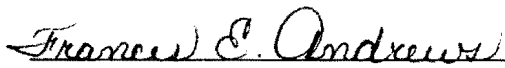


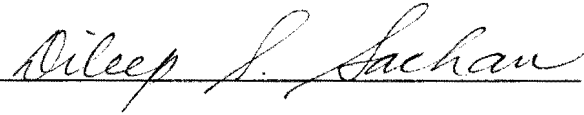
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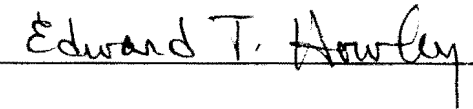
I am submitting herewith a dissertation written by Parvin Darabian entitled "The Effect of Feed Restriction, Exercise, and Aging on Lipogenesis in Rats." I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy with a major in Human Ecology.


Roy E. Beauchene
Major Professor

We have read this dissertation
and recommend its acceptance:







Accepted for the Council:


Vice Provost and
Dean of the Graduate School

THE EFFECT OF FEED RESTRICTION, EXERCISE,
AND AGING ON LIPOGENESIS IN RATS

A Dissertation
Presented for the
Doctor of Philosophy
Degree
The University of Tennessee, Knoxville

Parvin Darabian

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ABSTRACT

The effect of diet restriction, exercise and aging on body weight, epididymal and perirenal fat pad weights, liver and adipose lipogenesis and lipolysis in male Wistar rats were investigated. The study consisted of four experimental groups: ad libitum fed, sedentary (A); diet restricted by feeding on alternate days, sedentary (R); ad libitum-fed, exercised by swimming on alternate days (AE); diet restricted by feeding on alternate days, exercised by swimming on alternate days (RE).

Diet restriction as well as exercise resulted in a significantly lower mature body weight, epididymal and perirenal fat pad weights than ad libitum feeding. A significant decrease in these parameters was observed with age.

Hepatic and adipose lipogenesis was estimated by measuring the incorporation of acetate $1-^{14}\text{C}$ into fatty acids, and activity of glucose-6-phosphate dehydrogenase (G6PD) in vitro. Diet restriction resulted in an increased hepatic G6PD activity ($p < 0.005$) and adipose tissue fatty acid synthesis ($p < 0.01$) whereas exercise resulted in increased adipose tissue G6PD activity ($p < 0.05$), but it had no effect on lipogenic activity of either tissue. With age, however, fatty acid synthesis and G6PD activity were increased in both tissues.

Hepatic and adipose tissue lipolysis was estimated by measuring the amount of in vitro glycerol release of these tissues. Diet restriction resulted in an increased hepatic and adipose tissue glycerol release whereas exercise increased hepatic glycerol release, but did not affect adipose glycerol release. The amount of glycerol release of both tissues was increased with age.

In general, neither feed restriction nor exercise improved or prevented age-associated physiological changes. Correlations between physical parameters (body weight and fat pad weights) were significant. Liver fatty acid synthesis and adipose tissue fatty acid synthesis were correlated to both liver G6PD activity and adipose tissue G6PD activity in aging rats.

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CHAPTER I

INTRODUCTION

A large segment of the USA population is participating in exercise and reduced calorie intake regimens. A likely motivating factor is the current perception that these regimens have health and cosmetic benefits. Elevated body weight and/or obesity has been associated with increased morbidity and mortality in a number of studies (1-3), however, there has been controversy about these relationships (4). It has been demonstrated, however, that weight reduction can lead to improvement of some of the "risk factors" for several diseases associated with obesity (5,6).

Numerous studies have shown that a reduction in feed intake lowered body fat and extended the life span of laboratory animals (12-15). In rodents, feed restriction has been shown to reduce the incidence of disease, lengthen life span and maintain many physiological functions at levels normally observed in younger animals (7-11). Reducing the amount of feed intake for a short period of time daily leads to a decrease in body fat which could alter some metabolic reactions related to lipogenic activities (16-18).

Liver and adipose tissue lipogenesis can be affected by feed restriction and physical training. Restriction of the length of feeding time of rats to a period of 1-2 h per day

has been reported to increase activity of lipogenic pathways (16-21). Diets high in carbohydrate or fat (22) elevate or depress, respectively, fat synthesis in the liver and/or adipose tissues. Fasting has been shown to reduce the rate of fatty acid synthesis in the liver of the European eel (23).

It has been documented well that the amount of exercise engaged in by animals has a role in regulating their body weight and body fat content (14,24-28). Physical training has been shown to result in decreased weight, decreased body fat, and either increased or decreased synthesis and lipid degradation in the liver and/or in adipose tissue (29-34).

Researcher have reported recently that exercise trained animals have the potential of drawing a greater percentage of their energy needs from fat, thus, sparing limited carbohydrate stores (29,30,32). Other researchers have pointed out that exercise has a marked effect on the mobilization and oxidation of fatty acids (30,31). However, the effect of long-term exercise (i.e., throughout life) on lipid synthesis has not been investigated.

Alterations in liver and adipose tissue lipogenesis as a result of exercise and/or feed restriction have been shown to be related to the activity of specific enzymes such as glucose-6-phosphate dehydrogenase (G6PD) and malate dehydrogenase (MED) (19,22,23,30). G6PD catalyzes the dehydrogenation of glucose in the hexose monophosphate shunt

(HMP) and generates NADPH, which is required as a hydrogen source for fatty acid biosynthesis (29,30). Malic enzyme catalyzes the oxidation of malate which also provides NADPH (29,30).

The purpose of the present study was to elucidate the effects of exercise and/or feed restriction throughout life on liver and adipose tissue lipogenesis and lipolysis in rats. The hypothesis of the study were:

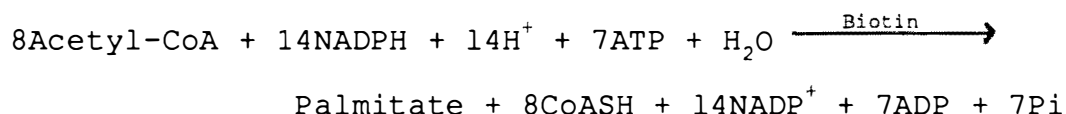
1. lower adult body weight and body adiposity would be obtained for diet restricted than for ad libitum-fed rats.
2. a decreased rate of lipogenesis and an increased rate of lipolysis would be observed in diet restricted animals.
3. lower adult body adiposity and weight and also lower lipogenesis but higher lipolysis would be obtained for exercised than for non-exercised rats.
4. lower adult body weight and adiposity, decreased rate of lipogenesis, and increased rate of lipolysis would be obtained in diet-restricted exercised animals than ad libitum non-exercised animals.
5. body weight, adiposity, and lipogenesis would decrease with age: lipolysis would increase with age.

CHAPTER II

REVIEW OF THE LITERATURE

Fatty Acid Biosynthesis:

The formation of long chain fatty acids from acetyl-CoA may be termed lipogenesis, and represents de novo synthesis. The synthesis occurs in the cytoplasm. The synthesis is catalyzed by two enzyme systems which function in sequence, namely acetyl-CoA carboxylase and fatty acid synthetase. Lipogenesis takes place in many organs such as liver, brain, kidney, and adipose tissue. The process requires NADPH, ATP, and Mn^{++} as cofactors and HCO_3 as source of CO_2 (35). The main end product is palmitate (24). As Figure 1 shows, acetyl-CoA is carboxylated to malonyl-CoA by the CO_2 provided by bicarbonate. In animals, all carbon atoms of fatty acids come from acetyl-CoA. The first controlling step is the carboxylation of acetyl-CoA to yield malonyl-CoA, catalyzed by acetyl-CoA carboxylase. The synthesis is initiated by a molecule of acetyl-CoA which functions as a primer. The carbon chain is then elongated by successive addition of two carbons from malonate which are derived from acetyl-CoA. The overall reaction may be indicated as:



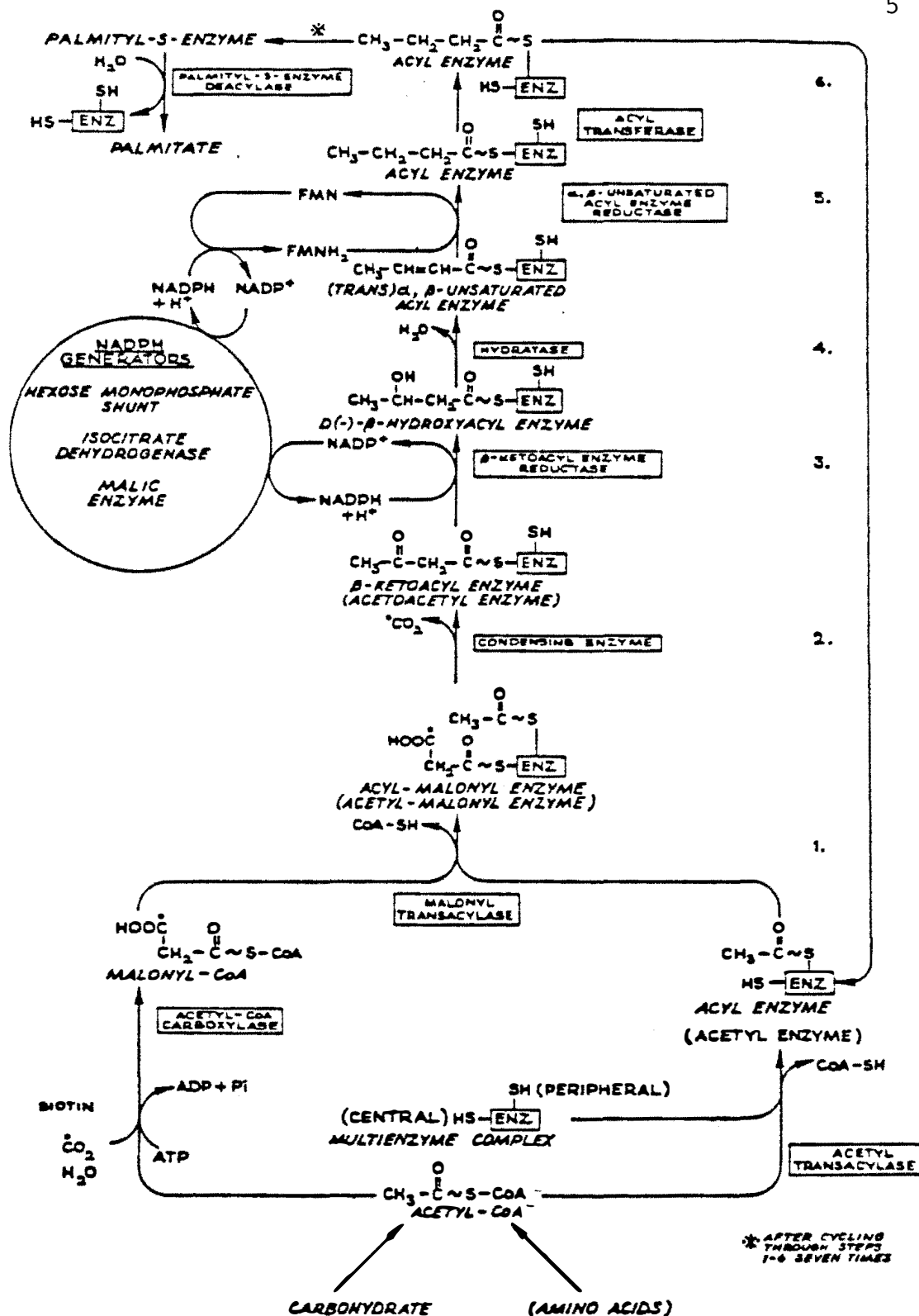
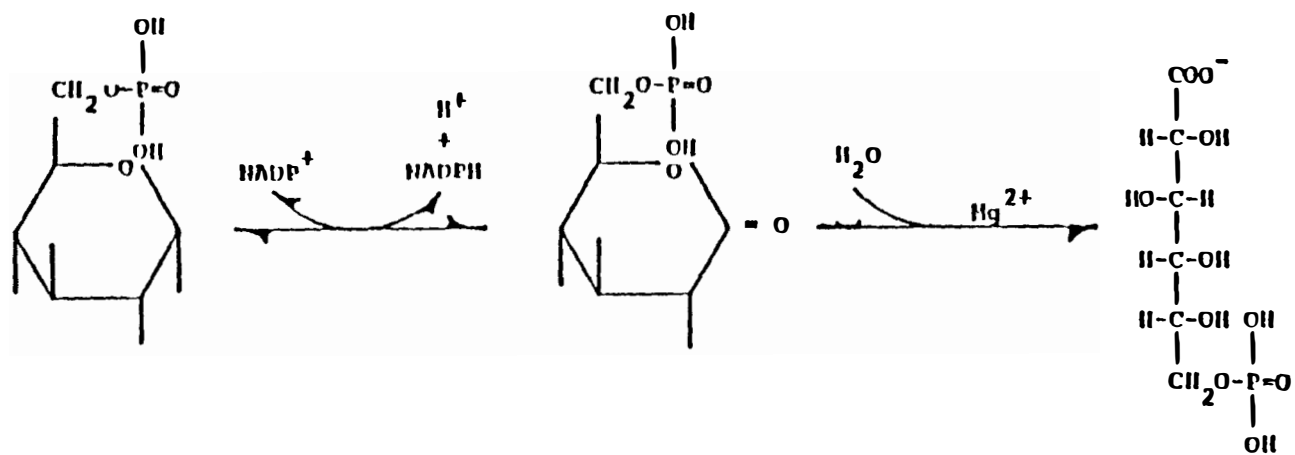


Figure 1. Fatty acid biosynthesis from Harper, H.A. (35)

The reducing equivalents for fatty acid synthesis are provided by NADPH, which is derived from glucose-6-phosphate (G6P) oxidation by the hexosemonophosphate shunt pathway (HMP). The HMP shunt and fatty acid synthesis pathways are found in the cytoplasm of the liver and adipose tissue cells, so there is no membrane-permeability barrier to the transfer of NADPH/NADP from one pathway to the other.

A change in the rate of fatty acid synthesis could result in a change in the rate of production of NADPH (35) because fatty acid synthesis requires NADPH as a reducing agent. Thus, measuring the activity of enzymes in the HMP shunt involved in the production of NADPH would be associated with lipogenic activity, i.e., the activity of G6PD could reflect rates of fatty acid synthesis.

G6PD catalyzes the first reaction in HMP, oxidizing G6P to 6-phosphogluconolactone (6PG) and reducing NADP to NADPH (Figure 2) (35,36). Therefore, 6PG is often referred to as the product of the G6PD catalyzed reaction (37). Essentially the reaction is irreversible (38). Almost all animal tissues, especially red blood cells, adipose tissue, and lactating mammary gland show high G6PD activity. Less G6PD activity occurs in the liver, pancreas, kidney, and lungs than in the mammary gland, red blood cells, and adipose tissue (37).



D-Glucose-6-phosphate

D-Glucose- δ -lactone-6-P

6-Phospho-D-glucose

Figure 2. Reaction Catalyzed by Glucose-6-Phosphate Dehydrogenase. From Harper, H.A. (35).

Fatty Acid Synthesis and Exercise:

A number of investigators have found decreased lipogenic activity as a result of exercise. Richards and Trayhurn (29) observed decreased rates of fatty acid synthesis in the liver and adipose tissue of male mice which swam 2 h daily for 28 days. When male rats were trained on a treadmill for 13 weeks, a decreased rate of lipogenesis was observed in liver and adipose tissues (39). Whether exercise was forced, as in treadmill running (39,40), and in swimming (29,32), or not forced, as animals having free access to a running wheel (31,41), similar decrements in fatty acid synthesis were observed.

Presumably, one of the reasons for decreased fatty acid synthesis after physical work is to spare dietary carbohydrate needed for muscle glycogen replenishment (32,42). Richards and Trayhurn (29) and Askew et al. (30) indicated that carbohydrate is shunted away from the synthesis of lipid in favor of glycogen storage after the training. In addition, rats may reduce fatty acid synthesis during exercise to facilitate conservation of blood glucose and to prolong endurance (25).

Tsai et al. (31) reported that exercise increased lipogenic activity in the liver of the female hamsters. Increases in lipogenesis activity were also observed by Tokuyama and Okuda (41) in female rats allowed free access to a running wheel for fifty days, and by Dohm et al. (43)

in male rats trained by treadmill running. The data of Tsai et al. (31) suggested that in trained hamsters fat metabolism had reached a new state of equilibrium, i.e., a high rate of lipolysis and fatty acid oxidation during activity and a high rate of lipogenesis during rest.

Richards et al. (29) investigated the effects of exercise training on in vivo fatty acids synthesis in mice. The synthesis 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 the liver and the adipose tissues.

Askew et al. (39) reported that exhaustive exercise decreased lipogenesis in the liver and adipose tissue of trained, but not in untrained rats. 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 differences observed were due to training induced metabolic changes or to the work load performed. Trained rats had approximately a two-fold increase in fatty acid oxidation and fatty acid mobilization potential compared to untrained rats. Decreased lipogenesis may be due to an increased level of acyl-CoA in adipose tissue of trained animals which would inhibit the activity of acetyl-CoA carboxylase (39).

Askew et al. (30) reported that long-term submaximal training increased the ability of the body to mobilize and to oxidize fatty acids. These investigators tested the

effect of diet, training, and exhaustive exercise on enzyme activities related to lipogenesis. They reported a significant diet-training interaction for the lipogenic enzymes in the liver but not in the adipose tissue of rats fed high and low carbohydrate diets. The reason for this differential response to training for the rats fed the high carbohydrate diet is not clear. The results of this study indicated that prior physical training can influence the response of hepatic lipogenic enzymes to diet. In trained rats, dietary glucose appears to be directed more toward the synthesis of glycogen rather than to lipids. This is a beneficial adaptation to physical training, according to Askew et al. (30), because there is a loss of energy to the animal during the conversion of glucose to fat.

In contrast to the above there have been studies showing increases in lipogenesis as a result of training. Tsai et al. (31) looked at the effect of termination of exercise on fatty acid synthesis in female hamsters. Hepatic lipogenesis in the trained animals was higher than in the untrained animals. There was a significant increase in the rate of fatty acid synthesis and in the activity of lipogenic-related enzymes in the liver during the exercise period. Therefore, in trained hamsters fat metabolism reached a new state of equilibrium, i.e., a high rate of lipolysis and of β -oxidation during physical activity and a

high rate of lipogenesis during rest resulting in replenishment of depleted tissue triglycerides.

Tohuyama and Okuda (41) also found a 3-fold increase in lipogenic activity in the liver and adipose tissue of exercised rats. Because the synthesis of fatty acids is stimulated by insulin, the concentration of this hormone in the serum was measured; no difference in insulin concentration was found between the groups. It was concluded that the increased fatty acid synthesis in the trained rats was secondary to increased food intake. The amount of body fat in trained rats was less than that of sedentary rats in spite of increased food intake and lipogenesis. Therefore, it was concluded that physical activity enhanced the turnover of fatty acids in the body.

In rats exercised by swimming, Scorpio et al. (32) found a decrease in fatty acid synthesis as indicated by a lower rate of hepatic acetyl-CoA carboxylase activity. Whatever the means by which acetyl-CoA carboxylase is regulated, these investigators viewed as a beneficial response. The regulation of fatty acid synthesis in the liver by exercise could contribute to at least two physiological responses. First, it would help spare dietary carbohydrate, crucial for muscle glycogen replenishment after endurance work. Second, it would complement hepatic fatty acid oxidation, which is increased

during exercise to provide energy for conversion of lactate and alanine to glucose in the liver.

Hartsook and Martin (40) evaluated the effects of simultaneously varying environmental temperature, percent of diet dilution with non-nutritive cellulose and minutes of daily exercise (treadmill running) on body weight gain and lipogenic related enzyme activities in male rats. G6PD and ME activities in the liver and adipose tissue were increased in a low exercise-high diet dilution regimen and also with high exercise-low diet dilution. However G6PD and ME activities in the liver and adipose tissue were depressed with the dilution condition. From these results the rate of fatty acid synthesis appeared to decrease as the level of exercise increased up to a certain point of exertion. Thereafter, the turnover of fatty acids increased and the net amount of fat deposited was continuously decreased. Applegate et al. (44) examined the effects of forced exercise on the development of diet-induced obesity and the reversibility of exercise-induced changes following retirement from exercise in Osborn-Mendel rats. These rats were exercised by running on a motordriven treadmill while fed a high fat diet to induce obesity. Exercise did significantly reduce adipose lipogenesis, thus demonstrating that moderate exercise can attenuate diet-induced obesity.

Thus, the results of various studies on the effect exercise has on lipogenesis are not consistent. Richards

and Trayhurn (29) suggested some reasons for these discrepancies. There have been differences in the species and/or sex, the time of day when the measurements of fatty acid synthesis were performed, and the particular tissue analyzed among the studies. Also different rates of fatty acid synthesis could be accounted for by variations in food intake and training, or by interaction between them. All these factors could be playing a role in causing the differences observed in the various studies. Therefore, the mechanisms by which exercise affects lipid synthesis are not clearly established.

Fatty Acid Synthesis and Dietary Restriction:

To examine the effect of feed restriction on lipogenesis, Leveille (19) investigated the effects of restricting access to food to a single 2-h period each day over an extended period of time. In vitro incorporation of glucose- u - ^{14}C into fatty acids of epididymal adipose tissue, as well as activity of lipogenic related enzymes such as G6PD in the liver and adipose tissue, were measured. Lipogenic capacity of adipose tissue from meal-fed animals was significantly greater than that of adipose tissue from nibbling rats throughout the 18-mo study period. Activity of G6PD was higher in adipose and liver tissue of meal-fed as compared to ad libitum-fed animals. Presumably, the increase in the activities of these enzymes is related to the increased requirement for NADPH to support fatty acid

synthesis (45). However, it is important to note that the activities of these enzymes do not regulate lipogenesis but serve as an index (19). A high degree of positive correlation between lipogenic capacity and the activity of G6PD has been observed. Leveille (19) pointed out that meal-fed animals were able to increase conversion of carbohydrate to fat at a greater rate than ad libitum-fed animals.

Stevenson et al. (20) investigated the effect of limiting feed intake to 2 h per day for 10 weeks on lipogenesis in the adipose tissue of rats. Their results showed that feed-restricted rats had significantly less body weight than ad libitum-fed rats. Acetate 1- ^{14}C incorporation into lipids in adipose tissue was significantly increased in restricted-fed as compared to ad libitum-fed animals. Total body fat of feed-restricted animals was less than that of control animals. Hollifield et al. (16) also reported that limiting feed intake to 2 h daily increased lipogenesis markedly, i.e., increased incorporation of acetate-1- C^{14} into lipids by adipose tissue. Thus it would appear that feed restriction slows the rate of body weight gain and decreases the amount of body fat through changes in the net metabolic balance of fat synthesis and degradation.

Fatty Acid Synthesis/Degradation and Aging:

Studies on the rate of fatty acid synthesis and/or degradation have been done on the isolated adipocytes from young and old rats. Jamdar et al. (46) reported that the rate of synthesis of glyceride-glycerol (a measure of triacylglycerol production) and of fatty acids was more rapid in larger (old rat) than in smaller (young rat) adipocytes. However, other investigators (47,48) found that in larger (56-60 weeks old rats) as compared with smaller (6-9 weeks old rats) adipocytes, the synthesis of fatty acids from acetate-1-¹⁴C, pyruvate, or glucose was reduced without any affect on the production of glyceride-glycerol. Gommers et al. (49) reported that in adipocytes of growing animals the metabolic activity is about 4 to 5 times greater than observed in adult animals. In fact, during the growing period the synthesis of fatty acids is very important and allows the enlargement of the cell. From maturity to senescence the decline in basal glucose utilization is achieved primarily by a decrease in fatty acid synthesis accompanied by a higher rate of lipolysis in animals (49). This reduction of carbohydrate metabolism could be a biochemical feedback that protects the cell against too much enlargement that threatens the survival of the cell itself (49).

Few data on the development of lipolytic activity have been reported. Most of the present knowledge of adipose

tissue lipolysis has been derived from studies of young adult or fully mature rodents (50-52) and humans (53,54). A basal level of lipolysis occurs continuously in fat cells of young adults and mature rats and the rate of lipolysis is increased in fasting (55). Because of the low level of glycerol kinase in adipose tissue, very little of the glycerol produced by intracellular lipolysis can be reutilized through rephosphorylation by the fat cells. Therefore, release of glycerol from adipose tissue is a good index of lipolysis (56).

Several studies have reported that age as well as fat cell size, may be an important determinant of basal lipolytic activity (57-59). Gruen et al. (50) reported that total glycerol release per epididymal fat pad of rats was increased as the rats reached older age up to 130 days. Also, they indicated that total glycerol release in epididymal fat pads of fasted rats was higher than that in fed animals. Bertrand et al. (59) indicated that lipolysis increased late in the life of rats. This age-related increase was not modulated by dietary restriction.

Klein et al. (60) investigated the regulation of lipolysis during short term fasting in humans. They reported that during fasting there is a decrease in insulin and an increase in epinephrine which stimulates lipolysis in adipose tissue resulting in an increased release of glycerol, which is converted to glucose in the liver, and

free fatty acids, which are oxidized for fuel. Apparently, body composition influences the sensitivity of adipose tissue, i.e., the rate of lipolysis per unit of fat mass is regulated to meet the needs of lean body mass.

In summary, there is lack of agreement among investigators as to whether exercise increased or decreased lipogenesis in the liver and/or adipose tissue. Also, it is not clear whether feed restriction elevated or reduced lipogenic activity in the liver and/or adipose tissue. Reduction in body weight and in body fat in exercised young laboratory animals are well documented, but whether similar changes occur in aged animals is not clear. Changes in lipogenesis and related enzymes activities with age are not conclusive. There has been no research testing the influence of both feed restriction and exercise on lipogenesis, lipolysis, and fat tissue accumulation throughout the life span of rats. It is, therefore, the object of this study to investigate the effect of dietary restriction and exercise on these parameters throughout the life span of rats.

CHAPTER III

EXPERIMENTAL PROCEDURE

General Outline of Research:

This study was initiated to assess the effect of long-term dietary restriction and exercise on adipose tissue mass and its relationship to lipogenesis and lipolysis in aging rats. Lipogenesis was estimated by measuring the in vitro rate of fatty acid synthesis and G6PD activity in the liver and the adipose tissue; lipolysis was estimated by measuring the release of glycerol in the liver and adipose tissue. Finally the gross balance of the two processes was estimated by measuring body fat accumulation perirenally and epididymally. The measurements were done on animals that were diet restricted or fed ad libitum and/or that were exercised or allowed to be sedentary.

Pathogen free weanling male rats of Wistar origin were obtained from Taconic Laboratory, German Town, 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 $22^{\circ} \pm 2^{\circ}\text{C}$, with a 12 h light-12 h dark cycle. Water was provided ad libitum. There were four experimental groups:

- A: Ad libitum-fed, non-exercised controls (n=35)
- R: Diet restricted by intermittent feeding, non-exercised (n=35)

AE: Ad libitum-fed, exercised by swimming on alternate days (n=35)

RE: Diet restricted by intermittent feeding, exercised by swimming on alternate days (n=35)

The animals were weighed weekly until 6 mos of age, biweekly up to 12 mos and monthly thereafter. The diet fed was ground Purina Rodent Chow. Diet restriction was achieved by feeding the rats on alternate days. Feed cups were placed in the cages at 9:00 a.m. and removed at 9:00 a.m. the following day.

The exercised rats swum in groups of 5 in 20 liter plastic barrels (50 cm X 68 cm) in the morning on alternate days. Water temperature was maintained between 33°-36°C. Swimming was begun at 6 weeks of age, initially for a 10 min period and increased 10 min daily until 3.0 h was reached at 3 mo of age. From 3 to 15 mo of age the rats swum 3 h on alternate days. At 15 mo of age, the swimming period was decreased to 2.0 h, at 20 mo to 1.5 h, and at 26 mo to 1.0 h on alternate days. The RE rats swum prior to being fed. Swimming was continued throughout the experimental period, i.e., until the animals were sacrificed for biochemical analysis. At 12-, 16-, 20-, 24-, and 28-mo of age, 6 rats randomly selected from each of the experimental groups were killed for biochemical determinations.

The R and RE rats were allowed to feed the day and night before the "kill" day; no rats swum on that day. Feed

cups were removed from all cages 2 h prior to the kill time. Rats were stunned by a blow to the head, followed by decapitation. The liver, epididymal and perirenal fat pads were removed and weighed. Liver and epididymal tissues were analyzed for enzyme activity, fatty acid synthesis, and glycerol release.

Preparation of Liver and Adipose Tissue Homogenates:

Immediately following excision, of the livers and the adipose tissues they were blotted with filter paper to remove excess blood and weighed. Approximately 250 mg of either the liver or the left epididymal fat pad tissue was added to EDTA-saline (see reagents) at a dilution of 1 mg tissue (wet weight) per 0.04 ml of EDTA saline. This was kept on an ice bath and homogenized for 2 min using a Potter-Elvehjem homogenizer. The homogenates were then centrifuged for 20 min at 200 x/g at 0°C. The clear supernatant fractions were decanted into vials and kept on ice until assayed (normally within an h).

Method for Enzyme Assay:

G6PD activities of rat liver and adipose tissue supernatant fractions were determined by the method of Lohr and Waller (37). The basis of this method is the spectrophotometric measurement of the rate of NADPH formation from the increase in absorbance at 340 nm. Actually, the NADPH produced in the analysis is the result

of dehydrogenation of G6P and a subsequent reaction, i.e., the dehydrogenation of 6-phosphogluconate (35). Nevertheless, the determination of NADPH is indicative of G6PD activity and the reducing power available for fat synthesis.

Reagents:

1. Triethanolamine buffer (0.05M; pH 7.5): 0.93 g triethanolamine hydrochloride and 0.2 g ethylene-diamine-tetra acetic acid ($\text{EDTA-Na}_2 \cdot \text{H}_2\text{O}$) was dissolved in 50 ml distilled water. This was adjusted to pH 7.5 with 0.1N NaOH and diluted to 100 ml with distilled water.

2. EDTA-saline: Physiological saline containing $6.6 \times 10^{-4}\text{M}$ EDTA was made by dissolving 0.25 g $\text{EDTA-Na}_2 \cdot \text{H}_2\text{O}$ in physiological saline and diluted to 1000 ml.

3. G6P (ca. $4 \times 10^{-2}\text{M}$ G6P): 130 mg Glucose-6-phosphate- Na_2 was dissolved in 10 ml distilled water.

4. NADP (ca. $3 \times 10^{-2}\text{M}$): 25 mg β -Nicotinamide adenine dinucleotidephosphate- NaH_2 was dissolved in 1.0 ml 1% NaHCO_3 solution.

Procedure:

The reaction mixture was prepared containing 2.40 ml triethanol-amine buffer, 0.50 ml supernatant of the liver or adipose homogenates, 0.05 ml G6P solution, and 0.05 ml NADP solution in a final volume of 3 ml. Blanks consisted of all the reagents except NADP.

The reaction was initiated by adding the G6P solution to the blank and sample tubes. The contents were poured into quartz cuvettes and mixed by pouring back into the original, then back to the cuvette. Then the absorbance (A) of the sample was measured against the blank at 340 nm in 2 min intervals for 10 min with a spectrophotometer (Beckman Model 34). Determinations were run in duplicate.

The enzyme activity of G6PD was expressed as nmoles NADPH produced per min/g of protein, as calculated from the extinction coefficient (E_{340}^{mM}) of NADPH as shown in the following equation:

$$\text{G6PD activity} = \frac{(\text{E/min}) \times 3}{6.2 (E_{340}^{mM}) \times (\text{g Pro}/0.5 \text{ ml supernatant fluid})}$$

Determination of Tissue Protein:

The protein content of the homogenate supernatant was determined by the method of Ohnishi and Barr (61) which is a modification of the Lowry method (62). The protein sample is mixed first with a dilute biuret reagent followed by 2N phenol reagent for color development.

Reagents:

1. Biuret reagent: 1.5 g $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 6 g $\text{NaKC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$, 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_2CO_3 (i.e., 1 volume of biuret reagent plus 7 volume of Na_2CO_3) at the time of analysis.

2. 2N Phenol reagent.

3. Bovine serum albumin (BSA) stock standard: 1 g bovine serum albumin was dissolved in and diluted to 100 ml with 0.4 N 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 min at room temperature. 0.1 ml of 2N phenol reagent was added while the solution was mixed using a vortex mixer. The protein standards containing bovine serum albumin received the same treatment as the supernatants from the samples.

After 30 min, the absorbance of the samples and standards was 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 concentration of the samples was calculated by the following formula:

Mg Protein/ml supernatant =

$$\frac{A_{\text{Sample}}}{A_{\text{Standard}}} \times 0.2 \text{ mg Protein/ml} \times \text{dilution factor}$$

Determination of Fatty Acid Synthesis:

The rate of fatty acid synthesis in the liver and adipose tissue was determined by the method of Leveille (63-65). The basis of this method is the incorporation of radioactive labeled carbon of acetate (acetate-1-¹⁴C) into fatty acid which is synthesized in vitro in the liver or adipose tissue by count per min/g of tissue (wet weight).

Reagents:

1. Ca⁺⁺ free Krebs ringer bicarbonate buffer:

a. NaCl, 0.154M: 9 g of NaCl was dissolved in 1000 ml of distilled water.

b. KCl, 0.154M: 11.5 g of KCl was dissolved in 1000 ml of distilled water.

c. KH₂PO₄, 0.154M: 21.1 g of KH₂PO₄ was dissolved in 1000 ml of distilled water.

d. MgSO₄•7H₂O, 0.154M: 38.2 g of MgSO₄•7H₂O was dissolved in 1000 ml of distilled water.

e. NaHCO₃, 0.154M: 13.0 g of NaHCO₃ was dissolved in 1000 ml of distilled water.

To prepare the buffer, the following amounts of the solutions above were mixed:

100 parts of solution a

4 parts of solution b

1 parts of solution c

1 parts of solution d

21 parts of solution e

The final volume of this mixed solution was gassed with a mixture of 5% CO₂ and 95% O₂ for 10 min and was kept in glass stoppered reagent bottle. The solution was prepared within 1 h of use and stored in a refrigerator.

2. Sodium acetate, 10 μM/ml of buffer, pH 7.4: 1.36 mg of CH₃COONa•3H₂O acetate was dissolved in 1 ml of the buffer.

3. Acetate-1-¹⁴C, .167 μCi/ml of buffer specific activity, 2.1 mCi/mm: 50 μCi acetate-1-¹⁴C was dissolved in 5 ml of distilled water and 50 μl of this solution was added to 2 ml of buffer.

4. H₂SO₄, .2N: 5.59 ml of concentrated H₂SO₄ was added slowly to 100 ml of distilled water and diluted to 1000 ml with distilled water.

5. Ethanolic KOH, 5%: 5 g KOH was dissolved in 100 ml of 95% ethanol.

6. Physiological saline: 9 g NaCl was dissolved in 1000 ml of distilled water.

7. Petroleum ether.

8. Hydrochloric acid, concentrated, about 12N.

9. Scintillation solution: 4 g of 2,5-Diphenyloxazole (PPO) and 0.15 g of 1,4-Bis(2-(5-Phenyloxazaly))Benzene (POPOP) were dissolved in 230 ml of ethanol and brought up to 1 liter with toluene.

Procedure:

Fresh thin peripheral portions of the left epididymal fat pads weighing 100 mg and of liver slices (left lateral lobe) weighing 200 mg were used to determine the in vitro lipogenesis. The tissues were incubated in 2.0 ml of Ca^{++} free Krebs ringer bicarbonate buffer, pH 7.4, containing 10 μmoles of sodium acetate and 0.167 μCi acetate $1\text{-}^{14}\text{C}$ per ml at 38°C for 3 h under 95% O_2 and 5% CO_2 in a shaking water bath (90 oscillations per minute). Incubation was carried out in flasks with rubber stoppers. At the end of the 3 h incubation period 0.5 ml of 0.2N H_2SO_4 was added to the medium and shaking was continued for 20 min to ensure termination of the reaction. The tissues were rinsed 5 times in cold saline and transferred to tubes containing 2 ml of 5% ethanolic KOH. The tissues were saponified by refluxing at 85°C for 30 min. Then 5 ml of distilled water was added and the nonsaponifiable lipid fraction was acidified with HCl and the fatty acids were removed by three successive 5 ml extractions with petroleum ether. The combined ether extracts were washed with water and the ether fraction was transferred to liquid scintillation vials. The ether was evaporated under a stream of nitrogen and the fatty acids were dissolved in 10 ml of scintillation solution. All samples were counted in a Beckman liquid scintillation counter (Model LS 100C). The control was treated the same as sample except the tissue was incubated

without the radioactive labeled acetate. All the determinations were run in duplicate and the rate of fatty acid synthesis was expressed as CPM/g fresh tissue (wet weight).

Determination of Lipolysis:

The rate of lipid degradation in the liver and adipose tissue was determined by the methods of Gruen et al. (50) and Buccolo et al. (66). The basis of these methods is the spectrophotometric measurement of glycerol which is released by the tissues as indicated by increased absorbance at 340 nm. A Stat-Pack enzymatic triglycerides-glycerol test (3 vial) kit prepared by Behring Diagnostics, 10933 North Torrey Pines Road, LaJolla, CA 92037, was used for this determination.

Reagents:

1. Krebs-Ringer bicarbonate buffer containing .4% CaCl_2 and 4% BSA pH 7.4.

- a. Krebs-Ringer buffer (including final concentration of .4% CaCl_2). The preparation of basic Krebs-Ringer was explained for fatty acid synthesis measurement.

- b. BSA, 4%: 4 g of Bovin Serum Albumin was dissolved in the final volume of the buffer.

2. Stat-Pack enzymatic triglycerides-glycerol test (3 vials).

Vial A (pH 7.1):

- a. Phosphate buffer, 1.0×10^{-1} M
- b. Adenosine triphosphate, 3.3×10^{-4} M
- c. Phosphoenolpyruvate, 5.3×10^{-4} M
- d. Pyruvate kinase, 2.1×10^3 μ M
- e. Lactate dehydrogenase, 7.2×10^2 μ M
- f. NADH, 2.2×10^2 M
- g. α -chymotrypsin, 9.8×10^3 μ M
- h. Magnesium, 1.6×10^{-3} M
- i. Stabilizer

Content of Vial A was dissolved in 15 ml of distilled water at the time of analysis.

Vial B:

- a. Glycerol kinase, 6.6×10^2 u/l
- b. Stabilizer

The content of this vial was dissolved in .5 ml of distilled water at the time of analysis.

Procedure:

Slices of liver or of epididymal fat pads (450-500 mg) were placed in a vial containing 3 ml of Krebs-Ringer buffer. The tissues were incubated for 2 h in a shaking water bath at 38°C under 95% O₂ and 5% CO₂. After 2 h, the tissues were removed and the medium was used for glycerol release measurement. Three ml of reagent from vial A was pipetted in each cuvette, the cuvettes were placed in a

water-bath for 3 min which was sufficient time to bring the reagent to 30°C. The samples were also preincubated for the same length of time in the same bath. Medium from each sample (200 μ l) was added to each cuvette. The content of cuvettes was mixed by inversion and the cuvettes were again placed in the water bath. After 5 min the cuvettes were inserted into the spectrophotometer (Beckman Model 34). Absorbances were measured at 340 nm versus the blank which contained all the reagents except the sample which was replaced by water. The initial absorbance (A_0) was read and immediately 50 μ l of glycerol kinase reagent (Vial B) was added to each cuvette. The content of the cuvettes was mixed and incubated at 30°C. Exactly 10 min after adding glycerol kinase, the final absorbance (A_{10}) was read. The amount of glycerol released (mg/dl) was calculated per kit instructions (66).

Statistical Methods:

The general linear model (GLM) procedures of SAS 1987 (67) were utilized to evaluate the effect of the dietary restriction, exercise, and age on the test responses. Type III sum of squares in multiple regression analysis were calculated to test the significance of the relationships. Diet, exercise, and age (class variables) were entered into the model statement first and the interactions of variables followed. Type I sum of squares for linear, quadratic and

cubic were calculated for the variable age. Pearson correlation coefficients were calculated to determine the relationships between physical and biochemical test responses within an experimental group. A probability level of less than 0.05 was considered statistically significant.

CHAPTER IV

RESULTS

Body Weight:

The mean body weights of the experimental groups at the time of sacrifice are shown in Figure 3; the statistical analysis of the data is presented in Table 1. The diet-restricted groups (R,RE) overall weighed less (28%, $p < 0.0001$) than the ad libitum-fed groups (A,AE). The body weight of AE overall was 6.2% less than that of A while that of RE was 0.6% more than R; the differences in body weight due to exercise were not significant ($p > 0.05$). Overall, the mean body weight of all groups decreased with age ($p < 0.05$). The initiation of body weight loss of A animals was delayed until 20 mo of age. The average decrement in body weight of all groups from 12 to 24 mo was 8.7%. Mean feed consumption of exercised animals was 12% greater than non-exercised animals, mean feed consumption of restricted animals was approximately 30% less than of ad libitum-fed animals.

Epididymal Fat Pad Weight:

Figure 4 presents the mean epididymal fat pad weights of the animals at the time of sacrifice. The results of the statistical analysis are shown in Table 2. The overall mean fat pad weights of the diet-restricted groups (R,RE) were approximately 50% less ($p < 0.0001$) than those of the ad libitum-fed groups (A,AE). The epididymal fat pad weights

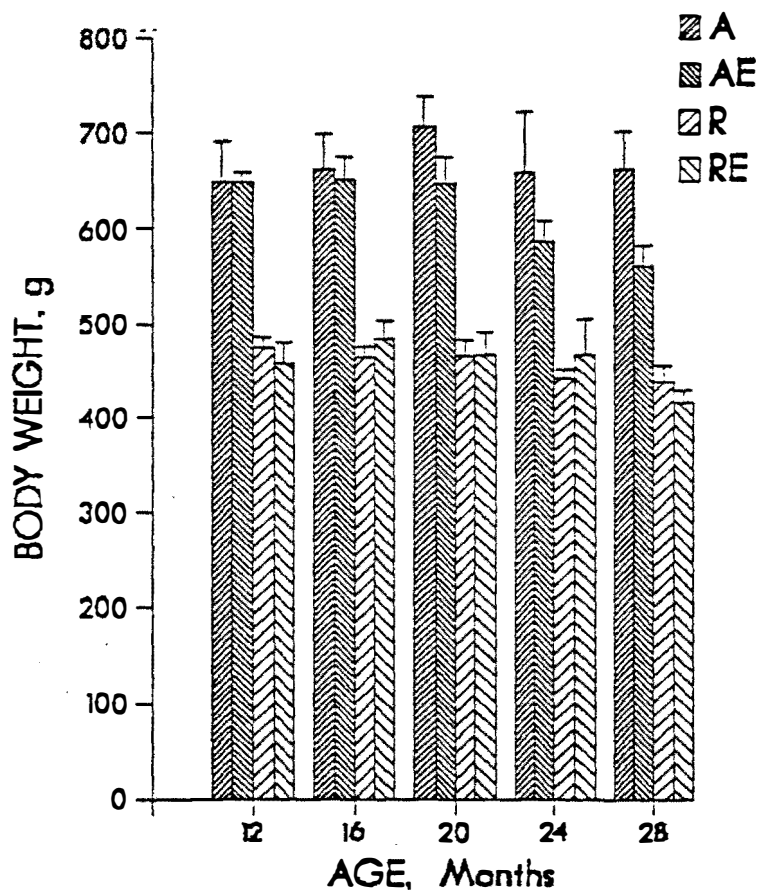


Figure 3. Body Weight Plotted Versus Age. (A = Ad libitum-fed; R = Restricted-fed; AE = Ad libitum-fed, exercised; RE = Restricted-fed; exercised. Values are mean $\pm$ SEM; in general $n=6$. Body weight is related to diet and exercise ($p<0.0001$), age ($p<0.05$), and diet*exercise ($p<0.05$) in multiple regression analysis.

Table 1. ANOVA Table for Body Weight

Variables	df	SS	F Values	P Values
<u>Type III SS</u>				
Diet	1	936270.1	196.0	0.0001
Exercise	1	10451.3	2.19	0.1423
Age	4	52816.4	2.77	0.0318
Diet * Age	4	7251.5	0.38	0.8224
Diet * Exercise	1	13470.7	2.82	0.0963
Age * Exercise	4	7286.1	0.38	0.8214
Diet * Age * Exercise	4	1023.9	0.54	0.7095
<u>Type I SS</u>				
Age				
Linear	1	29083.08	6.35	0.0132
Quadratic	1	19170.32	4.19	0.0432
Cubic	1	137.09	0.03	0.8629

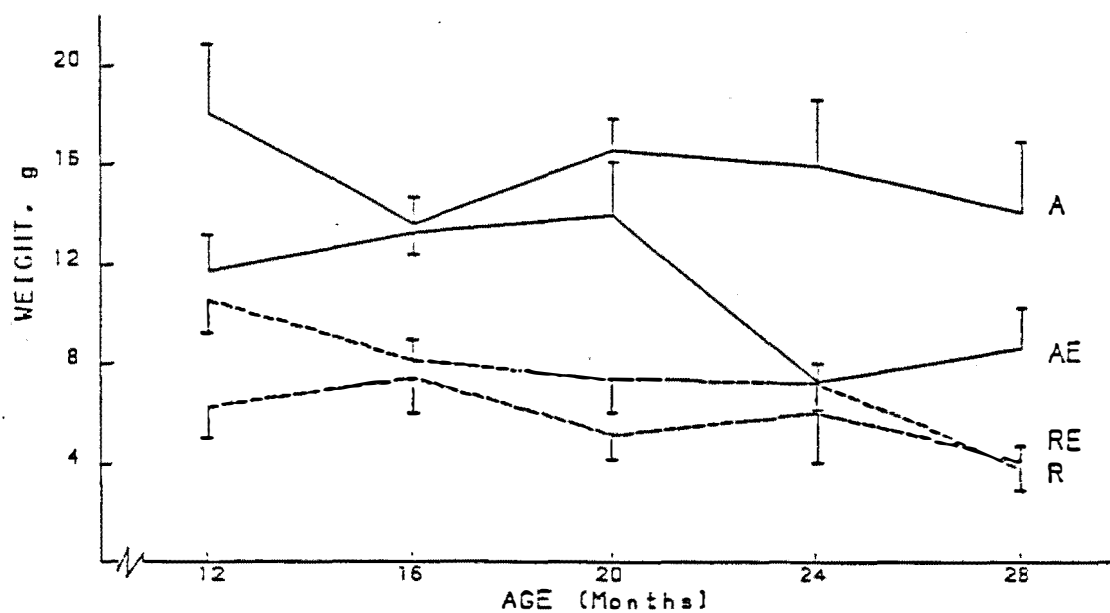


Figure 4. Epididymal Fat Pad Weight Plotted Versus Age. (A = Ad libitum-fed; R = Restricted-fed; AE = Ad libitum-fed, exercised; RE = Restricted-fed, exercised). Values are mean $\pm$ SEM; in general $n=6$. Epididymal fat pad weight is related to diet and exercise ($p<0.0001$), age ($p<0.05$), and diet*exercise ($p<0.05$) in multiple regression analysis.

Table 2. ANOVA Table for Epididymal Fat Pad Weight

Variables	df	SS	F Values	P Values
<u>Type III SS</u>				
Diet	1	1261.56	81.44	0.0001
Exercise	1	278.42	17.97	0.0001
Age	4	210.42	3.40	0.0121
Diet * Age	4	56.67	0.91	0.4587
Diet * Exercise	1	64.29	4.15	0.0444
Age * Exercise	4	93.51	1.51	0.2056
Diet * Age * Exercise	4	66.38	1.07	0.3750
<u>Type I SS</u>				
Age				
Linear	1	198.47	12.52	0.0006
Quadratic	1	9.76	0.62	0.4343
Cubic	1	2.34	0.15	0.7012

of the exercised groups (AE,RE) overall were 25% less ($p < 0.0001$) than those of the non-exercised groups (A,R). The overall epididymal fat pad weight of all groups decreased (34%, $p < 0.05$) with age; the decrement was linear ($p < 0.0006$). Interaction of diet and exercise was significant ($p < 0.05$), i.e., exercise decreased epididymal fat pad weight more in ad libitum-fed than in restricted-fed rats.

Perirenal Fat Pad Weight:

The mean perirenal fat pad weights are shown in Figure 5. Table 3 presents the statistical analysis of the data. Overall, perirenal fat pad weights of feed-restricted groups (R,RE) were less (68%, $p < 0.0001$) than those of ad libitum-fed groups (A,AE). The exercised groups (AE,RE) had less (35%, $p < 0.0001$) perirenal fat than non-exercised groups (A,R). Considering all groups there was a decrease (39%, $p < 0.05$) in perirenal fat weight from 12 to 28 mo of age. An interaction ($p < 0.05$) between diet and exercise was observed, i.e., the differences in fat pad weight associated with exercise were more pronounced in A than in R rats.

Liver G6PD Activity:

The overall liver G6PD activity of the diet restricted groups (R,RE) was 25% higher ($p < 0.005$) than that of ad libitum-fed (A,AE) groups (Table 4,5). The activity of hepatic G6PD was not significantly affected by exercise. Age affected G6PD activity ($p < 0.0005$), i.e., there was an

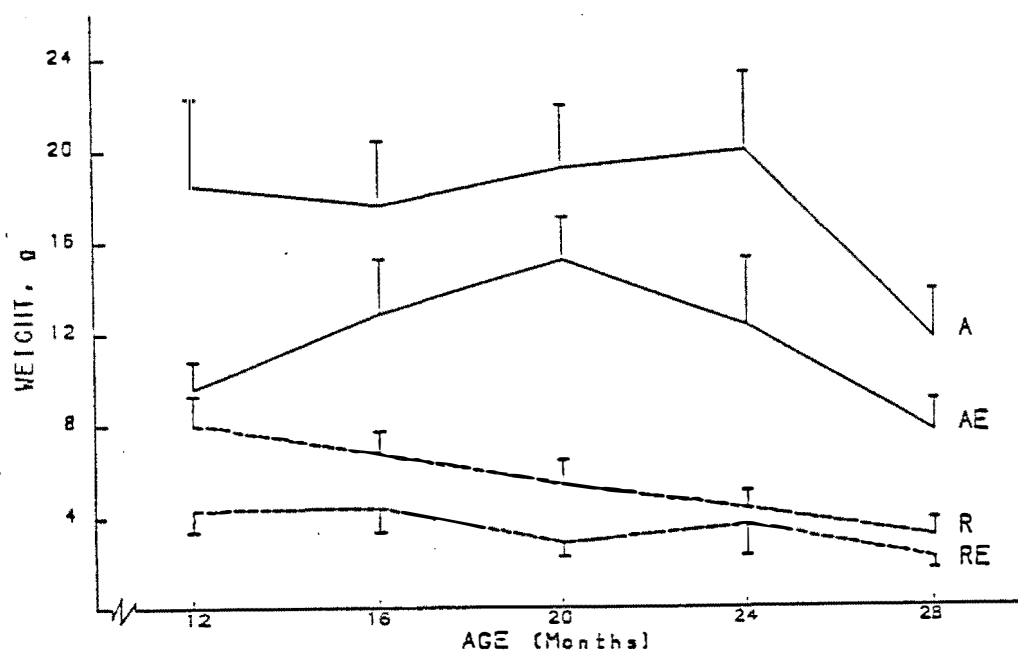


Figure 5. Perirenal Fat Pad Weight Plotted Versus Age. (A = Ad libitum-fed; R = Restricted-fed; AE = Ad libitum-fed, exercised; RE = Restricted-fed, exercised. Values are mean $\pm$ SEM; in general $n=6$. Perirenal fat pad weight is related to diet and exercise ($p<0.0001$), age ($p<0.05$), and diet*exercise ($p<0.05$) in multiple regression analysis.

Table 3. ANOVA Table for Perirenal Fat Pad Weight

Variables	df	SS	F Values	P Values
<u>Type III SS</u>				
Diet	1	2820.80	121.49	0.0001
Exercise	1	443.65	19.11	0.0001
Age	4	283.02	3.05	0.0206
Diet * Age	4	149.80	1.61	0.1772
Diet * Exercise	1	103.42	4.45	0.0374
Age * Exercise	4	45.50	0.49	0.7431
Diet * Age * Exercise	4	29.14	0.31	0.8681
<u>Type I SS</u>				
Age				
Linear	1	128.77	5.82	0.0176
Quadratic	1	130.43	5.89	0.0169
Cubic	1	25.11	1.13	0.2893

Table 4. Effect of Age, Diet Restriction, and Exercise on Liver Glucose-6-Phosphate Dehydrogenase Activity¹.

Group	Age in Months				
	12	16	20	24	28
A ²	21.07 ±4.50 (6) ⁶	27.57 ±2.79 (6)	48.22 ±4.50 (6)	45.64 ±12.79 (6)	28.34 ±4.50 (6)
R ³	31.61 ±8.19 (6)	60.94 ±3.74 (6)	48.80 ±1.54 (6)	42.19 ±9.14 (6)	33.78 ±9.00 (6)
AE ⁴	20.86 ±4.25 (6)	34.70 ±5.34 (6)	34.74 ±4.39 (5)	42.57 ±7.63 (6)	40.07 ±2.50 (3)
RE ⁵	31.04 ±5.34 (6)	41.09 ±3.49 (6)	45.15 ±2.33 (6)	66.78 ±8.88 (6)	30.18 ±8.31 (6)

¹Significant for diet ($p < 0.005$) and age ($P < 0.0005$) in multiple regression analysis.

²A = Ad libitum-fed, non-exercised.

³R = Restricted-fed, non-exercised.

⁴AE = Ad libitum-fed, exercised.

⁵RE = Restricted-fed, exercised.

⁶Values are means ± SEM (expressed as nmoles NADPH produced/min/g protein) for the number of animals shown in parenthesis.

Table 5. ANOVA Table for Liver Glucose-6-Phosphate-Dehydrogenase Activity

Variables	df	SS	F Values	P Values
<u>Type III SS</u>				
Diet	1	2178.83	9.10	0.0022
Exercise	1	0.254	0.00	0.8779
Age	4	7903.84	8.26	0.0003
Diet * Age	4	1405.17	1.47	0.2580
Diet * Exercise	1	7.71	0.03	0.8432
Age * Exercise	4	1436.26	1.50	0.2072
Diet * Age * Exercise	4	2675.54	2.78	0.0170
<u>Type I SS</u>				
Age				
Linear		1075.73	4.14	0.0443
Quadratic		6644.58	25.59	0.0001
Cubic		292.00	1.13	0.2908

increase in liver G6PD activity in each group with age and then a decrease (quadratic relationship with age, $p < 0.0001$). The interaction among diet, age, and exercise was significant ($p < 0.05$).

Adipose G6PD Activity:

The effect of diet on G6PD activity in adipose tissue was not significant (Table 6,7). Exercised groups (AE,RE) had 26% more ($p < 0.05$) adipose G6PD activity than that of non-exercised groups (A,R). There was a significant change in adipose G6PD activity with age ($p < 0.0001$). In general adipose G6PD activity peaked at 20 to 24 mo and then decreased. Both the quadratic (A^2 , $p < 0.0005$) and cubic (A^3 , $p < 0.0004$) relationships of age to G6PD activity were significant. The interactions between diet and age ($p < 0.005$) and among diet, age, and exercise ($p < 0.05$) were significant, i.e., adipose G6PD activity peaked at an earlier age (20 mo) in both A and AE animals than in RE animals (24 mo), while adipose G6PD enzyme activity in R rats did not change with age.

Liver Fatty Acid Synthesis:

The differences in the incorporation of acetate- $1-^{14}\text{C}$ into liver fatty acids resulting from either diet or exercise were not significant (Table 8,9). However, the incorporation of acetate- $1-^{14}\text{C}$ into liver fatty acids was affected by age ($p < 0.0001$). There was an overall 9-fold

Table 6. Effect of Age, Diet Restriction, and Exercise on Adipose Tissue Glucose-6-Phosphate Dehydrogenase Activity¹.

Group	Age in Months				
	12	16	20	24	28
A ²	14.95 ±1.56 (6) ⁶	19.12 ±2.03 (6)	60.19 ±8.05 (6)	38.22 ±8.87 (6)	12.28 ±2.58 (6)
R ³	33.98 ±13.45 (6)	15.30 ±2.21 (6)	26.02 ±5.50 (6)	21.87 ±8.36 (6)	26.01 ±0.50 (6)
AE ⁴	29.20 ±5.72 (6)	23.96 ±1.56 (6)	58.55 ±10.84 (5)	30.14 ±4.49 (6)	25.00 ±1.27 (3)
RE ⁵	36.04 ±11.32 (6)	20.12 ±1.67 (6)	41.27 ±0.64 (6)	53.48 ±10.04 (6)	18.09 ±4.49 (6)

¹Significant for age ($p < 0.0001$) and exercise ($p > 0.05$) in multiple regression analysis.

²A = Ad libitum-fed, non-exercised.

³R = Restricted-fed, non-exercised.

⁴AE = Ad libitum-fed, exercised.

⁵RE = Restricted-fed, exercised.

⁶Values are means ± SEM (expressed as nmoles NADPH produced/min/g protein) for the number of animals shown in parenthesis.

Table 7. ANOVA Table for Adipose Tissue G6PD Activity

Variables	df	SS	F Values	P Values
<u>Type III SS</u>				
Diet	1	102.08	0.35	0.5534
Exercise	1	1322.63	4.58	0.0348
Age	4	11554.12	10.01	0.0001
Diet * Age	4	4834.04	4.19	0.0036
Diet * Exercise	1	165.77	0.57	0.4503
Age * Exercise	4	274.44	0.24	0.9163
Diet * Age * Exercise	4	3289.35	2.85	0.0270
<u>Type I SS</u>				
Age				
Linear	1	10.06	0.03	0.8550
Quadratic	1	3942.55	13.05	0.0005
Cubic	1	4035.65	13.36	0.0004

Table 8. Effect of Age, Diet Restriction, and Exercise on Liver Fatty Acid Synthesis¹.

Group	Age in Months				
	12	16	20	24	28
A ²	158.80 ±52.41 (6) ⁶	422.60 ±135.83 (6)	587.09 ±181.42 (6)	961.52 ±375.43 (6)	733.94 ±238.41 (6)
R ³	98.31 ±19.63 (6)	460.77 ±122.66 (6)	378.15 ±121.26 (6)	1098.77 ±402.57 (6)	261.70 ±107.52 (6)
AE ⁴	108.26 ±25.17 (6)	210.56 ±53.00 (6)	292.76 ±108.78 (5)	1201.50 ±725.79 (6)	626.07 ±212.47 (3)
RE ⁵	99.26 ±21.02 (6)	296.47 ±93.53 (6)	299.35 ±122.13 (6)	994.39 ±356.50 (6)	189.65 ±42.47 (6)

¹Significant for age ($p < 0.0001$) in multiple regression analysis.

²A = Ad libitum-fed, non-exercised.

³R = Restricted-fed, non-exercised.

⁴AE = Ad libitum-fed, exercised.

⁵RE = Restricted-fed, exercised.

⁶Values are means ± SEM (expressed as incorporation of acetate-1-¹⁴C cpm/200 mg fresh tissue/3 hrs) for the number of animals shown in parenthesis.

Table 9. ANOVA Table for Liver Fatty Acid Synthesis

Variables	df	SS	F Values	P Values
<u>Type III SS</u>				
Diet	1	358859.17	0.96	0.3200
Exercise	1	201419.20	0.54	0.4600
Age	4	11982578.03	8.05	0.0001
Diet * Age	4	811154.52	0.55	0.7000
Diet * Exercise	1	11.879	0.00	0.9900
Age * Exercise	4	284426.69	0.19	0.9400
Diet * Age * Exercise	4	252877.88	0.17	0.9500
<u>Type I SS</u>				
Age				
Linear	1	4653347.17	13.35	0.0004
Quadratic	1	1907795.68	5.84	0.0212
Cubic	1	2930772.90	8.41	0.0046

increase in incorporation of acetate-1-¹⁴C by all groups from 12 to 24 mo of age. At 28 mo of age there was a sharp drop in fatty acid synthesis in all groups. The relationship of the fatty acid synthesis with age was linear ($p < 0.0005$), quadratic and cubic ($p < 0.005$). In general, from 12 to 20 mo there was a gradual increase in liver fatty acid synthesis; from 20 to 24 mo the rate of increase was accelerated; after 24 mo there was a sharp decrease in liver fatty acid synthesis.

Adipose Fatty Acid Synthesis:

Dietary restriction increased the incorporation of acetate-1-¹⁴C into fatty acids of adipose tissue (Table 10,11); i.e., overall incorporation was 64% higher in R than in A rats and 116% higher in RE than in AE animals ($p < 0.01$). Exercise did not have a significant effect on acetate-1-¹⁴C incorporation. The incorporation of acetate-1-¹⁴C into fatty acid in adipose tissue changed with age ($p < 0.0005$). Overall the incorporation were low at the youngest (12 mo) and oldest (28 mo) ages studied; the quadratic relationship with age was significant ($p < 0.0001$).

Liver Glycerol Release:

Dietary restriction overall increased (24%, $p < 0.005$) the amount of liver glycerol released (Table 12,13). Hepatic glycerol release was affected by exercise ($p < 0.05$). The exercised groups (AE,RE) showed a 44% increase in

Table 10. Effect of Age, Diet Restriction and Exercise on Adipose Tissue Fatty Acid Synthesis¹.

Group	Age in Months				
	12	16	20	24	28
A ²	379.1 ±126.6 (6) ⁶	1126.4 ±161.4 (6)	697.4 ±207.3 (6)	749.9 ±350.0 (6)	90.8 ±25.2 (6)
R ³	329.5 ±63.8 (6)	1428.4 ±210.6 (6)	747.0 ±220.2 (6)	2076.3 ±103.0 (6)	417.2 ±223.4 (6)
AE ⁴	220.3 ±42.2 (6)	591.08 ±83.5 (6)	483.4 ±153.9 (5)	519.9 ±106.8 (6)	103.5 ±24.5 (3)
RE ⁵	560.9 ±107.5 (6)	1253.7 ±331.2 (6)	885.62 ±144.6 (6)	1231.8 ±420.0 (6)	222.6 ±80.9 (6)

¹Significant for diet ($p < 0.01$) and age ($p > 0.0005$) in multiple regression analysis.

²A = Ad libitum-fed, non-exercised.

³R = Restricted-fed, non-exercised.

⁴AE = Ad libitum-fed, exercised.

⁵RE = Restricted-fed, exercised.

⁶Values are means ± SEM (expressed as incorporation of acetate-1-¹⁴C cpm/200 mg fresh tissue/3 hrs) for the number of animals shown in parenthesis.

Table 11. ANOVA Table for Adipose Tissue Fatty Acid Synthesis

Variables	df	SS	F Values	P Values
<u>Type III SS</u>				
Diet	1	4978619.85	7.47	0.0075
Exercise	1	1100953.48	1.65	0.2018
Age	4	15725722.37	5.90	0.0003
Diet * Age	4	3039442.13	1.14	0.3424
Diet * Exercise	1	22007.94	0.03	0.8562
Age * Exercise	4	1374988.07	0.52	0.7243
Diet * Age * Exercise	4	1195606.40	0.45	0.7733
<u>Type I SS</u>				
Age				
Linear	1	107239.14	0.17	0.6800
Quadratic	1	10573027.38	16.52	0.0001
Cubic	1	182920.87	0.29	0.5900

Table 12. Effect of Age, Diet Restriction and Exercise on Liver Glycerol Release¹.

Group	Age in Months				
	12	16	20	24	28
A ²	0.90 ±0.21 (6) ⁶	0.70 ±0.15 (6)	0.82 ±0.16 (6)	0.89 ±0.21 (6)	0.51 ±0.09 (6)
R ³	0.83 ±0.22 (6)	0.90 ±0.12 (6)	0.73 ±0.07 (6)	0.76 ±0.13 (6)	0.86 ±0.03 (6)
AE ⁴	0.94 ±0.14 (6)	0.95 ±0.04 (6)	1.12 ±0.10 (5)	1.22 ±0.22 (6)	0.52 ±0.03 (3)
RE ⁵	0.83 ±0.08 (6)	1.57 ±0.12 (6)	1.83 ±0.20 (6)	1.55 ±0.23 (6)	0.68 ±0.07 (6)

¹Significant for age ($p < 0.005$), diet ($p < 0.0001$), and exercise ($p < 0.01$) in multiple regression analysis.

²A = Ad libitum-fed, non-exercised.

³R = Restricted-fed, non-exercised.

⁴AE = Ad libitum-fed, exercised.

⁵RE = Restricted-fed, exercised.

⁶Values are means ± SEM (expressed as mg glycerol released/dl/250 mg tissue/2 hrs) for the number of animals shown in parenthesis.

Table 13. ANOVA Table for Liver Glycerol Release

Variables	df	SS	F Values	P Values
<u>Type III SS</u>				
Diet	1	1.53	10.10	0.0020
Exercise	1	2.47	12.27	0.0001
Age	4	2.34	3.86	0.0060
Diet * Age	4	1.13	1.87	0.1220
Diet * Exercise	1	0.32	2.17	0.1440
Age * Exercise	4	3.41	5.63	0.0004
Diet * Age * Exercise	4	1.41	2.33	0.0611
<u>Type I SS</u>				
Age				
Linear	1	0.1770	1.02	0.3140
Quadratic	1	2.7414	15.81	0.0001
Cubic	1	0.3105	1.79	0.1830

overall liver glycerol release as compared to non-exercised (A,R) groups. The effect of age on liver glycerol release was quadratic ($p < 0.0001$). In exercised groups (AE,RE) glycerol release increased from 12 to either 20 or 24 mo of age and then decreased. The interaction between age and exercise was highly significant ($p < 0.0005$). That is, the effect of exercise on glycerol release with age was much more pronounced in restricted than in ad libitum-fed animals.

Adipose Glycerol Release:

The amount of glycerol released by adipose tissue was affected by diet ($p < 0.005$) and age ($p < 0.0001$, Table 14,15); overall R and RE had 37% greater amount of glycerol released than A and AE. The effect of exercise was not significant, although AE and RE had 7% more glycerol released from adipose tissue than did A and R. With age, the amount of glycerol released increased until either 20 or 24 mo of age and then sharply decreased. Both the quadratic ($p < 0.0001$) and the cubic ($p < 0.007$) relationships of the amount of glycerol released with age were significant. In general, adipose glycerol release increased with age until 20 mo or 24 mo of age, depending on the experimental groups, and then decreased.

Table 14. Effect of Age, Diet Restriction, and Exercise on Adipose Tissue Glycerol Release¹.

Group	Age in Months				
	12	16	20	24	28
A ²	0.31 ±0.04 (6) ⁶	0.65 ±0.08 (6)	0.92 ±0.30 (6)	1.06 ±0.09 (6)	0.31 ±0.03 (6)
R ³	0.50 ±0.09 (6)	0.76 ±0.13 (6)	1.03 ±0.27 (6)	1.38 ±0.29 (6)	0.38 ±0.04 (6)
AE ⁴	0.54 ±0.10 (6)	0.69 ±0.14 (6)	0.67 ±0.21 (5)	0.80 ±0.15 (6)	0.26 ±0.008 (3)
RE ⁵	0.53 ±0.44 (6)	1.08 ±0.20 (6)	1.38 ±0.37 (6)	1.19 ±0.10 (6)	0.68 ±0.04 (6)

¹Significant for diet ($p < 0.005$) and age ($p < 0.0001$) in multiple regression analysis.

²A = Ad libitum-fed, non-exercised.

³R = Restricted-fed, non-exercised.

⁴AE = Ad libitum-fed, exercised.

⁵RE = Restricted-fed, exercised.

⁶Values are means ± SEM (expressed as mg glycerol released/dl/250 mg tissue/2 hrs) for the number of animals shown in parenthesis.

Table 15. ANOVA Table for Adipose Tissue Glycerol Release

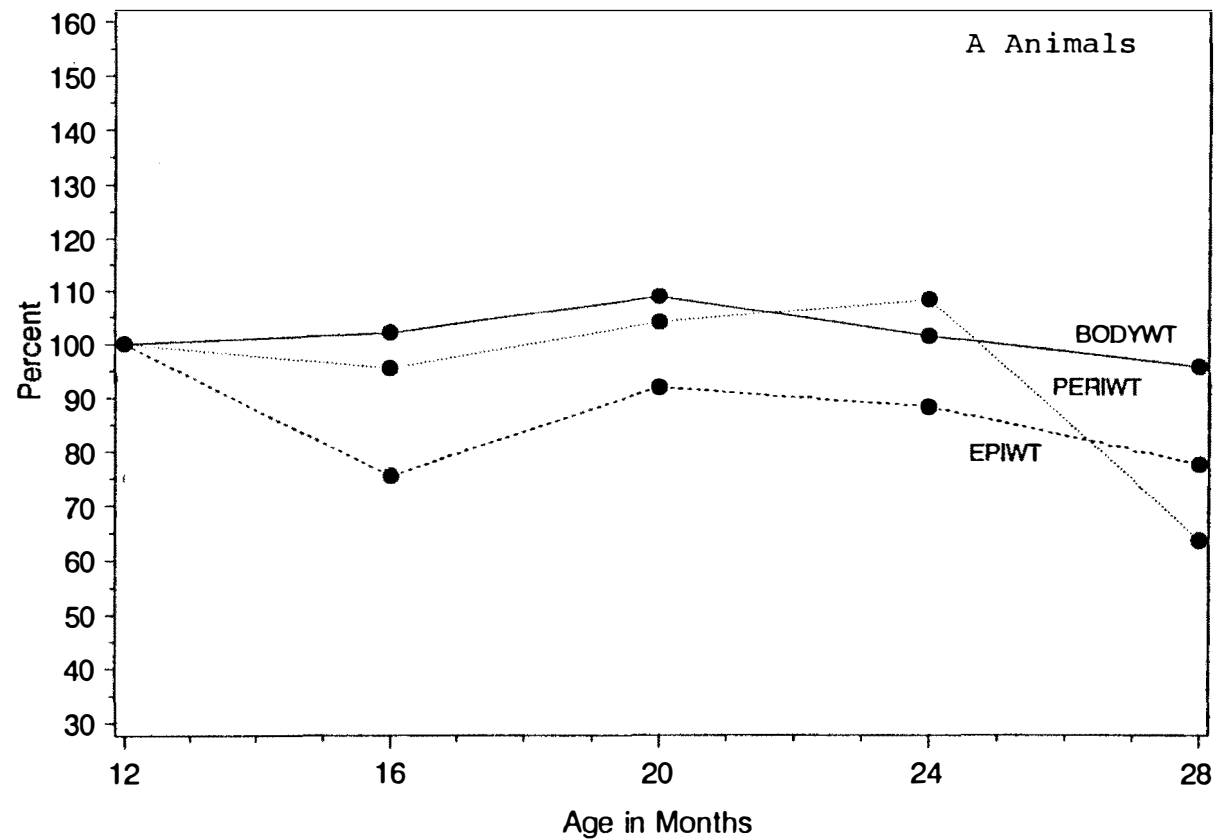
Variables	df	SS	F Values	P Values
<u>Type III SS</u>				
Diet	1	1.58	8.37	0.0047
Exercise	1	0.008	0.05	0.8290
Age	4	9.85	12.99	0.0001
Diet * Age	4	0.50	0.66	0.6194
Diet * Exercise	1	0.16	0.86	0.3570
Age * Exercise	4	0.60	0.80	0.5290
Diet * Age * Exercise	4	0.50	2.60	0.6200
<u>Type I SS</u>				
Age				
Linear	1	0.0352	0.19	0.6600
Quadratic	1	8.8267	47.53	0.0001
Cubic	1	1.4081	7.58	0.0070

Relationship of Lipogenesis/Lipolysis to Adiposity:

To estimate the relationship of adiposity to lipogenesis and lipolysis in each group, the mean value of each test response at the various ages was converted to percent of the test response mean value at 12 mo of age. Such a translation of the test responses for group A rats are presented in Figure 6A (physical data) and 6B (biochemical data).

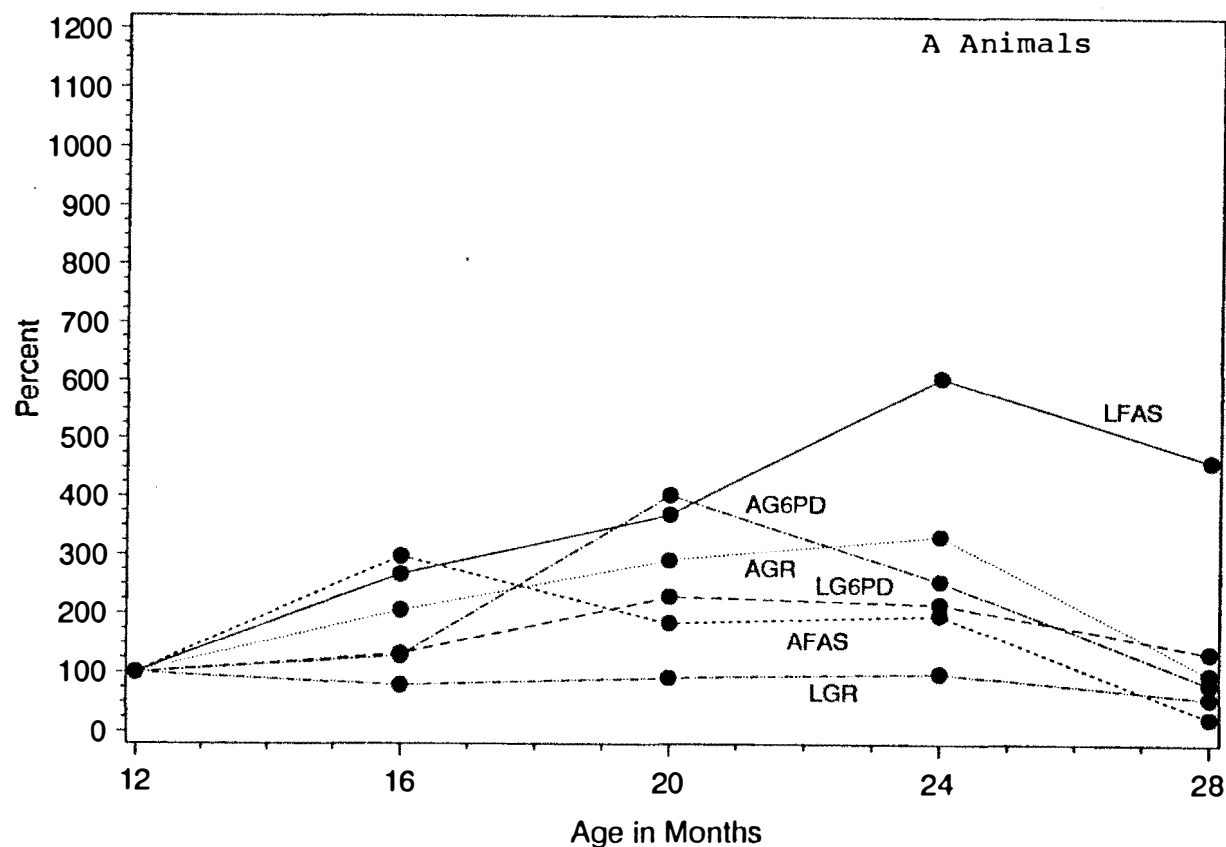
The percentages of body and perirenal fat pad weights, as compared to their 12 mo values of A animals increased until 20 or 24 mo of age, respectively, and then declined (Figure 6). The Pearson correlation coefficient between body weight and perirenal fat pad weight was 0.76 ($p < 0.0001$). Biochemical test responses peaked, in general, at the same ages (20-24 mo) as physical responses in A rats, but the magnitude of their increases tended to be much higher. Exceptions to this general pattern included epididymal fat pad mass which declined after 12 mo of age. Epididymal weight was negatively correlated to LFAS ($r = -0.40$, $p < 0.01$) and LFAS was negatively correlated to LGR ($r = -0.50$, $p < 0.01$). G6PD activities of liver and adipose tissue were correlated ($r = 0.70$, $p < 0.001$).

The percentage changes for AE rats are presented in Figure 7. Body weight of AE plateaued between 12 and 20 mo of age and then decreased. The mass of both fat pads increased until 20 mo, then fell precipitously as body



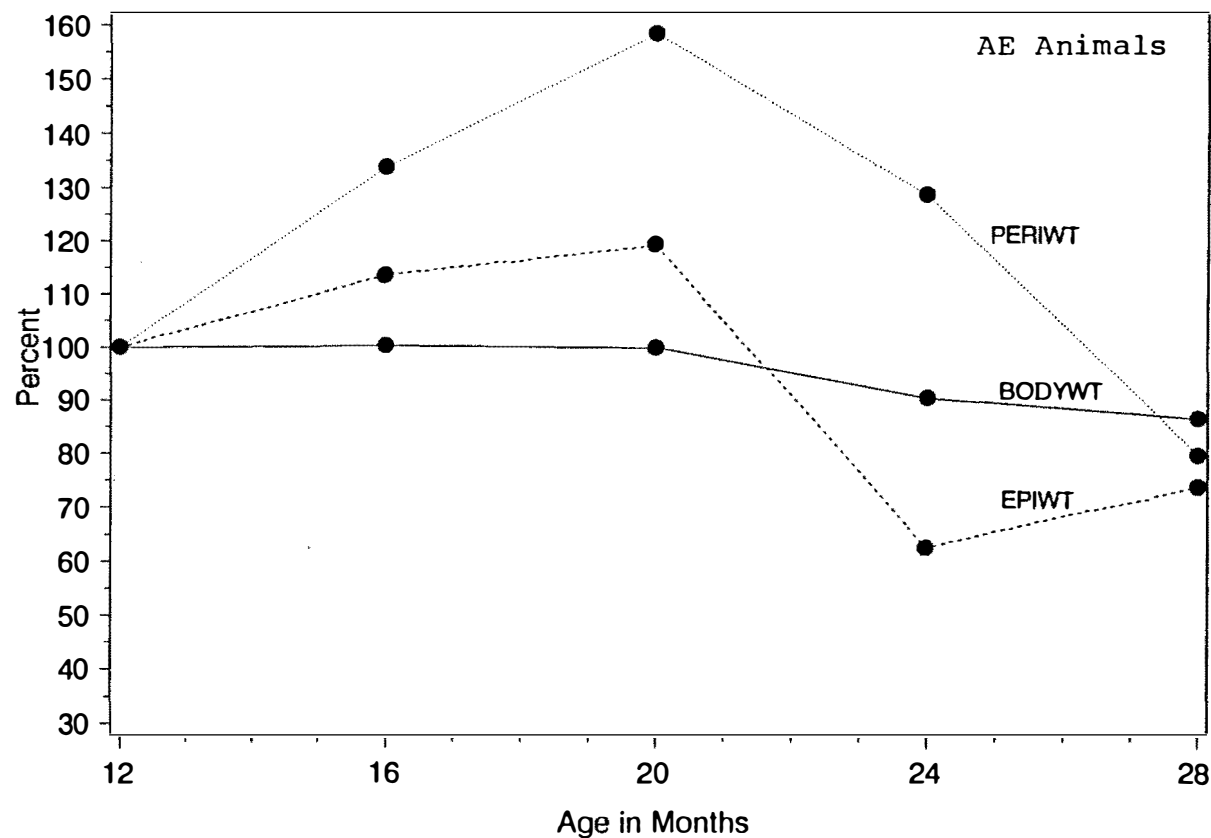
A. Body weights (BODYWT), perirenal fat pad weights (PERIWT), and epididymal fat pad weights (EPIWT) of A animals plotted versus age; values are expressed as a percent of 12 mo values.

Figure 6. The effect of age on test responses on A animals.



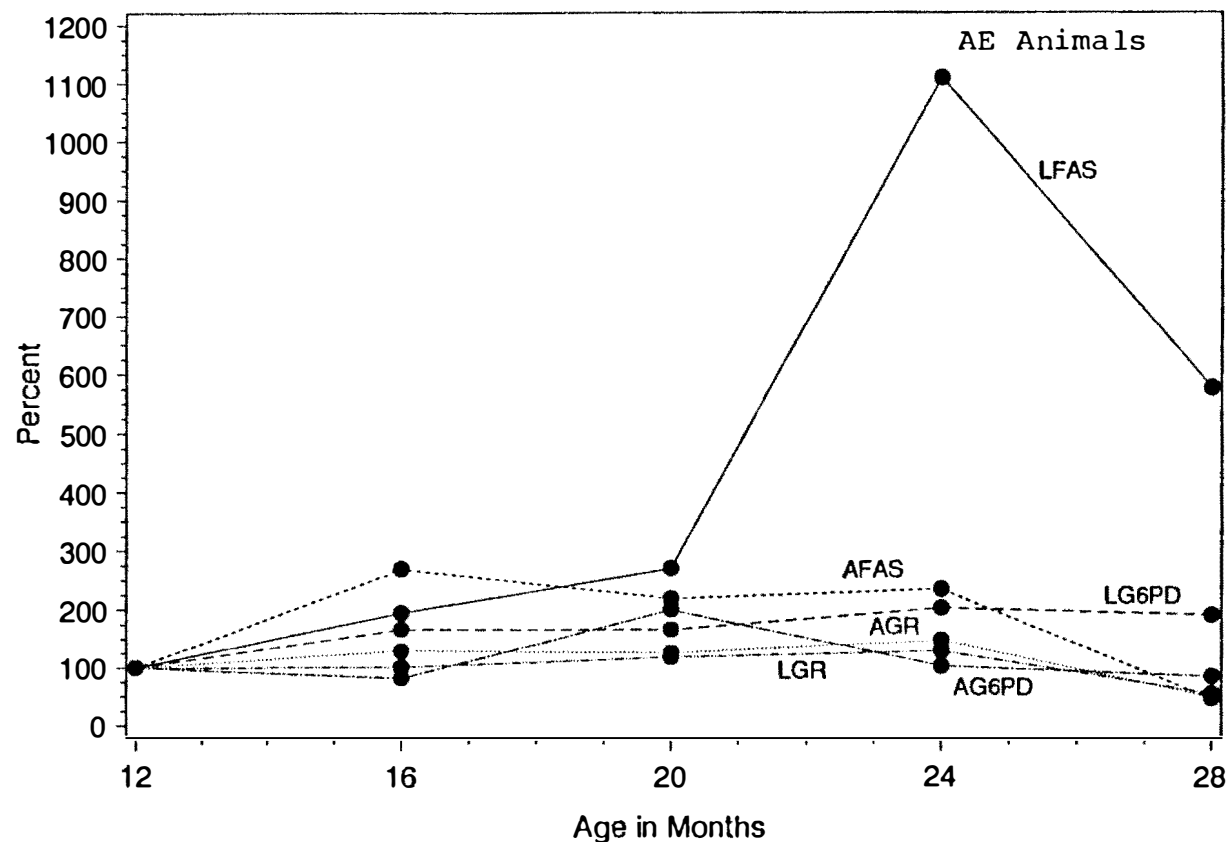
B. Liver fatty acid synthesis (LFAS), adipose fatty acid synthesis (AFAS), liver glucose-6-phosphate dehydrogenase (LG6PD), adipose glucose-6-phosphate dehydrogenase (AG6PD), liver glycerol release (LGR), and adipose glycerol release (AGR) of A animals plotted versus age; values are expressed as a percent of 12 mo values.

Figure 6. (continued)



A. Body weights (BODYWT), perirenal fat pad weights (PERIWT), and epididymal fat pad weights (EPIWT) of AE animals plotted versus age; values are expressed as a percent of 12 mo values.

Figure 7. The effect of age on test responses on AE animals.



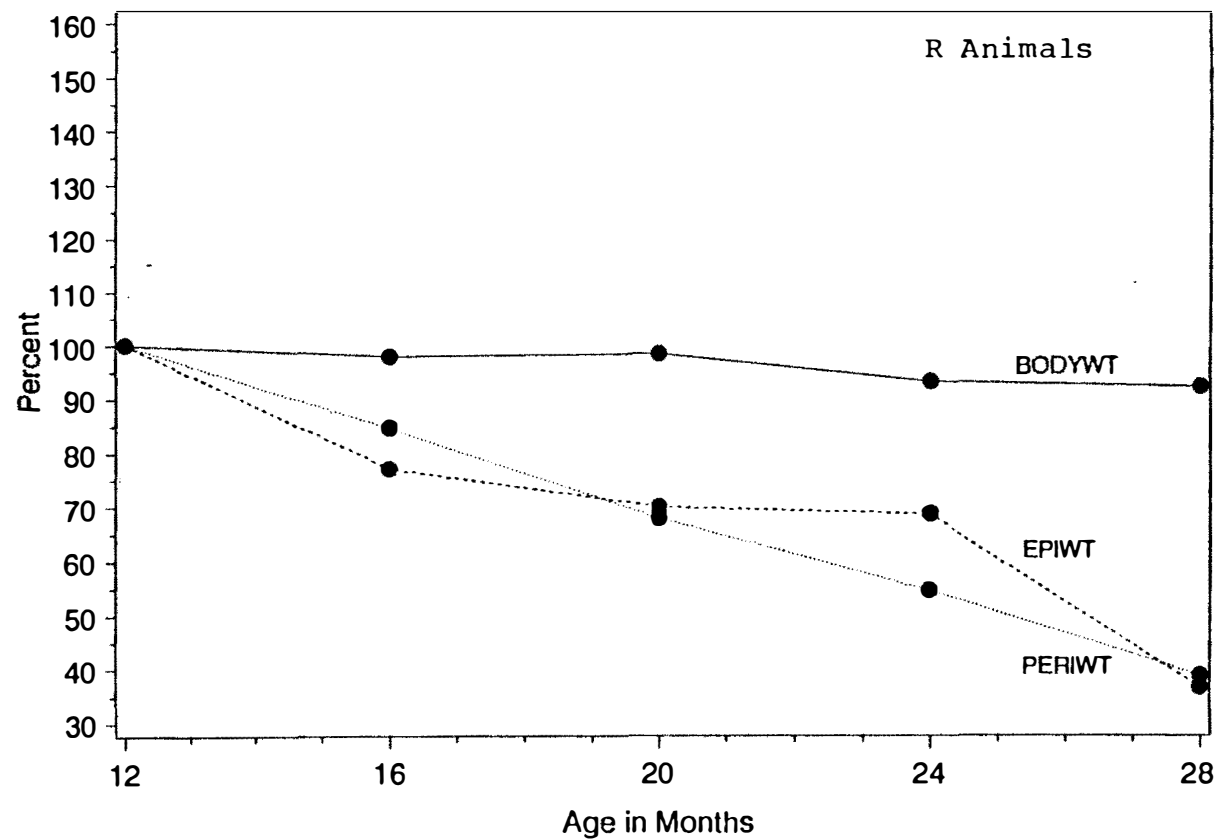
B. Liver fatty acid synthesis (LFAS), adipose fatty acid synthesis (AFAS), liver glucose-6-phosphate dehydrogenase (LG6PD), adipose glucose-6-phosphate dehydrogenase (AG6PD), liver glycerol release (LGR), and adipose glycerol release (AGR) of AE animals plotted versus age; values are expressed as a percent of 12 mo values.

Figure 7. (continued)

weight declined. Body weight was correlated to perirenal fat pad weight ($r=0.56$, $p<0.002$) and to epididymal fat pad weight ($r=0.60$, $p<0.001$). LFAS and LG6PD activity was correlated ($r=0.40$, $p<0.05$) in AE as was LGR and AFAS ($r=0.50$, $p<0.01$). Most all test responses showed decrements after 24 mo of age.

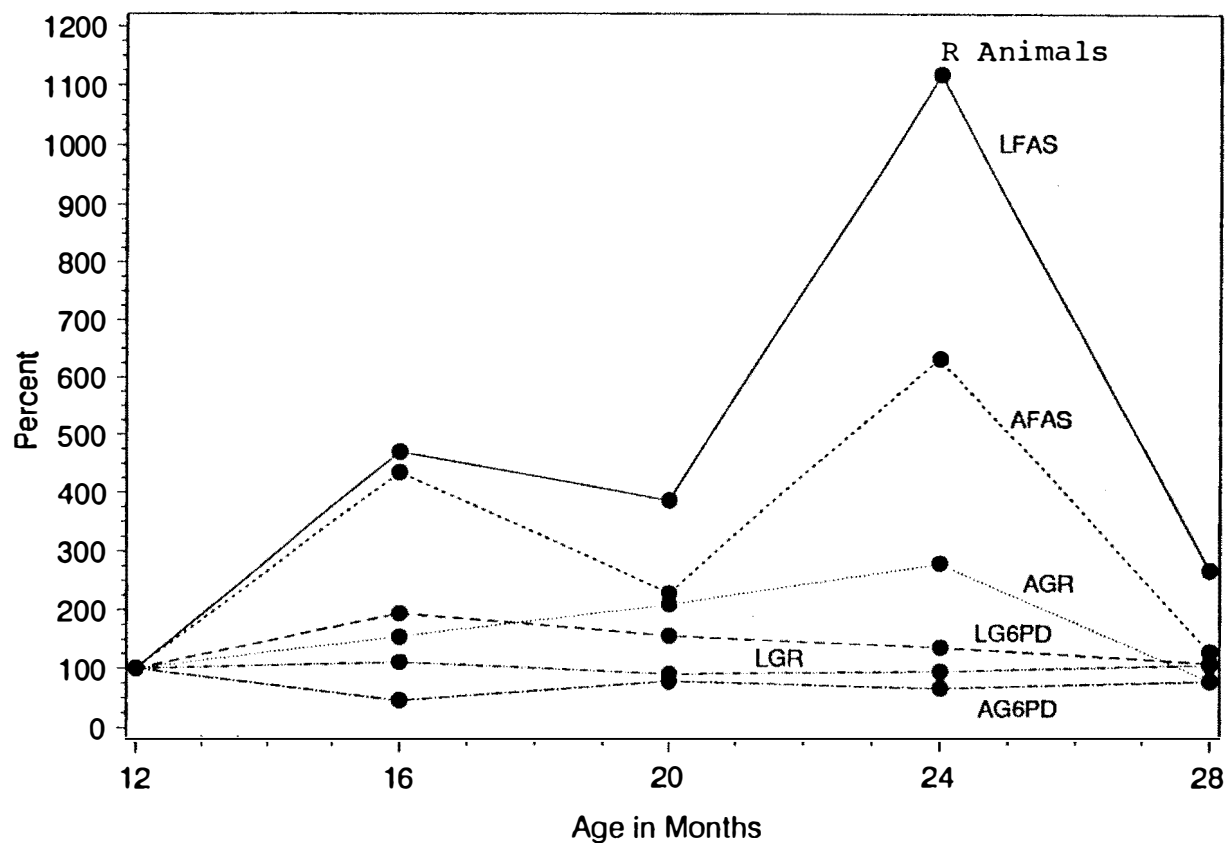
The body weights of R rats gradually decreased after 12 mo of age; fat pad weights decreased markedly after 12 mo of age (Figure 8). Body weight was correlated with perirenal ($r=0.60$, $p<0.001$) and epididymal fat pad weights ($r=0.57$, $p<0.001$). Liver and adipose fatty acid synthesis ($r=0.87$, $p<0.0001$) increased until 24 mo and then exhibited a marked decline. Fatty acid synthesis was negatively related to glycerol release in the liver ($r=0.36$, $p<0.05$).

RE animals showed decrements in weights of the body and fat pads with age (Figure 9). Body weight was correlated with perirenal ($r=0.62$, $p<0.001$) and epididymal ($r=0.53$, $p<0.005$) fat pad weights. The percentage decrements of the fat pads were more pronounced than those of body weight. As in other experimental groups, the biochemical responses of RE decreased after 24 mo of age. LFAS of RE was correlated positively with LG6PD ($r=0.50$, $p<0.005$), AFAS ($r=0.38$, $p<0.05$), AG6PD ($r=0.36$, $p<0.05$), and LGR ($r=0.50$, $p<0.005$). AFAS was correlated to glycerol release in adipose tissue ($r=0.45$, $p<0.01$) and in the liver ($r=0.55$, $p<0.001$).



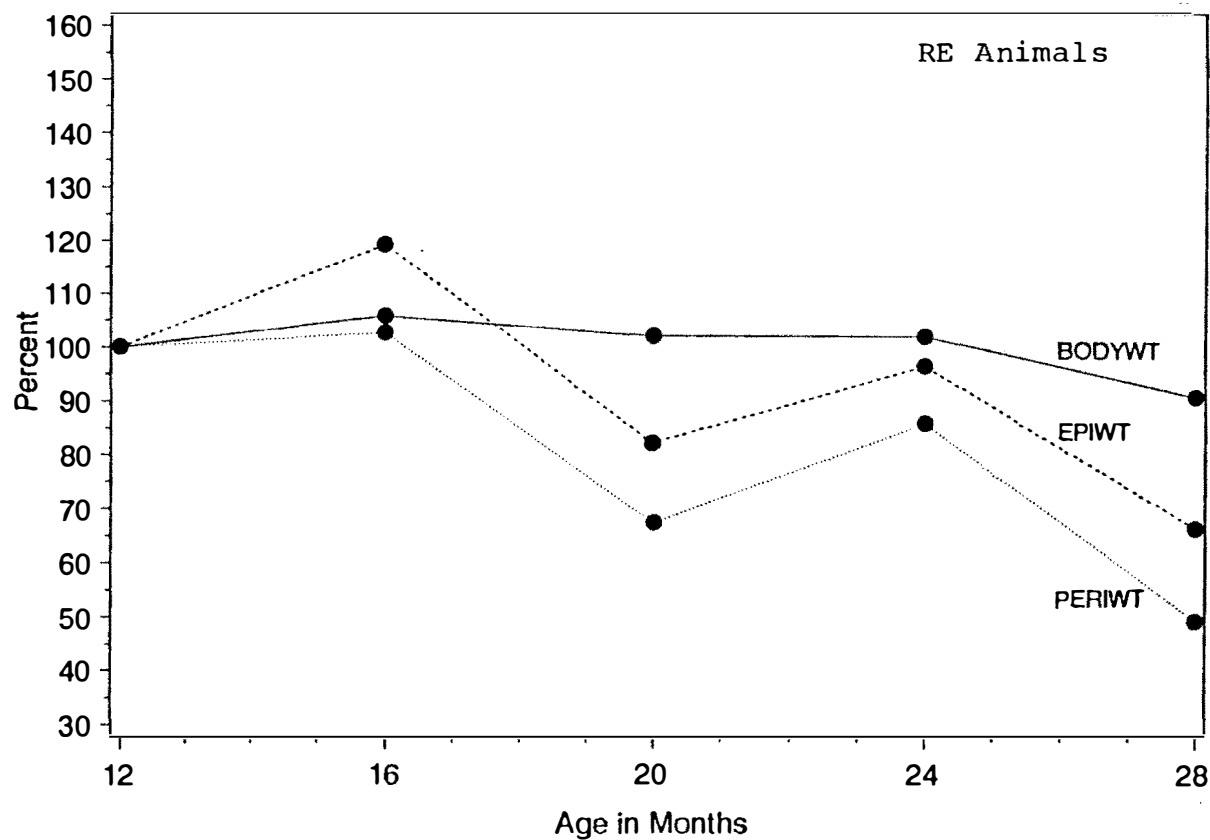
A. Body weights (BODYWT), perirenal fat pad weights (PERIWT), and epididymal fat pad weights (EPIWT) of R animals plotted versus age; values are expressed as a percent of 12 mo values.

Figure 8. The effect of age on test responses on R animals.



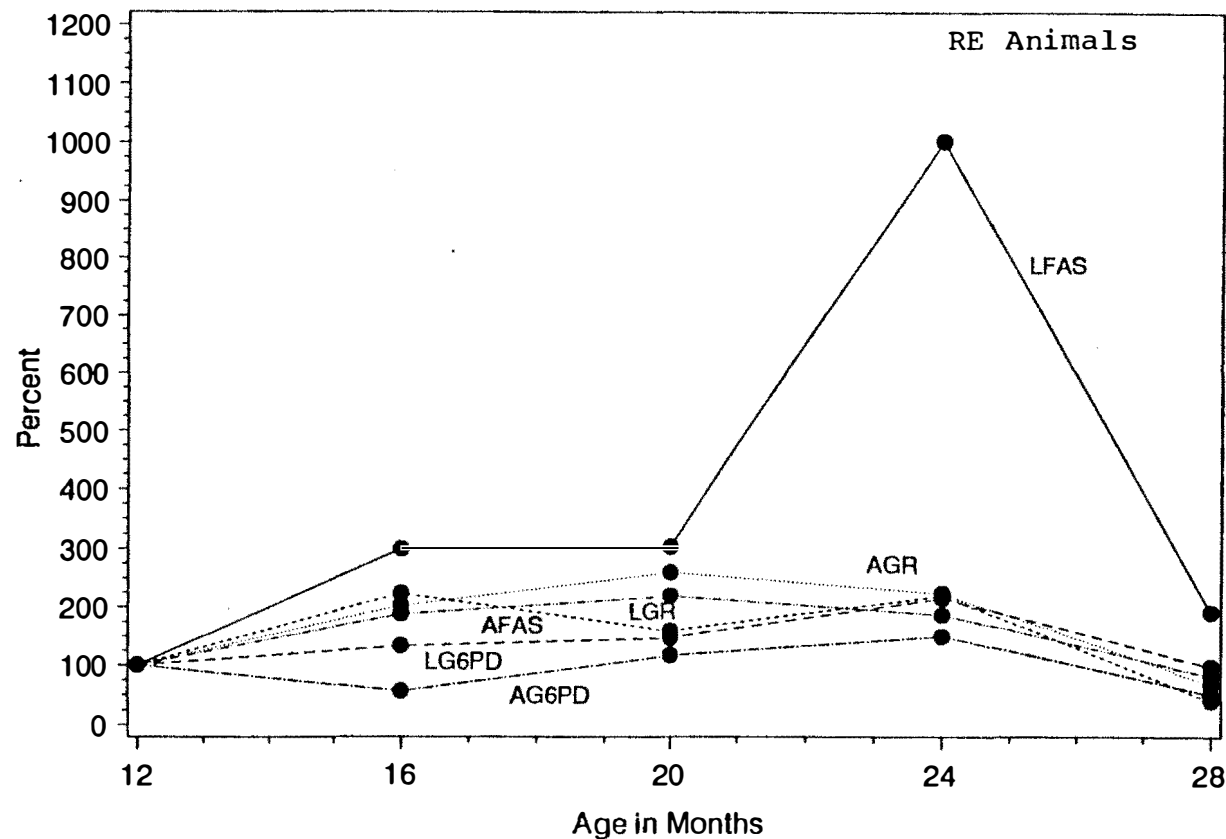
B. Liver fatty acid synthesis (LFAS), adipose fatty acid synthesis (AFAS), liver glucose-6-phosphate dehydrogenase (LG6PD), adipose glucose-6-phosphate dehydrogenase (AG6PD), liver glycerol release (LGR), and adipose glycerol release (AGR) of R animals plotted versus age; values are expressed as a percent of 12 mo values.

Figure 8. (continued)



A. Body weights (BODYWT), perirenal fat pad weights (PERIWT), and epididymal fat pad weights (EPIWT) of RE animals plotted versus age; values are expressed as a percent of 12 mo values.

Figure 9. The effect of age on test responses on RE animals.



B. Liver fatty acid synthesis (LFAS), adipose fatty acid synthesis (AFAS), liver glucose-6-phosphate dehydrogenase (LG6PD), adipose glucose-6-phosphate dehydrogenase (AG6PD), liver glycerol release (LGR), and adipose glycerol release (AGR) of RE animals plotted versus age; values are expressed as a percent of 12 mo values.

Figure 9. (continued)

CHAPTER V

DISCUSSION

The decrement in mature body weight and feed consumption, about 28% and 30%, respectively, associated with every-other-day feeding is similar to the results of others (12,15,19,69). The exercise regimen resulted in much smaller overall reduction (2.8 %) in body weight than did every-other-day feeding. Feed consumption of exercised rats averaged about 12% more than non-exercised rats. Pitts et al. (14) and Applegate et al. (44) have observed body weight reduction in exercised male rats, but not in female rats. Male rats tend to lose weight with exercise even when fed ad libitum, whereas female rats tend to increase their feed intake sufficiently to maintain body weight to the level of non-exercised animals.

The decrease in body weight with age is consistent with the findings of others (12,13,49). Feed restriction and exercise together (RE) reduced the magnitude of weight loss with age. However, in AE animals there was a tendency for weight loss to begin at an earlier age and to be of greater magnitude than in A animals.

The reductions in epididymal and perirenal fat pad weights with feed restriction, exercise, and age were in concordance with the findings of Bertrand et al. (12), Hollifield et al. (16), Pitts et al. (14), and Panomangalore

et al. (72). The strong positive correlation between fat pad weights and body weights agree with the findings of Gommer et al. (49) and Bertrand et al. (12). The large r value between fat pad weight and body weight of A animals (0.76) than that for the other groups (AE, 0.56; R, 0.60; RE, 0.62) indicates that either every-other-day feeding or the exercise regimen alters relative body composition and in a non-uniform manner.

The increase in adipose tissue fatty acid synthesis (AFAS) with feed restriction is consistent with the findings of Leveille (19,63,65), Stevenson et al. (20), and Hollifield (16). The lack of an effect of exercise on AFAS is inconsistent with the results of Tokayama et al. (41) who reported an increase in AFAS in rats with training and of Richard et al. (29) who reported decreases in AFAS in mice. The general increase in AFAS with age (from 12 to 24 mo) observed in the present study may be in disagreement with Leveille (19). The study of Leveille (19) however, used rats varying in age from 1 to 18 mo whereas in the present study the rats were much older, i.e., 12 to 28 mo of age. After 24 mo of age all groups in the present study did exhibit decreased AFAS.

Liver fatty acid synthesis (LFAS) was not affected by diet or exercise. However, Tsai et al. (31) and Tokayama et al. (41) Dellwo (69) have reported an increase, whereas Richard et al. (29) and Scorpio et al. (32) reported a

decrease in LFAS with exercise. Increased in hepatic fatty acid synthesis as a result of training may be due to an overall increase in mobilization and utilization of fatty acids which needs to be compensated for by increased lipogenesis between training sessions (31,41). Decreased in fatty acid synthesis as a result of exercise could be explained that carbohydrate is shunted away from the synthesis of lipid in favor of energy storage as glycogen. Findings of others (17,18,19,69,71) have indicated that diet restriction in the form of meal-feeding increased LFAS which is possibly due to the immediate adaptive changes that result in an increased rate of conversion of carbohydrate to fat. The pattern of fatty acid synthesis with age in liver and adipose tissue was similar, i.e., in general, increases in synthesis occurred from 12 to 24 mo followed by decreases at 28 mo. Sullivan et al. (71) showed a decrease in LFAS in rats from 5 to 14 weeks of age, and Story et al. (73) reported LFAS was not affected by age (2 to 24 mo). Of course, the age range of the animals in the former study is not comparable to that of the present study. The lack of agreement between the findings of Story et al. (73) and the present study could be due to strain differences, e.g. they used Fisher 344 rats.

The pattern of glucose-6-phosphate dehydrogenase activity (G6PD) in both the liver and adipose tissue, in general, was to increase from 12 to 24 mo of age and to

decrease at 28 mo. Leveille (19) reported a decrease in adipose G6PD activity in rats varying from 1 to 18 mo. The increased liver G6PD activity with feed restriction agrees with the findings of others (19,63,64). The lack of change of liver G6PD with exercise disagrees with the findings of Askew et al. (30,39), Woods et al. (33), and Walberg (34) who reported a decrease in liver G6PD activity with exercise whereas Delwo (69) and Tsai et al. (31) reported an increase in liver G6PD activity with exercise. Adipose tissue G6PD activity increased with exercise, but no change was observed with diet restriction. Askew et al. (30,39) reported that exercise decreased adipose G6PD activity, while Dohm et al. (43) reported an increase in the activity with exercise. The discrepancies among the studies might be explained when variations in the research are taken into account. For example, the type of exercise regimen, frequency and intensity of exercise, and tissues used for analysis all play a role in the results obtained in different studies.

Increased adipose tissue lipolysis, measured by glycerol release, as a result of feed restriction is in agreement with the findings of Gruen et al. (50) and Bertrand et al. (59). Klein et al. (60) reported an increase in lipolytic activity of fat mass in fasted humans. The increased lipolytic activity with age in the present study is consistent with the findings of Gruen et al. (50) and Bertrand et al. (59). Lack of effect of exercise on

adipose tissue lipolysis agrees with the findings of Walberg et al. (34). In the present study lipolytic activity of liver was increased as a result of feed restriction, exercise, and age. Glycerol release may not be a completely reliable indicator of lipolysis, for example if only 1 or 2 fatty acids were released from a triglyceride yielding a diglyceride or monoglyceride respectively, glycerol would not be released in the free form and thus, the procedure used (66) would underestimate lipolysis. Nevertheless, the partial hydrolysis of the triglyceride would release fatty acids for use as energy or for other purposes. This researcher is not aware of any reports in the literature which indicate lipolytic activity in the liver as affected by the independent variables of the present study.

Animals Responses to the Aging Process:

The expression of the data as a percent of the 12 mo values was utilized to attempt to interpret the relationship of the various test responses as they changed during aging within an experimental group. The positive correlation between fat pad weights and body weights in A animals indicates that changes in body weights reflect the changes in fat pad weights during aging. The negative correlation between LFAS and LGR indicates that with increased fat synthesis the lipolytic activity is depressed. That LFAS does not contribute directly to epididymal fat pad disposition is indicated by the negative correlation between

LFAS and epididymal fat pad weight. In A animals the decrease in the ratio of AFAS to AGR with age (Figure 6B), tends to parallel the decrements that occurred in body and fat pad weights with age (Figure 6A). The fall in adipose and hepatic G6PD activities after 20 mo of age would offer less reducing capacity for fat synthesis and this reduction coincided with the age at which body weight loss was initiated.

Although body weight in AE animals was essentially constant from 12 through 20 mo, the weights of the fat pads increased. After 20 mo body weight and fat pad weights decreased (Figure 7A). Since AFAS expressed as a percentage of 12 mo synthesis was greater than similar values for AGR through 20 mo of age (Figure 7B), it would be expected that a net accumulation of adipose tissue would occur as it did over this time period. During this same time period, LFAS, LG6PD, and AG6PD also tended to be greater than 12 mo values, thus favoring lipogenesis. After 24 mo of age biochemical values tended to fall whereas physical parameters showed decrements earlier, i.e., after 20 mo of age.

In R animals the correlation between body weight and fat pad weights is consistent with a marked decrease in all of these parameters beginning at 12 mo of age (Figure 8A). AFAS, LFAS and AGR all increased with age and peaked at 24 mo. However, the level of lipolytic activity (expressed as

a percent) never surpassed the level of lipogenic activity in either adipose tissue or the liver (Figure 8B). It might be expected that given the high lipogenic activity as compared to lipolytic activity that adiposity would have been increased in R animals during this time interval. It was not as indicated above. The disparity may exist because the in vitro measurements were performed on the animals in the "fed state", i.e., after the animals were exposed to feed the previous night. Even though the feed cups were removed 2 hrs before the animals were killed, the in vitro measurements probably reflect a period of most active anabolism of the animals. Had the measurements been made on a fast day the AFAS might have shown marked depression as indicated by Leveille (19). The high degree of correlation ($r=.87$) between LFAS and AFAS in R animals is presumably due to the tendency of both the liver and adipose tissue to utilize calorie intake for fat synthesis following the day of fast. Further evidence for cellular conditions favoring fat synthesis was the correlation ($r=.36$) between LFAS and LGR in R rats. Considering the positive relationship between LG6PD and AFAS in R animals, it could be assumed that increased liver reducing activity also favors adipose tissue synthesis of fat. In restricted fed animals (R), in spite of the relative high ratio of AFAS to AGR (Figure 8B) fat pad and body weights showed a rather continuous reduction with age (Figure 8A,B).

Body weight and fat pad weights decreased with age in RE animals and exhibited positive correlations. The level (percent of 12 mo) of AFAS exceeded AGR only at 16 mo, thus adipose lipolysis exceeded lipogenic activity sometime between 16 and 20 mo which coincided with the initiation of weight loss in these animals. The AG6PD activity exhibited levels mostly below 12 mo values (Figure 9A, B). The positive correlations between lipogenic factors, i.e., LFAS, LG6PD, AFAS, and AG6PD in RE animals indicated an active lipogenic process took place in both adipose tissue and liver during the "fed state". However, lipogenic activity was not of sufficient magnitude to prevent adipose tissue weight loss as the animals aged.

Since lipogenic activity increased with age, but epididymal and perirenal fat pad weights, in general, did not it could be postulated that the increased amounts of fatty acids synthesized were deposited as adipose tissue elsewhere and/or used for other purposes. For example, it is known that blood lipids increase with age (54,73). However, since body weight did not change substantially after 12 mo of age it is unlikely that deposition of fat as adipose tissue could explain the age-associated increase in vitro synthesis of fatty acids by the liver and adipose tissue. It is possible that incorporation of $1\text{-}^{14}\text{C}$ acetate in the fatty acids in vitro does not adequately represent the rate of fat synthesis in vivo. Thus, the author cannot

offer a completely adequate explanation as to why increases in fatty acid synthesis occurred with age.

The increase in fatty acid synthesis observed in feed restricted animals was probably due to the short-term effect of feed restriction itself, e.g., the increased synthesis reflected the "fed state" of the animals on the day they were killed. It could be assumed that the animals were attempting to replenish their fat stores that were depleted from the previous "day of fast".

With exercise, especially long-term moderate activity, the muscle shifts from the oxidation of carbohydrate to that of fat as an energy source (42). This is consistent with the reduction in adipose tissue observed in the exercised animals. It is theoretically possible that the amount of adipose tissue would not be reduced with exercise if lipogenesis was correspondingly increased. However, lipogenic activity of adipose tissue did not increase with exercise. The activity of G6PD in adipose tissue did increase in exercised animals. Perhaps the reducing power generated by this enzyme was being used directly for energy, i.e., for production of energy-rich phosphate.

In general, it is known that reduction in feed intake keeps animals physiologically younger, i.e., retards age-associated changes (9,12,45). The results of the present study showed that lipogenesis and lipolysis, as indicated by the biochemical measurements, increased with age, i.e., up

to 24 mo. Body weight and fat pad weights in general decreased with age. The biochemical test response used to indicate rates of lipogenesis or lipolysis did not change in a manner to indicate that restricted and/or exercised animals were biochemically younger than ad libitum fed animals.

The regulation of lipogenesis depends to a large extent on the availability of calories to the tissues involved in fatty acid synthesis and the ability of these tissues to metabolize the calories. Energy balance also serves in a regulatory capacity of lipogenesis (45). The specific chemical mechanisms by which these factors regulate the rate of fatty acid synthesis have yet to be defined.

CHAPTER VI

SUMMARY

Male weanling Wistar rats were fed ad libitum (A) or every-other-day feeding (R) from 6 weeks through 28 mo of age. A and R animals which were exercised by swimming 3 hr every-other-day were designated as AE and RE.

Mature body weights of restricted-fed animals were 28% less than those fed ad libitum. Exercise reduced mature body weight of A animals 6.2% whereas mature body weights of R animals were not affected by exercise. Epididymal and perirenal fat pad weights of restricted-fed animals were 59% less than those of ad libitum-fed rats. Exercise also reduced (30%) fat pad weights. Body weights and fat pad weights, in general, decreased with age.

Lipogenesis, as indicated by the in vitro incorporation of acetate $1\text{-}^{14}\text{C}$ into fatty acids was elevated in adipose tissue by dietary restriction and age. Exercise did not affect adipose tissue fatty acid synthesis. Liver fatty acid synthesis was increased with age, but it was not affected by either diet or exercise. G6PD activity was increased in the liver by dietary restriction and with age. Adipose G6PD activity increased with exercise and with age.

Lipolysis was estimated by in vitro glycerol release. Liver glycerol release was increased by dietary restriction, exercise and with aging. Adipose tissue glycerol release

was also increased by dietary restriction and with age, but it was not affected by exercise.

The age changes, in general, were quadratic, i.e., increases occurred until 20-24 mo of age and then decreased. A high positive correlation between body weight and fat pad weight was observed. In general, there was positive correlation between liver lipogenesis and liver G6PD; also, between adipose fatty acid synthesis and either liver G6PD or adipose G6PD.

The hypothesis of the study were:

1. lower adult body weight and body adiposity would be obtained for diet restricted than for ad libitum-fed rats. This was supported by the results of the present study.
2. a decreased rate of lipogenesis and an increased rate of lipolysis would be observed in diet restricted animals. The results indicated an increased rate of lipolysis and of lipogenesis was associated with diet restriction.
3. lower adult body weight, adiposity, and lipogenesis, but higher lipolysis would be obtained for exercised than for non-exercised rats. Lower body weight, adiposity and increased lipolysis was observed in exercised rats. The decrement in lipogenesis was not observed with exercise.

4. lower adult body weight and adiposity, decreased rate of lipogenesis, and increased rate of lipolysis would be observed in diet-restricted exercised animals than in ad-libitum non-exercised animals. The results of the present study were consistent with this hypothesis except that an increase in lipogenesis in diet-restricted exercised animals was observed.
5. body weight, adiposity and lipogenesis would decrease with age, but lipolysis would increase with age. The results supported lower body weight and adiposity with age and an increased rate of lipolysis, but did not support the hypothesized decrease in lipogenesis.

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