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Lipogenic enzymes in aging rats

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LIPOGENIC ENZYMES IN AGING RATS

by

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DEDICATION

This work is dedicated to my family, especially my mother, Mrs. Emma Nowacki, whose accomplishments and moral support have encouraged my academic pursuits.

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ABSTRACT

Four lipogenic enzymes were measured in liver and adipose tissues of 2-, 6-, 18- and 27-month-old male Fischer 344 rats. Adipose ATP-citrate lyase (EC 4.1.3.8; ATP-CL) activity increased 1.8-fold between 2 and 6 months of age. Hepatic ATP-CL activity underwent an overall 84% reduction between 2 and 27 months of age with maximum activity at 18 months. Adipose tissue fatty acid synthase (FAS) activity exhibited a linear 54% reduction between 2 and 27 months of age; while hepatic FAS activity declined, biphasically, to 14.2 IU/g protein by 27 months. Opposing age-related patterns of glucose-6-phosphate dehydrogenase (EC 1.1.1.49; G6PD) activity were observed in adipose tissue and liver. Adipose activity decreased between 2 and 6 months to 132.9 IU/g protein, and was unchanged thereafter; whereas liver G6PD activity increased to a maximum of 149.0 IU/g protein at 27 months. Malic enzyme (EC 1.1.1.40; ME) activity decreased between 2 and 6 months of age in both tissues. These data suggest diminished lipogenic capacity with age.

CHAPTER 1

INTRODUCTION

Since the early work of Chaikoff and his colleagues (Hill et al., 1958; 1960; Bortz, Abraham and Chaikoff, 1963), which examined the influence of dietary fats on liver lipogenesis, a number of studies have examined the effects of hormonal and dietary changes upon lipogenesis in rats (Iritani et al., 1980; Goodridge, 1987; Herzberg and Rogerson, 1988). The ingestion of dietary fat markedly inhibits fatty acid synthesis; that is, lipogenic enzyme activities are significantly depressed in rats fed a high-fat diet compared to rats fed a fat-free diet (Sabine, McGrath and Abraham, 1969; Carrozza et al., 1979). In addition, lipogenic enzyme levels are influenced by hormones: insulin and triiodothyronine (T3) increase activity levels (Goodridge, 1987); whereas glucagon decreases activity (Goodridge, 1986).

There has been extensive research into lipogenic activity with younger rats, such as the study by Gandemer, Pascal and Durand (1982) where the lipogenic capacity significantly increases in Wistar male rats between 19 and 50 days of age. Little work, however, has focused on lipogenic activity among older rats. The existing information, such as the Barakat et al. (1989) study seems to indicate that lipogenic activity decreases, in this case, in Fischer 344 rats of 6 and 27 months of age. Consequently, we examined the effects of age on lipogenic enzyme activities in a cross sectional study with Fischer 344 rats at 2, 6, 18

and 27 months of age. Enzyme activities measured were glucose-6-phosphate dehydrogenase (EC 1.1.1.49; G6PD), malic enzyme (EC 1.1.1.40; ME), ATP-citrate lyase (EC 4.1.3.8; ATP-CL) and fatty acid synthase (FAS), in liver and adipose tissues, because these are the primary sites of lipogenesis in the rat (Leveille, 1967).

CHAPTER 2

LITERATURE REVIEW

Lipogenesis

Lipogenesis is the synthesis of fatty acids, and its pathway is mainly diet-dependent. If the diet is high in fat, then the lipid in the diet is absorbed from the gut and transported in the blood in the form of chylomicrons or lipoproteins. These complexes are then broken down by lipoprotein lipase to fatty acids. These fatty acids are then able to enter the adipocyte and are either reesterified with glycerol to form triglyceride or oxidized to meet energy requirements. This process of breakdown outside the cell and reesterification inside the cell is necessary, because triglycerides are unable to cross the cellular membrane of the adipocyte.

When the diet consumed is mainly carbohydrate, then de novo lipogenesis is the predominant pathway of fatty acid synthesis. This process is simplified as glucose is converted to acetyl-CoA by glycolysis, and acetyl-CoA and malonyl-CoA condense in a step-wise process to synthesize fatty acids. This reaction is catalyzed by an enzyme complex, fatty acid synthase. De novo lipogenesis requires more energy than reesterification of dietary fat or lipids, even though the energy content of the triglyceride deposited is ultimately the same.

Fatty acid synthesis is not simply the reverse process of fatty acid oxidation, even though the starting material of synthesis is

identical to the product of oxidation, acetyl-CoA. In fact, the two processes take place in different portions of the cell; fatty acid synthesis takes place in the cytosol, while fatty acid oxidation is in the mitochondria.

The lipogenesis system has been found in the soluble fraction, cytosol, of many tissues such as liver, kidney, brain, lung, mammary gland, and adipose tissue. However, not all animals have the same lipogenesis organ sites. For instance, the primary site of lipogenesis for rodents such as rats and mice is both the liver and the adipose tissue, while the liver is the primary site for chickens, and the adipose tissue is the site of lipogenesis in ruminants. Gandemer, Durand and Pascal (1983) showed, by measuring the rate of the incorporation of the ^3H of ^3H_2O into fatty acids in many tissues and organs of 7-week-old male Wistar rats, the relative contribution of the main tissues and organs to whole body fatty acid synthesis. The liver was 42%; dissectable white adipose tissues, 27%; muscles, 18%; skin, 7%; and organs besides the liver, 6%. The Wistar rats were fed a low-fat diet, 0.3% fat (w/w) and 75% carbohydrate (w/w), to create the best conditions for observing de novo fatty acid synthesis.

Lipogenesis - Enzyme Mechanism

The primary function of lipogenesis is disposing of excess carbohydrate intake as fatty acids in the liver and as triacylglycerols in the adipose tissue. The overall process requires the cofactors NADPH, ATP, Mn^{2+} and HCO_3^- (as a source of CO_2). Acetyl-CoA is the substrate and free palmitate is the end product.

Bicarbonate and ATP are necessary cofactors for the key rate-limiting enzyme, acetyl-CoA carboxylase, which converts acetyl-CoA to malonyl-CoA. From the formation of malonyl-CoA, fatty acid synthase catalyzes the reaction of acetyl-CoA and malonyl-CoA to the synthesis of palmitate. The overall equation is:

$$CH_2CO*S*CoA + 7\ HOOC*CH_2CO*S*CoA + 14\ NADPH + 14\ H^+ \text{----}> \cdot$$

$$CH_3(CH_2)_{14}COOH + 7\ CO_2 + 6\ H_2O + 8\ CoAH*SH + 14\ NADP^+$$

NADPH, the hydrogen donor, or the source of reducing equivalents, is provided by the key enzymes glucose-6-phosphate dehydrogenase of the pentose phosphate pathway and by malic enzyme (NADP malate dehydrogenase) of the pyruvate-malate cycle, which converts malate to pyruvate. In addition, isocitrate dehydrogenase, which converts isocitrate to alpha-ketoglutarate, also has shown to supply NADPH, although this has not been of any substantial amount; it is primarily seen in ruminant animals.

Acetyl-CoA, the main building block for fatty acids, is formed from carbohydrate via the oxidation of pyruvate within the mitochondria. Pyruvate is decarboxylated to acetyl-CoA within the mitochondria; acetyl-CoA then combines with oxaloacetate to form citrate. Citrate is then able to translocate across the membrane into the extramitochondrial compartment, the cytosol. ATP-citrate lyase in the presence of ATP and CoA, catalyzes the cleavage of citrate into acetyl-CoA and oxaloacetate. Acetyl-CoA is then available for malonyl-CoA formation and synthesis to palmitate; while oxaloacetate can indirectly generate NADPH via NADH-linked malate dehydrogenase and malic enzyme. This NADPH is then available for lipogenesis (Mayes, 1988). Unlike monogastrics, ruminants

have very little ATP-citrate lyase and malic enzyme. Acetate is their main source of acetyl-CoA, and as mentioned above, NADPH is generated from isocitrate dehydrogenase in the cytosol. In rodents, such as rats and mice, glucose is the major source of carbon, acetyl-CoA, for fatty acid synthesis in adipose tissue; whereas, glycogen is an important precursor in the liver.

Regulation of Lipogenesis

Dietary Factors

The process of lipogenesis is concerned with the conversion of glucose and intermediates of glycolysis, such as pyruvate, lactate and acetyl-CoA, to fat. The main factor controlling the lipogenesis rate is the nutritional state of the organism and tissues, such as the liver and adipose tissue. As previously mentioned, this rate is increased in well-fed animals of carbohydrate diets (Hill et al., 1958; Leveille and Hanson, 1966; Leveille, 1967), and depressed in conditions in which plasma free fatty acid concentrations are increased, such as high-fat fed diets (Sabine et al., 1969; Clarke, Romsos and Leveille, 1977), restricted caloric intakes (Goodridge, 1968; Leveille, 1969) or an insulin deficiency state such as in diabetes mellitus (Goodridge and Adelman, 1976; Das, 1980; Wilson and McMurray, 1980).

Dietary restrictions and fasting markedly reduce lipogenesis. The fasting of 20-day-old chicks 1, 3 and 5 days markedly decreases citrate cleavage enzyme (ATP-citrate lyase) and malic enzyme activity levels and fatty acid synthesis. The activities of both enzymes were decreased on starvation, citrate cleavage enzyme activity decreased 65, 29 and 18% of

normal after 1, 3 and 5 days starvation, respectively; while after 1, 3 and 5 days of starvation, malic enzyme activity deceased to 17, 30 and 22% of normal, respectively (Goodridge, 1968). Similarly, Leveille (1969) observed a 76% decrease in hepatic malic enzyme activity after a 3-day fasting period in male chicks. The activity dropped rapidly following day 1 of the fast, and gradually declined thereafter. Upon refeeding, malic enzyme activity rapidly increased back to the normal levels. Rodents such as rats and mice also show similar responses to starvation (Jansen, Zanetti and Hutchison, 1966). Recently, Feuers et al. (1989) showed a 37% decline in hepatic malic enzyme activity with a 40% dietary restriction in 22-month-old male Fischer 344 rats.

In addition to dietary restriction, dietary fat intakes greatly inhibit lipogenesis; as dietary fat intake increases lipogenic enzyme activity levels respond in a parallel fashion (Yeh, Leveille and Wiley, 1970). Lipogenic enzyme activities and rates of fatty acid synthesis are not only regulated by the amount of dietary fat (Sabine et al., 1969; Yeh et al., 1970), but also by the type of dietary fat. Within the past 10-15 years, much attention has been focused on dietary fat intake, particularly the degree of fat saturation with respect to fatty acid synthesis. Clarke et al. (1977) showed that rats fed polyunsaturated fatty acids (C18:2 and C18:3) reduced the rate of hepatic fatty acid synthesis and the lipogenic enzyme activities of both fatty acid synthase (FAS) and malic enzyme (ME); whereas, the saturated fatty acids (C16:0 and C18:0) showed no effect on fatty acid synthesis. Neither saturated nor unsaturated fatty acids exhibited any affect on the rate of fatty acid synthesis or lipogenic

enzyme activity levels in adipose tissue. Triscari, Hamilton and Sullivan (1978) showed similar results in rats administered 5 g/kg of C18:0, C18:1 and C18:2. Unsaturated dietary fat (C18:1, C18:2) inhibited *in vivo* fatty acid synthesis, while the effects of feeding saturated fat (C18:0) showed no change. Thus concluding, the rate of lipogenesis is inversely proportional to the concentration of unsaturated dietary fat. Research of Herzberg and Janmohamed (1980) further support the findings of Triscari *et al*. (1978). They examined the effects of saturated *vs*. unsaturated dietary fat on lipogenesis in mouse liver. Mice were fed varying amounts of either maize oil (corn oil), which is rich in linoleic acid (C18:2), or tripalmitin (C16:0). Maize oil-fed animals more effectively reduced *in vivo* hepatic lipogenesis and hepatic levels of lipogenic enzymes: fatty acid synthase, glucose-6-phosphate dehydrogenase, malic enzyme and glucose kinase; than those fed tripalmitin diet.

The ability of a fatty acid to inhibit hepatic lipogenic enzyme activity and suppress lipogenesis is related to the degree and position of unsaturation of the fatty acid administered. Schwartz and Abraham (1982) examined the effects of specific polyunsaturated fatty acids by administering pure fatty acids: oleic (cis-Δ^{9}-18:1), ricinoleic (12-hydroxy-cis-Δ^{9}-18:1), linoleic (cis, cis-$\Delta^{9,12}$-C18:2), α-linolenic (cis, cis, cis-$\Delta^{9,12,15}$-18:3), columbinic (trans, cis, cis-$\Delta^{5,9,12}$-18:3) and arachidonic (all-cis$\Delta^{5,8,11,14}$-20:4), to mice maintained on 50% glucose diet. Results showed that an 18-carbon fatty acid must contain at least two unsaturated (double) bonds, in order to inhibit the activity and to prevent an accumulation of liver fatty acid synthesis in carbohydrate-fed

mouse liver. In addition, it appears that unsaturation at carbons 9 and 12 positions is more effective. Interestingly, Herzberg and Rogerson (1988) showed fatty acids in the (n-3) family are more potent inhibitors than the (n-6) fatty acids, with 20- and 22-carbon (n-3) fatty acids being the most effective.

The effects of dietary fats such as fish or marine oils are also of particular interest because of their high percentage of long chain and polyunsaturated fatty acids. Iritani et al. (1980) studied the effects of feeding rats for 2 weeks on 3% fat diets containing 0.5 or 1.0% corbicula, clam or oyster triglycerides or 1% corn oil. The activities of hepatic glucose-6-phosphate dehydrogenase, malic enzyme and acetyl-CoA carboxylase were markedly reduced in the rats fed shellfish oil, while rats fed corn oil showed no significant change in lipogenic enzyme activity levels. Thus, the long chain and polyunsaturated fatty acids, which are characteristics of shellfish, may play a role in the reduction of fatty acid synthesis.

Herzberg and Rogerson (1988) also showed similar results with administering dietary marine oil. They compared the effects of feeding rats three dietary fats, corn oil, tallow or marine oil, on hepatic lipogenic enzyme activity and lipogenesis in vivo. In both the fed and fasted animals, the lipogenic enzymes (fatty acid synthase, ATP-citrate lyase, malic enzyme and glucose-6-phosphate dehydrogenase), as well as lipogenesis in vivo, exhibited the same pattern of effects. Activity decreased in the order of tallow > corn oil > marine oil. Lipogenesis of fasted rats showed similar enzyme activity patterns compared to those of

fed animals, although they were lower overall. Dietary fish oil reduces hepatic lipogenesis in both fed and fasted rats, even though both weight gain and food consumption were unaffected by diet composition. This decrease in fatty acid synthesis may be due to a lowered availability of hepatic glycogen for fatty acid synthesis.

Attempts to understand further the mechanism of these effects of dietary saturated vs. unsaturated fats have led to the investigation of molecular research with mRNA levels of lipogenic enzymes. Cloned cDNA's have been isolated for lipogenic enzymes: malic enzyme, glucose-6-phosphate dehydrogenase, acetyl-CoA carboxylase and fatty acid synthase, and used to show that rates of enzyme synthesis correlate with abundances of these enzyme mRNA. Recently, various researchers (Jump et al., 1984; Goodridge, 1986; Katsurada et al., 1986a; Clarke, Armstrong and Jump, 1990a) have shown these increases in enzyme synthesis are related to increased lipogenic enzyme mRNA levels.

Katsurada et al. (1986a) showed carbohydrate and protein diets increase mRNA activities of both malic enzyme and glucose-6-phosphate dehydrogenase; while, as expected, fat feeding markedly decreased the mRNA activity levels of these enzymes. In addition, Goodridge and his colleagues have demonstrated similar results with malic enzyme and fatty acid synthase (Goodridge and Adelman, 1976; Goodridge, 1986). Using cDNA clones, Goodridge (1986) showed that as enzyme synthesis increases so does the levels of mRNA. The effects of dietary manipulation on the abundance of fatty acid synthase mRNA and malic enzyme mRNA in the liver are similar to those observed with lipogenic enzymes. Fatty acid synthase mRNA and

malic enzyme mRNA levels are high in animals fed a high-carbohydrate diet and low in starved or restricted animals (Goodridge and Adelman, 1976; Dozin, Rall and Nikodem, 1986; Goodridge, 1986; 1987). Pape, Lopez-Casillas and Kim (1988) have shown similar results with acetyl-CoA carboxylase under varying nutritional and hormonal conditions using cDNA probes. Changes in the level of acetyl-CoA carboxylase mRNA correspond to changes in activity and amount of acetyl-CoA carboxylase in both the epididymal fat tissue and liver. Thus, the physiological factors such as fasting and feeding, diabetes and insulin, and lactation, which affect fatty acid synthesis, alter the gene expression of acetyl-CoA carboxylase.

Protein S14 which has a tissue distribution similar to that of the lipogenic enzymes, is one of several lipogenic genes subject to coordinate regulation by dietary fats. Recently, Jump et al. (1984) have shown the factors that regulate S14 mRNA abundance are similar to those that control fatty acid synthase mRNA abundance. For instance, diet and hormones regulate S14 mRNA in the adipose tissue in the same manner they control lipogenic enzymes in the tissue. Because of this similarity, Clarke et al. (1990a) examined the possible relationship between S14 and fatty acid synthase, and thus the ability to use S14 gene as a model for studies of lipogenic proteins. Results showed a relatively high correlation between the mRNA of S14 and fatty acid synthase, ranging from 0.88 to 0.96. This correlation strongly supports S14 as a model for studying the regulatory region in lipogenic genes that may be responsive to nutrient control. With this in mind, Clarke et al. (1990b) also investigated the response of S14 mRNA and fatty acid synthase with respect to dietary polyunsaturated

fats. Dietary polyunsaturated fats reduced hepatic fatty acid synthase and S14 mRNA by 75 to 90%. Fish oils were more effective than safflower oil, while tripalmitin and triolein showed very little effect. S14 and fatty acid synthase mRNA levels appear to be coordinately regulated by dietary polyunsaturated fats in both mature and weanling rat, where fatty acid synthase appears to be more tightly regulated by the dietary polyunsaturated fats than is S14. In addition, the high correlation observed between the abundance of fatty acid synthase mRNA and S14 mRNA, strengthens the previous argument of Clarke et al. (1990a), in that the S14 protein product is involved in lipogenesis.

Much research deals with the hepatic lipogenesis, but as mentioned earlier, the adipose tissue is also a major site of lipogenesis in rats and other mammalian species. However, there is uncertainty about the role of dietary fat on the suppression of fatty acid synthesis in adipose tissue. For instance, Clarke et al. (1977) showed dietary fat, regardless of degree of saturation, had no effect on the regulation of lipogenesis in the adipose tissue. Nelson et al. (1987) showed similar results when rats were fed a high-fat diet of corn oil or hydrogenated soybean oil. The activities of fatty acid synthase, ATP-citrate lyase and glucose-6-phosphate dehydrogenase were not inhibited significantly by the addition of either of the fats to the diet; thus both saturated and polyunsaturated fatty acids did not suppress fatty acid synthesis in the adipose tissue. Because of these findings, it is suggested that other modulators of fatty acid synthesis, such as blood glucose levels, may play an important role in triggering fatty acid synthesis in the adipose tissue. It may be that

a fat-fed animal may synthesize fatty acids primarily in the adipose tissue, while a low or fat-free diet exhibits fatty acid synthesis primarily in the liver, with perhaps a contribution from adipose tissue (Gandemer et al., 1983). In addition, other factors such as diet, age, sex, strain and caloric intake may affect the site of fatty acid synthesis.

Hormonal Regulation

In addition to dietary factors, hormones also play a substantial role in the regulation of lipogenesis (Goodridge and Adelman, 1976; Das, 1980; Wilson and McMurray, 1980; Mariash et al., 1981). The hormones insulin, triiodothyronine (T3) and glucagon, are involved in mediating the effects of diet on metabolic pathways, particularly lipid metabolism. In well-fed animals the levels of insulin are elevated, especially if the diet is rich in carbohydrates. Insulin promotes glucose oxidation, glucose storage as glycogen and triglycerides, and protein synthesis. It does so by increasing the transport of glucose into the cell. Glucose is converted to pyruvate and glycerol 3-phosphate by glycolysis, and available pyruvate is utilized for fatty acid synthesis and glycerol 3-phosphate for esterification of the fatty acids. In addition, insulin activates acetyl-CoA carboxylase which depresses the level of intracellular cAMP, thus reducing long-chain acyl-CoA concentration, an inhibitor of lipogenesis (Mayes, 1988). Levels of T3 are also increased in well-fed conditions. T3 stimulates the accumulation of several enzymes, particularly enzymes of fat synthesis. In fact, T3 plays a significant role in regulating hepatic fatty acid synthesis. For

instance, Das (1980) showed that by administering L-triiodothyronine (T3) to diabetic rats, hepatic acetyl-CoA carboxylase and fatty acid synthase enzymes were restored to normal levels.

In addition, Goodridge and Adelman (1976) examined the effects of administering insulin, T3 and both insulin and T3. In avian hepatocytes, T3 stimulated more than a 50-fold increase in malic enzyme; whereas in serum-free media insulin had little effect by itself, but insulin with T3 amplified the effects by more than a 100-fold. Insulin and T3 also stimulated malic enzyme in rat hepatocytes in culture, however not to the same extent as that observed with chick-embryo hepatocytes (Wilson and McMurray, 1980; Mariash et al. 1981). Besides malic enzyme, fatty acid synthase and acetyl-CoA carboxylase are also stimulated by insulin and T3, but to a lesser extent than that of malic enzyme. In chick-embryo hepatocytes, fatty acid synthase is increased approximately 2-fold by insulin alone, 2-fold by T3 alone, and about 15-fold by the combination of insulin and T3; while acetyl-CoA carboxylase is increased 8-fold by the combination of insulin and T3 (Fischer and Goodridge, 1978).

Unlike insulin and T3, glucagon is stimulated by starvation and inhibited by feeding high-carbohydrate diets. In general, the metabolic effects of glucagon oppose those of insulin, in that the intracellular cAMP level is increased, acetyl-CoA carboxylase is inhibited, and thus lipogenesis activity reduced. Glucagon decreases the amount of fatty acid synthase, acetyl-CoA carboxylase (Fischer and Goodridge, 1978) and malic enzyme (Goodridge and Adelman, 1976) in avian hepatocytes treated with insulin and T3; however, the decreases in levels of fatty acid synthase

and acetyl-CoA carboxylase are much smaller than the decreases in malic enzyme. If both insulin and T3 are present together, glucagon inhibits the activities of acetyl-CoA carboxylase and fatty acid synthase by 73 and 78%, respectively (Fischer and Goodridge, 1978); while malic enzyme activity is almost completely blocked, 97% inhibited (Goodridge and Adelman, 1976). As with T3 and insulin, glucagon also affects the abundance of fatty acid synthase mRNA and malic enzyme mRNA, in that glucagon inhibits the accumulation of fatty acid synthase mRNA and malic enzyme mRNA (Goodridge, 1986).

Effects of Aging

With aging in healthy individuals, weight gain is a common occurrence; however the mechanism of aging is unclear. Since the early work of Ross (1969), it has been suggested that biological aging is a manifestation of naturally occurring changes in the enzyme activities. Leveille (1972) examined the effects of age with lipogenesis using male Sprague Dawley rats over an 18-month period. Adipose tissue fatty acid synthase, malic enzyme and glucose-6-phosphate dehydrogenase activity levels, all showed a significant decrease with aging. Adipose tissue in vitro fatty synthesis exhibited regression coefficients of -56.9 and -13.7 for meal-fed and nibbling animals, respectively. Meal-fed rats had regression coefficients of -7.1 and -8.6 for adipose tissue malic enzyme and glucose-6-phosphate dehydrogenase, respectively, while hepatic lipogenic enzyme activity levels were -1.3 for malic enzyme and -2.5 for glucose-6-phosphate dehydrogenase. The enzyme activities, and thus lipogenic capacity, were significantly influenced by age of the animal

with most parameters showing a significant decrease with age. In addition, the rate of change with time was generally greater for the meal-fed rats than for the nibbling animals.

Sullivan et al. (1971) also showed a decline in the rate of lipogenesis with age. As female Sprague Dawley rats increased in body weight, an inverse relationship was true of the rate of lipogenesis. And because the age of the rats was proportional to their body weight, it was concluded that lipogenesis decreases with aging. Unfortunately, the animals studied were only 5 to 7 weeks old, weighing 125 to 150 g, and 12 to 14 weeks of age, with body weights of 275 to 300 g, which does not provide a representative aging sample given the 27- to 32-month lifespan of the rat.

Contrary to previous studies, Gandemer et al. (1982) showed the rate of in vivo lipogenesis increased using male Wistar rats ages 19, 25, 34, 40, 50 and 80 days old. Hepatic lipogenesis increased 6-fold between 19 and 25 days reaching maximum in 50-day-old rats and remained high in young adults (80 days of age), but perirenal and subcutaneous adipose tissues in 80-day-old rats had low levels of fatty acid synthesis. The rest of the carcass had increased lipid synthesis after weaning, reached a maximum in 50-day-old rats, and decreased slightly in 80-day-old rats. Overall, de novo lipogenesis was highest at 50 days and changed only slightly in 80-day-old rats. In addition, total lipids and fatty acids in liver and perirenal adipose tissue increased between 19 and 50 days, then moderately increased between 50 and 80 days (Gandemer et al., 1982).

Based on these results, it would appear that when rats are young and still growing, lipogenesis increases. However once rats have reached maturity, fatty acid synthesis begins to decline sharply at first, and more gradually throughout their lifespan. For instance, in adipose tissue of male Sprague Dawley rats the activity of malic enzyme declined sharply between 1 and 4 months, followed by relatively minor reduction between 4 months and 1 year (Kaiser et al., 1983).

Similarly, recent work of Barakat et al. (1989) observed in male Fischer 344 rats a lower lipogenic potential of liver and adipose tissue in aged rats, 27-months-old, than younger rats, 6-months-old. This was evident by a decrease in the activity levels of several lipogenic enzymes. Activity levels of hepatic acetyl-CoA carboxylase, fatty acid synthase and malic enzyme were significantly decreased in aged rats, while aging had no effect on hepatic ATP-citrate lyase activities. In adipose tissue, aging resulted in significant decreases in acetyl-CoA carboxylase and ATP-citrate lyase activities, and no effect on fatty acid synthase and malic enzyme activity levels. In contrast, glucose-6-phosphate dehydrogenase activity levels in both liver and adipose tissue increased with aging.

It is believed that with aging there is a gradual accumulation of body fat, however studies on the subject are inconclusive. Bertrand et al. (1980) showed an increase in fat mass between 6- and 12-month-old male Fischer 344 rats which remained constant until 18 months of age and declined with advancing age. In general, adipose tissue mass increases with increasing age in Fischer 344 rats until about 75% of their lifespan, after which it decreases. On the other hand, McDonald et al. (1989)

showed, based on data from aging male and female Fischer 344 rats (5, 23 and 27 months of age) fed a high-fat and low-fat diet, that age, gender and diet have no significant effect on the ability to regulate body composition. The major differential effect of diet on body mass in both high-fat and low-fat diets occurred during the first week, which reflects a rapid increase during the first week of feeding. However, there was no significant diet effect on growth rate in both the male and female 5-month-old rats from week two to the end of the experiment. Lean body mass percent was not altered due to diet or age and it did not decline as a function of aging in either male or female Fischer 344 rats.

Summary

Much of the past research on the rate of lipogenesis has been with young animals (Sullivan et al., 1971; Leveille, 1972; Gandemer et al., 1982), and only a few studies have been done on rats as old as 18 months (Leveille, 1972), and 27 months (Barakat et al., 1989). The present study was designed to determine the effects of aging on lipogenic enzyme function in rats ranging from 2 to 27 months of age. Research conducted with rodents, such as rats, provides control over diet, physical activity, and nongenetic diseases. Since diseases, infection, organ impairment, such as the renal failure common in aged Fischer 344 rats, especially after 24 months of age (Iwasaki et al., 1988), and altered nutritional state may affect lipogenic enzyme activity, the study employed the Fischer 344 strain of aging rat. These animals have been bred and maintained under nutritionally balanced and barrier-reared conditions to minimize the effect of disease in order to permit a study of the aging process itself.

CHAPTER 3

MATERIALS AND METHODS

Animals and Diets

Male Fischer CD 344 rats, pathogen-free, were purchased (ages: 2 mo from Charles River and 6, 18 and 27 mo from the National Institute of Aging colony derived from Charles River stock). Rats were housed individually in metabolic cages with a 12-h light-dark cycle for 10 days. Rats of each age were divided into a control group which was fed ad libitum, and a restricted group which was pair-fed to the amount of food per gram of body weight consumed ad libitum by the 27-month-old rats (3.8 grams of food per 100 grams of body weight). All rats were fed an unrefined rodent diet (Wayne Rodent Blox®, 8604-00; Table 1) that was ground and mixed with chromium oxide as an inert marker to a final chromium oxide concentration of 0.2%. Water was available ad libitum.

Food consumption and body weights were measured daily. Due to the fact that some lipogenic enzymes and that fatty acid synthesis show considerable variation throughout the daily 24 h cycle (Carrozza et al., 1979), rats were killed by CO_2 inhalation at 0830 to 0930 h on day 10. Portions of the liver and adipose tissues were immediately removed and put on ice. Approximately one gram of each tissue was weighed and homogenized in a volume of cold buffer (pH 7.6) containing 0.15 M potassium chloride, 1.0 mM magnesium chloride, 0.5 mM dithiothreitol and 10 mM N-acetyl cysteine (Herzberg and Rogerson, 1988). Liver homogenates were prepared

with a Potter-Elvehjem tissue grinder; and adipose tissue was homogenized using a polytron (homogenizer model PT 10/35, Brinkmann Instruments, Inc., Westbury, NY). Homogenates were ultracentrifuged (model L8-70M with rotor Ti 70.1, Beckman Instruments, Inc., Palo Alto, CA) at 250,000 × *g* for 40 min at 4°C. The supernatant fraction (cytosol) was used for quantitation of enzyme activities.

Enzyme Activity Assays

Fatty acid synthase (FAS) was determined spectrophotometrically from the rate of malonyl-CoA-dependent NADPH oxidation (Gibson and Hubbard, 1960; Arslanian and Wakil, 1975) with 0.0625 M potassium phosphate buffer (pH 6.5), 10 mM dithioerythritol, 150 μM NADPH, 25 μM acetyl coenzyme A, and 250 μM malonyl coenzyme A combined as the assay mixture. Glucose-6-phosphate dehydrogenase (D-glucose-6-phosphate:$NADP^+$ 1-oxidoreductase, EC 1.1.1.49; G6PD) was measured from the rate of glucose-6-phosphate-dependent reduction of NADP (Bergmeyer, Gawehn and Grassl, 1974; Lohr and Waller, 1974). The assay mixture included 0.087 M triethanolamine buffer (pH 7.6), 3.33 mM magnesium chloride, 1.17 mM glucose-6-phosphate (monosodium salt), and 430 μM NADP. Malate dehydrogenase (decarboxylating) ($NADP^+$) (L-malate:$NADP^+$ oxidoreductase (oxaloacetate-decarboxylating), EC 1.1.1.40; malic enzyme (ME)) was measured from the rate of malate-dependent NADP reduction by the method of Yeh *et al*. (1970) using an assay mixture which contained 0.042 M glycylglycine buffer (pH 7.4), 8.33 mM manganese chloride, 367 μM malate, and 83 μM NADP. ATP-citrate (*pro-3S*) lyase (ATP:citrate oxaloacetate-lyase, EC 4.1.3.8; ATP-CL) was determined by the method described by Srere

(1959) and Yeh et al. (1970). Supernatant and 5.0 mM ATP were added to an assay mixture of 0.1 M tris buffer (pH 7.4), 20 mM sodium citrate, 10 mM magnesium chloride, 10 mM dithioerythritol, 330 μM coenzyme A, 140 μM NADH, and 25 μM malate dehydrogenase. All enzyme assays were conducted at 37°C, and followed at 340 nm using a kinetic spectrophotometer (Ultrospec model 4053, Pharmacia LKB, Piscataway, NJ). Activities were expressed as μmoles of substrate converted to product per min per g protein which is equal to International Units per g protein (IU/g). Expressing liver and adipose tissue enzyme activities on a fresh weight or body weight basis is an unreliable method, because of the diffuse nature of the tissue and its variable triglyceride content. Therefore, liver and adipose tissue enzyme activities were expressed on a tissue protein basis only. Protein concentration of the supernatants was determined using the Folin phenol reagent and a bovine serum albumin standard as described by Lowry et al. (1951). Chemicals and biochemicals were purchased from Sigma Chemical Co., St. Louis, MO.

Statistical Analysis

Data were analyzed by one-way analysis of variance (ANOVA) and the means separated using least significant difference (LSD), with significance at $P<0.05$ (Steel and Torrie, 1960).

TABLE 1. Composition of Wayne Rodent Blox®, 8604-00

Nutrient	Diet %	Diet kcal	Metabolizable energy % of total kcal
Protein	25.93	1.04	32.24
Fat	3.98	0.36	11.14
Carbohydrate	45.54	1.82	56.62

CHAPTER 4

RESULTS

In the present study only the 2-month-old rats exhibited significant differences in lipogenic enzyme activities between the control and restricted groups (Tables 2 and 3) and only for hepatic G6PD and ME and adipose FAS and ME. The differences were due to the degree of restriction. Rats were subjected to food restriction based on the food intake of 27-month-old rats, which was 3.8% of their body weight in grams. This restriction was a decrease from ad libitum consumption of only 3% for the 18-month-old rats, and 25% and 56% for the 6- and 2-month-old rats, respectively (Table 4). Body weights of the 2-, 6- and 18-month-old food-restricted rats were significantly lower compared to those of their respective control groups (Table 4). Because the enzyme activities obtained for 6-, 18- and 27-month-old rats for control and restricted groups were not different ($P>0.05$) the data were combined for further evaluations. Only the values for the 2-month-old control group were used in subsequent analyses.

Body weights of all age groups were significantly different. Rats reached their maximum weight at 18 months and dropped slightly by 27 months. Body weights for the 2-, 6-, 18- and 27-month-old rats were 206, 340, 441 and 408 grams, respectively (Table 5 and Figure 1).

Food intakes were slightly higher for the younger rats, 2 and 6 months of age, 16.6 and 16.7 grams per day, respectively, than for the

older rats, 18 and 27 months of age, 14.5 and 14.0 grams per day, respectively (Figure 2). When food intakes were expressed per kg of physiological body weight (PBW = $BW_g^{.75}$) for each age group, a significant difference was observed between the growing rats, 2- and 6-month-old, and the older rats, 18- and 27-month-old. Food intakes expressed per $BW_{kg}^{.75}$ (Table 5) were 54.72 ± 5.58 and 37.63 ± 6.94 grams for the 2- and 6-month-old rats, respectively and 26.77 ± 3.56 and 27.61 ± 2.92 grams for the 18- and 27-month-old rats, respectively. Food intakes expressed per kg body weight showed similar patterns; food intakes were highest in 2-month-old rats and decreased significantly with age until 18 months old and remained at that level in 27-month-old rats (Table 5).

The lipogenic enzyme activity levels of FAS, ATP-CL, ME and G6PD of the liver and adipose tissue all showed significant differences between the four age groups; however, not in a similar fashion (Table 6). Hepatic G6PD activity levels exhibited a significant increase by 2-fold at 27 months of age, while opposite results were obtained in adipose G6PD with a significant decrease in activity level after 2 months of age and remained constant thereafter (Figure 3).

Both hepatic FAS and ATP-CL activity levels showed similar biphasic responses, with rats having higher activities at 2 months, dropping slightly at 6 months, increasing to their highest level at 18 months and dropping again to low levels at 27 months. Adipose tissue FAS and ATP-CL enzyme activities showed a significant, but gradual, decline with aging rats (Figures 4 and 5). Adipose tissue FAS showed a linear reduction of 54% with age between 2 to 27 months (Figure 4). Significant

decreases were also observed in ME activities in which both the liver and adipose tissue behaved similarly. ME activity levels decreased significantly between 2 and 6 months, and remained at low levels at 18 and 27 months of age (Figure 6).

For all age groups, lipogenic enzyme activities were generally higher in adipose tissue than liver (Table 6). Younger rats, 2 and 6 month, exhibited higher levels of adipose tissue lipogenic enzyme activities than older rats of 18 and 27 months. Adipose tissue activity levels of G6PD and ME were highest at 2 months of age, while FAS and ATP-CL activities reached maximum levels in both 2- and 6-month-old rats.

Unlike the adipose tissue, hepatic lipogenic enzymes did not exhibit similarities between enzyme activity levels and age groups. Hepatic FAS reached maximum activity at 18 months of age, while ATP-CL had highest activity levels at 2 and 18 months. The activity levels of hepatic G6PD reached maximum at 27 months and hepatic ME activities were highest in 2-month-old rats.

TABLE 2. Hepatic lipogenic enzyme activities

Feeding level	Age (months) 2[1]	6[2]	18[3]	27[4]
	Fatty acid synthase (IU/g protein)			
Control	25.73 ± 0.88	12.42 ± 1.25	37.11 ± 5.97	12.41 ± 2.43
Restricted	29.12 ± 1.96	13.89 ± 1.05	40.12 ± 5.33	15.98 ± 4.04
	Glucose-6-phosphate dehydrogenase (IU/g protein)			
Control	70.43[a] ± 7.19	61.64 ± 7.55	78.76 ± 8.20	140.40 ± 16.09
Restricted	46.37[b] ± 2.90	68.77 ± 6.25	90.83 ± 17.25	157.59 ± 27.07
	Malic enzyme (IU/g protein)			
Control	30.71[a] ± 3.45	16.73 ± 2.22	13.52 ± 1.40	14.95 ± 3.73
Restricted	16.18[b] ± 2.10	15.63 ± 1.50	13.84 ± 1.79	13.67 ± 3.19
	ATP-citrate lyase (IU/g protein)			
Control	33.03 ± 3.01	14.43 ± 6.95	32.36 ± 5.01	5.42 ± 2.86
Restricted	26.57 ± 1.81	13.84 ± 6.56	37.55 + 5.29	5.61 ± 1.98

[a,b]Means within a column within an enzyme with no common superscripts differ significantly ($P<0.05$).

[1]n=6 for control and restricted treatments

[2]n=5 for control and restricted treatments

[3]n=7 for control treatment; n=9 for restricted treatment

[4]n=4 for control and restricted treatments

TABLE 3. Adipose tissue lipogenic enzyme activities

Feeding level	Age (months) 2[1]	6[2]	18[3]	27[4]
	Fatty acid synthase (IU/g protein)			
Control	36.28[a] ± 4.51	35.89 ± 5.40	29.21 ± 7.37	13.66 ± 3.95
Restricted	20.07[b] ± 2.80	30.57 ± 5.24	19.90 ± 3.74	19.36 ± 2.99
	Glucose-6-phosphate dehydrogenase (IU/g protein)			
Control	218.93 ± 26.32	132.60 ± 6.57	127.03 ± 4.89	171.03 ± 45.95
Restricted	174.59 ± 18.51	133.15 ± 5.83	114.78 ± 3.96	107.02 ± 12.05
	Malic enzyme (IU/g protein)			
Control	240.42[a] ± 36.50	25.55 ± 3.64	19.71 ± 3.53	14.71 ± 3.82
Restricted	120.09[b] ± 24.93	49.99 ± 11.89	13.05 ± 2.71	12.40 ± 4.26
	ATP-citrate lyase (IU/g protein)			
Control	96.35 ± 18.63	168.97 ± 52.56	61.79 ± 47.90	33.16 ± 13.81
Restricted	74.38 ± 33.33	171.10 ± 66.34	39.93 ± 30.26	43.43 ± 18.18

[a,b]Means within a column within an enzyme with no common superscripts differ significantly ($P<0.05$).

[1]n=6 for control and restricted treatments

[2]n=5 for control and restricted treatments

[3]n=7 for control treatment; n=9 for restricted treatment

[4]n=4 for control treatment; n=3 for restricted treatment

TABLE 4. Average body weights and food intake of ad libitum-fed and restricted-fed rats

Feeding level	Age (months)			
	2[1]	6[2]	18[3]	27[1]
	Body weight (g)			
Control	242[a] ± 17	370[a] ± 6	452[a] ± 8	413[a] ± 15
Restricted	185[b] ± 13	328[b] ± 19	430[b] ± 13	400[a] ± 5
	Food intake (g/day)			
Control	17.3[a] ± 1.0	16.9[a] ± 0.7	15.0[a] ± 0.5	14.5[a] ± 0.5
Restricted	7.5[b] ± 0.5	12.6[b] ± 0.6	14.5[a] ± 0.7	12.8[b] ± 0.9

[a,b]Means within a column within a parameter with no common superscripts differ significantly ($P<0.05$).

[1]n=6 for control and restricted treatments

[2]n=5 for control and restricted treatments

[3]n=7 for control treatment; n=9 for restricted treatment

TABLE 5. Effect of age on body weight and food intake

	Age (months)			
	2[1]	6[2]	18[3]	27[4]
Body weight, g	206[d] ± 20	340[c] ± 10	441[a] ± 10	408[b] ± 10
Feed intake, g	16.6[a] ± 1.22	16.7[a] ± 0.92	14.5[b] ± 0.47	14.0[b] ± 0.33
Feed intake, g/kg BW[5]	82.0[a] ± 4.83	49.4[b] ± 3.06	32.9[c] ± 1.05	34.6[c] ± 1.06
Feed intake, g/PBW[6]	54.7[a] ± 2.50	37.6[b] ± 2.19	26.7[c] ± 0.84	27.6[c] ± 0.78

[a,b]Means within a row with no common superscripts differ significantly ($P<0.05$).

[1]n=5

[2]n=10

[3]n=18

[4]n=14

[5]BW = body weight

[6]PBW = physiological body weight ($BW_{kg}^{.75}$)

TABLE 6. Effects of age on lipogenic enzyme activities

Feeding level	Age (months) 2[1]	6[2]	18[3]	27[4]
LIVER	(IU/g protein)			
Fatty acid synthase	25.73[b] ± 0.88	13.16[c] ± 0.85	38.81[a] ± 4.00	14.20[c] ± 2.47
Glucose-6-phosphate dehydrogenase	70.43[b] ± 7.19	65.20[b] ± 5.03	85.55[b] ± 10.77	148.99[a] ± 16.03
Malic enzyme	30.71[a] ± 3.45	16.18[b] ± 1.35	13.70[b] ± 1.18	14.31[b] ± 2.47
ATP-citrate lyase	33.03[a] ± 3.01	14.14[b] ± 4.78	35.28[a] ± 3.75	5.42[b] ± 1.74
ADIPOSE TISSUE	(IU/g protein)			
Fatty acid synthase	36.28[a] ± 4.51	33.23[a] ± 3.85	23.97[ab] ± 4.02	16.51[b] ± 2.74
Glucose-6-phosphate dehydrogenase	218.93[a] ± 26.32	132.88[b] ± 4.39	120.14[b] ± 3.44	143.60[b] ± 29.31
Malic enzyme	240.42[a] ± 36.50	37.77[b] ± 7.32	15.97[b] ± 2.33	13.31[b] ± 2.90
ATP-citrate lyase	96.35[ab] ± 18.63	170.04[a] ± 42.32	49.50[b] ± 27.13	37.56[b] ± 11.26

[a,b]Means within a row with no common superscripts differ significantly ($P<0.05$).

[1]n=6

[2]n=10

[3]n=16

[4]n=8 for liver; n=7 for adipose tissue

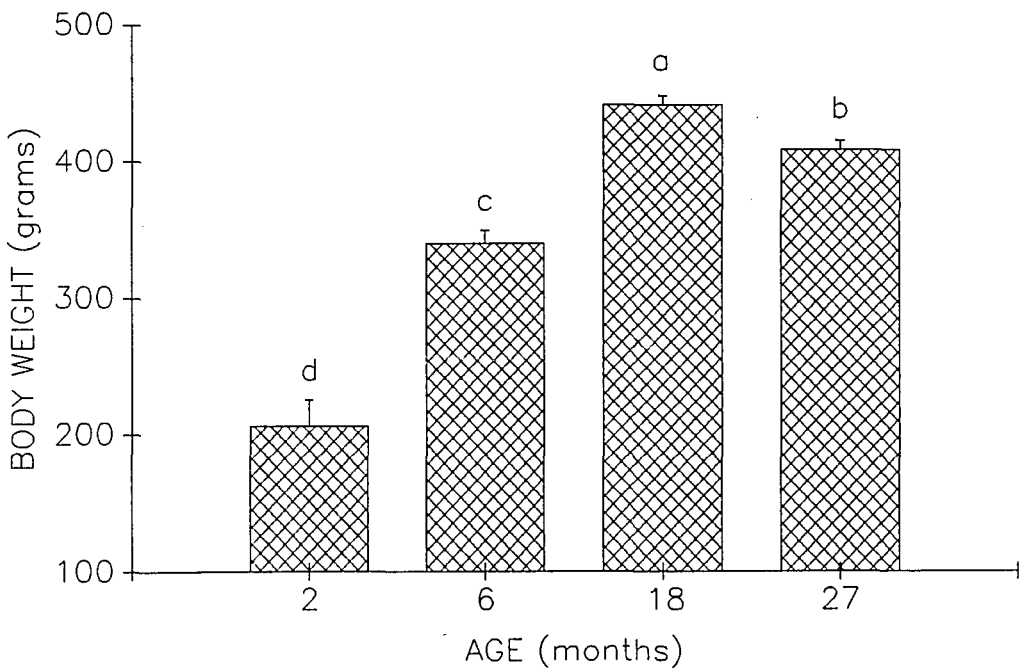

FIGURE 1. Effect of age on body weight of male Fischer 344 rats.

[a-d]Means with no common superscripts differ significantly ($P<0.05$).

n=5 for 2 months; n=10 for 6 months; n=18 for 18 months; n=14 for 27 months.

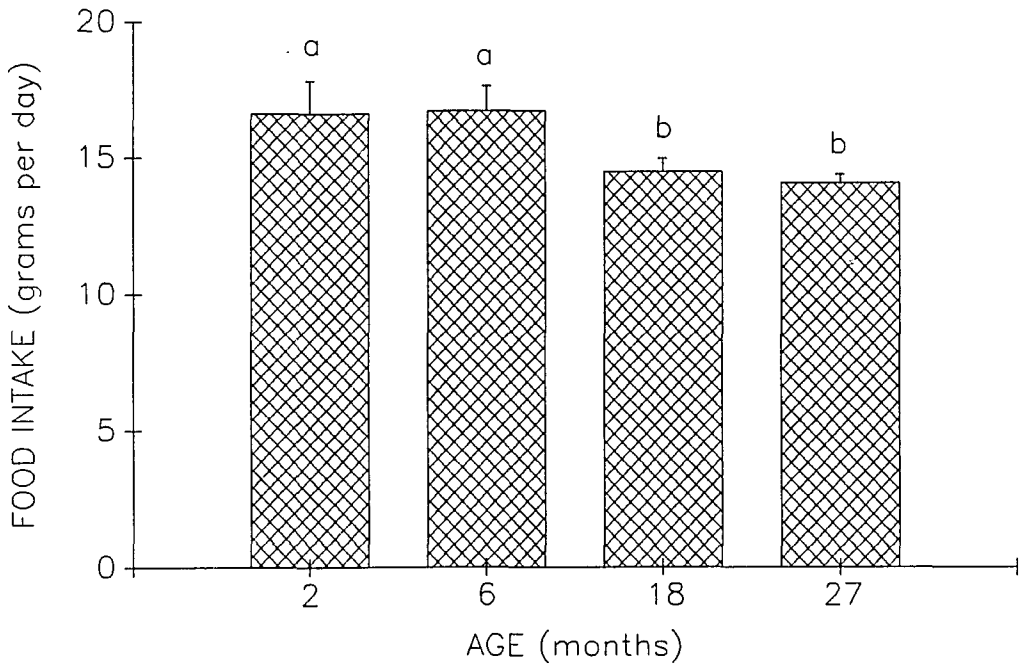

FIGURE 2. Effect of age on food intake of male Fischer 344 rats.

[a,b]Means with no common superscripts differ significantly ($P<0.05$).

n=5 for 2 months; n=10 for 6 months; n=18 for 18 months; n=14 for 27 months.

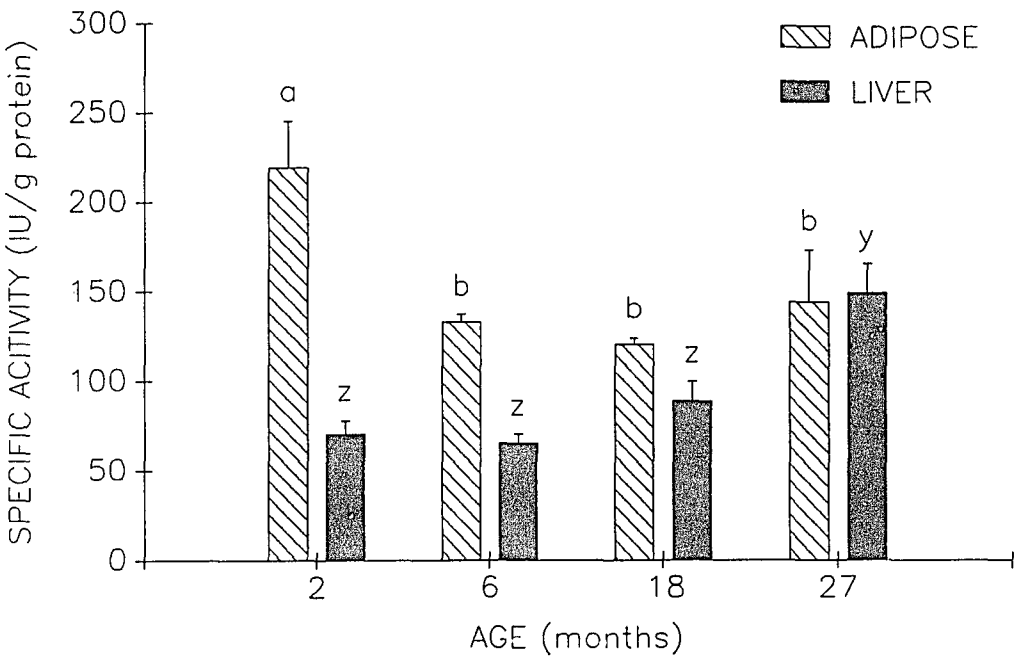

FIGURE 3. Influence of age on the specific activity of glucose-6-phosphate dehydrogenase (EC 1.1.1.49) in adipose and hepatic tissues of male Fischer 344 rats.

[a,b; y,z]Means within a tissue with no common superscripts differ significantly ($P<0.05$).

n=5 for 2 months; n=6 for 6 months; n=18 for 18 months; n=8 for liver and n=7 for adipose tissue for 27 months.

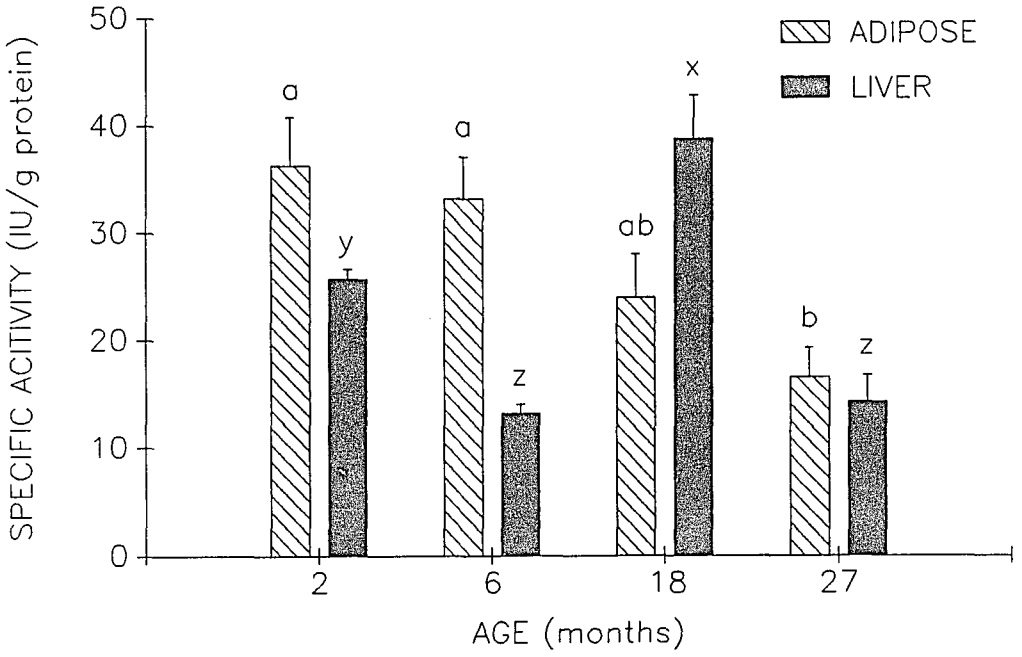

FIGURE 4. Influence of age on the specific activity of fatty acid synthase in adipose and hepatic tissues of male Fischer 344 rats.

[a,b; x-z]Means within a tissue with no common superscripts differ significantly ($P<0.05$).

n=5 for 2 months; n=6 for 6 months; n=18 for 18 months; n=8 for liver and n=7 for adipose tissue for 27 months.

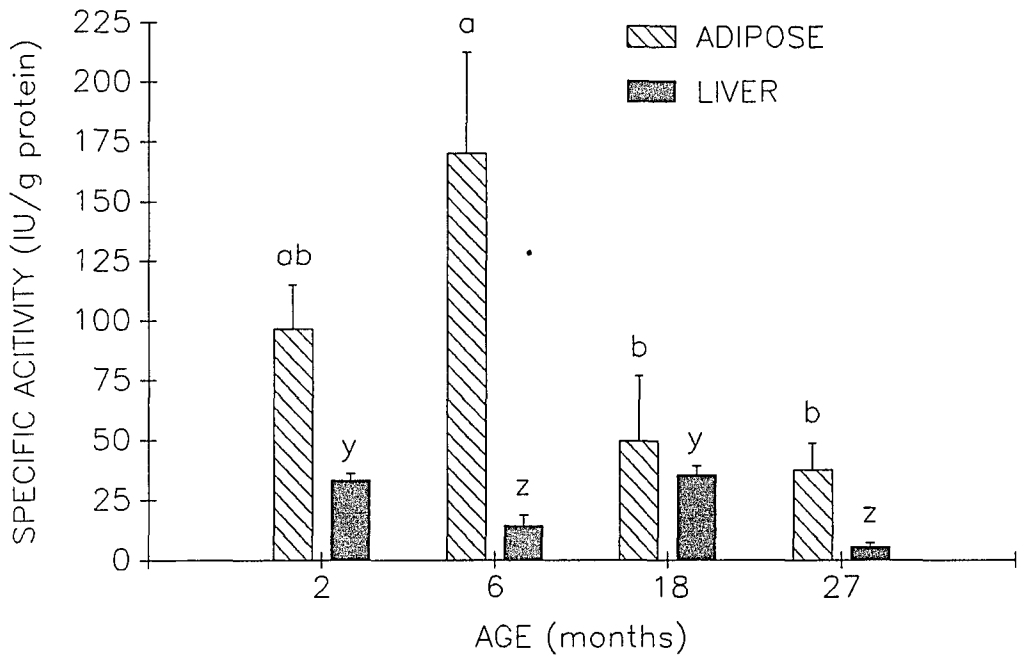

FIGURE 5. Influence of age on the specific activity of ATP-citrate lyase (EC 4.1.3.8) in adipose and hepatic tissues of male Fischer 344 rats.

[a,b; y,z]Means within a tissue with no common superscripts differ significantly ($P<0.05$).

n=5 for 2 months; n=6 for 6 months; n=18 for 18 months; n=8 for liver and n=7 for adipose tissue for 27 months.

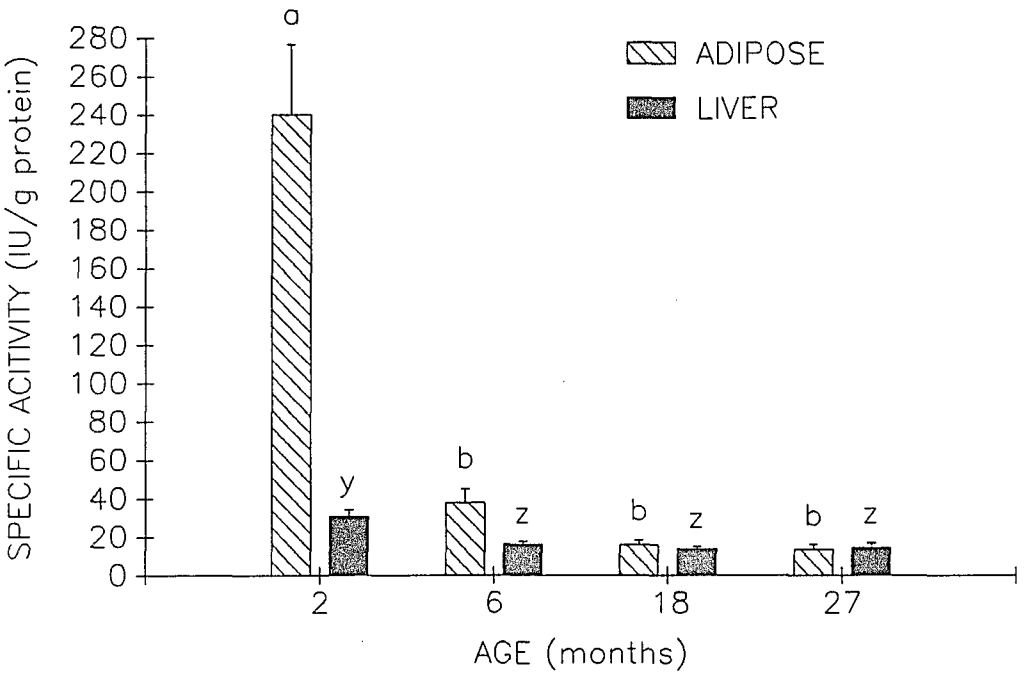

FIGURE 6. Influence of age on the specific activity of malic enzyme (EC 1.1.1.40) in adipose and hepatic tissues of male Fischer 344 rats.

[a,b; y,z]Means within a tissue with no common superscripts differ significantly ($P<0.05$).

n=5 for 2 months; n=6 for 6 months; n=18 for 18 months; n=8 for liver and n=7 for adipose tissue for 27 months.

CHAPTER 5

DISCUSSION

Dietary restrictions inhibit lipogenic enzymes (Goodridge, 1968; Leveille, 1969; Feuers et al., 1989); however, in the present study only the 2-month-old rats exhibited significant differences between the control and restricted groups (Tables 2 and 3). The differences were due to the degree of restriction. It is not surprising that 6- and 18-month-old food restricted rats showed no significant differences because the level of restriction was low compared to ad libitum consumption. This is in agreement with a study by Katsurada et al. (1986a), which shows no significant effects on lipogenic enzyme activities when rats are restricted by 20% of ad libitum food consumption. The lack of significant differences in lipogenic enzyme activity levels between control and restricted groups with food intake restricted to as low as 25% of ad libitum, suggests that the differences in enzyme activities observed between age groups were a result of aging, rather than nutritional status.

Lipogenic enzyme activities, with the exception of hepatic G6PD, all showed a significant decrease between 2 and 27 months of age. These findings of the reduction in lipogenic enzyme activities with aging in Fischer 344 rats are in agreement with those of Barakat et al. (1989). In fact, Barakat et al. (1989) also observed increases in G6PD activity levels with age in both liver and adipose tissue, while we only observed an increase in liver (Figure 3). Furthermore, as observed by Barakat et

al. (1989), no significant differences were found in adipose tissue ME or G6PD between 6- and 27-month-old rats (Figures 3 and 6). In contrast, Leveille (1972) reported a decrease in lipogenic enzyme activity level between 6 and 18 months of age in Sprague Dawley rats. Leveille's (1972) results with 2- and 6-month-old rats were similar with those we have obtained, in that adipose tissue ME and G6PD activity levels significantly decreased between 2 and 6 months.

While adipose tissue G6PD activity decreased gradually with age, the opposite was true for the hepatic G6PD activity levels. In addition, ATP-CL and FAS activities, with the exception of 27-month-old rats, also had opposite patterns of enzyme activity for liver and adipose tissue (Table 6). It would appear that for these particular enzymes (G6PD, ATP-CL and FAS), the lipogenic pattern with aging is different between liver and adipose tissue. Only ME showed similar patterns of lipogenic enzyme activities in liver and adipose tissue.

The higher activity levels obtained in adipose tissue in comparison with those in liver contradict the findings of Gandemer et al. (1983). Their study indicated that, in rats fed a low-fat diet (0.3%), 27% of the total fatty acid synthesis was in adipose tissue and 42% was in the liver. However, our findings are in agreement with that of Leveille (1972), in that adipose tissue activity levels were higher than those of liver in his study with Sprague Dawley rats at 1 to 18 months of age. These differences may be due to strain and possibly age, because Gandemer et al. (1983) used male Wistar rats, ages 19 through 80 days. Although, the recent work of Barakat et al. (1989) with 6- and 27-month-old Fischer

344 rats showed lipogenic activity levels to be higher in liver than adipose tissue.

Carrozza et al. (1979) found that lipogenic enzyme activities, particularly G6PD and ME, did not always correlate positively with rates of fatty acid synthesis. On the other hand, Leveille (1972) observed a high correlation between lipogenic capacity and the activity of ME or G6PD in adipose tissue ($r = 0.949$) in Sprague Dawley rats. Therefore, because of the high degree of correlation observed between the activities of ME and G6PD, these enzyme activity levels may serve as a good index to lipogenesis. Based on our findings of ME and G6PD activity levels in adipose tissue (Figures 3 and 6); fatty acid synthesis in Fischer 344 rats may decrease between ages 2 and 6 months and remains low up to 27 months.

Enzyme activity levels reflect maximum capacity and not necessarily the actual synthesis that occurs (Tepperman and Tepperman, 1965). The study conducted by Sullivan et al. (1971) on rat liver showed that in vitro rates of lipogenesis were 13 to 15 times the in vivo rate. This suggests that, in addition to lipogenic enzyme activities, other regulators function in control of lipogenesis. Rates of fatty acid synthesis cannot always be extrapolated from the activity levels of the various lipogenic enzymes.

Katsurada, Fukuda and Iritani (1986b) found that acetyl-CoA carboxylase and fatty acid synthase control the rate of fatty acid synthesis due to substrate levels (ie. citrate, acetyl-CoA and malonyl-CoA) found in the liver. Thus, the activities observed with FAS and ATP-CL may accurately reflect the actual rate of fatty acid synthesis. Based

on the hypothesis of Katsurada et al. (1986b), our results for hepatic FAS and ATP-CL activities would suggest that Fischer 344 rats synthesize fat at a higher rate while they are young and growing (2-month-old), decrease once they have reached full growth (6-month-old), increase again when at a mature level (18-month-old) and then decrease again in old age (27-month-old), (Figures 4 and 5). The increase in FAS activity seen in 18-month-old rats may explain the increase in body weights (Figure 1), even though it is assumed they have reached full growth by 6 months. Furthermore, the decline in FAS activity levels observed in 27-month-old rats may explain in part the loss in body weight with advanced age.

Even though the 18-month-old rats had significantly lower food intake per kg body weight, they still had the largest body weight of the rats studied (Table 5). Thus, higher liver FAS and ATP-CL activities obtained in the 18-month-old rats, would suggest that a portion of food energy was used for fatty acid synthesis. Genetically obese Zucker rats, which have higher body fat content than lean rats, exhibit higher hepatic fatty acid synthesis rates and enzymatic activities than lean rats (Berke and Kaplan, 1983; Simmons and Tyzbir, 1990). Body composition studies would help clarify the actual relationships between enzyme activities and fat deposition in these animals. When the rats in this study were killed the heavier weight rats (18- and 27-month-old) visually appeared to have greater amounts of adipose tissue than the lighter weight rats (2- and 6-month-old); however, one cannot make any such concluding statements because body composition studies were not conducted. Although we did not carry out carcass analyses, Bertrand et al. (1980) showed that the highest

amounts of epididymal and perirenal fat depots are in 18-month-old Fischer 344 rats compared with younger animals, with a decline at 24 and 27 months of age. The results obtained by Bertrand et al. (1980) may explain the biphasic response observed in the activities of hepatic lipogenic enzymes, ATP-CL and FAS. The overall age-related reduction observed in hepatic enzymes may be reflected in body fat changes (Bertrand et al., 1980).

Sullivan et al. (1971) showed that female Sprague Dawley rats had hepatic lipogenesis rates which were inversely related to body weight. Because the age of these animals is proportional to their body weight, Sullivan et al. (1971) concluded that as animals get older lipogenic enzyme activity levels decrease. When our results were expressed in relation to body weight, the 2- and 6-month-old rats had results similar to those found by Sullivan et al. (1971). However, the 18- and 27-month-old animals, which had higher body weights (Figure 1), did not exhibit lower levels of enzyme activity; in fact, the 18-month-old rats, which exhibited the highest body weight, had the highest hepatic FAS and ATP-CL activity levels (Figures 4 and 5). Furthermore, body weights showed a significant increase between 6 and 18 months, while no significant differences were observed in G6PD and ME activities in either liver and adipose tissue (Figures 3 and 6). Thus, the concluding statement made by Sullivan et al. (1971) may not be universal among rat sub-strains; we found that lipid synthesis was not the inverse of body weight, in advanced age. Unfortunately, Sullivan et al. (1971) only studied rats 125 to 150 g, 5 to 7 weeks of age and 275 to 300 g animals, 12 to 14 weeks of age;

therefore, it appears to be an insufficient cross-section for extrapolation to aging animals.

Factors such as strain, age, sex and nutritional status affect lipogenesis (Leveille, 1969; 1972; Nace, Szepesi and Michaelis, 1979; Gandemer et al. 1982), it is interesting to explore the use of the Fischer 344 rat as a model for aging studies. The Fischer rat is a major animal model for aging research because of its genetic homogeneity and because it does not develop the obesity that is known to occur with advancing age in many rat strains (National Research Council, 1981). Therefore, the results observed for lipogenic enzyme activities may be applicable only to the Fischer 344 rat.

CHAPTER 6

CONCLUSIONS

The results of our study suggest that aging significantly reduces lipogenic enzyme activities in liver and adipose tissue, and thus fatty acid synthesis in rats. This was most evident for the adipose tissue FAS activity, which declined linearly with age. Body composition studies would have been helpful in interpreting the data. In light of the recent work of Goodridge (1986) and Clarke et al. (1990a) showing that fatty acid synthesis is highly correlated with lipogenic enzyme mRNA and S14 mRNA levels, it would be interesting to examine the effects of these parameters with respect to aging and differences that may exist between strains of rats. It should also be mentioned that lipogenic enzymes and fatty acid synthesis are under hormonal control. Hormones such as insulin, glucagon, thyroxine and growth hormones mediate some of the effects of aging in relation to lipogenic enzyme activities (Goodridge and Adelman, 1976; Fischer and Goodridge, 1978; Goodridge 1986). Thus, further work is needed, especially on body composition and hormonal mechanisms, in aging rats with respect to lipogenesis.

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