The Effect of Exercise on Food Intake, Growth, Body Composition and Liver Lipogenesis in the Rat

Madeline Dellwo

University of Tennessee, Knoxville
To the Graduate Council:

I am submitting herewith a thesis written by Madeline Dellwo entitled "The Effect of Exercise on Food Intake, Growth, Body Composition and Liver Lipogenesis in the Rat." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Nutrition.

Roy E. Beauchene, Major Professor

We have read this thesis and recommend its acceptance:

Accepted for the Council:
Carolyn R. Hodges
Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)
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THE EFFECT OF EXERCISE ON FOOD INTAKE,
GROWTH, BODY COMPOSITION AND LIVER LIPOGENESIS
IN THE RAT

A Thesis
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Master of Science
Degree
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Madeline Dellwo
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Growth, feed consumption, body composition and hepatic lipogenesis were studied in male Wistar rats that were exercised or feed restricted. The study consisted of 5 groups, with 10 animals per group: Sedentary, ad libitum-fed control group (A); Exercised by swimming for 3 hours/day, ad libitum fed (E3.0); Exercised by swimming for 1.5 hours/day, ad libitum fed (E1.5); Sedentary, diet restricted to match weight of E3.0 (R3.0); Sedentary, diet restricted to match weight of E1.5 (R1.5).

The body weights and feed consumption of the two exercised groups were never significantly different. Therefore, it became unnecessary to treat the two diet-restricted groups separately in an effort to match their body weights to those of the exercised groups. Thus, R1.5 and R3.0 were combined into one group (R) for statistical purposes. It was also observed that the test responses for the 2 exercised groups were not significantly different, hence, these 2 groups were also combined for statistical comparisons (E = E1.5 + E3.0).

Hepatic lipogenesis was estimated by tritium ($^3\text{H}_2\text{O}$) uptake of hepatic fatty acids and glucose-6-phosphate dehydrogenase activity (G6PD). Exercise resulted in enhanced fatty acid synthesis as seen by increased G6PD activity ($p<0.05$) and tritium uptake. Diet restriction produced a non-significant increase in estimated lipogenesis. This was probably due to a meal-feeding effect.

Exercise significantly decreased body weight, in spite of the fact that the feed consumption of E was approximately the same as A. To maintain the same body weight as E, feed intake of R animals was
decreased by 30%. Growth curves for all the experimental groups were of similar shape, though R and E were at a lower level than A.

Body composition measurements were performed in which protein, fat, moisture and ash were analyzed separately. These analyses were expressed in absolute and relative amounts. Exercised rats had a significant decrease in both absolute and relative amounts of body fat as compared to A, and significantly less relative amounts than R, even though E and R were weight-matched. Diet restricted animals also had less absolute and relative amounts of fat than A, though not significantly so. Exercise and caloric restriction produced no significant differences between any of the groups in absolute amounts of protein. Surprisingly, R did have a significantly greater percent protein than E and A.

E had more absolute and relative amounts of moisture than any of the other groups. Though increased body moisture is usually indicative of increased lean body mass, this was not true in this study. Ash showed no significant differences among groups.

In general, exercise resulted in increased hepatic lipogenesis and affected body size and body composition, mainly by decreasing body fat. Diet restriction caused similar alterations, though not to the same degree. Since exercised animals had approximately the same feed consumption, but increased liver lipogenesis and less body fat as compared to control rats, it was concluded that exercise altered lipid metabolism by increasing overall mobilization and utilization of body fatty acids.
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CHAPTER I

INTRODUCTION

The primary method for weight control has traditionally been achieved through dietary changes. However, even though a calorie-restricted diet may result in a loss of weight, body composition on a percent basis after weight loss is usually indistinguishable from free-eating obese animals (1). It has become apparent that factors other than dietary intake contribute to body composition and to the metabolism of fatty acids.

The amount of exercise engaged in by animals is of major importance in regulating their body fat content (2). While inactivity is often associated with obesity (2), there are many studies (3,4,5) showing that exercise results in decreased total body fat (3) and sometimes in increased lean body mass (3,5). Walberg et al (1) demonstrated that exercise favorably altered body composition, adipose tissue cellularity and plasma insulin levels of the obese rat. These investigators also showed that exercise plus food restriction more dramatically affected the body composition and adipose cellularity and resulted in more permanent effects on adipose cellularity than exercise alone.

The role of exercise training in the regulation of body weight has been well documented (6,7). In rats, forced exercise training leads to a decreased weight gain in the fat depots, suggesting an enhancement of lipid degradation and/or an inhibition of its synthesis (9,11).
Recent studies on the effects of exercise training on lipid metabolism have shown that trained animals have the potential of deriving a greater proportion of their energy needs from fat, thus sparing limited carbohydrate stores (8, 9, 10). Several investigators have pointed out the marked effect training has on the mobilization and oxidation of fatty acids (10, 11). However, the effects of training on lipid synthesis are less well documented and more controversial.

The purpose of the present study was to elucidate the effects of exercise training and caloric restriction on feed intake, body weight, body composition and hepatic lipogenesis of rats. It was hypothesized that lower body weight, lower feed consumption, lower body adiposity, and a higher rate of hepatic fat synthesis would be obtained for exercised rats than for non-exercised rats; also for non-exercised, restricted-fed rats as compared to non-exercised, ad libitum-fed rats.
CHAPTER II

REVIEW OF THE LITERATURE

Biosynthesis of Fatty Acids

The de novo synthesis of fatty acids from acetyl-CoA occurs in an active extramitochondrial system. This system takes place in the soluble fraction of many tissues, such as brain, liver, lung, adipose tissue, kidney, and mammary glands (12). It requires NADPH, ATP, Mn++ as cofactors, and HCO₃⁻ as a source of CO₂. Free palmitate is the main end product (13). As shown in Figure 1, acetyl-CoA is carboxylated to malonyl-CoA by the CO₂ provided from bicarbonate. Two enzyme systems, acetyl-CoA carboxylase and fatty acid synthetase, function in sequence to catalyze lipogenesis. Some important features of these reactions are (12):

a. Lipogenesis occurs in the cytoplasm, whereas oxidation takes place in the mitochondria.

b. All carbon atoms of the fatty acids come from acetyl-CoA (obtained from the oxidation of carbohydrates or amino acids).

c. The first, rate-controlling step is the carboxylation of acetyl-CoA to yield malonyl-CoA, catalyzed by acetyl-CoA carboxylase.

d. The synthesis is begun by a molecule of acetyl-CoA which functions as a primer. The carbon chain is then elongated by successive additions of 2 carbons of malonate which are originally derived from acetyl-CoA.

e. The overall reaction for the synthesis of palmitate starting from acetyl-CoA is:

\[
8 \text{acetyl-CoA} + 14 \text{NADPH} + 14 \text{H}^+ + 7 \text{ATP} + \text{H}_2\text{O} \longrightarrow \text{Palmitate} + 8 \text{CoASH} + 14 \text{NADP}^+ + 7 \text{ADP} + 7 \text{Pi}
\]

NADPH serves as the hydrogen donor for both reductions during the biosynthesis of fatty acids. The oxidative reactions of the hexose
Figure 1. Fatty Acid Biosynthesis
From Harper, H. A. (13)
monophosphate (HMP) shunt are the chief source of NADPH. Tissues such as the liver, lactating mammary glands and adipose tissue, specialize in both active lipogenesis and the HMP shunt (13). The HMP shunt pathway, as well as the fatty acid synthesis pathway, are found in the extramitochondrial region of the cell, so there are no membranes or permeability barriers to the transfer of NADPH/NADP from one pathway to the other.

**Glucose-6-phosphate Dehydrogenase.**

Since fatty acid biosynthesis requires NADPH as a reducing agent, a change in rate of fatty acid biosynthesis should result in a change in the rate of production of NADPH (12). Therefore, it is possible to measure the activity of enzymes in the HMP Shunt involved in the reduction of NADP to NADPH to estimate rates of lipogenesis. These enzymes, of which glucose-6-phosphate dehydrogenase is one, thus reflect changes in fatty acid synthesis, but do not themselves cause the lipogenic changes (25).

Glucose-6-Phosphate dehydrogenase (G6PD) catalyzes the first step in the HMP Shunt, oxidizing glucose-6-phosphate to 6-phosphogluconate and reducing NADP to NADPH (14) (Figure 2).

This reaction requires nicotinamide adenine dinucleotide phosphate (NADP) as a cofactor. NADP is reduced to NADPH at a rate that is proportional to G6PD activity. The 6-phosphoglucono-lactone is spontaneously converted to 6-phosphogluconate (6PG) (15), thus 6PG is often referred to as the product of the G6PD catalyzed reaction (16). This is essentially an irreversible reaction (15, 17), as the almost complete conversion of G6P to 6PG is thermodynamically favored (18).
Figure 2. Reaction Catalyzed by Glucose-6-Phosphate Dehydrogenase
From Harper, H. A. (13)
The rapid hydrolysis of the lactone to 6PG results in a large free energy change making the reconversion of 6PG to G6P virtually impossible (19).

G6PD activity has been demonstrated in almost all animal tissues, but especially in blood cells, adipose tissue, and in lactating mammary glands (16). Less occurs in the liver, pancreas, kidney, and lung. Only traces of G6PD are found in skeletal and heart muscle, and none in the serum.

**Tritiated Water**

A frequently used determination of in vivo fatty acid synthesis is to measure the amount of radioactivity incorporated into fatty acids during their synthesis (20). Tritium-labeled water is often the medium employed, since the conversion of carbohydrate precursors into fatty acids yields a constant ratio of hydrogen incorporated into the fatty acid synthesized (21).

Jungas (20) performed extensive studies to determine the reliability of its use. It was found that tritium is, in fact, only incorporated into tissue fatty acids during their synthesis. Gas chromatography revealed that the average chain length of the newly synthesized fatty acid was 16 (22). Fourteen tritium atoms were incorporated into each new palmitate acid residue, or an average of one for each methylene group present.

Further analyses were performed to determine where the tritium was incorporated on the carbon chain (20). This was done by degradation of the carbon chain and measuring the tritium on each individual methylene group. The results clearly indicated that the tritium incorporation is
nonuniform along the carbon chain. Approximately 1.84 tritium atoms were found associated with the even numbered carbon atoms, whereas about 0.74 atoms were present on the odd numbered positions.

Junga's data demonstrated that the incorporation of tritium from tritiated water into fatty acids may be used as an accurate measure of the total rate of fatty acid synthesis in normal rats. Approximately 46%-56% of the hydrogen of newly synthesized fatty acids was derived from water, the rest coming from NADPH.

**Alterations in Rate of Lipogenesis**

The rate of fatty acid synthesis can be affected by many factors. It is very low in physiological states associated with carbohydrate deprivation, such as diabetes mellitus or starvation (23, 24). Meal feeding of rats alters lipogenic activity (25) as do diets low in protein (24). Exercise affects fatty acid synthesis (8-11,27,28); however, the extent and direction of these effects is uncertain.

To fully understand the literature, it is helpful to define the terminology used regarding exercise. In this review, "training" implies endurance exercise performed regularly, over a period of time, to exert a training effect. Exhaustive exercise usually refers to one bout of exercise, performed until the animal is no longer able to continue. When an animal can choose to exercise, such as when it is allowed free access to a running wheel, it is said that the animal is allowed ad libitum exercise. However, if the animal has no control over the exercise, as with the use of an electric treadmill or swimming, this is referred to as forced exercise.
Many investigators have found decreased lipogenic activity as a result of exercise. Richard and Trayhurn (9) observed decreased rates of fatty acid synthesis in all tissues, during exercise and at rest, in male mice swum for 28 days. When male rats were trained on a treadmill for 13 weeks, decreased rates of lipogenesis were again seen (10). Whether exercise was forced, as in treadmill running (10, 26) or in swimming (8, 9), or if the animals had free access to a running wheel (11, 27), similar decreases in fatty acid synthesis were found.

One of the most frequent postulates for decreased fatty acid synthesis is the benefit of sparing dietary carbohydrate crucial for muscle glycogen replenishment after endurance work (8). Richard and Trayhurn (9) and Askew et al. (10) stated that carbohydrate is shunted away from the synthesis of lipids in favor of energy storage as glycogen. Rats may adapt to physical training by decreasing lipogenesis during exhaustive exercise to facilitate the conserving of blood glucose, and in that way, prolong endurance (10).

Tsai et al. (11) investigated the effects of cessation of exercise on fatty acid synthesis in female hamsters and found increased lipogenic activity. This was also observed by Tokuyama and Okuda (27) when female rats were allowed free access to a running wheel for fifty days, and by Dohm et al. (42) in male rats trained by treadmill running. The data of Tsai et al (11) suggested that, in trained hamsters, fat metabolism had reached a new state of equilibrium due to increased catecholamine levels during exercise and increased insulin during resting. However, Tokuyama and Okuda (27) found no difference in the concentration of serum insulin after exercise training. They
therefore postulated that the increased lipogenesis was secondary to increased food intake and thus a greater overall utilization of fatty acids.

It is necessary to examine the literature more closely to understand why various discrepancies appear in the studies. Richard and Trayhurn (9) investigated the effects of exercise training on in vivo fatty acid synthesis in mice at rest and during exercise. This was determined by measuring the incorporation of tritiated water into fatty acids. The results indicated that long-term exercise decreased the rate of lipogenesis in various tissues. However, the mechanisms by which exercise training affected fatty acid synthesis were not clearly recognized. It is possible that the reduced rates of synthesis were secondary to acute changes which take place during a single occurrence of exhaustive exercise. Changes in the plasma levels of glucagon, catecholamines, and insulin can create hormonal conditions during exercise that are conducive to lipolysis and inhibitory to lipogenesis. The concentrations of these hormones return to normal values within a few hours following the cessation of exercise. Therefore, it is unlikely that they still exert a direct inhibitory effect on fatty acid synthesis 24 hours after the end of the last training session. Nevertheless, it is possible that other acute metabolic changes take place during exercise which could influence lipogenesis long term.

The effect of physical training and exhaustive exercise on fatty acid synthesis was investigated by Askew et al. (28). They found that exhaustive exercise decreased lipogenesis in trained rats, but not in the untrained animals. However, because the untrained rats were unable
to run as fast or as long as the trained rats, it is difficult to state whether the difference is due to training induced metabolic changes, or merely the workload performed. Nevertheless, the possibility exists that the differences in lipogenesis in the trained versus the untrained rats was due to hormonal influences. Characteristic of states in which fatty acid synthesis is decreased is a high rate of fatty acid oxidation. Trained rats have approximately a two-fold increase in fatty acid oxidation and fatty acid mobilization potential compared to untrained rats. High levels of fatty acyl-CoA in adipose tissue of exhausted trained rats may also act to decrease lipogenesis by direct inhibition of acetyl-CoA carboxylase. Whatever the mechanism, Askew et al. suggested such an adaptation is useful in that this would most likely result in direct oxidation of glucose, which would be energetically more efficient to the exercised animal than converting its energy to fat prior to oxidation. This would also permit a certain degree of blood glucose conservation.

Askew et al. (10) recognized that during submaximal exercise most of the energy is supplied by the oxidation of fat. Therefore, long-term exercise increased the ability of the body to mobilize and to oxidize fatty acids, but its influence on synthetic metabolic processes has not been clarified. For this reason, Askew designed an experiment to test the effects of diet, training, and exhaustive exercise on enzyme activities related to lipogenesis. They found significant diet-training interactions for the lipogenic enzymes in the liver tissue of rats fed a high carbohydrate diet. This may relate to an interaction between the stimulation of lipogenesis by the high
carbohydrate diet and the effect of daily exercise on hormonal secretions. Epinephrine released in response to the stress of exercise may suppress or inhibit insulin secretion. This would shunt glucose to the muscle tissue for oxidation. The results of this study indicated that prior physical training can influence the response of hepatic lipogenic enzymes to diet. Dietary glucose in trained rats appears to be directed more toward the synthesis of glycogen rather than lipids. This is a more beneficial adaptation to training, according to Askew et al. (10), than increasing lipogenesis since there is a loss of energy to the animal during the conversion of glucose to fat.

In contrast to the above, however, there have been some studies showing increases in lipogenesis as a result of training. Tsai et al (11) looked at the effect of retirement from exercise on fatty acid synthesis in female hamsters. Compared to sedentary controls, hepatic lipogenesis in the trained animals was 1.63 times the control value during exercise, 1.78 times at 8 days after retirement and 1.40 times at 41 days after retirement. There was a significant increase in the rate of fatty acid synthesis and the activity of lipogenesis or lipogenic-related enzymes in the liver during the exercise period. The increased lipogenic capacity is probably the result of enhanced tissue sensitivity to insulin due to exercise, plus increased circulating insulin levels during the resting phase of exercised hamsters. Thus, in trained hamsters, fat metabolism reaches a new state of equilibrium, i.e., a high rate of lipolysis and of beta-oxidation during activity due to increased catecholamines, and a high rate of lipogenesis during
resting due to insulin, leading to the replenishment of depleted tissue stores of triglycerides.

Tokuyama and Okuda (27) also found increased lipogenic activity with training. Rats with free access to a running wheel for 50 days had a rate of fatty acid synthesis three times higher than sedentary rats. Because the synthesis of fatty acids is stimulated by insulin, the concentration of serum insulin was also analyzed, but no differences were found between the groups. It was postulated that the increased fatty acid synthesis in the trained rats is secondary to increased food intake. The amount of body fat in trained rats was less than sedentary rats, in spite of the increased food intake and increased lipogenesis. Therefore, it was concluded that the overall utilization of fatty acids must be increased, i.e., training enhances the turnover of fatty acids in the body.

Again, contrary to the findings above, in rats exercised by swimming, Scorpio, et al. (8) found a decrease in fatty acid biosynthesis as indicated by a lower rate of hepatic acetyl-CoA carboxylase activity. Some possibilities regarding regulation of acetyl-CoA carboxylase were suggested: a) exercise produces a noncompetitive type inhibitor of the enzyme; b) exercise decreases the concentration of the enzyme; or c) exercise inactivates the enzyme, possibly by covalent modification. Whatever the means by which acetyl-CoA carboxylase is regulated, these authors see it as a beneficial response. It would, first of all, help spare dietary carbohydrate crucial for muscle glycogen replenishment after endurance work. Secondly, it would complement hepatic fatty acid oxidation,
which is increased with exercise. This would, thus, provide energy
for conversion of lactate and alanine to glucose in the liver.

It is known that cold temperatures induce a depression in liver
lipogenesis. Hartsook and Martin (26) evaluated the effects of
simultaneously varying the environmental temperature, percentage of
diet dilution with non-nutritive cellulose and minutes of daily
treadmill running on body weight gain and lipogenic enzymes. G6PD
activity was increased in a low exercise-high dilution diet regimen and
also with a high exercise-low diet dilution. However, its activity was
depressed with low exercise-low dilution and with high exercise-high
dilution situations. Environmental temperature had no effect on G6PD
activity in this study, although it did effect other lipogenic enzymes.

From these observations, the rate of fatty acid synthesis appears to
decrease as the level of exercise increases, up to a certain point of
exertion. Thereafter, Hartsook and Martin (26) observed that the
turnover of fatty acids increases and the net amount of fat deposited
is continuously decreased. The role of endocrine control of these
metabolic responses to exercise and diet dilution remains to be
determined. It has been shown that exercise results in an increase in
growth hormone secretion. The increased growth hormone levels could
explain the increase in fatty acid turnover due to its lipolytic
activity. The depression in insulin, in response to exercise, would
potentiate the lipolytic response to growth hormone. These
investigators suggested that to determine how these hormones interact
in the exercised animal, it is necessary to measure endocrine changes
in an experimental design similar to this one.
To examine the effects of forced exercise on the development of diet-induced obesity, and then assess the reversibility of exercise-induced changes following retirement from exercise, Applegate et al. (29) used Osborne-Mendel rats, which have been shown to become readily obese. These rats were exercised by running on a motor-driven treadmill while fed a high fat diet to induce obesity. Exercise did significantly reduce total fat pad lipogenesis, thus demonstrating that a moderate exercise program can attenuate diet-induced obesity. The effects were not long lasting, though, as they were reversed within 2 weeks of exercise termination. A rapid increase in food intake, weight gain and lipogenesis occurred after cessation of training. The exaggerated reversal with detraining is perhaps due to the enhanced insulin sensitivity with exercise, though other factors need to be analyzed.

As can be seen, the results of various studies are not consistent as to what effect exercise has on lipogenesis. Richard and Trayhurn (9) suggested some reasons for these discrepancies. There have been differences in the sex of the animals, the species, the timing of measurements of fatty acid synthesis, and the particular depot analyzed between the various studies. Different rates of fatty acid synthesis could be accounted for by some variations in food intake caused by sex, species, and training or by interactions among them. All these factors could be playing a role in causing the differences in the results obtained. Therefore, the mechanisms by which exercise affects fatty acid synthesis are not clearly understood.
Exercise plays an important role in long-term regulation of body weight and composition. Mayer (6) hypothesized that the combination of diet and exercise may be the best way to regulate body weight. As a result of his work, the effects of exercise on body composition has been looked at extensively.

Carcass analysis on male mice, performed by Bulbulian et al. (30), showed decreases in percent body fat with increases in exercise. There was a small but non-significant decrease in feed intake. A possible explanation for the loss of body fat may be that a training effect occurs with long-duration exercise, allowing the animal to become progressively more capable of oxidizing fat. Long-term exercise also appeared to have a catabolic effect on the lean body mass, as suggested by a drop in protein content in these mice. The latter was unexplained, but the mice seemed to reach a low plateau of body fat at approximately 12%. When the mice reached this plateau, it is possible that protein was used as an alternative source of energy.

Rats were initiated to a running regimen at 7 weeks of age or younger in research done by Pitts (7). The exercise was associated with a decrease in the fat-free body mass (FFBM) in males and an increase in females. It was also found that initiation of the activity at 11 weeks of age or older did not change FFBM. Activity at all ages reduced body fat in either sex. There were several other notable variations in response to exercise between the sexes. For example, males did not increase their level of food consumption with exercise, whereas the females did. Body weights of exercised males were 120
grams below that of controls, while those of exercised females remained close to control values. Looking at body composition in general, fat showed greater lability than FFBM, which was much more stable.

Some interesting generalizations emerged from this study: all exercise modes decreased body fat; all exercise modes changed FFBM if initiated at 7 weeks of age, though males had smaller changes than females; if exercise was initiated at the 21st week, instead of the 11th week, FFBM remained constant. This might be due to the possibility that at seven to nine weeks of age, the increase of total DNA (believed to reflect hyperplasia) falls off. Sex differences remain to be explained. Energy budgets clearly differ between the two sexes.

A consideration that Pitts (7) felt should be taken into account with his study is that swimming of rats is potentially severe and often excessive. This may result in psychological stress and overtax the thermoregulatory mechanism. Such stress may lead to an inability to regulate the FFBM and may explain divergent results in studies in which rats who were exercised by swimming as opposed to other means.

Oscai et al. (31) subjected eight-day-old rats to a program of swimming over a period of 14 - 16 weeks for 6 hours per day. The exercised rats gained weight more slowly than those not exercised. This was due solely to an increase in expenditure of calories, as food intake remained unaffected by the exercise. Exercise also resulted in a significant decrease in the total body content of fat, plus a reduction in lean body mass. A non-exercised group was weight-matched with the exercised group by restricting their diet. The weight-matched
group had a decrease in both fat and lean body mass. Though the weights were the same, the body composition of the two groups was different. The weight-matched group had almost twice as much fat as the exercised group. It was concluded that exercise was effective in reducing the rate of accumulation of cells in epididymal fat pads of rats, which could have interesting implications with respect to the development of obesity.

Similar results were found by Pitts and Bull (32). They exercised rats on a treadmill and determined that exercise decreased the masses of both the fat and the fat-free compartments below values for sedentary groups. There was no rectilinear growth in the exercised groups, which most probably reflected an absence of fat accretion. It was also found that exercise begun at 5 - 7 weeks of age resulted in a reduced lean body mass. However, when exercise was initiated at 21 - 23 weeks, lean body mass was unaffected. The effects of exercise on body composition which were seen at the end of the exercise period (141 days) disappeared by the 293rd day of age, leaving exercised and non-exercised groups with statistically similar body compositions.

No hypertrophy of the limb and girdle muscles was seen when Oscai et al. (33) examined the body composition of female rats subjected to a program of swimming. This is in spite of the fact that these are the muscles directly involved with the exercise. These muscles are also weight-bearing, i.e., they support the animal's body weight during normal activities. Thus, the evidence suggests that the effect of exercise on muscle size is determined more by forcefulness than by the frequency of muscle contractions. Surprisingly, the exercised animals
did have a greater lean body mass than the sedentary animals. The increases in LBM came mainly from skin and subcutaneous tissue. This finding was unexpected and was not explained. There was also hypertrophy in the muscles responsible for stabilizing the head, shoulders, spine, pelvis and tail. The latter are, normally, non-weight-bearing muscles, so it can be postulated that the isometric contractions involved in stabilizing these muscles during swimming do provide a stimulus for hypertrophy.

To examine the effects of fat intake and exercise on the body composition, Hanson et al. (34) swam rats for two 30-minute periods each day for six weeks and fed them either a high- or a low-fat diet. Exercise resulted in a significantly different body composition at the ad libitum intake level, as animals laid down less fat in proportion to total body weight gain. Of interest, though, is that exercise coupled with caloric restriction did not alter relative body composition of animals losing weight. Thus, the composition of weight loss seemed to be the same as that originally gained. This would be expected of weight loss due to caloric restriction alone, but is not usually seen when exercise accompanies caloric restriction. No explanations were provided. An increase in the metabolic activity in rats forced to exercise was also seen, as indicated by their enlarged livers, hearts, and adrenal glands, particularly in relation to body size. It was suggested that the larger livers might be a result of increased glucocorticoid secretion from the enlarged adrenals, and thus be an indirect stress response.
In contrast to the above study, Walberg et al. (1) demonstrated that exercise altered body composition, and that exercise plus food restriction was even more effective. The latter treatment also resulted in more permanent effects on adipose cellularity than exercise alone. In this study, seven-week-old female lean and obese Zucker rats were swim trained or kept sedentary for eight weeks. Another group was exercised plus food restricted. The exercise regimen stimulated an increase in the food intake in both obese and lean rats, but this was not sufficient to compensate for the higher energy expended since both exercised groups gained less body weight than sedentary controls. However, virtually all changes resulting from exercise were found to be transient. Body fat of exercised rats surpassed that of sedentary by the end of four months of retirement.

In conclusion, the consensus among researchers appears to be that exercise does, in fact, decrease absolute and relative amounts of body fat in the animal model (30,31,32,34), though males tend to lose more fat than females (7). However, there seems to be no agreement on whether hypertrophy of the muscles occurs with exercise. Changes in fat free body mass (FFBM) appear to be dependent on gender, type of animal model, age, and type of exercise (7). Nevertheless, it is apparent from the literature that exercise results in changes in body composition that are different from diet restriction alone.
CHAPTER III

EXPERIMENTAL PROCEDURE

Basic Design

In this study, hepatic lipogenesis and body composition were measured on exercised and non-exercised animals. Male rats of Wistar origin were obtained from Taconic Laboratories, Germantown, New York. Animals were housed individually in wire mesh stainless steel cages (20 cm x 20 cm x 26 cm). The animal facility was maintained at 22±2°C, with a 12 hour light - 12 hour dark cycle. Water was provided ad libitum.

There were 5 experimental groups:

A: Non-exercised; ad libitum fed control group
E3.0: Exercised by swimming for 3 hours/day; ad libitum fed
E1.5: Exercised by swimming for 1.5 hours/day; ad libitum fed
R3.0: Non-exercised; diet restricted to match weight of E3.0
R1.5: Non-exercised; diet restricted to match weight of E1.5

The animals were weighed twice weekly to assure that the groups were weight-matched throughout the experiment. Feed intakes were measured weekly. The amount of diet fed to R1.5 and R3.0 was adjusted weekly to counteract excessive gains or losses in weight. The diet fed was ground Purina Rat Chow.

The exercised animals were swum in groups of five in 32 gallon plastic barrels (50 cm x 68 cm) for either 1.5 (E1.5) or 3.0 (E3.0) hours/day. Water temperature was maintained between 34°C - 37°C. The exercise regimen was begun at 6 weeks of age and continued for 6 months. When the animals were 7.5 months of age all of the rats were sacrificed.
One hour before sacrifice, rats were injected with tritiated water (1 mCi/100 gm body weight). Rats were sacrificed by decapitation. Livers were removed, weighed and analyzed for G6PD and \(^3\text{H}_2\text{O}\) uptake. Carcasses were frozen at -15°C until analyzed for body composition.

**Preparation of Liver Homogenates**

Immediately following excisions of livers, they were blotted with filter paper to remove excess blood, and weighed. Approximately 0.5 g liver tissue was added to ethylene-diamine-tetra-acetic acid (EDTA) saline at a dilution of 1 mg tissue wet weight/0.04 ml EDTA saline. This was kept in an ice bath and homogenized for two minutes on a motor driven homogenizer with a teflon pestle. The homogenates were then centrifuged for 20 minutes at 0°C and 2000 x g. The clear supernatant fractions were decanted into vials after centrifugation and kept on ice until assayed. The assays were always done immediately following sacrifice. The remaining portion of the liver was frozen in liquid nitrogen and stored at -15°C until measured for tritium uptake at a later date.

**Methods for Enzyme Assay**

G6PD activity of rat liver supernatant fraction was determined by the method of Lohr and Waller (16). The basis of this method is the spectrophotometric measurement of the rate of NADPH formation from the increase in absorbance at 340 nm.

**Reagents.**

1. Triethanolamine buffer (0.05 M; pH 7.5): 0.93 g triethanolamine hydrochloride and 0.2 g ethylene-diamine-tetra-acetic
acid (EDTA-Na₂H₂·2H₂O) was dissolved in ca. 50 ml distilled water. This was adjusted to pH 7.5 with 0.1 N NaOH and diluted to 100 ml with distilled water.

2. EDTA-saline: Physiological saline containing 6.6x10⁻⁴ M EDTA was made by dissolving 0.25 g EDTA-Na₂H₂·2H₂O in physiological saline and diluting to 1000 ml.

3. Glucose-6-Phosphate (ca. 4x10⁻²M G6P): 130 mg G6P-Na₂ was dissolved in 10 ml distilled water.

4. Nicotinamide adenine dinucleotide phosphate (ca. 3 x 10⁻²M): 25 mg β-NADP-NaH₂ was dissolved in 1.0 ml 1% NaHCO₃ solution.

Procedure. The reaction mixture was prepared containing 2.40 ml triethanolamine buffer, 0.50 ml supernatant of the liver homogenate, 0.05 ml NADP solution and 0.05 ml G-6-P solution, in a final volume of 3 ml. Blanks consisted of all the reagents except NADP.

The reaction was initiated by adding the G-6-P solution to blank and sample tubes. The contents were poured into silica cuvettes and mixed by pouring back into the original tube, then back to the silica cuvette. Then the absorbance (A) of the sample was measured against the blank at 340 nm in two minute intervals for 10 minutes with a Beckman spectrophotometer (Model 34, Palo Alto, California). Determinations were run in duplicate.

The enzyme activity of G6PD was expressed as µmoles NADPH produced per min/g of protein as calculated from the extinction coefficient (E₉₄₀⁰⁰) of NADPH as shown in the following equation:

\[ \text{G6PD Activity} = \frac{(E/\text{min}) \times 3 - 6.2 \times (E_{940} \times (\text{g pro/0.5 ml supernatant fluid}))}{E_{940}} \]
Determination of Tissue Protein Levels

The protein content of the homogenate supernatant was determined by the method of Ohnishi and Barr (35). This method is a modification of the Lowry method (36) whereby the protein sample is mixed first with a diluted biuret reagent and later with 2 N phenol reagent for color development.

Reagents.

1. Biuret reagent: 1.5 g CuSO$_4$$\cdot$5H$_2$O, 6 g NaKC$_4$H$_4$O$_6$$\cdot$4H$_2$O, and 30 g NaOH and 1 g KI were dissolved, in order, and diluted to 1 liter with distilled water and stored in a polyethylene bottle. This was diluted eight times with 2.3% Na$_2$CO$_3$ (i.e., 1 volume of biuret reagent plus 7 volumes of Na$_2$CO$_3$) at the time of analysis.

2. 2 N phenol reagent.

3. Bovine serum albumin stock standard: 1 g bovine serum albumin was dissolved in and diluted to 100 ml with 0.4 NaOH. Working standards were prepared daily by diluting this to a concentration of 0.02 g bovine serum albumin per 100 ml of 0.4 N NaOH.

Procedure. To 0.8 ml of supernatant, 3.2 ml of the diluted biuret solution was added. The mixture was allowed to stand for 10 minutes at room temperature. 0.1 ml of 2N phenol reagent (undiluted) was added while the solution was mixed using a vortex mixer. The protein standard containing bovine serum albumin received the same treatment as the supernatant from the samples.

After 30 minutes, the absorbance of the samples and standards were read in a spectrophotometer at 550 nm against a blank containing all
reagents except 0.8 ml water was used in place of the supernatant.

The protein concentrations of the samples were calculated by the following formula:

\[
\text{mg protein/ml supernatant fluid} = \frac{A_{\text{sample}}}{A_{\text{standard}}} \times 0.2 \text{ mg pro/ml} \times 10 \text{ (dilution factor)}
\]

**Measurement of Tritiated Water Uptake**

The method used to measure the rate of *in vivo* hepatic lipogenesis with tritiated water was that described by Lowenstein et al (21).

**Reagents.**

1. Chloroform-methanol (2:1, by volume): 1000 ml of chloroform was mixed with 500 ml of methanol.
2. NaOH (5N): 200 g NaOH was gradually dissolved in distilled water and diluted to 1000 ml.
3. Hexane (Boiling range: 68.2-68.8°C).
4. H₂SO₄ (10N): 138 ml of concentrated H₂SO₄ was added slowly to 300 ml distilled water and then diluted to 500 ml.
5. Scintillation fluid: 33 g PPO (2,5-Diphenyl-oxazole) and 1.0 g POPOP (1,4-Bis (2- (5-Phenylloxazolyl) Benzene) was added to 4 liters of scintillation grade toluene (Fisher Scientific, Co.).

**Procedure.** A 1 g sample of liver tissue was dropped directly into 19 ml chloroform-methanol. The liver sample was then homogenized using a Polytron (Brinkmann Instruments, Westberg, New York).

The chloroform-methanol extract was allowed to stand until the insoluble material had settled, then the clear supernatant was filtered. The filtrate was evaporated to dryness in a stream of
nitrogen. The residue was saponified in 2 ml of 5N NaOH in a water bath held at 80°C for 3 hours. Following this, the solution was diluted with 5 ml H₂O and was extracted 3 times with 8 ml hexane. Theaqueous layer remaining after the hexane extractions was acidified with 1.25 ml of 10N H₂SO₄. This was followed by 3 more extractions with 8 ml hexane. The latter hexane extracts were combined and evaporated at 40°C in a stream of nitrogen. The residue (which contained only the free fatty acids) was counted in 10 ml of toluene scintillation fluid at a channel setting of 400 on the Beckmann Scintillation Counter (LS 3801) for 10 minutes and recorded as counts per minute (cpm).

The rates of fatty acid synthesis was expressed as cpm/g fresh tissue.

**Analysis of Body Composition**

Analysis of body composition was carried out by a modification of the method of Hartsook and Hershberger (37). All analyses were performed in duplicate.

**Preparation of Rat Homogenate**

Carcasses were removed from the freezer and allowed to thaw for 24 hours in a refrigerator. The liver, removed earlier for previously described analyses, was not included in the body composition analysis. The gastrointestinal (GI) tract was rinsed free of its contents and then the carcass of each animal weighed, placed in a 1000 ml beaker and autoclaved for 30 minutes at 10 psi (115°C). After autoclaving, the carcass was placed in a tared blender and distilled water was added in an amount 1.2 times the weight of the carcass and blended at low speed
for 15 minutes. Aliquots were taken from the homogenate for protein and moisture determinations immediately after the blender was stopped in order to obtain a representative sample. The samples taken for the moisture analyses were used also for fat and ash analyses.

Aliquots for all the analyses were corrected for the water added during preparation of the homogenate by dividing the body weight by the total weight of the homogenate, or

\[
\text{Moisture Correction Factor (MCF)} = \frac{\text{body weight}}{\text{homogenate weight}}
\]

This factor, when multiplied by the weight of the aliquot, equaled the weight of the wet carcass contained in the aliquot.

**Moisture Determination**

Twenty five grams of the homogenate were transferred to a preweighed aluminum pan and the sample allowed to dry in a convection oven at 55°C for 6 hours. The pan was then transferred to a vacuum oven and dried for an additional 20 hours at 75°C. The weight of the aluminum pan was subtracted from the weight of the dish with the dried residue to obtain the dried residue weight.

\[
\% \text{ Moisture} = 100 - \left(\frac{\text{weight of dried residue}}{\text{weight of wet homogenate}}\right)(\text{MCF}) \times 100
\]

**Protein Determination**

Approximately 2-3g of fresh homogenate were placed on preweighed weighing papers and weighed. These were stored in a refrigerator at 4°C until analysis. The Macro-Kjeldahl (37) procedure was used to analyze the samples: the homogenate and paper were digested, distilled, and then titrated to ascertain the amount of nitrogen in each sample.
Reagents.

1. Boric Acid: 55g of $\text{H}_3\text{BO}_3$ was added to 1000 ml of distilled water and heated to boiling until the boric acid was in solution. The solution was prepared as needed.

2. Hydrochloric Acid, standardized: 10 liters of 0.2N HCl was prepared and standardized against a primary standard solution of $\text{Na}_2\text{CO}_3$.

3. Saturated Sodium Hydroxide (50%): to 500g of NaOH was added enough water to make 1 liter.

4. Mixed Indicator: Equal quantities of solutions of 0.2% methyl red and 0.1% methyl blue were mixed. The solvent was 95% ethyl alcohol for either indicator solution.

Procedure. The papers with the homogenates were placed into separate Kjeldahl flasks. To each flask was added 5g sodium sulfate, 25 ml concentrated sulfuric acid, a Hengar crystal, a small crystal of copper sulfate, and 2 glass beads. The samples were heated on a digesting rack under a hood for 1 hour. When the flasks were cooled, 200 ml of distilled water was added to the solution in the flasks and the flasks allowed to cool again. Seventy five ml of saturated NaOH was slowly layered into each cooled Kjeldahl flask. The flasks were quickly attached to the distillation rack and then rotated gently. Erlenmeyer flasks containing 50 ml of boric acid and 2 - 3 drops of mixed indicator (purple in acidic, green in basic solution) were adjusted to the distillation rack so that the tips of the delivery tubes of the condensers were immersed into the solution. Distillation continued for 10 minutes after the solution in the Erlenmeyer flasks
changed from purple to green. The distillate was titrated with standardized HCl and the percent nitrogen in each sample was calculated using the following equation:

\[ \% \text{ Protein} = (\%N \times 6.25) \]

where \( gN = (\text{meq HCl}) (\text{meq weight N}) \) and \( \%N = 100 \times gN / g \text{ body tissue} \)

Fat Analysis

The dried residue from the moisture determination was extracted in a Soxhlet apparatus for 12 hours with chloroform:methanol (2:1) solution. The chloroform:methanol extract containing the fat was transferred to a tared beaker and allowed to dry over night (15 hours) in a convection oven at 55°C. Fat weight was determined by difference.

\[ \% \text{ Fat} = \left( \frac{g \text{ fat}}{g \text{ dried residue}} \right) (% \text{ dry weight of the carcass}) \]

where, \( % \text{ dry weight of the carcass} = (100 - % \text{ moisture}) \).

Ash Analysis

The residue remaining after fat extraction was analyzed for ash. The residue was placed in a tared crucible and ashed at 550°C for 24 hours in a muffle furnace. The difference between crucible weight and crucible weight with ash equaled weight of ash.

\[ \% \text{ Ash} = \left( \frac{\text{ash weight/corrected sample wet weight}}{} \right) \times 100 \]

where, corrected sample wet weight = sample weight x MCF.

Statistical Methods

The general linear model (GLM) procedures of SAS 1982 (38) were utilized to evaluate the effects of exercise and diet restriction on body weight, feed intake, hepatic lipogenesis and body composition.
The least squares option in GLM was utilized to derive group means and standard errors. To test for statistical significance of the differences among means, a modification of Duncans multiple range test was employed (44). Thus, mean values of exercised animals (E) were compared with those of restricted-fed animals (R) and also with those of ad libitum-fed animals (A). Likewise, the means of R and A groups were compared. A probability level of less than 0.05 was considered statistically significant.
Growth curves of all the experimental groups are presented in Figure 3. The mean body weights of the 3-hour exercise group (E3.0) were not significantly different than that of the 1.5-hour exercise group (E1.5) at the time of sacrifice. The ad libitum-fed control group (A) was 26 g (5%) greater than the two exercised groups (E)(p<0.05). The two diet restricted groups (R3.0 and R1.5) were weight-matched with E3.0 and E1.5, respectively, so the weights of the diet restricted groups (R) were approximately the same as those of the exercised groups. The growth curves for the E and R groups were of similar shape to A but at a 5% - 10% lower level (p<0.05).

Mean feed consumption (Figure 4) was not significantly different between E and A, and averaged 30 - 32 g/day at 30 weeks of age. The feed intake of R was restricted to closely control body weight, so the animals were limited as to maximum amount of feed consumed. R's intake varied at approximately 1/3rd less than that of ad libitum-fed animals (about 20 - 22 g/day;p<0.05) to approximate the same body weight as E. However, there was no difference in the diets of R1.5 and R3.0.

Comparisons Between E3.0 and E1.5

There were several fatalities among the rats that swam, due to various causes. Initially, there were 10 animals in each of the 5 experimental groups. However, by the end of the study, the n of E3.0 was 9 and of E1.5 was 5. The one death in E3.0 was the result of an infection, and one in E1.5 was caused by drowning. Four animals in the
Figure 3. Growth curve of animals between the ages of 6 weeks to 30 weeks. A = Ad libitum-fed controls; E3.0 and E1.5 - Rats swum 3 hours and 1.5 hours, respectively; R3.0 and R1.5 = Rats diet restricted to match weights of E3.0 and E1.5, respectively.
Figure 4. Feed consumption in g/day. A = Ad libitum-fed controls; E3.0 and E1.5 - Rats swum 3 hours and 1.5 hours, respectively; R3.0 and R1.5 = Rats diet restricted to match weights of E3.0 and E1.5, respectively.
same group (E1.5) died suddenly within minutes of each other. It is believed that this occurred as a result of the water being too warm.

Very few differences were observed when comparing the data from the two exercised groups, E3.0 and E1.5 (Table 1). Feed consumption was nearly the same between the two groups throughout the study and weights never diverged significantly. There were no significant differences in any of the body components except for ash. Lipogenic activity, as measured by G6PD activity and tritium uptake, showed no significant differences between E1.5 and E3.0, though tritium uptake tended to be greater in E1.5.

Since it was observed, for the most part, that the test responses were not significantly different between animals in the two exercise groups, these two groups were collapsed (E = E1.5 + E3.0) for purposes of further statistical comparisons with the other treatment groups, i.e., A and R. Additionally, the two diet restricted groups (R1.5 and R3.0), actually received the same treatment throughout the study. Hence, they were also analyzed as one group for statistical comparisons (R = R1.5 + R3.0). The original rationale for having the two diet-restricted groups was to match the mean body weight of R1.5 to that of E1.5 and of R3.0 to that of E3.0 by reducing the feed offered to the restricted groups. However, since the weights of the two exercise groups never diverged significantly, there was never any need to vary the diets between R1.5 and R3.0. Therefore, they were, in effect, treated as one group rather than two and, thus, there was no longer a theoretical basis for maintaining the animals in two groups (Table 2).
<table>
<thead>
<tr>
<th>Test Response</th>
<th>3.0¹ n=9</th>
<th>1.5² n=5</th>
<th>Significance³ (p values)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed Intake/day, g</td>
<td>31.7 ± 1.1⁴</td>
<td>32.6 ± 1.1</td>
<td>0.608</td>
</tr>
<tr>
<td>Body Weight, g</td>
<td>482 ± 21</td>
<td>471 ± 22</td>
<td>0.723</td>
</tr>
</tbody>
</table>

**BODY COMPOSITION**

| Absolute Values | | |
|-----------------|-----------------|-----------------|-----------------|
| Protein, g | 84.2 ± 5.6 | 81.4 ± 5.1 | 0.716 |
| Fat, g | 60.3 ± 6.2 | 67.0 ± 9.2 | 0.565 |
| Moisture, g | 299 ± 11 | 292 ± 12 | 0.666 |
| Ash, g | 14.0 ± 0.8 | 11.7 ± 0.7 | 0.047⁵ |

| Relative Values | | |
|-----------------|-----------------|-----------------|-----------------|
| Protein, % | 18.4 ± 0.7 | 18.2 ± 0.7 | 0.915 |
| Fat, % | 13.0 ± 0.9 | 14.9 ± 1.6 | 0.344 |
| Moisture, % | 65.7 ± 0.8 | 65.6 ± 0.4 | 0.969 |
| Ash, % | 3.11 ± 0.2 | 2.63 ± 0.14 | 0.076 |

**LIPOGENESIS**

| G-6-P Dehydrogenase | 16.6 ± 2.7 | 19.9 ± 2.4 | 0.372 |
| Tritium uptake, cpm/g fresh tissue | 1044 ± 98 | 1412 ± 132 | 0.054 |

¹Exercised by swimming 3 hours/day.
²Exercised by swimming 1.5 hours/day.
³Determined by "t" test.
⁴Values are means ± SEM.
⁵p<0.05
TABLE 2
EFFECT OF EXERCISE AND DIET RESTRICTION ON FEED INTAKE AND MEAN TERMINAL BODY WEIGHTS

<table>
<thead>
<tr>
<th></th>
<th>A&lt;sup&gt;1&lt;/sup&gt; n=10</th>
<th>E&lt;sup&gt;2&lt;/sup&gt; n=14</th>
<th>R&lt;sup&gt;3&lt;/sup&gt; n=20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed Intake/day, g</td>
<td>30.5 ± 1.0&lt;sup&gt;a(4,5)&lt;/sup&gt;</td>
<td>32.1 ± 0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.4 ± 0.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Body Weight, g</td>
<td>533 ± 14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>478 ± 14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>480 ± 7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup>A = Ad libitum-fed, non-exercised rats.

<sup>2</sup>E = Rats exercised by swimming 1.5 and 3.0 hours/day, ad libitum-fed.

<sup>3</sup>R = Restricted-fed, non-exercised rats.

<sup>4</sup>Values are means ± SEM.

<sup>5</sup>Means in a row with the same superscript are not significantly different (p<0.05) by the Duncan's multiple range test.
**Body Composition**

The data on body composition for the various groups are shown in relative and absolute amounts in Table 3.

**Protein**

Exercise and caloric restriction produced no significant differences on absolute values of body protein between the groups. However, when expressed in relative amounts, R had a greater percent protein than E and A ($p<0.05$). No significant differences were found between E and A, whether looking at absolute or relative values of body protein. Nevertheless, when comparing absolute values, exercised animals had 10% less protein than A. Diet restricted animals averaged only 1% less body protein than A.

**Fat**

Exercised rats had significantly less absolute amounts of fat than A (29%; $p<0.05$) and, though not significantly so, less than diet-restricted rats (16%), even though body weights were approximately the same (Table 2). When expressed in relative amounts E had significantly less fat than R ($p<0.05$) and A ($p<0.05$). Diet restricted animals also had less absolute amounts of fat than A (16%; NS), but when in terms of relative amounts, this difference was very small.

**Moisture**

The exercised animals showed significantly greater percent moisture than R ($p<0.05$) and A ($p<0.05$). However, when expressed in absolute amounts, E still had more moisture than R, though not significantly so (4.5%; NS), but had less moisture than A (3.0%; NS).
### TABLE 3
EFFECT OF EXERCISE AND DIET RESTRICTION ON BODY COMPOSITION

<table>
<thead>
<tr>
<th></th>
<th>A&lt;sup&gt;1&lt;/sup&gt; n=10</th>
<th>E&lt;sup&gt;2&lt;/sup&gt; n=14</th>
<th>R&lt;sup&gt;3&lt;/sup&gt; n=20</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Absolute Values</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein, g</td>
<td>92.7 ± 2.5&lt;sup&gt;a&lt;/sup&gt;(4,5)</td>
<td>83.2 ± 3.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>91.8 ± 3.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fat, g</td>
<td>88.7 ± 6.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>62.7 ± 5.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>74.4 ± 4.6&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Moisture, g</td>
<td>306 ± 6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>297 ± 7&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>279 ± 4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ash, g</td>
<td>14.5 ± 0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.2 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.6 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Relative Values</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein, %</td>
<td>18.6 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.3 ± 0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20.4 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fat, %</td>
<td>17.7 ± 1.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.7 ± 0.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.6 ± 0.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Moisture, %</td>
<td>61.6 ± 1.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>65.6 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>62.4 ± 0.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ash, %</td>
<td>2.9 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.9 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.8 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup>A = Ad libitum-fed, non-exercised rats.

<sup>2</sup>E = Rats exercised by swimming 1.5 and 3.0 hours/day, ad libitum-fed.

<sup>3</sup>R = Restricted-fed, non-exercised rats.

<sup>4</sup>Values are means ± SEM.

<sup>5</sup>Means in a row with the same superscript are not significantly different (p<0.05) by the Duncan's multiple range test.
had less absolute amounts of moisture than A (7%; p<0.05) but when expressed in relative amounts, R and A were not significantly different.

Ash

There were no significant differences found among groups in ash, when expressed in absolute or relative amounts. A trend does appear, though, that the diet restricted animals have less ash than the other treatment groups.

Glucose-6-phosphate Dehydrogenase

Table 4 presents the effects of exercise or caloric restriction on liver activity of G6PD. G6PD activity was significantly higher in E than in A (40%; p<0.05). Liver G6PD activity of R rats was 26% higher than that of A, but the difference was not significant. There was no significant difference between E and R, though E had 21% greater activity than R. Mean G6PD activity levels ranged from 10.5 umoles NADPH/min/g protein in A to 19.0 umoles NADPH/min/g protein in E.

Uptake of Tritium Water

The uptake of tritium by newly synthesized fatty acids in the liver is shown in Table 4. There were no significant differences among groups. However, E was found to have the greatest rate of tritium uptake, at 15% greater than A, whereas R had the least uptake, at a 3% slower rate than A. The mean tritium uptake ranged from 805 cpm/g fresh tissue in R to 1413 cpm/g fresh tissue in E.
**TABLE 4**

EFFECT OF EXERCISE AND DIET RESTRICTION ON HEPATIC G6PD ACTIVITY AND INCORPORATION OF TRITIUM INTO HEPATIC FATTY ACIDS

<table>
<thead>
<tr>
<th></th>
<th>A&lt;sup&gt;1&lt;/sup&gt; n=10</th>
<th>E&lt;sup&gt;2&lt;/sup&gt; n=14</th>
<th>R&lt;sup&gt;3&lt;/sup&gt; n=20</th>
</tr>
</thead>
<tbody>
<tr>
<td>G6PD&lt;sup&gt;4&lt;/sup&gt;</td>
<td>10.6 ± 1.9&lt;sup&gt;b(5,6)&lt;/sup&gt;</td>
<td>17.8 ± 1.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.4 ± 1.7&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>&lt;sup&gt;3&lt;/sup&gt;H&lt;sub&gt;2&lt;/sub&gt;O, cpm/g fresh tissue</td>
<td>1006 ± 131&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1176 ± 90&lt;sup&gt;a&lt;/sup&gt;</td>
<td>981 ± 107&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

1<sup>a</sup> = Ad libitum-fed, non-exercised rats.
2<sup>E</sup> = Rats exercised by swimming 1.5 and 3.0 hours/day, ad libitum-fed.
3<sup>R</sup> = Restricted-fed, non-exercised rats.
4 µmoles NADPH/min g protein
5 Values are means ± SEM.
6 Means in a row with the same superscript are not significantly different (p<0.05) by the Duncan's multiple range test.
CHAPTER V

DISCUSSION

The results indicate that exercise caused increased hepatic lipogenic activity. This is in agreement with Tokuyama and Okuda (27) and Tsai et al. (11) who showed increased hepatic lipogenesis as a result of training. It was postulated that this was due to an overall increase in mobilization and utilization of fatty acids, which needs to be compensated for by increased lipogenesis between training sessions. However, these studies, as well as the present study, are inconsistent with the findings of others (1-5,7-10). Richard and Trayhurn (9) reported decreased hepatic and adipose fatty acid synthesis with exercise, explaining that carbohydrate is shunted away from the synthesis of lipids in favor of energy storage as glycogen.

The discrepancies between these studies might be explained when variations in the research are taken into account. Borer and Kelch (46) used hamsters, which have been shown to have increased levels of insulin after retirement from exercise. This would result in increased fatty acid synthesis (46). Food intake increased as a result of training in female animals, as used by Tokuyama and Tsai (27). This could be partly responsible for the increased lipogenic activity found in their studies. However, forced exercise in male rodents has been reported by Oscai and Holloszy (48) to decrease feed intake, which might account for decreased fatty acid synthesis in their investigations.

The relationship of the present findings to these observations is unclear, since in the present investigation E male rats showed no
significant changes in feed consumption and, nevertheless, weighed significantly less (approximately 8% less; \( p < 0.05 \)) than ad libitum-fed controls. It is possible that, though the exercise was forced, it was not sufficient enough to result in the decreased feed intakes seen by Oscai and Holloszy (48), who swam their animals with attached weights.

Though hormone levels were not measured in the current study, our findings provide support for the hypothesis of Tsai et al. (11) that the increased lipogenic capacity resulting from exercise is possibly due to enhanced tissue sensitivity to insulin. This could also be due to increases in circulating insulin during the resting phase of exercised hamsters, as seen by Borer and Kelch (46). This would contribute to the heightened rate of lipogenesis, which might serve to replenish tissues depleted of triglycerides.

An unexpected finding in the present study was the somewhat enhanced lipogenic activity of the diet restricted rats. This is not in agreement with studies that allow "nibbling" in restricted fed rats (25). However, as was shown by the work of Leveille (25), the meal-feeding of rats (that is, the limitation of access to food to a short period of time every day) causes adaptive changes which result in an increased rate of conversion of carbohydrate to fat. This would be demonstrated by a greater rate of fatty acid synthesis, indicated by \( ^3 \)H incorporation into fatty acids and G6PD activity as occurred in this study. Though there was no intentional meal-feeding practiced in the present study, the animals were fed only a small amount of feed daily. They tended to consume the feed rapidly, which left them with no food
for the remainder of the day, and possibly resulted in a meal-feeding effect.

Exercised animals exhibited a slower growth rate than the ad libitum-fed controls. Even though the exercised animals ate about the same amount as A, body weights of E were approximately 8% less. This is consistent with most findings (31,32), though studies using female rats do not show this effect (7,29). It seems male rats tend to lose weight with exercise, whereas female rats tend to increase feed intake to maintain body weight to the level of sedentary animals (7,29). Why this occurs has not been explained.

The comparison of body components of different experimental groups based on percentages has been criticized (40). Therefore, both percentages and absolute amounts were used to express the data in this study. For example, when looking at body fat, E and R differed significantly in relative amounts of fat. However, when expressed in absolute amounts there was no longer any significant difference between these two groups. It is, therefore, helpful to look at the two values together to avoid confusion.

Weil and Wallace (39) proposed the theory of compositional homeostasis, that is, the proportions of body components remain constant regardless of diet or size of the animals. The compositional data obtained in this study do not support this theory. Instead, the results of the present study indicate that not only will exercise create changes in proportions of body components, but that diet restriction also resulted in proportional changes of the body components, as discussed below.
In agreement with others (30,31,32,34), exercise significantly decreased body fat in the present investigation. While caloric restriction also reduced the absolute amount of body fat as compared to controls, relative body fat of R did not differ significantly from A but was significantly greater than E. This indicates that weight loss of components other than fat occurred in R rat.

A surprising finding in the present study was that R had a significantly greater percentage of body protein, as compared to E and A, though it was not significant when expressed in absolute amounts. This is in contrast to E, which did not differ significantly from A. These findings are not consistent with Hanson (34), who found that diet restriction usually resulted in losses of body protein in proportion to other losses. However, Beauchene et al. (45) observed that R rats had a higher percentage of body protein than A rats, which was in agreement with the present study. Also, Hill et al. (41) found that caloric restriction with adequate protein had little influence on the carcass muscle and significantly decreased percent fat. Their data indicated that protein content of the restricted diet was adequate for normal protein synthesis in muscle and liver since ribosomal activity (as determined by RNA concentration) was unaffected. Therefore, with the loss of percent fat, the percent of body protein would increase proportionately, as seen in the present study.

It also has been reported that exercise will show hypertrophy of the muscles, resulting in increased body protein (7), or, no hypertrophy in the exercised muscles but increases in protein tissues in other parts of the body (33). The present study found that exercise
resulted in no changes in relative amounts of protein, but less absolute amounts of protein, as compared to ad libitum-feeding. This is in agreement with Bulbulian et al. (30) and it can be assumed that no hypertrophy occurred in this study. Pitts (7) discussed the possibility that exercise can cause an actual loss in body protein, which he attributed to stress. This might provide an additional explanation as to why E was found to have less protein than R, as discussed above.

Exercise led to an increase in body moisture, both in absolute amounts and when expressed as a percentage of body weight. E was significantly greater than A in terms of relative amounts, though these two groups did not differ significantly when comparing absolute amounts. Moisture is usually related to the amount of lean body mass (LBM) (34). However, in spite of increased moisture, E did not show significant increases in LBM over the other treatment groups in this study, so it seems unanswerable as to why the moisture increases were observed. It is possible, however, that a portion of this increase could be due to enhanced glycogen storage in muscle and liver tissues. Training can cause muscle glycogen stores to almost double (47), and for each gram of glycogen stored, three grams of water is also stored. It is unknown if this is a factor in the current study.

Ash showed no significant differences between groups, which is consistent with most studies (7,29,34). However, a tendency appeared for the R animals to have less ash, when expressed both in terms of absolute and relative amounts, than A or E rats. Thus, since the ash of E tended to be higher than R, yet the body weights were nearly
identical, it would appear that exercise might have contributed to maintaining body ash.

The loss of 4 animals due to warm water was of great concern. Water was usually maintained at 34-37°C. However, on the day of the accident, water was unintentionally allowed to get as high as 41°C. Subsequent to this mishap, the investigator became aware of just how critical the water temperature is through a study performed by Baker and Horvath (43). These researchers analyzed the effects of water temperature on swimming rats and found that these animals have a very narrow range of tolerance. At 42°C, the rats became exhausted after only a few minutes and began to sink to the bottom. This is apparently what happened in the present study. Extreme care was taken after the loss of these animals in order to prevent the water from rising above 37°C.
CHAPTER VI

SUMMARY

The effects of exercise and diet restriction on feed intake, body weight, body composition and hepatic lipogenesis were investigated. There were 5 experimental groups: Sedentary, ad libitum fed control group (A); Exercise by swimming for 3 hours/day, ad libitum fed (E3.0); Exercise by swimming for 1.5 hours/day, ad libitum fed (E1.5); Sedentary, diet restricted to match weight of E3.0 (R3.0); Sedentary, diet restricted to match weight of E1.5 (R1.5). Feed intakes were measured weekly and weights measured twice weekly. Body composition was reflected in absolute and relative amounts of moisture, protein, fat, and ash. Hepatic lipogenesis was determined by glucose-6-phosphate dehydrogenase (G6PD) activity and $^3$H uptake.

Exercised animals ate approximately the same amount as the controls, but weighed about 8% less. Diet restricted animals had to eat 30% less to maintain the same body weights as the exercised animals. The food consumption and body weights of the two exercised groups never diverged significantly. Growth curves for the E and R groups were of similar shape to A, but at a 5-10% lower level.

As would be expected, exercised rats had significantly less absolute and relative amounts of body fat than A ($p<0.05$) and less relative amounts than R ($p<0.05$). Diet restricted animals also had less absolute and relative amounts of fat than A, though not significantly so. Exercise and caloric restriction produced no significant differences between any of the groups in absolute amounts
of protein. R, however, did have a greater percent protein than E and A (p<0.05).

E had more moisture than any of the other groups (p<0.05) in absolute and relative terms. This is usually indicative of increased lean body mass, though this did not occur in the current study. Ash showed no significant differences between groups in either absolute or relative amounts.

Exercise produced a significant increase in G6PD activity (p<0.05) and a non-significant increase in $^3$H uptake. This indicates enhanced fatty acid synthesis resulting from exercise. Diet restricted animals also showed increased lipogenesis, most likely due to a meal-feeding effect.

Exercise, therefore, increased lipogenesis, and affected body size and body composition, mainly by decreasing body fat. Diet restriction caused similar alterations, though not to the same degree. Since the exercised animals ate the same amount as controls, but had less body fat, these findings support the hypothesis that exercise alters lipid metabolism by increasing overall mobilization and utilization of fatty acids in the body.
LITERATURE CITED
LITERATURE CITED


VITA

Madeline Dellwo was born in Spokane, Washington, where she attended Holy Names Academy Grade School and High School. In August, 1977, she graduated from the Coordinated Undergraduate Program in Dietetics at the University of Washington, with a Bachelor of Science degree in Nutrition Science. She then worked for several years as a clinical and administrative dietitian in places such as Malaysia, Seattle, and Saudi Arabia. In September of 1984, she began graduate work in Nutrition at the University of Tennessee, where she received a Master of Science degree in December, 1986. During her graduate program, she served as a graduate teaching assistant and a graduate research assistant. After receipt of her M.S. degree, she continued graduate work at the University of Tennessee as a Doctoral Student.