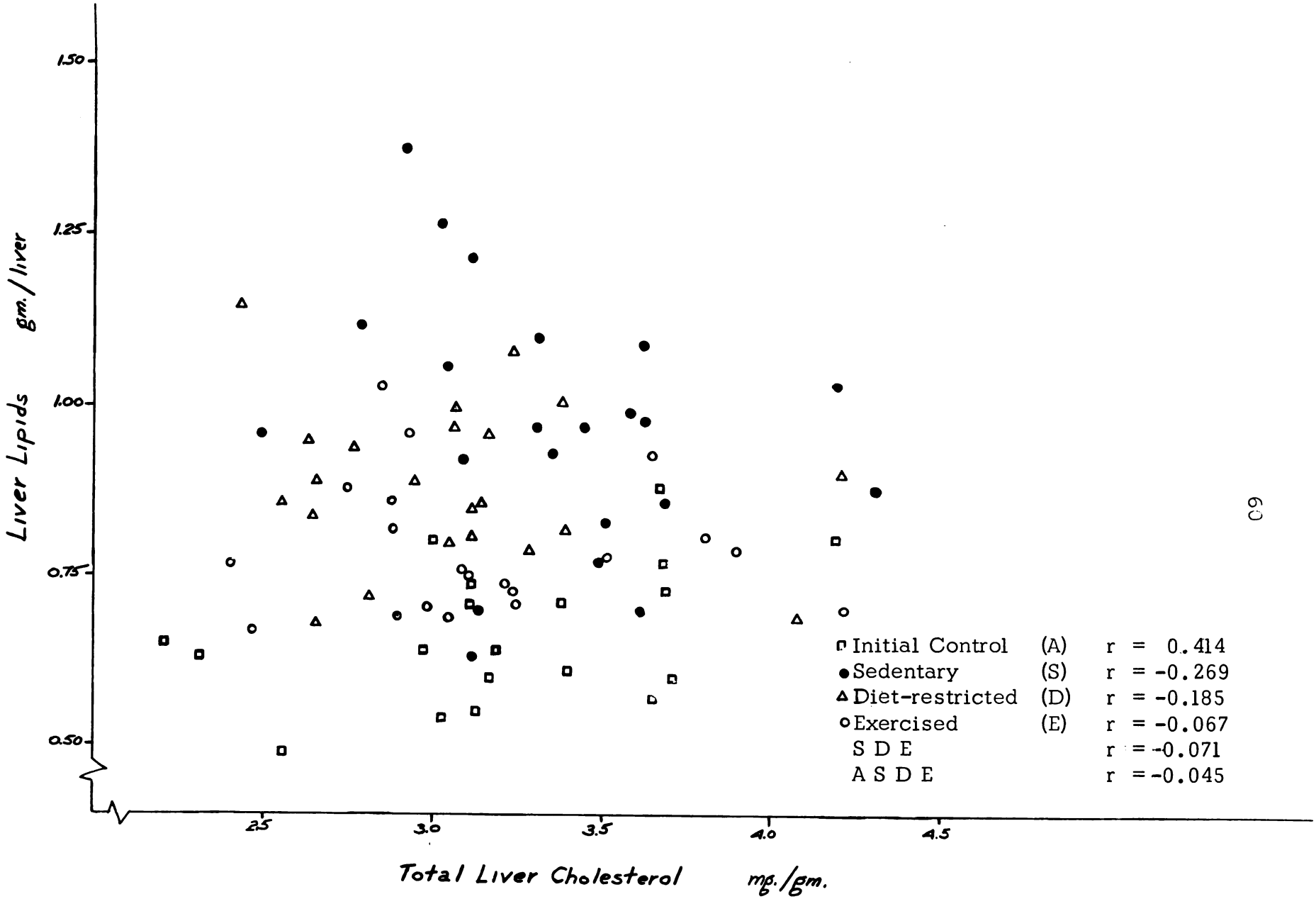

FIG. 9. Total liver cholesterol (mg./gm.) plotted against liver lipids (gm./liver).

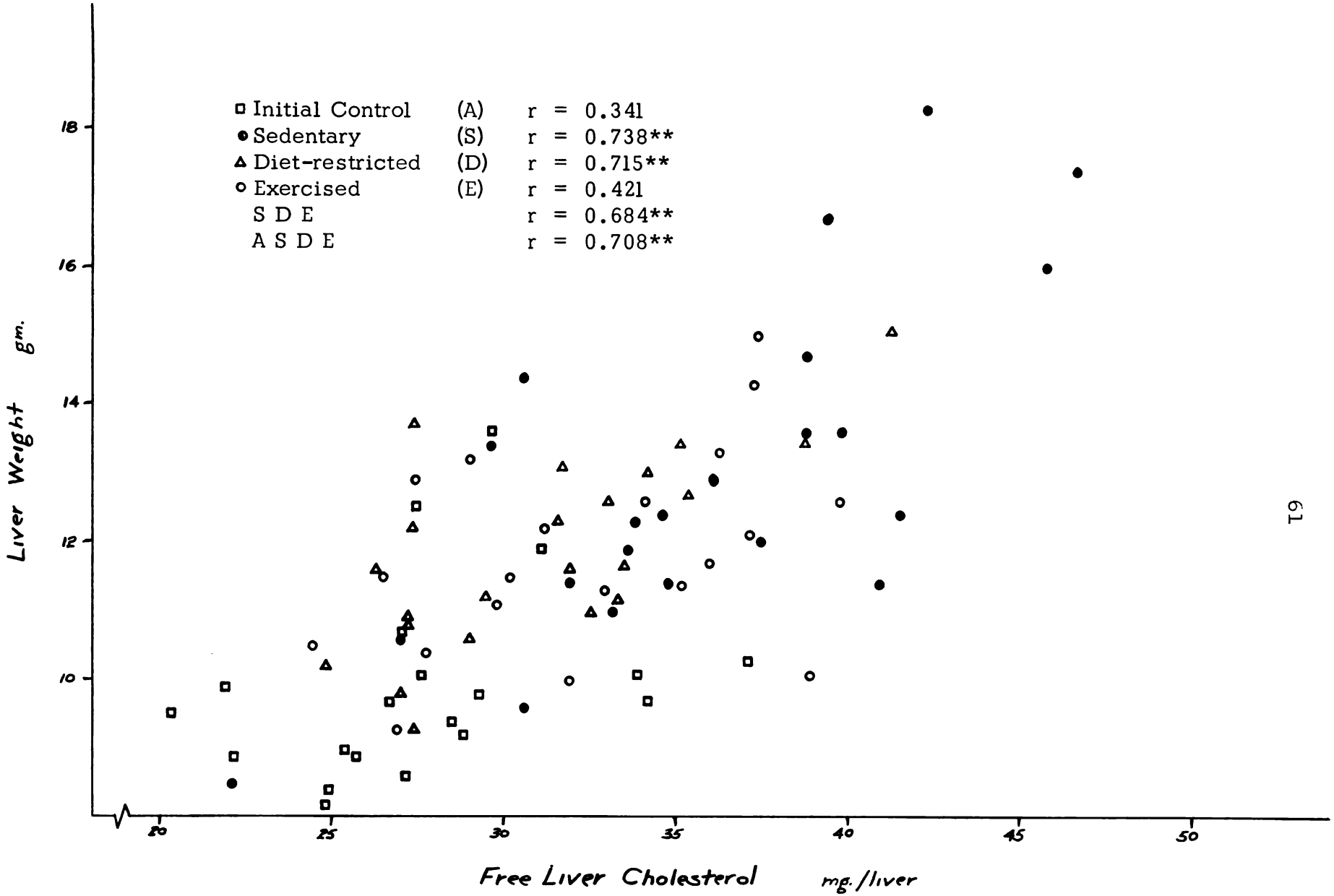

FIG. 10. Free liver cholesterol (mg./liver) plotted against liver weight (gm.).

was correlated with the liver lipids in gm./liver (r = 0.563 to 0.692) except in Group D (Fig. 11). No positive correlation was observed between free liver cholesterol in mg./gm. of liver and liver lipids in gm./liver (Fig. 12). When free liver cholesterol in mg./gm. of liver was plotted against the per cent of liver lipids (Fig. 13) a positive correlation was observed for all animals (r = 0.285) and within Groups A (r = 0.665) and E (r = 0.489). Total liver cholesterol in mg./liver was correlated with free liver cholesterol in mg./liver (Fig. 14) within all groups and for the combined groups (r = 0.894). Similarly, when total liver cholesterol in mg./gm. of liver was plotted against free liver cholesterol in mg./gm. of liver (Fig. 15) a positive correlation (r = 0.916 to 0.632) was observed. Total liver cholesterol in mg./liver and total serum cholesterol were correlated for combined Groups A, S, D and E (r = 0.428) and within Group S (r = 0.510). No correlation was observed for the older animals or within Groups A, D and E, as shown in Fig. 16.

Okey et al.[26] found diet-restriction produced higher serum cholesterol and lower liver cholesterol in both intact and castrated controls than in ad libitum-fed rats. The current study agreed with the findings of Okey et al.[26] on serum cholesterol (Johnson[21]) and liver cholesterol.

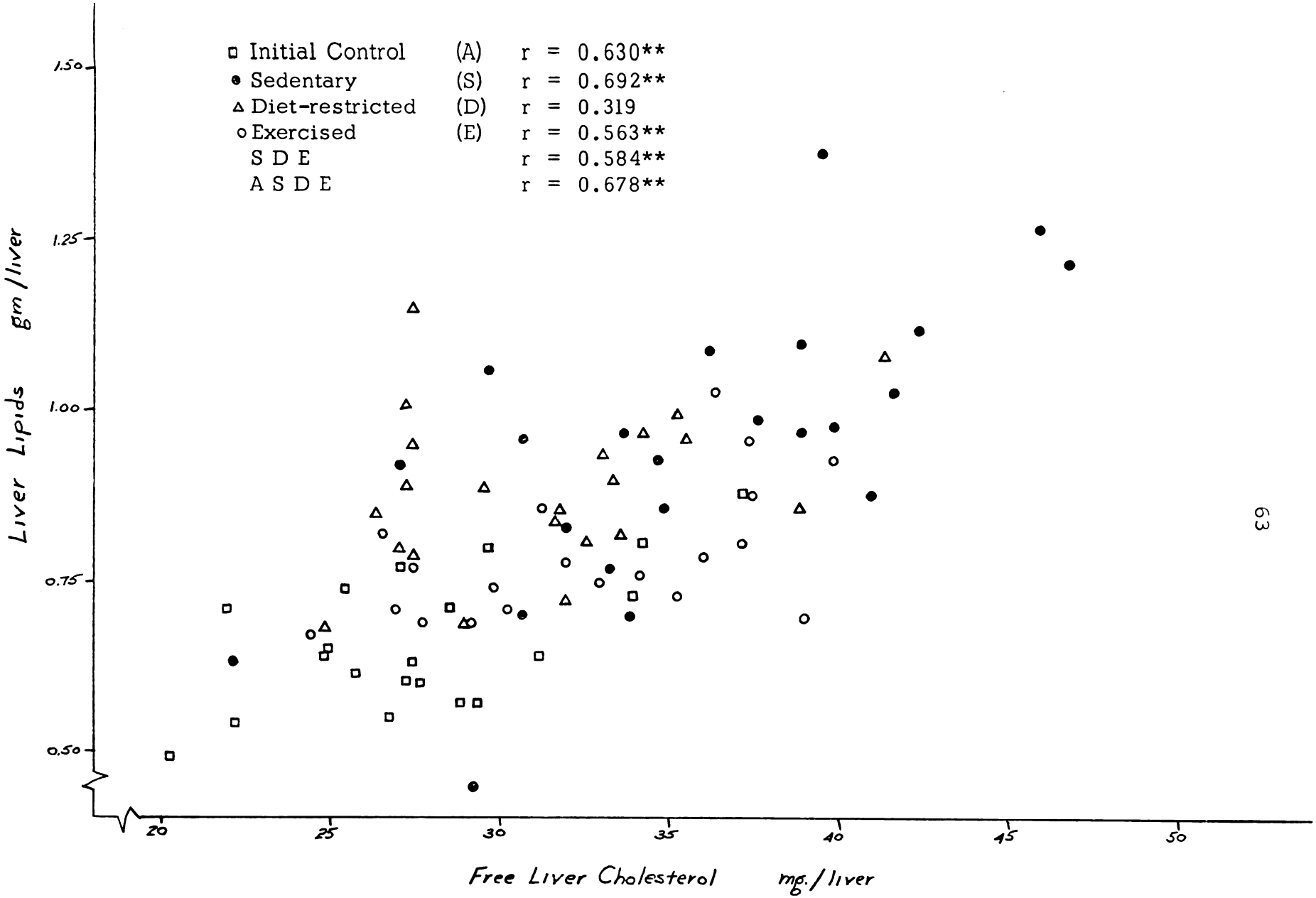

FIG. 11. Free liver cholesterol (mg./liver) plotted against liver lipids (gm./liver).

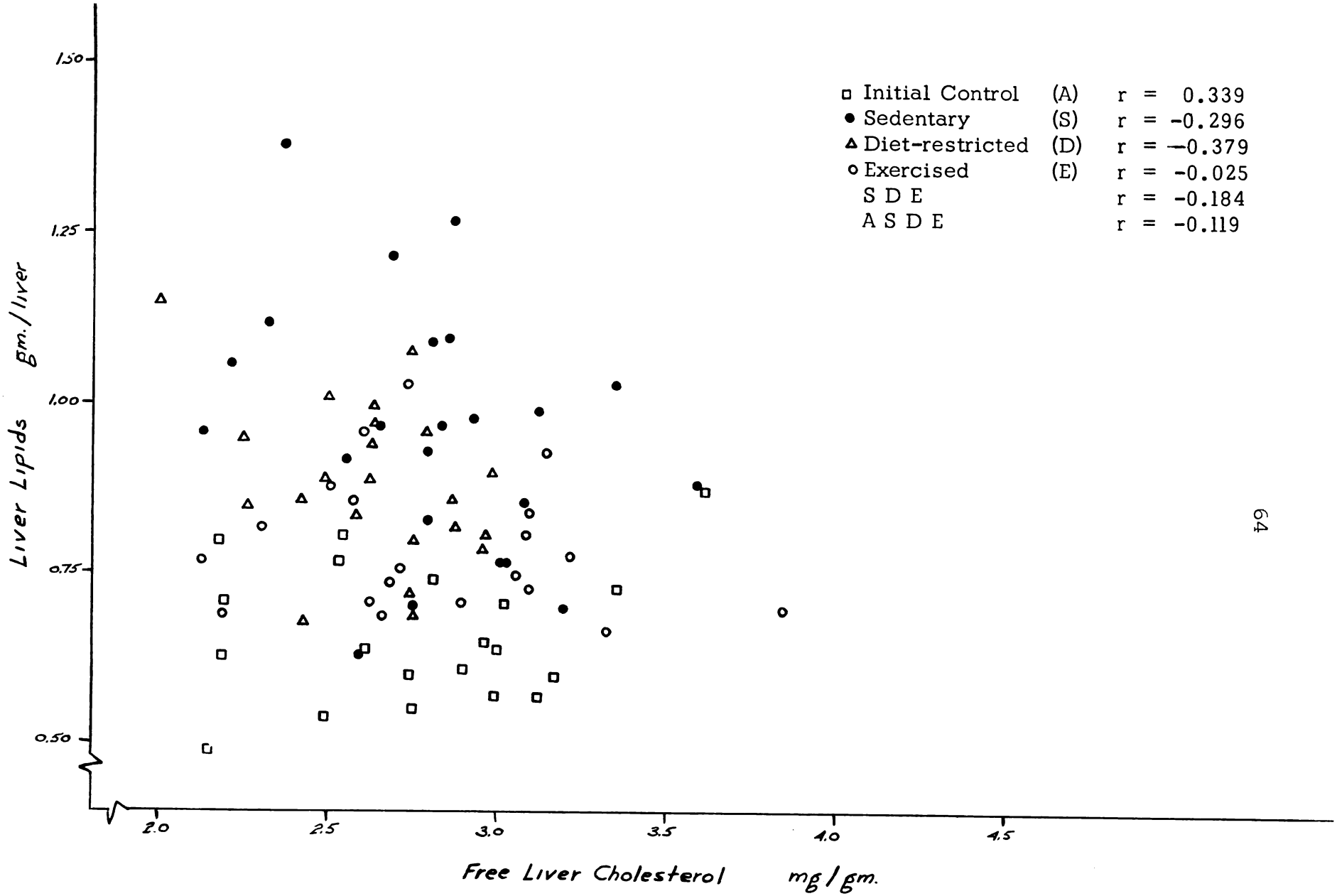

FIG. 12. Free liver cholesterol (mg./gm.) plotted against liver lipids (gm./liver).

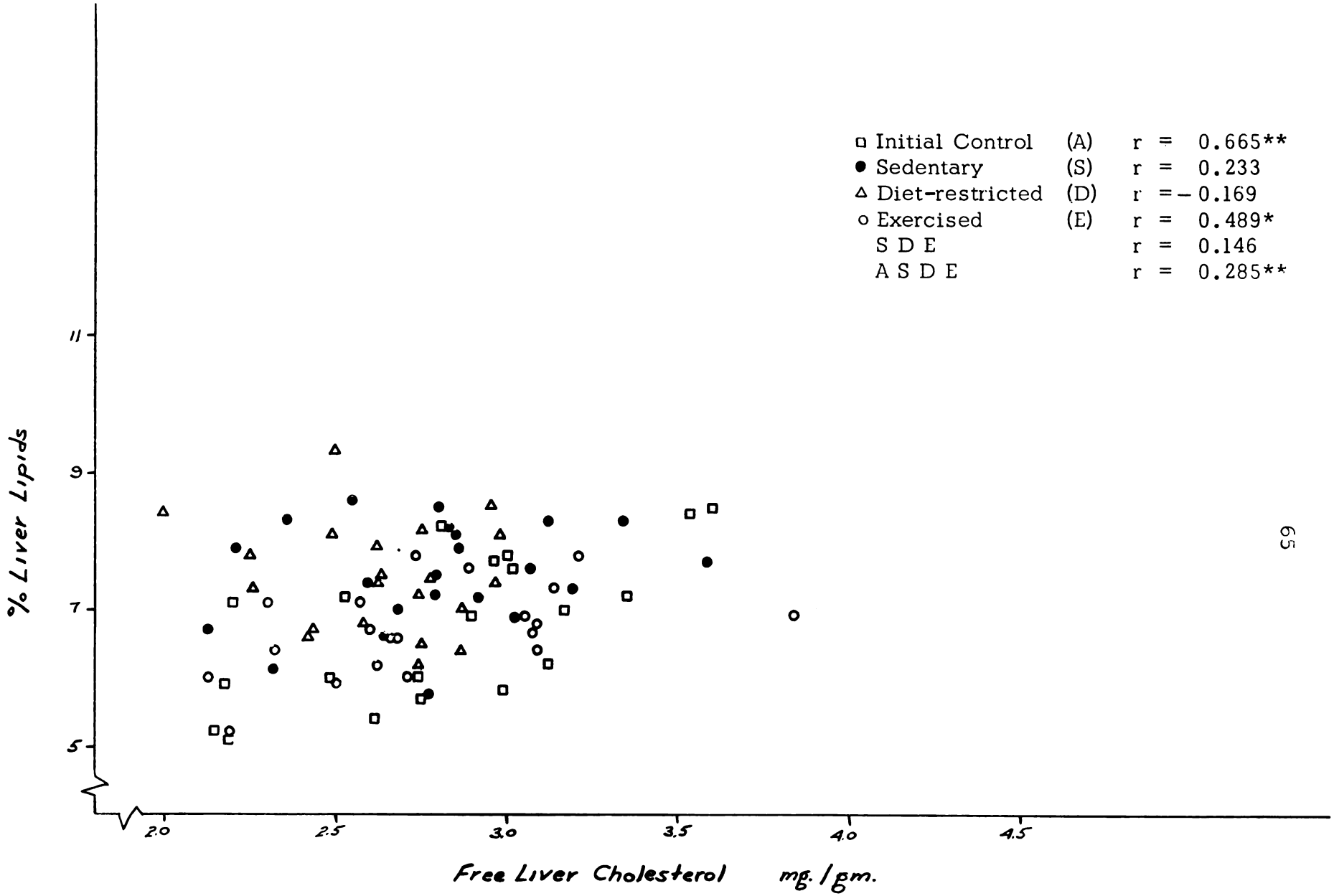

FIG. 13. Free liver cholesterol (mg./gm.) plotted against per cent liver lipids .

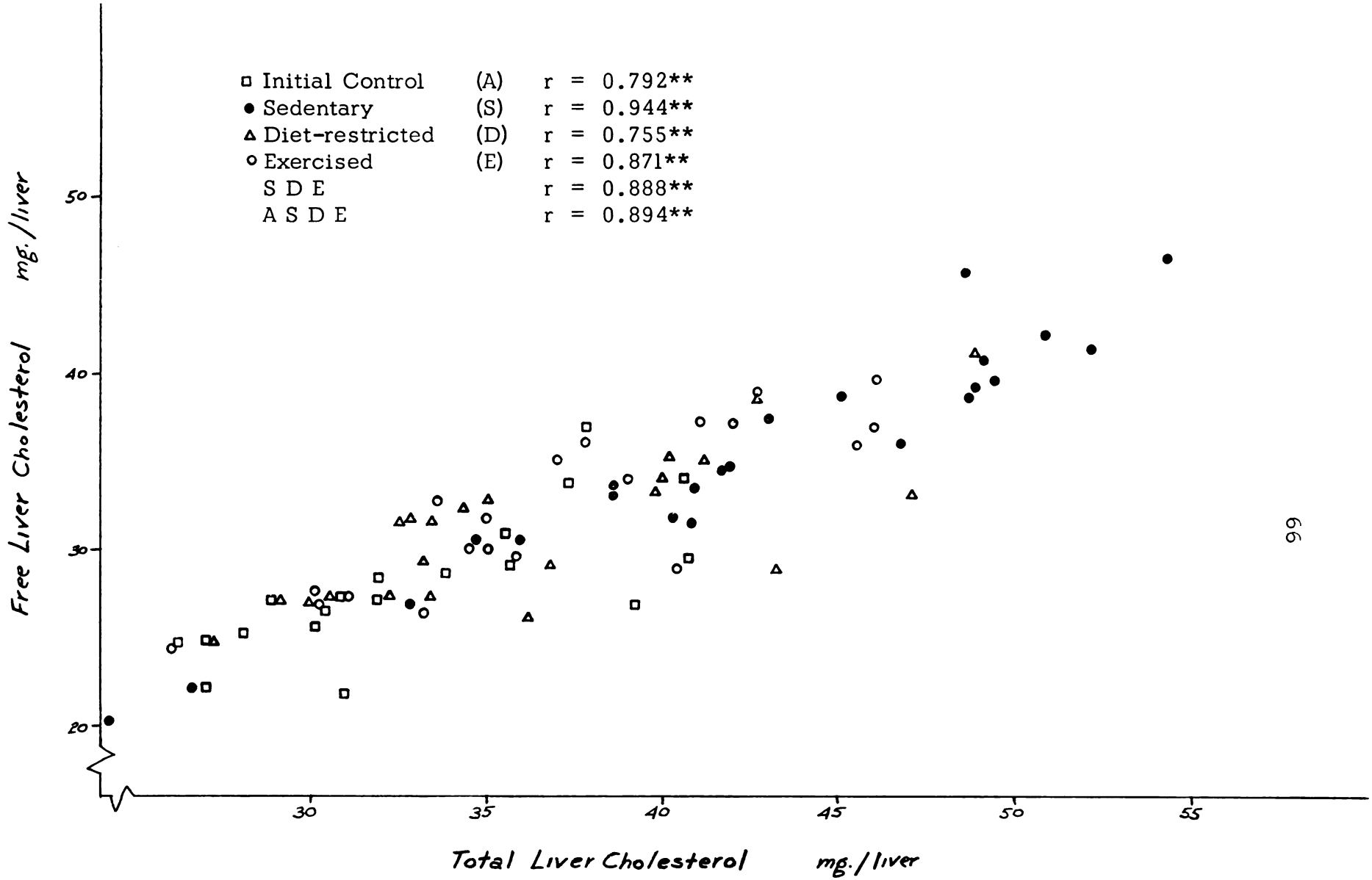

FIG. 14. Total liver cholesterol (mg./liver) plotted against free liver cholesterol (mg./liver).

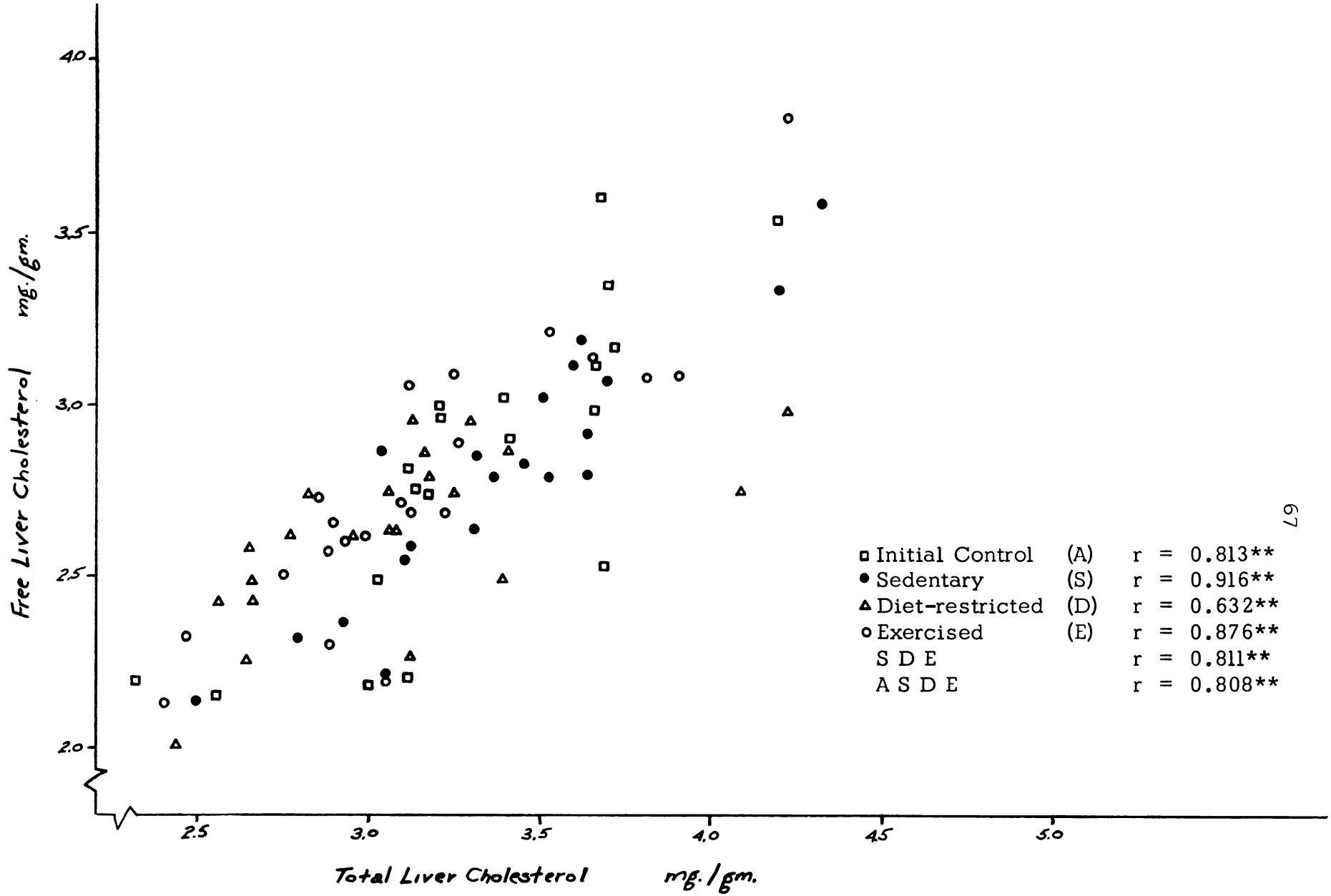

FIG. 15. Total liver cholesterol (mg./gm.) plotted against free liver cholesterol (mg./gm.).

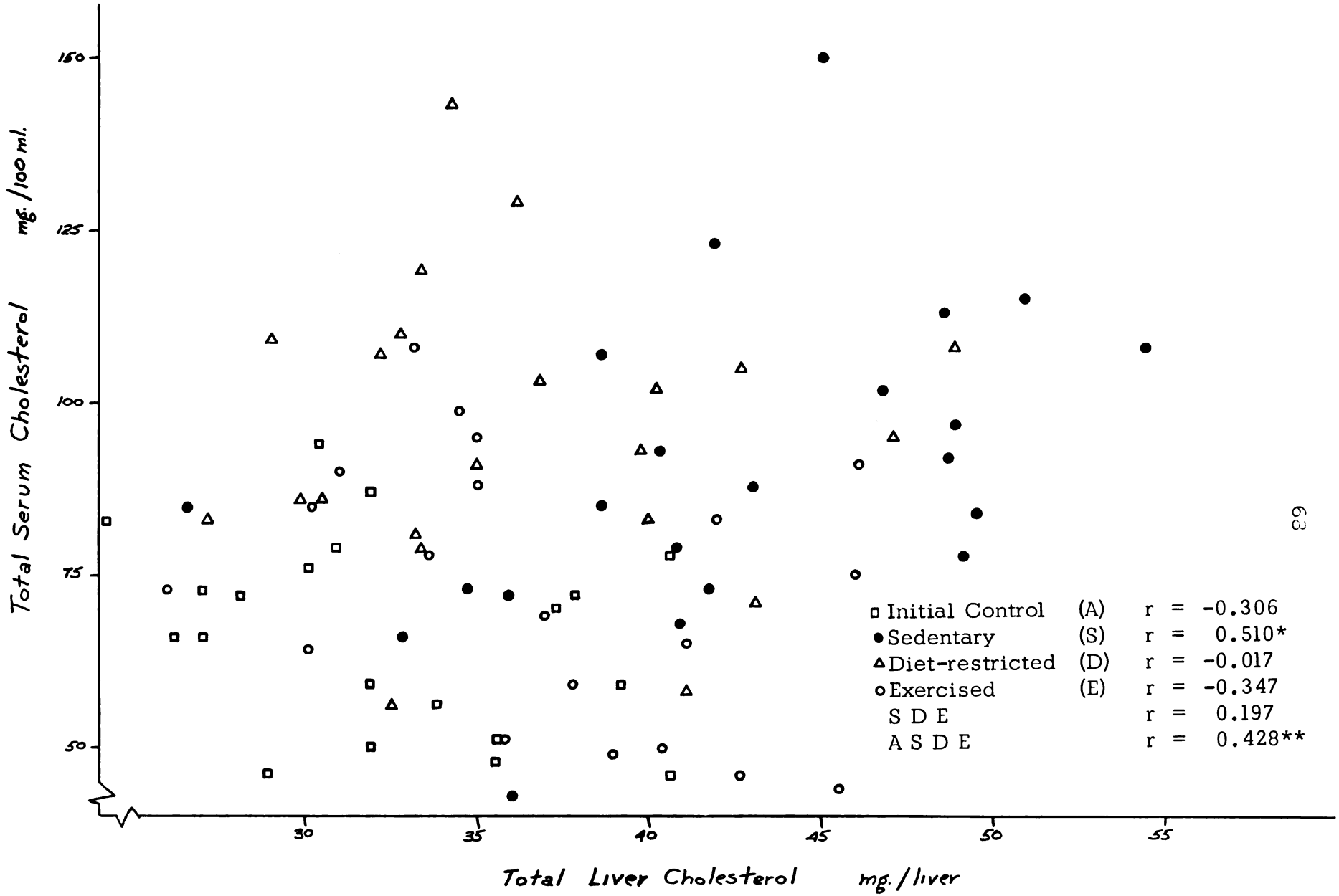

FIG. 16. Total liver cholesterol (mg./liver) plotted against total serum cholesterol (mg./100 ml.).

It seemed that caloric restriction effected a mobilization of liver cholesterol to the blood which resulted in elevated serum cholesterol. This evidence supports the theory serum cholesterol may not be a good index of tissue cholesterol retention. Although exercise was more effective than caloric restriction in controlling serum cholesterol concentrations, both exercise and caloric restriction exerted a similar influence on liver cholesterol.

SUMMARY AND CONCLUSIONS

The purpose of this study was to investigate the effect of exercise and caloric restriction on liver cholesterol in the rat. Eighty-eight male rats (Carworth) were divided into four groups equated on the basis of body weight. The initial controls (Group A) were sacrificed at the beginning of the experimental period. The remaining animals were maintained on a stock diet for fifteen weeks. The sedentary controls (Group S) were fed ad libitum; the exercise animals (Group E) were fed ad libitum and subjected to an exercise regimen; the diet-restricted animals (Group D) were sedentary and were fed restricted rations in order to maintain body weights comparable to those of the exercise animals.

The livers were macerated and the lipids were extracted with ethyl alcohol and ethyl ether. The liver lipids were determined gravimetrically after evaporation of the solvents from aliquots of the extracts. Free and total cholesterols were measured in suitably treated aliquots of the lipid extracts. The cholesterol was precipitated as the digitonide and analysed colorimetrically.

Liver weights of Group A (mean, 9.92 gm.) were significantly less than the older animals of Groups S, D and E. The livers of the sedentary group (mean, 12.99 gm.) were larger than those of the other two groups. No difference in liver weights was observed between the exercise (mean, 11.82 gm.) and the diet-restricted animals (mean, 11.89 gm.). The correlation coefficient for the combined groups ($r = 0.630^{**}$) indicated a positive relationship between body weight and liver weight.

The analysis of variance for liver lipids in mg./liver showed significant differences due to treatment. The means were 0.662 gm., 0.971, 0.885, and 0.789 for Groups A, S, D and E, respectively. The sedentary (7.50 per cent) and the diet-restricted rats (7.46 per cent) had significantly higher percentages of liver lipids than either the initial control (6.74 per cent) or the exercise animals (6.70 per cent). There appeared to be an accumulation of fat in the livers of the diet-restricted and sedentary groups. A highly significant correlation was observed between liver lipids (gm./liver) and the liver weight for all groups combined ($r = 0.788$), for the older animals ($r = 0.766$) and within all groups except A.

No significant differences in total and free liver cholesterol in mg./liver were observed between the diet-

restricted and exercise groups. The total and free cholesterol concentrations of the younger animals were less than those of the groups of older animals; the sedentary rats had higher liver cholesterol concentrations than any of the other animals. The total cholesterol in mg./liver averaged 32.5, 43.2, 36.3, and 37.1 for Groups A, S, D and E, respectively; the free cholesterol in mg./liver, 27.7, 35.9, 31.2 and 32.5 for the groups in the same order. Differences in total and free liver cholesterol in mg./liver were attributed to the size of the liver.

No correlations between total serum cholesterol and total liver cholesterol were observed. Although exercise was more effective than caloric restriction in controlling serum cholesterol concentrations, both exercise and caloric restriction exerted a similar influence on liver cholesterol. It appeared that caloric restriction mobilized liver cholesterol to the serum causing elevated serum cholesterol concentrations.

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