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# Growth and protein metabolism in exercised, estrogen-fed and developing female Zucker rats

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by

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# INTRODUCTION

## Preamble

The genetic obesity of the Zucker rat results from an autosomal recessive mutation (fa/fa) (Zucker and Zucker 1961). The affected rats become obese and defend their obese body composition despite a variety of treatments. These include food restriction (Bray et al. 1973, Cleary et al. 1980), jejunoileal bypass surgery (Greenwood et al. 1982), and treatment with a fatty acid synthesis inhibitor (Greenwood et al. 1981).

The metabolic defect underlying the obesity is as yet unknown. However, it has been suggested that the obesity is a consequence of subnormal protein synthesis, which results in increased shunting of nutrients into fat synthesis. Obese male Zucker rats deposit less body protein than lean ones, and when the rats are pair-fed, the difference becomes more pronounced (Pullar and Webster 1974, Cleary and Vasselli 1981). Adult obese male rats deposited a smaller percentage of a labelled amino acid dose as lean tissue than lean rats did (Dunn and Hartsook, 1980). Furthermore, obese weanling male Zucker rats incorporated less $^{3}$H-phenylalanine into muscle protein than their lean counterparts (Reeds et al. 1982).

However, although both male and female Zucker rats develop obesity, only the obese male Zucker rat has subnormal lean body mass. Body protein content of obese female Zucker rats is normal or above-normal at 24, 33, 50, 66 and 98 days of age (Radcliffe and Webster 1976).

Furthermore, 19 week old obese female Zucker rats had above-normal body protein content (Walberg et al. 1984). Obese female Zucker rats deposited protein at rates similar to those of lean rats even when dietary protein content was below optimum levels (Radcliffe and Webster 1979). Even pair-feeding of obese female Zucker rats to lean rats did not lower their lean body weight (Bray et al. 1973). This evidence suggests that, compared to obese male rats, obese female Zucker rats are better able to defend lean body mass.

Many of the characteristics of the obese female Zucker rat are similar to those of ovariectomized rats. These include hyperphagia and obesity (Radcliffe and Webster 1976) as well as functional sterility and delayed vaginal opening (Bray et al. 1976). Furthermore, the above-normal lean body mass of obese female rats is similar to the increase in lean body mass associated with ovariectomy in normal rats (Dohm and Beecher 1981, Shaw et al. 1983). These findings suggest subnormal estrogen status of obese female Zucker rats.

Because fa/fa rats of both sexes develop obesity, the underlying metabolic defect is apparently identical in male and female obese Zucker rats. Therefore, any explanation for the obesity must be consistent with the known characteristics of both male and female rats.

## Statement of Rationale

### Experiment 1: Effects of exercise on growth and 3-methylhistidine excretion of female Zucker rats

Forced exercise can be used as a stress to growth and protein metabolism. Male rats usually respond to exercise with decreased food intake, body weight and lean body weight. In contrast, female rats respond to exercise with increased food intake, body weight and lean body weight.

As previously discussed, defective regulation of protein metabolism has been suggested as an underlying cause of the obesity of the Zucker rat. Experiment 1 employs exercise as a stress to growth so that this hypothesis can be tested in female rats. Urinary 3-methylhistidine (3-MH) was measured to determine whether or not any exercise-induced change in lean body mass is mediated through a change in muscle protein catabolism.

### Experiment 2: Effects of ethynyl estradiol on body composition growth and muscle protein catabolism of female Zucker rats

The results of Experiment 1 showed that obese female Zucker rats resemble ovariectomized rat in as much as both groups have above-normal lean body mass and respond similarly to exercise. If obese female Zucker rats have subnormal estrogen status, then they may be more sensitive to estrogen administration than lean rats are. Experiment 2 was designed to test if estrogen administration would normalize the

above-normal lean body mass, carcass fat content and organ weights associated with the obesity of female Zucker rats. Urinary 3-MH was measured to determine if any effect of estrogen on lean body mass was achieved through a change in muscle protein catabolism.

Experiment 3: Development and protein metabolism of female Zucker rats

A subnormal protein synthesis rate and lean body mass of weanling obese male Zucker rats may be caused by the metabolic defect underlying the obesity. However, female rats achieve normal or above-normal lean body mass despite the development of obesity. Experiment 3 was designed to determine the rate of protein synthesis of female Zucker rats at weaning and at onset of sexual maturity. Urinary 3-MH was measured to determine if the ability of the female Zucker rat to maintain normal lean body mass in early life is associated with alterations in muscle protein catabolism.

# REVIEW OF LITERATURE

## The Zucker Rat as a Model of Obesity

The obese Zucker rat is a mutant, first reported by Zucker and Zucker (1961), which appeared in a cross between Sherman and Merck stock M rats. The obesity results from an autosomal recessive mutation (fa/fa).

The Zucker rat is used as a model of juvenile onset obesity. Many symptoms of the obesity syndrome are similar in both the Zucker rat and humans with juvenile onset obesity. These include hyperplastic-hypertrophic adipose tissue, hypertriglyceridemia (Bray and York 1979), hyperinsulinemia (Zucker and Antoniades 1972), insulin resistance (Martin and Gahagan 1976), elevated lipoprotein lipase activity (Boulange et al. 1979), subnormal physical activity (Habery et al. 1980) and hyperphagia at an early age (Stern and Johnson 1977).

The inability of the male Zucker rat to maintain normal lean body mass is in contrast to the symptoms of juvenile onset obesity. Most human obesity, including the juvenile onset type, is associated with above-normal lean body mass (Forbes and Welle 1983) whereas the obesity syndrome of the male Zucker rat is associated with subnormal lean body mass (Zucker 1967, Pullar and Webster 1974). Because the female Zucker rat achieves normal or above-normal lean body weight (Radcliffe and Webster 1976, 1978, 1979), perhaps the female, rather than the male, Zucker rat is a better model for juvenile onset obesity.

Obese Zucker rats defend their high body fat content despite a variety of treatments. These include food restriction (Bray et al. 1973, Cleary et al. 1980), treatment with a fatty acid synthesis inhibitor (Greenwood et al. 1981), and jejunoileal bypass surgery (Greenwood et al. 1982).

A problem encountered in research on the Zucker rat is separating the symptoms of the underlying cause of the obesity from the symptoms of the obesity itself, which develops at an early age. Although the affected rats are not visibly obese until four weeks of age, they can be identified by abnormally low oxygen consumption as early as 7 days of age (Planche et al. 1983). Also, body fat content is measurably increased by 13 days of age (Bell and Stern 1977), and adipocyte hypertrophy is detectable by 5 to 7 days of age (Boulange et al. 1979). The obese rats become hyperphagic before weaning when presented with solid food, at about age 16 to 18 days (Stern and Johnson 1977).

Obese Zucker rats are fat because of a metabolic defect in which abnormally high proportions of nutrients are shunted to fat rather than to lean (Deb et al. 1976, Pullar and Webster 1974, Zucker 1975). The nature of the defect has not been identified. However, some characteristics of the Zucker rat have been shown not to be the underlying cause of the obesity. Although obesity and hyperphagia occur together in these animals, hyperphagia is not the cause of the obesity (Bray and York 1979, Cleary et al. 1980, Deb et al. 1976, Stolz and Martin 1982). Even if obese rats are pair-fed with lean controls

obesity will still develop. Although the pair-fed obese rats contain less body fat than ad libitum fed obese rats, they are visibly obese and contain more body fat than lean controls (Cleary et al. 1980).

Insulin resistance has also been proposed as the defect underlying the obesity of Zucker rats. When diabetes was induced in obese and lean rats with streptozotocin and all rats received the same level of exogenous insulin, increased hepatic lipogenesis and increased percent body fat in the obese rats were not normalized, although body weight and food intake were equal in both genotypes (Stolz and Martin 1982). The authors concluded that the conversion of more dietary energy to lipid in obese rats than in lean ones is independent of food intake and blood insulin levels.

Many explanations of how various characteristics of the Zucker rat relate to the development of obesity are proposed in the literature. Consequently, a complete review of these relationships is impractical for the purposes of this thesis. A schematic representation of some of these relationships is presented in Figure 1. Citations at various steps are provided to indicate studies in which these relationships are discussed in detail.

## Relationship of hyperphagia and body protein content to the obesity of the Zucker rat

As previously discussed, hyperphagia is not the cause of obesity in the Zucker rat. However, because the same underlying defect may cause hyperphagia and obesity, appetite regulation of Zucker rats has been studied.

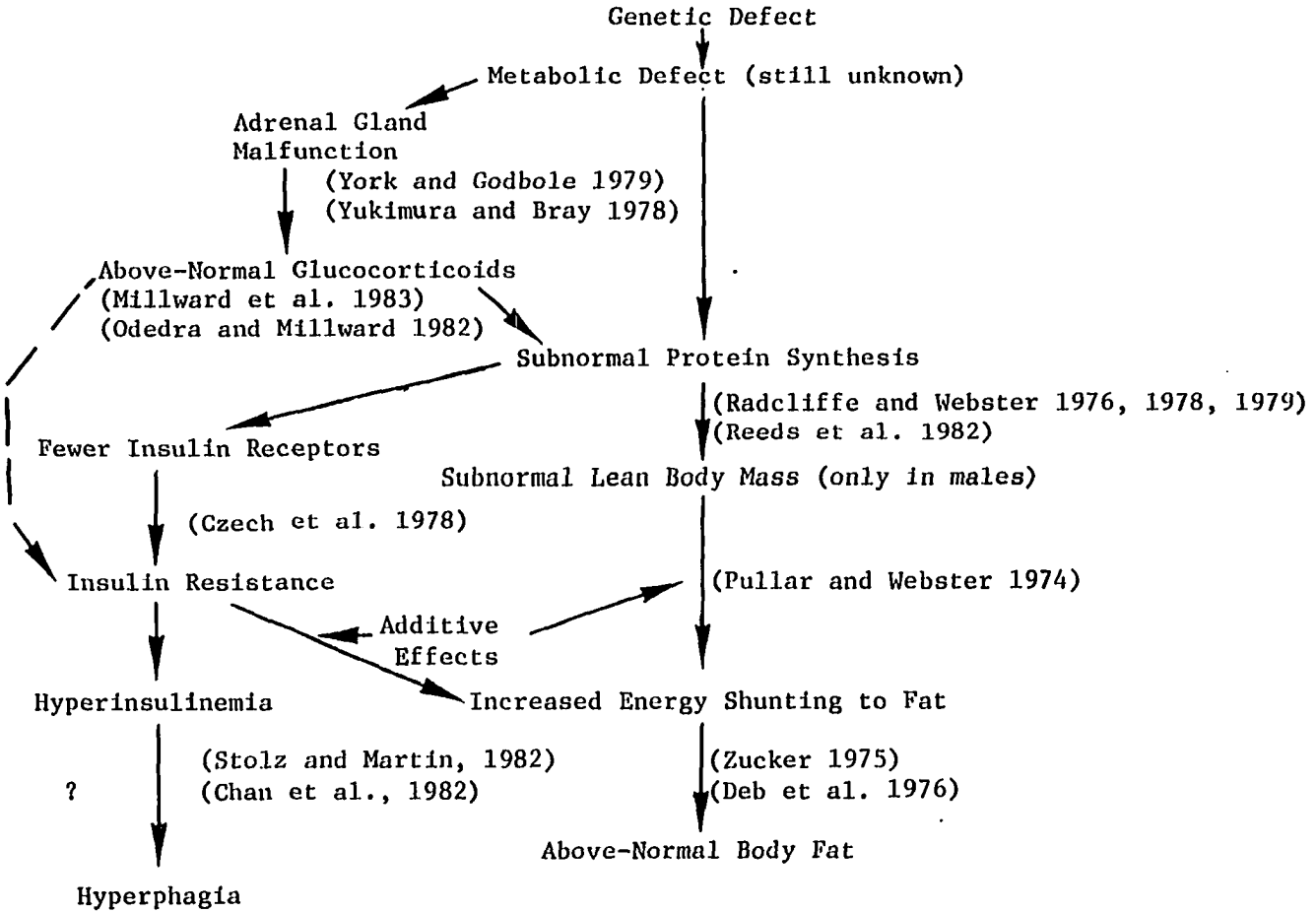


FIGURE 1. Relationships of some characteristics of the Zucker rat to the development of obesity

A basis for studies on appetite regulation has been the difference in body protein content of lean and obese male Zucker rats. The absolute amount of protein and ash are similar for both genotypes from 13 to 16 days of age, although body fat is increased in the obese rat as early as 13 days of age (Bell and Stern 1977). However, after 23 days of age, male obese rats, even when they were allowed to eat ad libitum, contained less body protein than lean controls (Reeds et al. 1982). These results led Pullar and Webster (1974) to suggest that obese Zucker rats deposit protein at a slower rate than lean rats do, and therefore overeat in an attempt to attain normal skeletal and muscle growth. In three separate studies (Radcliffe and Webster 1976, 1977 and 1978), using both male and female Zucker rats on a range of diets which varied in protein content, protein source, or both, the rate of protein deposition was lower in the obese males than in the lean males; in contrast, females of both genotypes maintained identical body protein content. One problem with this hypothesis is that even when fed a protein deficient diet the obese female rats deposited as much protein as the lean females did. This finding is inconsistent with the hypothesis that hyperphagia in obese Zucker rats represents an attempt to compensate for impaired ability to deposit protein. The authors nevertheless defended their hypothesis by stating that, because males deposit protein at a faster rate than females, protein deposition rate to determine food intake occurs in both sexes but this relationship is obvious only in males.

To test the hypothesis that obese rats overeat to obtain sufficient dietary protein, Castonguay et al. (1982) presented a cornstarch-vitamin mix, casein and corn oil, each in a separate container, to 10 week old lean and obese male Zucker rats. The rats were allowed to compose their own diet for nine days. Obese rats ate less casein, similar amounts of cornstarch mix and more corn oil than their lean counterparts. These results were not consistent with the hypothesis that dietary protein is critically involved in appetite regulation in the Zucker rat.

Other theories on appetite regulation have been presented. One is that obese rats are hyperphagic because of an abnormally high set point for food intake inhibition (Bray and York 1972). Another is that both obese and lean Zucker rats control food intake by sensing the heat increment of the diet rather than the composition or gross energy content of the diet (Jenkins and Hershberger 1978). The results of these studies on appetite regulation support the conclusion that the hyperphagia of obese Zucker rats cannot be explained by any particular diet composition.

## Evidence for Subnormal Estrogen Status of Female Zucker Rats

In normal adult female rats, removal of endogenous estrogen by ovariectomy results in hyperphagia and body fat gain (Kakolewski et al. 1968, Tarttelin and Gorski 1973), both of which can be reversed by estrogen administration (Landau and Zucker 1976, Hervey and Hervey 1981). Ovariectomy of normal rats also increases lean body mass

compared to that of intact female rats (Dohm and Beecher 1981, Harris et al. 1984).

Many of the characteristics of the obese female Zucker rat are similar to those of ovariectomized rats, thus suggesting subnormal estrogen status. These characteristics include hyperphagia, obesity and above-normal lean body mass (Radcliffe and Webster 1976, 1979, Shaw et al. 1983). Also, obese female Zucker rats exhibit decreased ovarian and uterine weights, functional sterility, delayed vaginal opening and ovarian cycle irregularities compared to lean female Zucker rats (Saiduddin et al. 1973, Bray et al. 1976). On the basis of these observations, Saiduddin et al. (1973) suggested that the Zucker rat has subnormal estrogen levels. However, the blood of both lean and obese rats contains normal concentrations of ovarian hormones (Hervey et al. 1982). A decreased sensitivity of the reproductive tissues to estrogen could explain the reproduction abnormalities of obese Zucker rats. However, Bray et al. (1976) found that uterine tissues of both genotypes were equally sensitive to stimulation of growth by estrogen. When the degree of obesity was decreased by pair-feeding, the low weights of the uterus, ovary and pituitary gland of the obese female Zucker rats did not increase, indicating that the obesity itself is not the primary cause of subnormal reproductive function of obese Zucker rats (Bray et al. 1973).

## Protein Turnover in Rats

Protein turnover depends on both protein synthesis and protein degradation, which are under different control (Waterlow et al. 1978). The difference between the two rates determines the magnitude of net protein deposition or loss. In the case of the growing rat, the protein turnover rate in the whole body is three times greater than the rate of protein accumulation in a tissue (Buckley and Milligan 1978). However, the rates of protein synthesis and degradation, as well as control of protein accumulation, vary widely between tissues. For example, in rapidly growing animals and during compensatory growth after fasting, growth of skeletal muscle is associated with an increase in protein degradation but an even greater increase in protein synthesis, resulting in net protein accumulation (Waterlow et al. 1978, Buckley and Milligan 1978). However, under similar conditions, liver growth results primarily from reduced protein degradation (Conde and Scornick 1976, Hutson and Mortimore 1982).

Studies performed on perfused tissues in vitro have yielded valuable information on protein turnover. However, in vitro conditions during measurement of protein synthesis and degradation sometimes result in negative nitrogen balance, probably due to the lack of innervation and hormonal stimuli present in vivo (Li and Goldberg 1976, Durschlag and Layman 1983). For this reason, emphasis has been placed on the development of techniques which would allow protein synthesis and degradation to be studied in the whole animal.

## Protein synthesis

Regulation of protein synthesis The amount of protein produced could be regulated by alterations in the amount of messenger ribonucleic acid (RNA), number of ribosomes, rate of chain initiation or elongation, amount of energy available, and amino acid pool size within the cell (Waterlow et al. 1978). Long-term adaptations in protein synthesis rates in various tissues are related to the RNA content of the cells (Millward et al. 1973), whereas short-term adaptation of muscle protein synthesis involves no change in RNA content of the cell.

Protein synthesis in muscle responds rapidly to alteration in nutritional state in rats. When rats are receiving frequent meals, there is no diurnal variation in protein synthesis or protein turnover (Buckley and Milligan 1978). Once food intake ceases, protein synthesis and amino acid oxidation decrease (Clugston and Garlick 1982, Rennie et al. 1982). The reduced rate of protein synthesis occurs only after the stomach is empty, and this reduction can be reversed completely in 60 minutes with refeeding (Garlick et al. 1983). For a review of the effects of dietary alteration on protein synthesis see Waterlow et al. (1978).

Hormones, especially insulin and corticosteroids, alter rates of protein synthesis. Insulin is necessary for optimal protein synthesis in muscle (Goldberg 1979, Odedra et al. 1982). Changes in protein synthesis in vivo are associated with parallel changes in insulin levels (Millward et al. 1974). Corticosterone administration decreases

skeletal muscle protein synthesis (Millward et al. 1976, Rannels et al. 1978) and increases protein degradation (Tomas et al. 1979). Corticosterone decreases protein synthesis in muscle even when insulin levels are high (Odedra and Millward 1982, Odedra et al. 1983). Millward et al. (1983) suggest that short-term regulation of muscle protein synthesis involves insulin, corticosterone and another unknown anabolic factor.

However, the control mechanism of the long-term regulation of protein synthesis, such as the difference in protein deposition between males and females, is still unknown.

Measurement of protein synthesis As stated previously, most research on protein synthesis has been done in vitro or the rate of protein synthesis has been calculated from the amount of protein deposited. The techniques of constant infusion and single dose of labelled amino acids have made accurate measurement of protein synthesis in vivo possible (Waterlow et al. 1978). The single dose technique involves a single intravenous injection of a mixture of labeled and unlabelled amino acid so that cellular amino acid pools are quickly flooded. Equilibrium of blood and tissue amino acid levels is rapidly reached and is followed by a slow but linear decline in plasma and tissue levels of the free amino acid. By measuring this linear decline and the amount of amino acid incorporated into protein, a rate of protein synthesis can be calculated (Garlick et al. 1980). The major advantage of this method is that it requires less time than the constant

infusion technique, which requires six hours to complete. Also, in small laboratory animals, the use of the single dose method avoids the effects of stress due to restraint or anesthesia which are required for the constant infusion method.

Protein degradation and 3-methylhistidine excretion

The urinary excretion of 3-methylhistidine (3-MH) has been widely used as an indicator of muscle protein catabolism (Ward and Buttery 1980, Young and Munro 1978). Tallan et al. (1954) originally identified this amino acid as a normal constituent of human urine. Later, 3-MH was identified as a component of actin (Asatoor and Armstrong 1967) and of myosin (Johnson et al. 1967). The 3-MH content of actin is constant from all sources measured thus far (Trayer et al. 1968, Young and Munro 1978). In contrast, 3-MH is a component of myosin in fast-twitch (white) muscle fibers but not of myosin in cardiac muscle and slow twitch (red) muscle fibers (Kuehl and Adelstein 1970, Trayer et al. 1968).

The formation of 3-MH occurs by methylation of histidine residues of newly synthesized protein (Krzysik et al. 1971, Reporter 1973). Because no tRNA charging with 3-MH occurs, 3-MH is not re-utilized in protein synthesis (Young et al. 1970). Rapid and quantitative excretion of 3-MH via the urine has been demonstrated in rats (Young et al. 1972) and in humans (Long et al. 1975). The 3-MH released during muscle protein catabolism is excreted in the urine as either unchanged 3-MH or as N-acetyl 3-MH. The N-acetyl form of 3-MH is predominant in the urine

of rats (Young et al. 1972). However, in humans, only 5% of the total excretion is the N-acetyl form, and the remainder is excreted as unchanged 3-MH (Long et al. 1975). Because 3-MH is not reutilized and is quantitatively excreted in the urine, the accurate measurement of 3-MH released from muscle in vivo requires that the diet of the animal or subject be 3-MH-free.

Urinary 3-MH as an indicator of skeletal muscle catabolism

The use of urinary 3-MH as an indicator of skeletal muscle protein degradation is now widespread (Young and Munro 1978, Ward and Buttery 1980). Although 3-MH excretion has been validated as a product of muscle protein catabolism, its use as an indicator of skeletal muscle protein catabolism specifically is a controversial matter. Skeletal muscle is the major source of 3-MH in the total body 3-MH pool (Haverberg et al. 1975, Nishizawa et al. 1977a). The gastrointestinal tract and skin contribute 10 to 17% of the total body 3-MH pool (Nishizawa et al. 1977a, 1977b). Other tissues such as heart, lung, liver, etc., contain 3-MH but the relative contribution of 3-MH in these tissues to the total body pool is small (Haverberg et al. 1975).

The assumption that skeletal muscle is the major contributor to urinary 3-MH because it is the major component of the total body 3-MH pool could be invalid because 3-MH release depends upon 3-MH turnover rate, as well as amount, in various tissues. A small tissue pool of 3-MH with a high turnover rate could be a major source of urinary 3-MH (Millward et al. 1980). In an attempt to determine the relative

contribution of skeletal muscle, gastrointestinal tract and skin to urinary 3-MH, the rates of protein synthesis, 3-MH synthesis and 3-MH turnover have been measured by various methods. In several studies, one group of investigators determined that the contribution of skeletal muscle ranged from 24 percent to 74 percent of the total urinary 3-MH excretion in the rat (Bates and Millward 1981, Millward and Bates 1983, Rennie and Millward 1983). Harris (1981) and Wassner and Li (1982) discussed sources of variation in results of the above studies, including differences in age and sex of the rats, methods of measurement, and assumptions made in calculations. Other investigators estimated that skeletal muscle contributed approximately 75% of the total urinary 3-MH excretion both in the rat (Nishizawa et al. 1977a) and in the human (Afting et al. 1981). Wassner and Li (1982) measured the fractional catabolic rate of 3-MH in various perfused tissues and determined that the gastrointestinal tract contributed 40% of the total urinary 3-MH excretion in the male rat. Because of the conflicting evidence presented by various investigators, studies in which 3-MH excretion is measured should be interpreted cautiously.

## Protein Metabolism in the Zucker Rat

Subnormal protein synthesis, which results in increased shunting of nutrients into fat synthesis, has been suggested as an underlying factor in the development of obesity in the Zucker rat. The obese male Zucker rat deposits less body protein than the lean male rat, and when pair-fed, the difference becomes more pronounced (Pullar and Webster 1974,

Cleary and Vasselli 1981). Adult male obese rats injected with $^{14}C$-labelled amino acids deposit a smaller percentage of the total dose as lean tissue and a greater percentage of the total dose as lipid than lean rats. Obese rats excreted more 3-MH/g body protein than lean rats (Dunn and Hartsook 1980). However, in this study the labelled amino acid dose was based on body weight, not on estimated body protein content, and calculations were not corrected for the amount of label presented to lean tissues. Also, measurement of total $^{14}C$ in the tissue did not discriminate between free and protein-bound label. In contrast, as a result of measurements of carcass composition and nitrogen retention, Pullar and Webster (1974);D suggested that fractional rates of protein deposition are similar in both genotypes.

The conflicting evidence regarding altered protein synthesis in adult male Zucker rats led Reeds et al. (1982) to study protein synthesis in weanling male rats. The authors suggest that the metabolism of weanling rats is not yet dominated by long-term obese body composition. Protein synthesis was measured in 18 and 27 day old lean and obese males using intraperitoneal injection of a single dose of labelled phenylalanine. Obese rats synthesized less muscle protein than lean rats, but the difference was greater at 18 days than at 27 days of age. However, there were no differences in liver and intestine protein synthesis rates between genotypes. One reason for this might be the use of intraperitoneal injection instead of intravenous injection. Intraperitoneal injection of the labelled amino acid might result in

adsorption of the label onto the surface of the visceral organs, thus delaying attainment of equilibrium between blood and tissue. This would alter the linear decline of tissue label levels on which the accuracy of this method depends (Garlick et al. 1980). Reeds et al. (1982) suggested that the obese rat has a period of subnormal protein synthesis during early life. After this early phase, the obese rat synthesizes protein at the normal rate, but cannot further increase protein synthesis to achieve normal protein deposition. The early difference in protein synthesis rate could increase energy available for fat storage in obese rats and therefore may in part explain their obesity. However, the protein synthesis rates of male Zucker rats over 27 days of age, and female Zucker rats of all ages, are unknown. The development of obesity in female Zucker rats, despite their ability to maintain normal lean body weight throughout life, is not explained by this hypothesis. Because both male and female Zucker rats become obese, the underlying metabolic defect is apparently the same in both sexes. Therefore, any explanation of the obesity must accommodate the characteristics of both sexes.

## Exercise Studies in Rats

Forced exercise in small laboratory animals is used to determine the effects of physical training on endurance and other physiological characteristics as well as the effects of drugs and diets on performance. Because different methods of exercise produce different

physiological and psychological stresses, it is difficult to compare one type of exercise with the other. This difficulty is illustrated by two studies in which female rats were exercised by treadmill running or by swimming (Schiable et al. 1981, Schiable and Scheuer 1981). Both studies included intensive long-term exercise programs in which exercise increased the heart weight, stroke work, stroke volume, coronary flow and cardiac oxygen consumption of male rats. The treadmill exercised female rats showed no change in cardiac weight or function; however, swim-trained female rats showed significant increases in heart weight and cardiac function compared with sedentary controls. Difficulties also arise in ensuring that each swimming rat within an experiment performs the same amount of work, and in comparing the amount of work done by swimming rats and by running rats. For these reasons, this discussion will include results obtained only with treadmill exercised rats.

Food intake and body composition of treadmill exercised rats

Male rats exercised daily on a treadmill from 6 to 18 weeks of age gained less weight and attained lower final body weights than sedentary controls do (Schiable et al. 1981). Male rats subjected to bouts of exercise every three days ate less, and as duration of exercise increased, the percent body fat decreased (Stevenson et al. 1966). Dohm et al. (1977a) compared sedentary male rats with rats exercised at three different intensities. Exercised rats had decreased body weight, body fat content and body protein content compared with sedentary rats;

these changes were independent of exercise intensity. A study involving sedentary and exercised castrated and intact male rats demonstrated that exercise and castration had similar effects on decreasing food intake and weight gain (Dohm and Beecher 1981).

In contrast to male rats, young female rats respond to exercise by increasing food intake and gaining weight at the same rate as sedentary controls (Nance et al. 1977, Dohm and Beecher 1981, Tapscott et al. 1982). Female rats also respond to exercise by increasing their lean body weight (Dohm and Beecher 1981, Tapscott et al. 1982). However, Schiable et al. (1981) found that exercise decreased weight gain in female rats, although less markedly than in male rats.

In summary, exercise usually decreases food intake, body weight gain, and lean body mass in male rats, but has the opposite effects in female rats. In both sexes, exercise decreases the absolute amount as well as the percentage of carcass fat.

Effects of exercise on protein turnover in rats

The exercise-induced growth inhibition in male rats might be caused by appetite suppression by exercise. However, when sedentary and exercised male rats were pair-fed, growth was still inhibited by exercise, and this inhibition was greater than could be explained by the calculated energy expenditure of the exercise. Also, the growth inhibition by exercise was not altered when exercise intensity was increased (Dohm et al. 1977a).

Male rats responded to a bout of exercise by decreasing in vivo protein synthesis during exercise (Dohm et al. 1982a). Dohm et al. (1985) suggest that the degree of depression of in vivo muscle protein synthesis is proportional to both the duration and intensity of exercise.

Reports of increased urinary nitrogen and less positive nitrogen balance in exercised rats led to the suggestion that exercise may result in greater protein catabolism, especially in male rats (Dohm et al. 1977b). This hypothesis is consistent with the finding of increased urea excretion in exercised male rats (Dohm et al. 1977b, Refsum and Stromme 1974) but not in exercised female rats (Dohm et al. 1978).

Tapscott et al. (1982) measured protein synthesis and degradation rates by perfusing hindquarters of exercised and sedentary male and female rats with a solution containing $^{3}$H-tyrosine. Both protein synthesis and degradation were more rapid in sedentary females than in sedentary males. However, the difference in degradation rate between the sexes was greater than the difference in rate of synthesis; this could result in a decreased deposition of protein in females and may partly explain their lower growth rate compared with that of male rats. Exercise resulted in increased protein degradation and unchanged protein synthesis in male rats. In contrast, exercise had no effect on protein synthesis or degradation in females. This lack of effect in the female rat may partly explain the opposite growth responses to exercise by male and female rats.

Few reports are available on the effect of exercise in rats on 3-methylhistidine (3-MH) excretion, which is assumed to be proportional to muscle protein catabolism. A bout of exercise has been shown to increase the 3-MH excretion of male rats for 48 hours following exercise (Dohm et al. 1978a). Long-term treadmill training of male rats also resulted in increased 3-MH excretion compared with that of sedentary controls (Radha and Bessman 1983). However, no data are available on 3-MH excretion by treadmill trained female rats.

Effects of exercise on food intake and body composition of Zucker rats

Because few reports on exercised Zucker rats are available, the effects of exercise in these animals are not well-established. When obese and lean exercised male Zucker rats and obese sedentary males were all pair-fed to lean sedentary controls, exercise decreased body fat in both genotypes (Deb and Martin 1975). The lean male rats in this particular study responded atypically to exercise in that neither food intake nor body protein content decreased with exercise. As expected, the obese rats had less body protein than the lean rats. However, the obese exercised rats had greater body protein than obese sedentary rats. This effect is the opposite of the decrease in lean body mass usually seen in exercised male rats of other strains. Exercise had no effect on adipose cellularity or on the hyperinsulinemia that is characteristic of the Zucker rat (Deb and Martin 1975). In another study, both lean and obese male Zucker rats responded normally to exercise by decreasing food intake, weight gain and lean body mass (Wardlaw 1984). Furthermore,

exercise did not decrease the high percent body fat of obese male Zucker rats. The different effects of exercise in these studies may be explained by the fact that rats in the latter study were not pair-fed to sedentary controls.

In another study, lean male Zucker rats responded normally to exercise by decreasing food intake, body weight and carcass protein and fat content (Seelbach et al. 1985). In contrast, the same training program for obese Zucker rats resulted in no change in food intake and body protein content compared to sedentary counterparts. Exercise slightly decreased, but did not normalize, carcass fat content of obese Zucker rats. The different responses of lean and obese Zucker rats to exercise may also be explained by the different training programs of each study. The training program of Wardlaw (1984) was least strenuous while that of Seelbach et al. (1985) was most strenuous.

Moderate treadmill training in adult (25 week old) obese female Zucker rats had no effect on body weight, food intake, or skeletal muscle mass (Becker-Zimmerman et al. 1982). In the same study, a more intensive exercise program, started at seven weeks of age in obese female Zucker rats, produced effects which in general were more marked than those observed in the adult rats. Although there were no differences in food intake or skeletal muscle mass between exercised and sedentary young females, the exercised rats had decreased body weight. However, the effects of exercise on body composition, food intake and glucose tolerance in the lean female Zucker rat are largely unknown.

Because lean female Zucker rats were not included in this study, it is impossible to compare the obese animals to the lean ones or to know whether the effects of exercise are the same in both genotypes.

In another study, both lean and obese female Zucker rats were exercised from 5 to 11 weeks of age (Wardzala et al. 1982). The lean rats responded normally to exercise by increasing final body weight compared to that of sedentary counterparts. However, exercise had no effect on final body weight of obese rats. The results of this study did not include any information on food intake or body composition of exercised female Zucker rats.

## Interrelationship of Protein Metabolism, Corticosterone and Obesity in the Zucker Rat

### Effects of corticosterone in normal rats

Corticosterone is the major glucocorticoid produced by the adrenal gland in the rat. Elevated blood concentrations of corticosterone results in net muscle protein loss (Steele 1975). It has been established that corticosterone supresses muscle protein synthesis (Millward et al. 1976, Rannels et al. 1978). However, the effect of corticosterone on muscle protein degradation is controversial. Subcutaneous injection of corticosterone increased 3-MH excretion in rats (Tomas et al. 1979, Santidrian et al. 1981, Tomas et al. 1984b). In contrast, intraperitoneal injection of corticosterone did not change protein degradation rates (Millward et al. 1976, Shoji and Pennington

1977). Santidrian et al. (1981) suggest that the route of corticosterone administration may cause the discrepancy in these results.

Although muscle protein synthesis is decreased with corticosterone administration, liver weight and total protein content of liver is increased (Tomas et al. 1984b). Corticosterone administration also results in elevated plasma levels of protein and glucose. Tomas et al. (1984b) suggest that the decreased synthesis and increased catabolism of muscle protein associated with corticosterone administration results in greater nutrient availability to the liver for synthesis and storage.

Corticosterone administration also increases plasma insulin levels, which antagonizes the effects of corticosterone on protein turnover. Corticosterone administration increased plasma insulin levels in rats (Steele 1975, Tomas et al. 1979). This effect of corticosterone may be the result of decreased protein synthesis, which may decrease the production of insulin receptors. Tomas (1982) found that the elevated plasma insulin levels associated with corticosterone administration diminished the effects of corticosterone. This antagonism is primarily achieved by insulin moderating the increased muscle protein degradation, but also by limiting decreased protein synthesis (Millward et al. 1983, Tomas et al. 1984a). However, in diabetic rats insulin provided only minor protection against the effects of corticosterone (Odedra and Millward 1982). Therefore, high insulin levels may diminish, but will not prevent the effects of corticosterone administration.

Relationships of corticosterone to the obesity of the Zucker rat

The obese Zucker rat has elevated plasma levels of corticosterone and insulin (Martin et al. 1978, Zucker and Antoniades 1972). Furthermore, the normal diurnal variation in plasma corticosterone levels is absent in the obese Zucker rat (Martin et al. 1978). This evidence suggests adrenal gland malfunction in the obese Zucker rat.

The ability of elevated plasma corticosterone to decrease muscle protein synthesis is consistent with the characteristics of the obese male Zucker rat. The adult obese male Zucker rat has subnormal lean body mass (Pullar and Webster 1974, Radcliffe and Webster 1979). The weanling obese male rat exhibits subnormal muscle protein content at 21 days of age and subnormal muscle protein synthesis rates at 18 days of age (Reeds et al. 1982). Also, hyperinsulinemia and increased liver weight, which are consequences of elevated corticosterone levels, are present in the obese Zucker rat. Furthermore, Czech et al. (1978) suggests that the decreased number of muscle insulin receptors in the obese Zucker rat may be related to the ability of corticosterone to decrease protein synthesis.

Although fat cell size may be increased in the obese Zucker rat by 7 days of age (Planche et al. 1983), the overt symptoms of obesity appear at weaning. These symptoms include hyperphagia (Planche et al. 1983), hyperinsulinemia (Zucker and Antoniades 1972) and visibly obese body composition. However, the onset of subnormal protein synthesis in the male obese Zucker rat precedes weaning (Reeds et al. 1982).

Furthermore, it is estimated that full maturity of the adrenal gland is reached at 14 to 20 days of age in the rat (Henning 1978). Therefore, full adrenal gland function is achieved just prior to the onset of many of the obesity symptoms.

Effect of adrenalectomy on the obese Zucker rat

Adrenalectomized 10 week old obese Zucker rats had decreased food intake, weight gain and body fat compared to intact obese Zucker rats (Yukimura and Bray 1978). However, the body fat content was not reduced to normal lean levels. Adrenalectomy of 5 week old obese Zucker rats resulted in normal weight gain and reduced (but not normal) food intake, body fat, serum insulin and fatty acid synthesis rates (York and Godbole 1979, Holt et al. 1983). Adrenalectomy of obese Zucker rats has also been shown to correct other symptoms of the obesity. These symptoms include brown adipose tissue mass and thermogenesis (Holt et al. 1983, Marchington et al. 1983) and adipocyte cell size (Freedman et al. 1985). The obese Zucker rats of these studies had markedly elevated body fat content by the time of adrenalectomy, which may explain the failure of adrenalectomy to completely normalize the symptoms of obesity.

In contrast to older obese rats, adrenalectomy of 21 day old obese rats abolished obesity, hyperphagia and hyperinsulinemia (Fletcher 1985). Although corticosterone administration to these rats reversed most of the effects of adrenalectomy, it had no effect on food intake. Fletcher (1985) suggests that adrenal-derived factors, other than corticosterone, may be necessary for full expression of the obesity

syndrome. Although lean body mass of the obese rats was not reported, it may be assumed that if body weight and body fat is normal in adrenalectomized fa/fa rats, then lean body mass must also be normal. Therefore, adrenalectomy can correct many of the symptoms of the obesity of the Zucker rat.

In contrast to the theory that adrenal malfunction may cause obesity in the Zucker rat, Bray and Fisler (1985) suggest that a hypothalamic defect may cause the obesity, in which elevated corticosterone levels are necessary for the full expression of the symptoms. This theory is supported by the fact that elevated corticosterone levels result in obesity in other rats, not just the genetically obese Zucker rat (Sclafani 1984). Furthermore, adrenalectomy of ventromedial hypothalamus lesioned rats will prevent the characteristic increases in body fat and food intake (Bruce et al. 1982). Also, hypophysectomy slows the progress of obesity in the Zucker rat (Powley and Morton 1976). This evidence suggests that defective hypothalamic regulation may be the primary cause of obesity in the Zucker rat.

The hypothesis that adrenal gland malfunction is an underlying factor in the obesity of Zucker rats is inconsistent with the characteristics of female Zucker rats. The ability of female Zucker rats to achieve normal or above-normal lean body mass suggests that either protein synthesis is increased or protein catabolism is decreased in these rats. Both of these implied conditions are the opposite of the

effects of high plasma corticosterone levels in rats. Because both male and female rats become obese, the underlying metabolic defect is apparently the same in both sexes. Therefore, either adrenal gland malfunction is not an underlying factor in the obesity or the obese female rat overcomes some of the effects of adrenal gland malfunction, thereby maintaining normal lean body mass despite the development of obesity.

# EXPERIMENT 1: EFFECTS OF EXERCISE ON GROWTH AND 3-METHYLHISTIDINE EXCRETION OF FEMALE ZUCKER RATS

## Introduction

The obese Zucker rat is a mutant, first reported by Zucker and Zucker (1961). Because body fat content is measurably greater in homozygotes than in heterozygote siblings by 13 days of age (Bell and Stern, 1977), and the adipose tissue is hypertrophic-hyperplastic, the Zucker rat is a useful model of juvenile onset obesity in humans. Obese Zucker rats are fat because of a metabolic pattern in which an abnormally high proportion of energy is converted to fat rather than to lean tissue. Although the metabolic defect has not been identified, the use of treadmill exercise as a stress to lean tissue may clarify the nature of this defect.

Forced exercise in small laboratory animals is used to determine the effects of physical training on growth, body composition, protein catabolism and other physiological characteristics. Male rats respond to forced exercise by decreasing food intake, weight gain and lean body mass (Dohm et al. 1977a, Schiable et al. 1981). Exercised male rats also increase whole body protein breakdown even though protein synthesis is unchanged (Tapscott et al. 1982). In contrast to male rats, young female rats respond to exercise by increasing food intake and lean body weight and gaining weight at the same rate as sedentary controls (Nance et al. 1977, Dohm and Beecher 1981, Tapscott et al. 1982). Exercise had

no significant effect on protein synthesis or degradation in female rats (Tapscott et al. 1982). This lack of effect in the female rat may partly explain the opposite growth responses to exercise by male and female rats.

Few reports of the effects of forced exercise in Zucker rats are available. Lean and obese male Zucker rats respond to exercise in the same way as other strains with respect to food intake, weight gain and lean body mass (Wardlaw 1984). Treadmill training of 7 and 12 week old obese female Zucker rats produced no change in food intake (Becker-Zimmerman et al. 1982, Wardzala et al. 1982) or skeletal muscle mass (Becker-Zimmerman et al. 1982) and only a slight decrease in body weight (Becker-Zimmerman et al. 1982, Wardzala et al. 1982) compared to sedentary obese female rats.

The urinary excretion of 3-methylhistidine (3-MH) has been widely used as an indicator of muscle protein breakdown (Ward and Buttery 1980, Young and Munro 1978). Long-term treadmill training of male rats resulted in increased 3-MH excretion compared with that of sedentary controls (Kasparek et al. 1980). However, no data are available on 3-MH excretion by treadmill trained female rats.

The purpose of the present study was to determine if lean and obese female Zucker rats respond to exercise as normal female rats do and to determine the effects of exercise on the obesity and muscle protein breakdown rate of female Zucker rats.

## Methods

Female lean and obese Zucker rats were obtained from the animal breeding colony of the Food and Nutrition Department of Iowa State University, either at weaning or at 6 weeks of age. The rats were caged individually in a room lighted from 0600 to 1800 hours with a temperature range of 22-24 C. Standard pelleted laboratory animal diet and water were provided ad libitum.

Rats in exercised groups were forced to exercise daily on a treadmill. The treadmill consisted of eight compartments, with a shock grid at the rear of each, suspended above a motor driven tread. By the end of the conditioning period, the rats ran willingly without the use of electric shock. During an initial ten-day conditioning period, the duration and speed of the treadmill exercise was gradually increased until the full intensity of the training program was reached. The training program consisted of daily exercise from 80 days of age to 120 days of age for 30 minutes at 17 meters/min and 8 degrees incline for lean rats. It was found that obese rats had great difficulty in attempting to run at 17m/min; therefore, for obese rats, treadmill speed was decreased to 12 m/min. The duration of exercise was adjusted as necessary to compensate for the greater body weight of the obese animals by the following formula: Duration for obese rats(min) = (Avg. B.W.lean)x(17m/min)x(30min)/ (Avg. B.W. obese)x(12m/min).

All rats were weighed daily, before exercise for the exercised groups. Five days before the last day of exercise, all rats were caged

in metabolism cages and fed the control 3-MH-free diet (Table 1). Food intake was measured for the last three days of exercise and a 24 hour urine collection was completed on the last day of exercise. All rats were killed on the last day of exercise.

Carcass weight was measured after decapitation and evisceration. Carcasses were homogenized in a Waring blender with an equal weight of ice water. Carcass fat was determined on duplicate aliquots of carcass homogenate by hexane extraction on a Goldfisch extraction apparatus (Mickelsen and Anderson 1959). Carcass lean was calculated as the difference between carcass weight and carcass fat. Percent carcass fat was calculated as the percent of carcass weight as carcass fat. Urinary 3-methylhistidine was determined on urine collected during the last 24 hours of the study. Before analysis, urine samples were hydrolyzed in 6N HCl at 100C for 24 hours. Urine samples were analyzed by the Biochemistry-Biophysics Department, Iowa State university, using a Durrum D-400 amino acid analyzer with a 0.6 cm x 27 cm Durrum DC-6A column. A flow rate of 32 ml/hr and temperature of 61C were used with detection of post-column ninhydrin derivatives. The following buffer solutions were used for 3-MH elution. Buffer A (0.2N lithium citrate with 1.2N lithium chloride adjusted to a pH of 4.5) for 65 minutes. Buffer B (0.2N lithium hydroxide) for 40 minutes, then the column was reequilibrated with Buffer A for 50 minutes. 3-MH was eluted at 83 minutes and total run time was 125 minutes. Analysis of variance was used for all statistical analyses (Steel and Torrie 1980).

TABLE 1. Composition of 3-MH-free diet [a]

| | g/100g diet |
|---|---|
| Casein | 20.0 |
| DL-Methionine | 0.3 |
| Briggs Salt Mix | 5.0 |
| AIN Vitamin Mix | 2.0 |
| Safflower Oil | 5.0 |
| Fiber (Cellufil) | 3.7 |
| Cornstarch | 64.0 |

[a]All ingredients purchased from United Biochemical Corporation.

All experimental animals and proposed experimental proceedures involving the animals were given prior approval by the Laboratory Animal Facilities Committee.

## Results

### Weight gain and food intake

Exercise had no significant effect on body weights of either lean or obese rats during the full exercise program (Figure 2). However, during an initial 10 day conditioning period, exercise decreased weight gain of both obese and lean rats (Table 2). During the period of full exercise, exercise significantly ($p<0.05$) decreased the total weight gain of obese rats but not of lean rats. As shown in Table 2, food intake was not affected by exercise. As expected, the food intake, when expressed as g/100g body weight/day was lower for obese rats than for lean rats.

### Body composition and organ weights

Lean rats responded to exercise by increasing lean carcass weight. In contrast, exercise resulted in a slight, and not significant, decrease in lean carcass weight of obese rats (Table 3). Lean carcass weight of lean sedentary rats was significantly ($p<0.005$) less than that of lean exercised rats and of both groups of obese rats. Exercise decreased the percent carcass fat of lean rats, but not of obese rats (Table 3). Heart, liver and spleen weights were unaffected by exercise

in either lean or obese rats. However, the organ weights of all obese rats were significantly ($p<0.001$) greater than those of all lean rats. This genotype effect was evident in absolute organ weights (Table 4) and when organ weights were expressed relative to lean carcass weight (Table 5).

Urinary 3-methylhistidine

As shown in Table 6, both exercised and sedentary obese rats excreted significantly ($p<0.001$) greater amounts of 3-MH than either exercised or sedentary lean rats. Exercise significantly ($p<0.01$) decreased urinary 3-MH excretion in lean rats but had no effect in obese rats.

## Discussion

Weight gain and food intake

Young female exercised rats normally increase food intake and gain weight at a rate equal to that of sedentary female rats (Nance et al. 1977, Dohm and Beecher 1981, Tapscott et al. 1982). Lean female exercised and sedentary rats in this study also gained weight at similar rates (Table 2). Although the exercised obese rats gained less weight than sedentary obese rats, the final body weight of exercised and sedentary obese rats was not significantly different because of the large variation in body weight of the obese rats (Table 3). The decreased weight gain during conditioning in both exercised groups has not been reported by other investigators (Table 2). However, data on

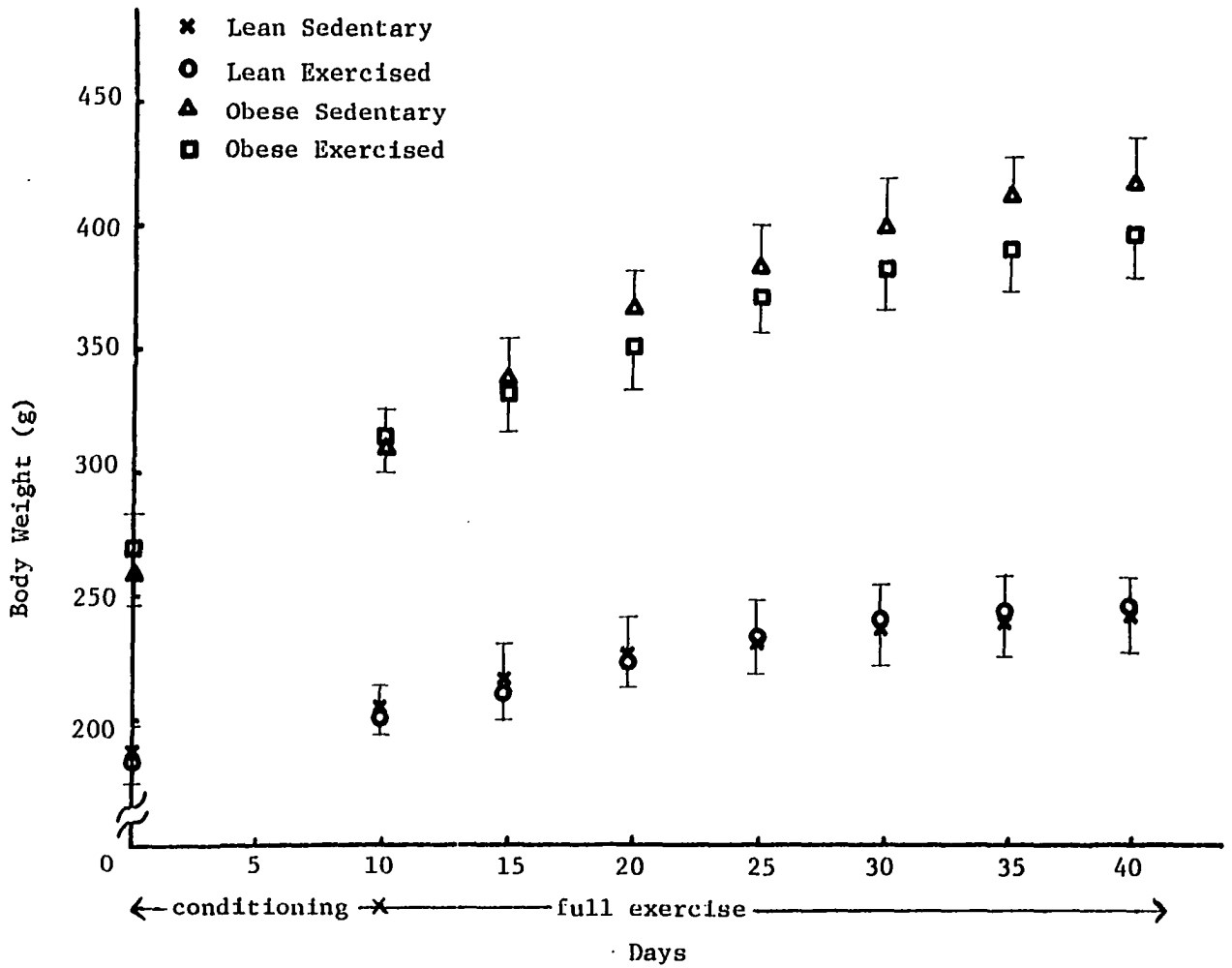


FIGURE 2. Body weights of exercised and sedentary female Zucker rats

TABLE 2. Weight gain and food intake of exercised and sedentary female Zucker rats

| Genotype | Treatment[a] | Weight Gain Conditioning | Total Weight Gain Full Exercise | Food Intake |
|---|---|---|---|---|
| | | g | g | g/100g bw/day |
| Lean | Sed | 20.8$^{A}$ | 35.6$^{A}$ | 6.6$^{A}$ |
| Lean | Exer | 15.0$^{B}$ | 41.3$^{A}$ | 6.5$^{A}$ |
| Obese | Sed | 50.3$^{C}$ | 106.0$^{B}$ | 5.2$^{B}$ |
| Obese | Exer | 41.8$^{C}$ | 83.9$^{C}$ | 5.2$^{B}$ |
| L.S.D.[b] | | 3.8 | 7.6 | 0.4 |

[a] n=12 for all groups.

[b] Least Significant Difference.

A-C Different superscript within column indicates significant difference ($p < 0.001$).

the conditioning period (if any) have not been separated from the data on full training presented in other studies of exercised female rats.

Although female rats generally increase food intake in response to exercise, no such response was evident in this study, possibly because the rats were no longer rapidly growing when food intake was measured. However, in a study of 10 week old female Zucker rats, exercise increased the food intake of lean rats but had no effect on the food intake of obese rats (Wardzala et al. 1982). This inability of exercise to change food intake of obese female Zucker rats has also been reported in 8 and 25 week old rats (Becker-Zimmerman et al. 1982).

Body composition and organ weights

Lean rats responded normally to exercise with decreased percent carcass fat (Table 3). In contrast, the percent carcass fat of obese rats was unchanged by exercise. The obese Zucker rat defends its high body fat content despite food restriction (Bray et al. 1973) and jejunoileal bypass surgery (Greenwood et al. 1982). Furthermore, exercise had little effect on the obese body composition of obese male Zucker rats (Wardlaw 1984, Seelbach et al. 1985). Therefore, the inability of exercise to decrease the high body fat content of obese female Zucker rats is consistent with the resistant nature of the obesity.

Lean female rats responded normally to exercise by increasing lean carcass weight (Table 3). However, exercise did not change the lean

TABLE 3. Body composition in exercised and sedentary female Zucker rats

| Genotype | Treatment[a] | Final Body Weight | Carcass Lean | Carcass Fat |
|---|---|---|---|---|
| | | g | g | % |
| Lean | Sed | $243.5^{A}$ | $173.5^{A}$ | $14.3^{A}$ |
| Lean | Exer | $247.0^{A}$ | $192.0^{B}$ | $5.9^{B,C}$ |
| Obese | Sed | $419.0^{B}$ | $191.0^{B}$ | $45.7^{D}$ |
| Obese | Exer | $398.0^{B}$ | $184.5^{B}$ | $44.5^{D}$ |
| L.S.D.[b] | | 16.9 | 12.9 | 2.9 |

[a] n=12 for all groups.

[b] Least Significant Difference.

A-D Different superscript within column indicates significant difference ($p < 0.01$).

carcass weight of obese rats. The inability of exercise either to decrease carcass fat or increase lean carcass weight of obese rats supports the theory that their basic metabolic lesion is related to defective regulation of lean tissue growth.

The lean carcass weight of all obese rats was greater than that of lean sedentary rats. This genotypic difference is consistent with other studies in which obese female Zucker rats had above-normal carcass protein content (Radcliffe and Webster 1979, Walberg et al. 1984). These results are similar to the characteristics of ovariectomized rats. Ovariectomized sedentary rats have greater lean body mass than that of intact sedentary rats (Dohm and Beecher 1981, Harris et al. 1984). Furthermore, both obese female Zucker rats (Table 3) and ovariectomized rats (Dohm and Beecher 1981) fail to increase lean body mass in response to exercise. This evidence supports the suggestion that obese female Zucker rats have subnormal estrogen status.

Obese Zucker rats have been reported to have enlarged livers (Bray and York 1979, Cleary and Vasselli 1981). The increased liver weight is due not only to an increase in fat deposition but also to an increased protein content (Cleary and Vasselli 1981, Kaminski et al. 1984). The higher protein:DNA ratio in the liver of obese rats may be related to their increased lipogenic activity in the liver (Cleary and Vasselli 1981). The ability of exercise to decrease liver weight of obese female rats (Table 4) may reflect a decrease in energy available for lipogenesis. However, in a study of 10 week old female Zucker rats,

TABLE 4. Organ weights of exercised and sedentary female Zucker rats

| Genotype | Treatment[a] | Heart | Liver | Spleen |
|---|---|---|---|---|
| | | mg | g | mg |
| Lean | Sed | 780.0[A] | 8.18[A] | 432.0[A] |
| Lean | Exer | 837.0[A] | 8.54[A] | 439.0[A] |
| Obese | Sed | 964.0[B] | 14.42[B] | 542.0[B] |
| Obese | Exer | 962.0[B] | 12.98[B] | 516.0[B] |
| L.S.D.[b] | | 61.4 | 1.08 | 67.3 |

[a] n=12 for all groups.

[b] Least Significant Difference.

[A-B] Different superscript indicates significant difference ($p < 0.001$).

exercise did not change the liver weight of obese rats although it did increase the liver weight of lean rats (Wardzala et al. 1982). The failure of exercise to decrease liver weight in 10 week old obese female rats is similar to the lack of effect of exercise on liver weights of male obese rats (Deb and Martin 1975). It is likely that 10 week old obese female rats have not yet reached sexual maturity (Bray and York 1979). The different stage of sexual maturity of the rats studied by Wardzala may explain the different responses of obese Zucker rats to exercise.

The increased heart weight of exercised male and female rats compared to sedentary rats has been well-documented (Dohm and Beecher 1981, Schiable et al. 1981). The lean female Zucker rats of this study responded normally to exercise with a slight increase in heart weight (Table 4). In contrast, exercise did not increase heart weight of obese rats. Male Zucker rats, whether lean or obese, have been shown to respond to exercise by increasing heart weight relative to lean carcass weight; however, absolute heart weight did not increase (Wardlaw 1984). In another study, the heart weight of 10 week old obese female rats was also unaffected by exercise (Wardzala et al. 1982). The greater heart weight of both male and female obese rats compared to lean rats may suggest that the heart is already so stressed with maintenance of blood flow to a larger circulatory system that the added stress of exercise did not result in further hypertrophy of the heart. Alternatively, the metabolic defect of the obese Zucker rat may prevent heart hypertrophy from occurring.

TABLE 5. Organ weight to lean carcass weight ratio in exercised and sedentary female Zucker rats

| Genotype | Treatment[a] | Heart | Liver | Spleen |
|---|---|---|---|---|
| | | (mg/100g CL)[b] | (g/100g CL)[b] | (mg/100g CL)[b] |
| Lean | Sed | 452[A] | 4.72[A] | 251[A] |
| Lean | Exer | 438[A] | 4.45[A] | 229[A] |
| Obese | Sed | 509[B] | 7.61[B] | 284[B] |
| Obese | Exer | 522[B] | 7.02[B] | 276[B] |
| L.S.D.[c] | | 30 | 0.42 | 28 |

[a] n=12 for all groups.

[b] Carcass lean.

[c] Least Significant Difference.

[A-B] Different superscript indicates significant difference ($p < 0.001$).

Urinary 3-methylhistidine

This is the first report of urinary 3-MH excretion of female Zucker rats (Table 6). Other investigators have reported that male lean and obese Zucker rats excrete similar amounts of 3-MH (Dunn and Hartsook 1980, Houtz and Hartsook 1982). However, when these data are expressed as μ-moles 3-MH/100g lean body weight, the obese male rat excretes more 3-MH than its lean counterpart does because of the subnormal lean body weight of male obese Zucker rats. In this study, even though obese female Zucker rats maintained lean carcass weight similar to that of lean rats, urinary excretion of 3-MH was higher in the obese rats.

Increased excretion of 3-MH and urea by male rats and humans after a bout of exercise to exhaustion has been reported (Dohm et al. 1982a). Endurance training in humans results in decreased 3-MH concentrations in muscle, plasma and urine, suggesting a decrease in myofibrillar protein breakdown, although whole body protein breakdown increases (Radha and Bessman 1983, Rennie et al. 1981). Endurance trained male rats increase 3-MH excretion (Kasparek et al. 1980) as well as whole body protein breakdown (Tapscott et al. 1982). In contrast, exercise did not increase nitrogen excretion or whole body protein degradation in female rats (Tapscott et al. 1982).

This study indicates that lean female rats decrease 3-MH excretion and presumably decrease myofibrillar protein breakdown in response to endurance training. In contrast, the excretion of 3-MH in obese female

TABLE 6. Urinary excretion of 3-MH of exercised and sedentary female Zucker rats

| Genotype | Treatment | n | 3-MH |
|---|---|---|---|
| | | | (nM/100g CL)[a] |
| Lean | Sed | 7 | 902[A] |
| Lean | Exer | 5 | 796[B] |
| Obese | Sed | 7 | 1022[C] |
| Obese | Exer | 5 | 1073[C] |
| L.S.D.[b] | | | 64 |

[a]Carcass lean.

[b]Least Significant Difference.

[A-C]Different superscript indicates significant difference ($p < 0.001$).

rats is unaffected by exercise. In lean female rats, the ability of exercise to increase lean body weight may be related to its ability to decrease 3-MH excretion. The inability of exercise to increase lean carcass weights and heart weights of obese female Zucker rats appears to support the concept that the metabolic defect of obese Zucker rats is related to abnormal regulation of lean tissue growth. However, the ability of the obese female Zucker rat to maintain above-normal lean carcass weight despite elevated 3-MH excretion suggests elevated protein synthesis rates, which may be a consequence of subnormal estrogen status.

# EXPERIMENT 2: EFFECTS OF ETHYNYL ESTRADIOL ON BODY COMPOSITION, GROWTH AND 3-METHYLHISTIDINE EXCRETION OF FEMALE ZUCKER RATS

## Introduction

In normal adult female rats, removal of endogenous estrogen by ovariectomy results in hyperphagia and body fat gain (Kakolewski et al. 1968, Tarttelin and Gorski 1973), both of which can be reversed by estrogen administration (Landau and Zucker 1976, Hervey and Hervey 1981). Ovariectomy of normal rats also increases lean body mass compared to that of intact female rats (Dohm and Beecher 1981, Harris et al. 1984).

In general, estrogen administration to intact normal rats decreases body weight, carcass fat and lean carcass weight by decreasing food intake (Wade 1976, Wade and Gray 1979). However, estrogens can decrease lean carcass weight without reducing food intake (Wade 1976). Estrogen administration usually decreases overall protein synthesis (Robertson 1967, Gaafar et al. 1973), although it may also induce the synthesis of specific proteins.

Many of the characteristics of the obese female Zucker rat are similar to those of ovariectomized rats, thus suggesting subnormal estrogen status. These characteristics include hyperphagia, obesity and above-normal lean body mass (Radcliffe and Webster 1976, 1979). Furthermore, exercise did not increase lean carcass weight of obese female Zucker rats (see discussion, Experiment 1). This lack of

response to exercise is similar to that of exercised ovariectomized rats (Dohm and Beecher 1981).

If subnormal estrogen status is a factor in the differences in body composition (especially lean carcass weight) between lean and obese female Zucker rats, then estrogen administration may normalize some of these differences. The purposes of this study are to determine; 1) the response to estrogen feeding in both genotypes, 2) the magnitude of any response with respect to increasing estrogen dosages and 3) the relative sensitivity of lean and obese female Zucker rats to estrogen feeding.

## Methods

Female lean and obese Zucker rats were obtained from the animal breeding colony of the Food and Nutrition Department of Iowa State University at 8 weeks of age. Rats were caged individually under the same conditions described in Experiment 1. At ten weeks of age, rats were assigned to the following groups;

Group 1 - Lean, control diet

Group 2 - Lean, control diet plus 50 ug ethynyl estradiol (EE)/kg diet

Group 3 - Lean, control diet plus 100 ug EE/kg diet

Group 4 - Lean, control diet plus 200 ug EE/kg diet

Group 5 - Obese, control diet

Group 6 - Obese, control diet plus 50 ug EE/kg diet

Group 7 - Obese, control diet plus 100 ug EE/kg diet

Group 8 - Obese, control diet plus 200 ug EE/kg diet

The composition of the control diet is given in Table 1. Ethynyl estradiol was added to the control diet by first mixing it with sucrose (1:1000), then the sucrose-ethynyl estradiol mixture was added to the diet, and mixed thoroughly with the dry ingredients. Approximately 1 liter of tap water per Kg of dry diet was added and mixed to a consistent slurry. The mixture was quickly transferred to waxed paper lined trays, allowed to solidify, scored into squares and dried in a forced-air drying oven at 50 C for 48 hours.

After one week of the experiment, food intake was measured to confirm that EE feeding had not significantly altered food intake, thereby altering the EE dosage received. Rats were weighed three times weekly on an Ohaus balance model 700. During the last two days of the experiment, rats were housed in individual metabolism cages and 24 hour urine samples were collected. After the collection period, the metabolism funnels and screens were washed down with distilled water. The urine was filtered, measured and stored in tightly capped sample bottles at 0 C. All rats were killed on the day the urine collection ended. Carcass analyses were performed as described in Experiment 1.

Urinary 3-MH was determined using High Pressure Liquid Chromatography by the method of Wassner et al. (1980) with the following equipment: Beckman model 110A HPLC, LDC/Milton Roy fluoroMonitor III filter fluorescence detector (254 nm excitation, 418-700 nm emission); Altex Ultrasphere ODS column, 4.6 x 25mm, 5u particle size; DuPont permaphase ODS guard column. Adaptation of this method to rat urine

samples required that the urine was first hydrolyzed in 6N HCl at 120 C. for 3 hours to free the acetylated form of 3-MH present in rat urine, then neutralized with 3N NaOH. The sodium borate buffer was prepared to a pH of 9.5 rather than 11.5 as used by Wassner et al. (1980). After fluorescamine derivatives were prepared, samples were not neutralized before injection onto the HPLC column. A gradient of 25% to 40% Acetonitrile-Sodium Phosphate Buffer at 1.5 ml/min starting 5 minutes after injection for 15 minutes was used as the mobile phase.

## Results

### Body weight and weight gain

Ethynyl estradiol (EE) feeding significantly ($p<0.001$) decreased the final body weights of both lean and obese female Zucker rats compared to that of non-EE-fed counterparts (Figure 3). Although final body weight declined linearly with increasing EE dosage in both genotypes, the magnitude of this decline was greater in obese rats than lean ones.

Both lean and obese female Zucker rats decreased weight gain in response to EE feeding when compared to non-EE fed counterparts (Table 7). However, EE-fed obese female Zucker rats decreased weight gain at an earlier age and at lower EE levels than lean rats did.

### Body composition and organ weights

Lean rats responded to EE feeding by significantly ($p<0.05$) decreasing percent carcass fat to 60% of that of non-EE-fed lean rats

(Figure 5). However, the percent carcass fat of EE-fed and non-EE-fed obese female Zucker rats were nearly identical (Figure 5).

Lean carcass weight of non-EE-fed obese rats was significantly ($p<0.0005$) greater that that of non-EE-fed lean rats (Figure 4). Estrogen feeding reduced significantly ($p<0.0001$) lean carcass weight in both lean and obese rats. However, EE feeding affected lean carcass weight more markedly in obese female Zucker rats. In lean rats receiving the highest EE dosage, lean carcass weight decreased to 90% of that of non-EE-fed counterparts. However, in obese rats receiving the highest EE dosage, lean carcass weight decreased to 80% of that of non-EE-fed obese rats.

Heart weight (Table 8) and heart weight relative to lean carcass weight (Figure 6) decreased linearly with increasing EE dosage in both lean and obese female Zucker rats. Liver weight (Table 8) and liver weight relative to lean carcass weight (Figure 7) decreased with EE feeding in both lean and obese rats and this decrease was of greater magnitude in obese rats. Estrogen fed lean rats decreased liver weight relative to lean carcass weight to 95% of that of non-EE-fed lean rats and EE-fed obese rats decreased liver weight relative to lean carcass weight to 65% of that of non-EE-fed obese rats. Estrogen feeding had no effect on spleen weight (Table 8) or spleen weight relative to lean carcass weight (Figure 8) of lean rats. In contrast, EE-fed obese rats significantly ($p<0.0001$) decreased spleen weight and spleen weight to lean carcass weight compared to that of non-EE-fed obese rats.

Urinary 3-methylhistidine excretion

Non-EE-fed obese female Zucker rats excreted significantly ($p<0.001$) greater amounts of 3-methylhistidine (3-MH) than non-EE-fed lean rats did (Table 9). However, EE feeding did not alter urinary 3-MH excretion of either lean or obese rats.

## Discussion

Body weight and weight gain

In this study, estrogen feeding decreased weight gain (Table 7) and final body weight (Figure 3) of both lean and obese rats. These results are consistent with the known effects of estrogen administration in rats (Wade 1975, 1976). Estrogen inhibited weight gain at an earlier age and at a lower dose in obese rats than in lean rats. Estrogen also lowered final body weight of obese rats more than that of lean ones. These findings indicate a greater sensitivity of obese rats than of lean ones to estrogen, thus suggesting that obese female Zucker rats have subnormal estrogen status.

It has been suggested that estrogen deficiency increases body weight by altering a metabolic "set point" through an unknown mechanism. This conclusion was drawn from a study in which high body weight ovariectomized rats were more sensitive to estrogen administration than their low body weight counterparts (Zucker and Antoniades 1972). This evidence may appear to explain the increased sensitivity to EE feeding of obese female Zucker rats. However, the high body weight rats in the

TABLE 7. Weight gain of EE-fed lean and obese female Zucker rats (n=9)

| Genotype | Treatment | Weekly Weight Gain (g) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Lean | 0 | $21.1^{A}$ | $24.5^{A}$ | $13.5^{A}$ | $8.2^{A}$ | $5.0^{A}$ | $6.5^{A}$ | $7.7^{A}$ | $4.8^{A}$ |
| | 50 | $19.8^{A}$ | $20.0^{A,B}$ | $13.6^{A}$ | $4.5^{B}$ | $2.3^{A}$ | $3.0^{A}$ | $6.7^{A}$ | $2.1^{A}$ |
| | 100 | $21.5^{A}$ | $14.3^{B,C}$ | $8.8^{B}$ | $4.3^{B}$ | $2.8^{A}$ | $4.5^{A}$ | $6.4^{A}$ | $3.5^{A}$ |
| | 200 | $16.9^{A}$ | $11.5^{C}$ | $10.4^{A,B}$ | $3.4^{B}$ | $4.5^{A}$ | $0.7^{B}$ | $3.2^{B}$ | $4.2^{A}$ |
| Obese | 0 | $46.8^{B}$ | $46.8^{D}$ | $45.0^{C}$ | $30.3^{C}$ | $24.0^{B}$ | $21.9^{C}$ | $15.7^{C}$ | $15.3^{B}$ |
| | 50 | $36.3^{C}$ | $43.2^{D,E}$ | $35.3^{D}$ | $22.2^{D}$ | $20.8^{C}$ | $18.2^{C}$ | $13.0^{C,D}$ | $12.7^{B}$ |
| | 100 | $35.9^{C}$ | $38.7^{E}$ | $29.4^{E}$ | $19.0^{D,E}$ | $21.4^{C}$ | $14.9^{D}$ | $12.7^{D}$ | $13.3^{B}$ |
| | 200 | $25.7^{D}$ | $28.2^{F}$ | $26.1^{E}$ | $16.1^{E}$ | $16.7^{D}$ | $16.1^{C,D}$ | $12.2^{D}$ | $13.4^{B}$ |
| L.S.D.[a] | | 5.1 | 6.0 | 4.7 | 3.7 | 2.8 | 3.8 | 3.0 | 8.0 |

[a]Least Significant Difference.

$^{A-F}$Different superscripts within column indicates significant difference ($p < 0.05$).

experiment of Zucker (1972) were obtained by decreased litter size from birth to weaning. Rats treated in this manner achieve sexual maturity earlier than control rats do. In contrast, obese female Zucker rats reach sexual maturity later than lean rats do (Bray and York 1979). Furthermore, sensitivity to estrogen was more highly correlated with age than with body weight (Zucker and Antoniades 1972). This evidence suggests that factors other than high body weight may be responsible for the greater sensitivity to estrogen of obese female Zucker rats.

In response to the "set point" theory, Wade and Gray (1979) suggest that increased body weight and food intake associated with estrogen deficiency are consequences, rather than causes, of metabolic shifts. It has been shown that estrogen deficiency (ovariectomy) increases adipose tissue lipoprotein lipase levels, which in turn increases body fat storage (Ferreri and Naito 1978). These characteristics of ovariectomized rats are similar to those of obese female Zucker rats (which may have subnormal estrogen status) (Gray and Greenwood 1984).

It is unlikely that subnormal estrogen status is responsible for the shifts in lipid metabolism in the obese Zucker rat. The effects of ovariectomy in normal rats last only a few weeks, then food intake, body weight gain and lipoprotein lipase levels return to near normal (Tarttelin and Gorski 1973, Ferrari and Naito 1978). In contrast, food intake, body weight gain and lipoprotein lipase levels remain above-normal throughout the life of obese Zucker rats (Bray and York 1979). Furthermore, estrogen administration prevents the effects of ovariectomy

TABLE 8. Organ weights of EE-fed lean and obese Zucker rats (n=9)

| Genotype | Treatment | Heart Weight | Liver Weight | Spleen Weight |
|---|---|---|---|---|
| | | g | g | mg |
| Lean | 0 | $0.87^{A}$ | $7.1^{A}$ | $375.0^{A}$ |
| | 50 | $0.85^{A}$ | $6.5^{A}$ | $377.0^{A}$ |
| | 100 | $0.78^{B}$ | $6.3^{A}$ | $367.0^{A}$ |
| | 200 | $0.72^{C}$ | $5.9^{A}$ | $350.0^{A}$ |
| Obese | 0 | $1.27^{D}$ | $20.1^{B}$ | $663.0^{B}$ |
| | 50 | $1.08^{E}$ | $12.6^{C}$ | $476.0^{C}$ |
| | 100 | $1.00^{F}$ | $11.9^{C}$ | $437.0^{C}$ |
| | 200 | $0.91^{G}$ | $10.4^{D}$ | $380.0^{D}$ |
| L.S.D.[a] | | 0.06 | 1.58 | 41.51 |

[a]Least Significant Difference.

[A-G]Different superscripts within column indicates significant difference ($p < 0.05$).

in normal rats (Wade and Gray 1979). In contrast, ovariectomy, with or without estrogen, or estrogen administration alone, have little effect on food intake, body fat content or lipoprotein lipase levels in obese female Zucker rats (Gale and VanItallie 1979, Gray and Greenwood 1984). However, the lack of effect of estrogen status on food intake and body fat regulation in the obese female Zucker rat does not rule out the possibility of an estrogen effect on protein metabolism in these rats.

Body composition and organ weights

Lean rats responded normally to EE feeding by decreasing total carcass fat compared to non-EE-fed controls. However, although EE-fed obese rats had reduced total carcass fat, the percent carcass fat (Figure 5) and, therefore, the degree of obesity, remained similar to that of non-EE-fed obese rats. The failure of estrogen to normalize the body composition of the obese rats is consistent with the results of other studies which showed that these rats remained obese despite a variety of treatments. These include food restriction (Bray et al. 1973, Cleary et al. 1980), treatment with a fatty acid synthesis inhibitor (Greenwood et al. 1981), and jejunoileal bypass surgery (Greenwood et al. 1982) as well as exercise (see discussion, Experiment 1).

Estrogen administration reduced lean carcass weight in both lean and obese rats (Figure 4). This is consistent with the effects of estrogen in other rat strains. It does not appear to be the same as the

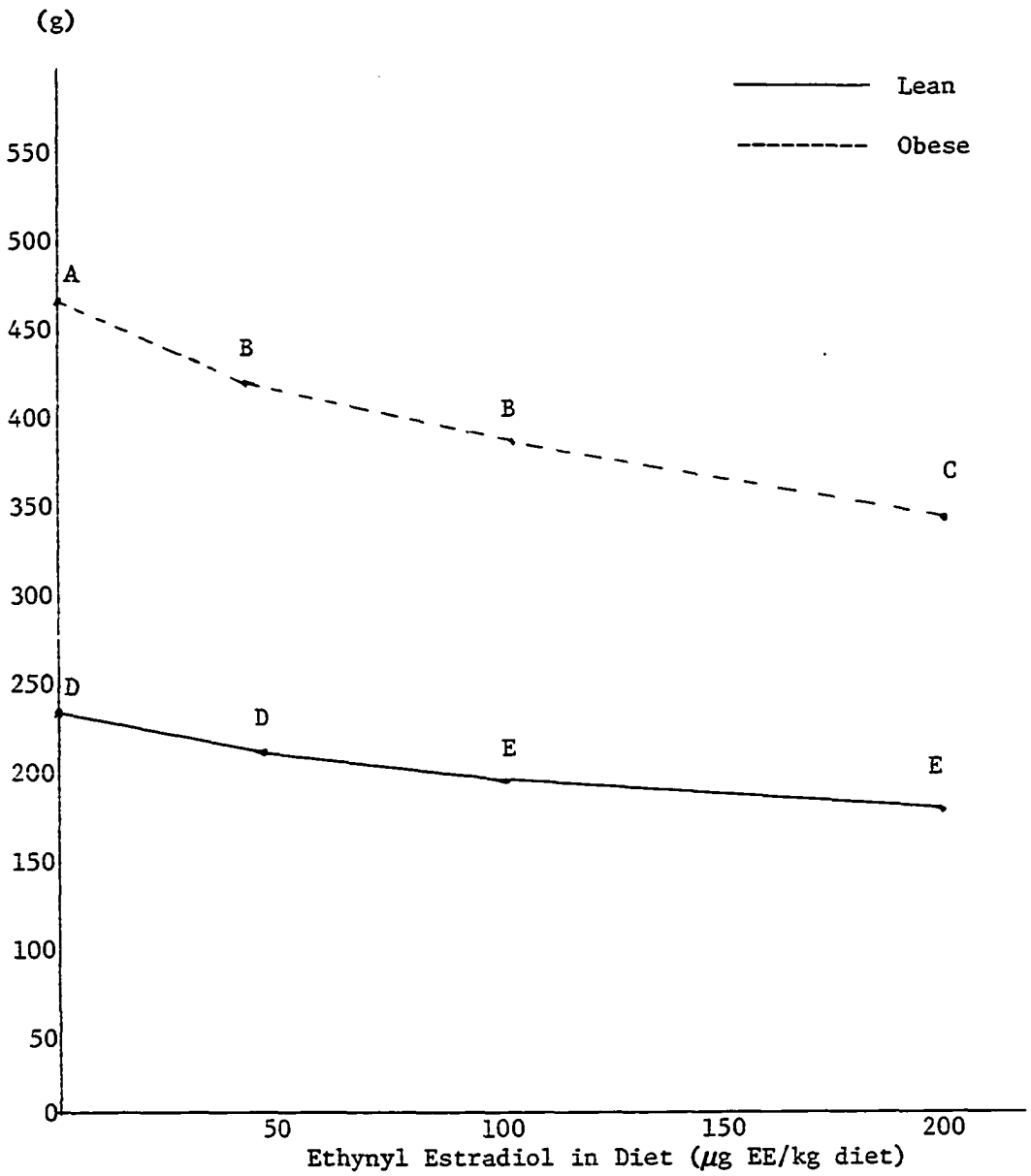


FIGURE 3. Final body weight of EE-fed lean and obese female Zucker rats (Least Significant Difference = 22.64. A-E, different superscript indicates significant differences, $p < 0.001$)

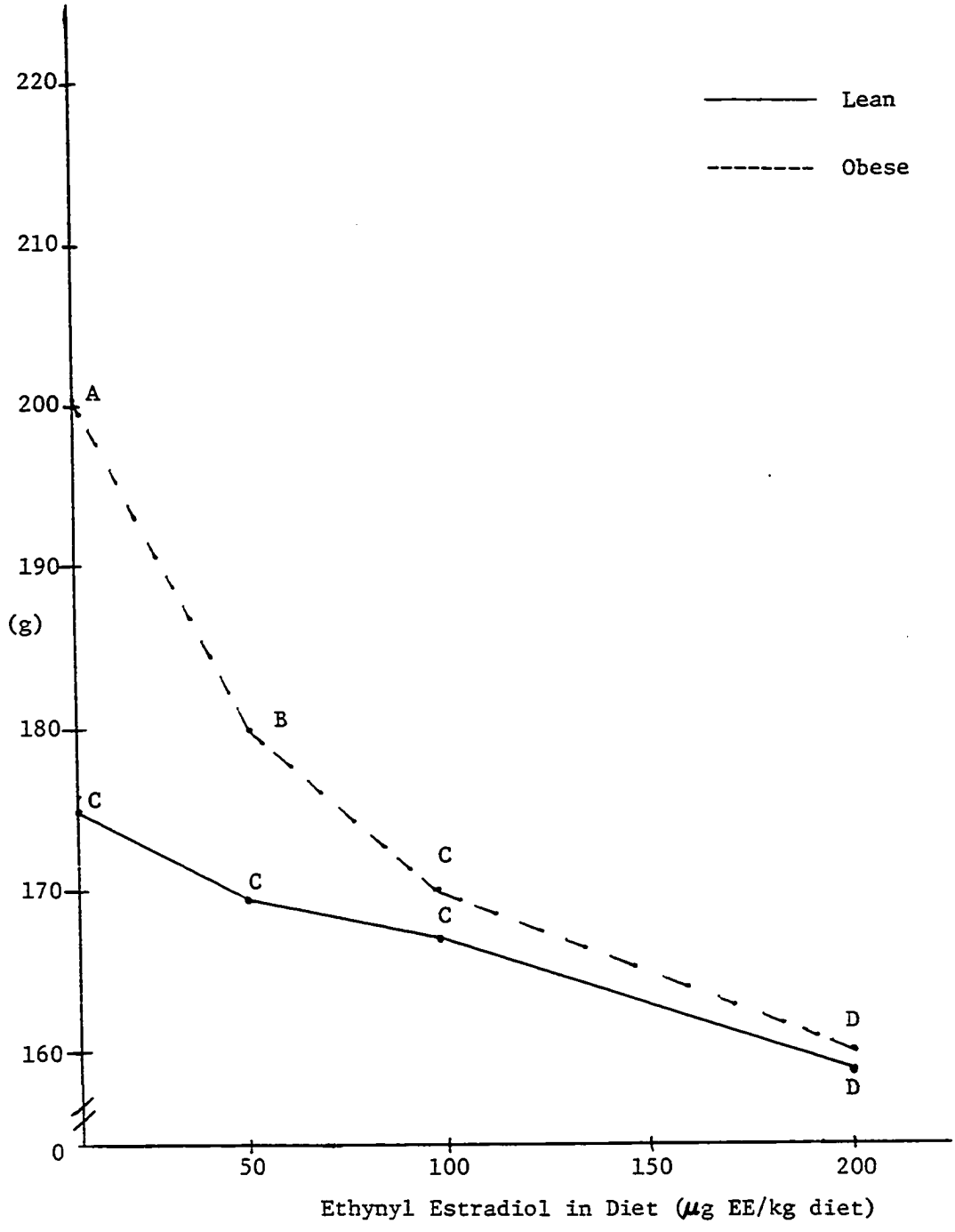


FIGURE 4. Lean carcass weight of EE-fed female Zucker rats (Least Significant Difference = 10.06. A-D, different superscript indicates significant difference, $p < 0.001$)

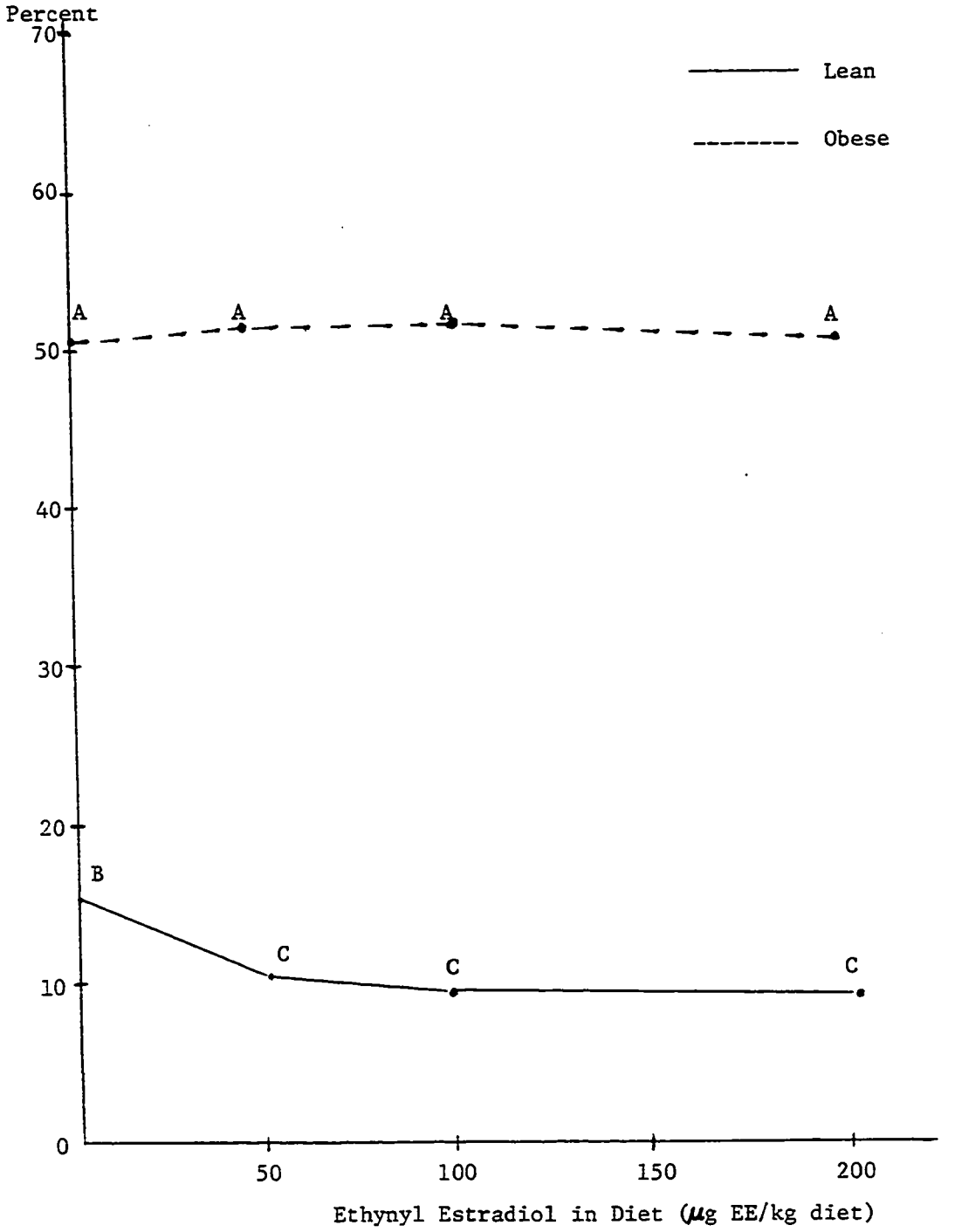


FIGURE 5. Percent carcass fat of EE-fed female Zucker rats (Least Significant Difference = 1.81. A-C, different superscript indicates significant difference, $p < 0.001$)

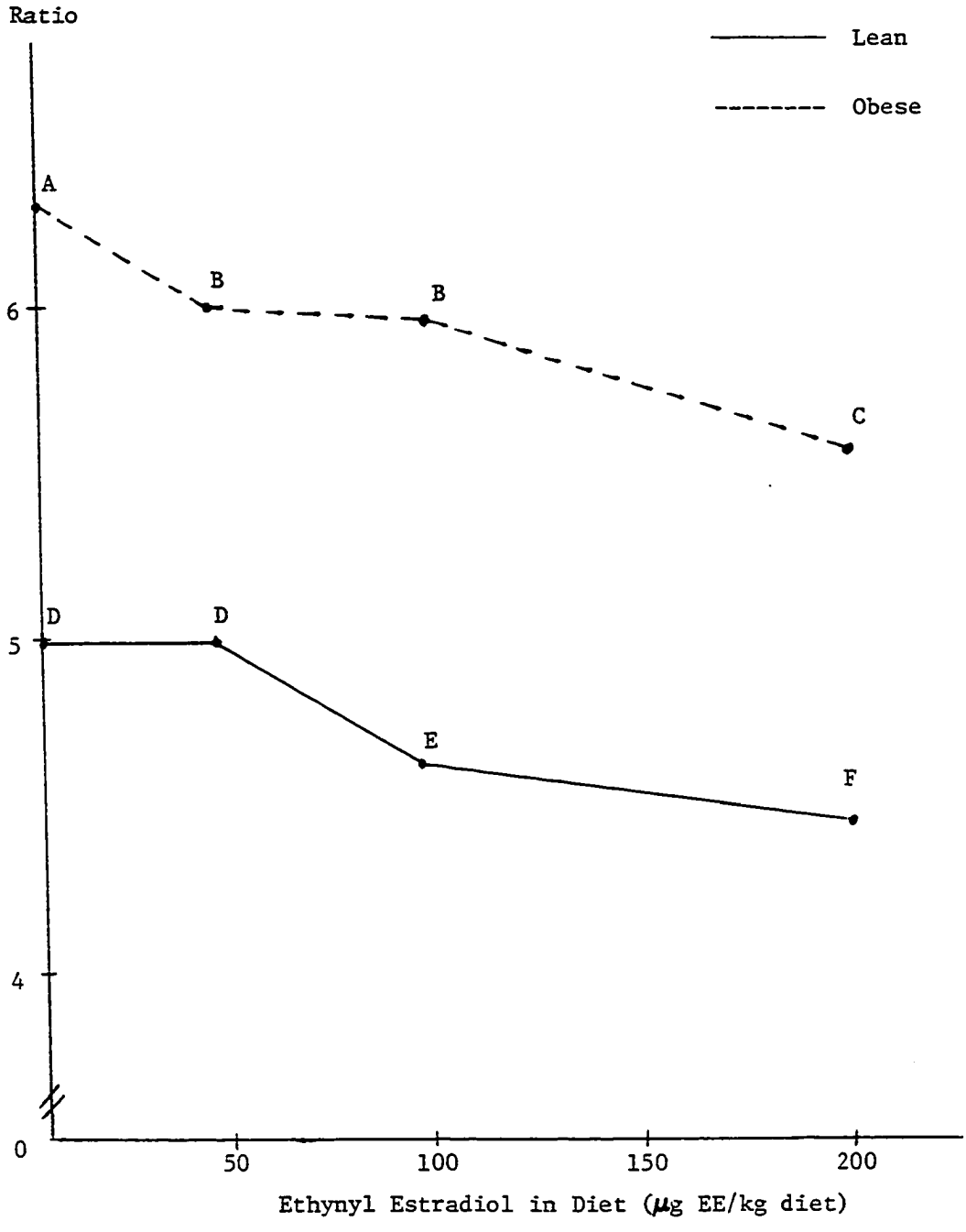


FIGURE 6. Heart weight to lean carcass weight ratio of EE-fed female Zucker rats (Least Significant Difference = 0.01. A-F, different superscript indicates significant difference, $p < 0.001$)

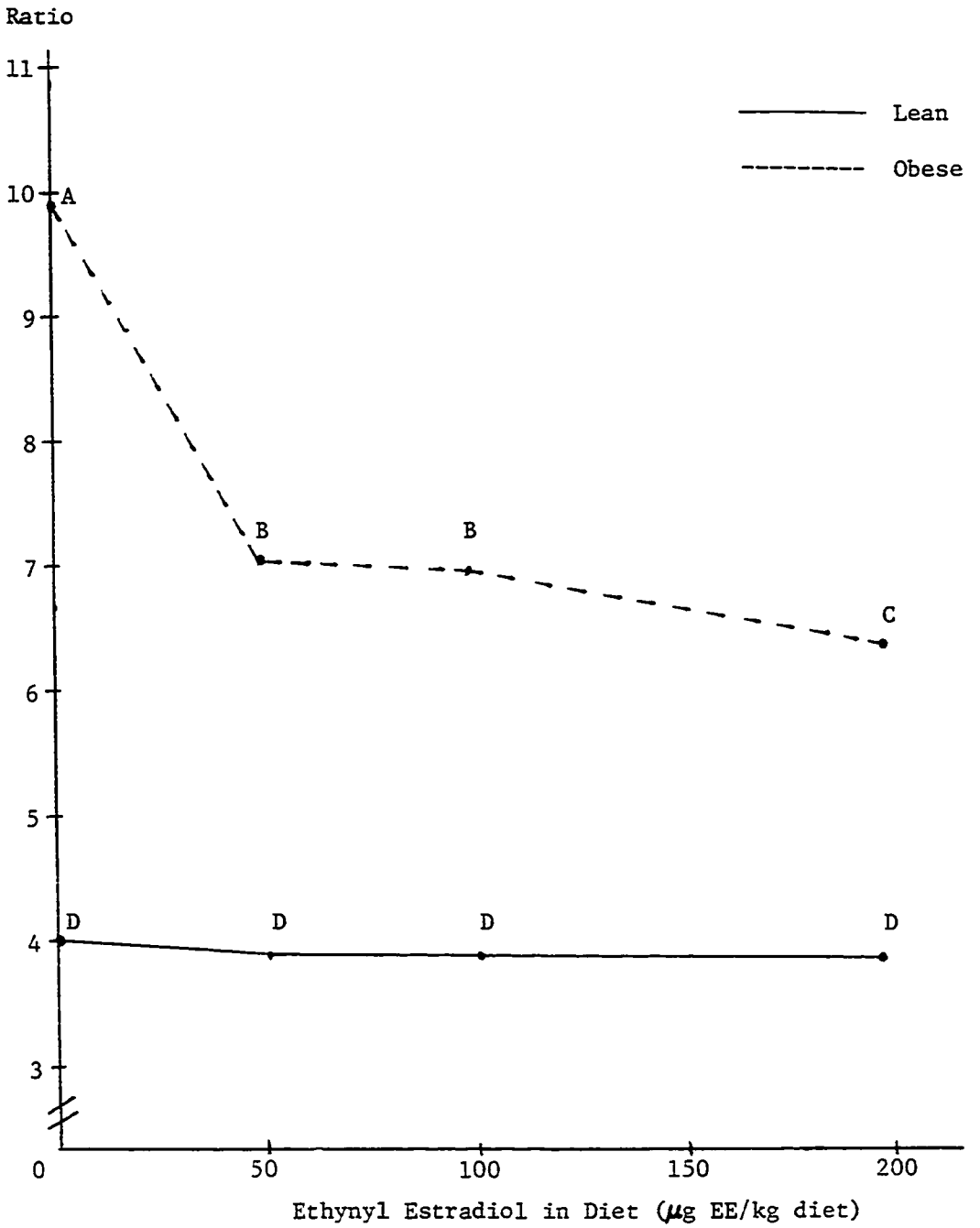


FIGURE 7. Liver weight to lean carcass weight ratio of EE-fed female Zucker rats (Least Significant Difference = 0.21. A-D, different superscript indicates significant difference, $p < 0.0001$)

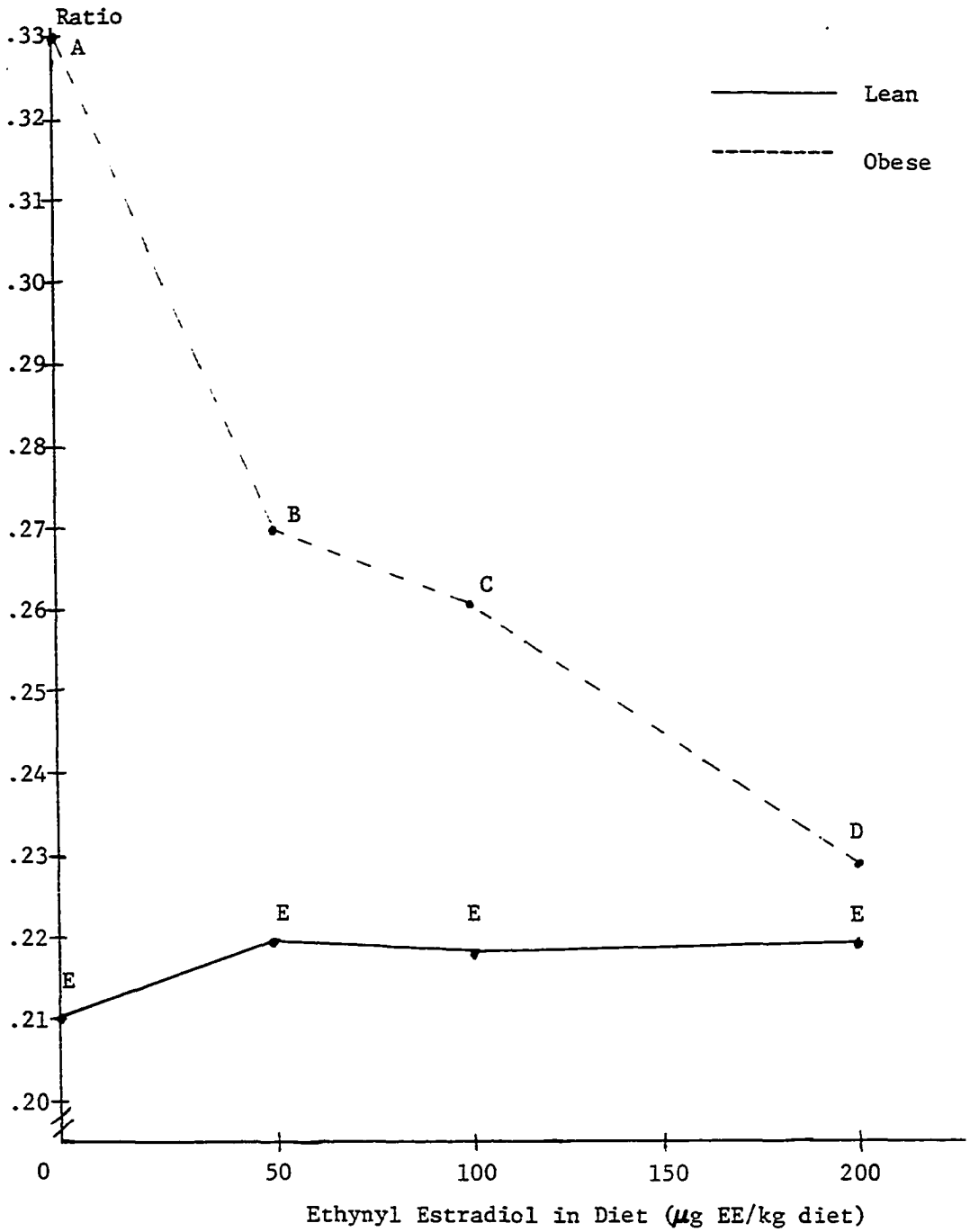


FIGURE 8. Spleen weight to lean carcass weight ratio of EE-fed female Zucker rats (Least Significant Difference = 0.22. A-E, different superscript indicates significant difference, $p < 0.001$)

TABLE 9. Urinary 3-MH excretion of EE-fed lean and obese female Zucker rats

| Genotype | EE Level | Urinary 3-MH nM/100 g Carcass Lean |
|---|---|---|
| Lean | 0 | 781[A] |
| | 200 | 812[A] |
| Obese | 0 | 1102[B] |
| | 200 | 1150[B] |
| L.S.D.[a] | | 93 |

[a]Least Significant Difference.

[A-B]Different superscript indicates significant difference ($p < 0.001$).

effect of simple food restriction. Although pair feeding of obese female Zucker rats somewhat reduced weight gain and carcass fat content, the fat-free carcass weight of the pair-fed rats remained similar to that of ad libitum fed obese rats (Bray et al. 1973). Only when weight gain was abolished by further reducing food intake (to two-thirds that of lean rats) was fat-free carcass weight reduced (to 75% of that of ad libitum fed obese rats). In contrast, pair-feeding of obese male Zucker rats to lean controls resulted in a significant reduction of lean carcass weight compared to that of ad libitum fed obese male rats (Milam et al. 1982, Zucker 1967). Therefore, it appears that the obese female Zucker rat is better able to defend lean body weight than is the obese male Zucker rat.

The more marked effect of EE feeding on lean carcass weight of obese rats than on that of lean rats has not been previously reported. These data, like the previously-discussed results on weight gains, support the suggestion that obese female rats have subnormal estrogen status. Estrogen deficiency (ovariectomy) increased lean carcass weight of female rats (Dohm and Beecher 1981, Harris et al. 1984). Thus, subnormal estrogen status may be a contributing factor in the elevated lean carcass weight of obese female rats.

Liver weights of EE-fed rats were lower than those of non-EE-fed rats, regardless of genotype (Figure 7). However, spleen weights of EE-fed rats were lower than those of non-EE-fed rats in obese rats only

(Figure 8). In obese rats, especially, decreased organ weight may merely reflect decreased fat content of the organs. However, the reduction of liver weight of both genotypes was approximately the same at all EE levels. In contrast, the decrease in spleen weight of obese rats is linear with increasing EE dosage. It is unlikely that fat content of organs would respond differently in this manner.

Fat storage in the liver is much more extensive than fat storage in the spleen. Therefore, the potential for decreased fat content is greater in liver than in spleen. However, EE feeding decreased weight of the spleen more markedly than of the liver. Gross visual inspection of spleens from obese rats revealed no visible fat deposition, although such deposition was routinely noted in hearts and livers of obese rats. Furthermore, the total lipid content of livers from adult lean and obese female Zucker rats has been reported as 40 and 59 mg/g liver, respectively (Kaminski et al. 1984). This difference is hardly sufficient to account for the difference in liver weight between genotypes or EE levels. This suggests that protein content, as well as fat content of organs is reduced with EE feeding.

In contrast to the effects of estrogen on liver and spleen, EE feeding decreased heart weight relative to lean carcass weight to a similar degree in both genotypes (Figure 6). This decrease was linear with increasing EE dosage in both genotypes. Thus, estrogen effects varied from organ to organ as well as differing in the two genotypes.

Food restriction of obese female Zucker rats did not significantly decrease the weights of liver, kidney or thyroid gland compared to those of ad libitum fed obese rats (Bray et al. 1973). Therefore, it appears that the estrogen effects in this study cannot be explained by decreased food intake alone.

In contrast to food restricted obese female rats, food restricted obese male Zucker rats decreased the weights and protein contents of liver and kidney (Cleary and Vasselli, 1981). As discussed previously in this section, obese female Zucker rats are better able to defend lean carcass weight under conditions of food restriction than are their male counterparts. Apparently, this ability extends to the organs as well.

Amounts of nutrients available to the cells can be decreased by means other than food restriction. For example, severely limiting nutrient absorption is one of the primary results of jejunoileal bypass surgery. Jejunoileal bypass surgery of obese female Zucker rats decreased carcass protein content as well as weights and protein contents of liver, heart, kidney and muscle compared to that of sham operated counterparts (Greenwood et al. 1982). These results, which are similar to the estrogen effects in the present study, might appear to suggest that food intake restriction could explain the estrogen effects. However, Greenwood et al. (1982) discuss evidence that bypass surgery can produce symptoms of protein malnutrition as well as symptoms of food restriction. This evidence suggests that estrogen treatment may decrease organ weights and lean carcass weight by altering protein metabolism independenly of food intake restriction.

Urinary 3-methylhistidine

The urinary excretion of 3-methylhistidine (3-MH) is widely used as an indicator of muscle protein breakdown (Ward and Buttery 1980, Young and Munro 1978). Obese non-EE-fed rats excreted more 3-MH than their lean counterparts did (Table 9). This finding is consistent with the results of Experiment 1 as well as with the results of other studies of Zucker rats (see discussion, Experiment 1). However, EE feeding had no effect on 3-MH excretion in either genotype. This suggests that the ability of estrogen to decrease lean carcass weight is not accomplished through increasing muscle protein degradation.

In summary, the decreased body weight and weight gain of EE-fed lean and obese rats compared to that of non-EE-fed counterparts are similar to the effects of food restriction. Furthermore, the ability of estrogen to decrease lean carcass weight and organ weights, especially in obese rats, suggests an additional estrogen effect on protein metabolism. The failure of estrogen to change urinary 3-MH excretion in either genotype suggests that the ability of estrogen to decrease lean carcass weight is not accomplished through increasing muscle protein degradation. The greater sensitivity of obese rats to estrogen feeding implies that subnormal estrogen status may be a contributing factor to the elevated lean carcass weight of the obese female rat.

As discussed previously, estrogen administration decreases overall protein synthesis. Therefore, the ability of estrogen to decrease lean carcass weight may be accomplished through decreasing protein synthesis.

In contrast, subnormal estrogen status may increase lean carcass weight through removing an inhibition on protein synthesis. In rats maintaining lean carcass weight, an increase in protein synthesis could reasonably be followed by an increase in protein degradation and, presumably, urinary 3-MH excretion.

# EXPERIMENT 3: DEVELOPMENT AND PROTEIN METABOLISM OF FEMALE ZUCKER RATS

## Introduction

The genetically obese Zucker rat is widely used as a model for juvenile onset obesity (Bray and York 1979). However, the metabolic defect causing the obesity is as yet unknown. Subnormal protein synthesis, which results in increased shunting of nutrients into fat synthesis, has been suggested as an underlying factor in the development of obesity in the Zucker rat. The obese male Zucker rat deposits less body protein than the lean male rat (Pullar and Webster 1974, Dunn and Hartsook 1980). Obese weanling male rats synthesized less muscle protein at 18 and 27 days of age (Reeds et al. 1982). Also, there was no difference in the calculated rate of protein degradation between the genotypes. The authors suggested that this early difference in protein synthesis rate could increase energy available for fat storage in obese rats and, therefore, may in part explain their obesity.

There is conflicting evidence of subnormal protein synthesis in adult male obese Zucker rat. Adult male obese rats injected with $^{14}C$-labeled amino acids deposited a smaller percentage of the total dose as lean tissue than the lean rats did (Dunn and Hartsook 1980). However, in this study, the labelled amino acid dose was based on body weight, not on estimated body protein content. Also, measurement of total tissue $^{14}C$ did not discriminate between free and protein-bound label. In contrast, as a result of measurements of carcass composition and

nitrogen retention, it is suggested that fractional rates of protein deposition are similar in both genotypes in both male and female Zucker rats (Pullar and Webster 1974, Radcliffe and Webster 1979).

The suggestion that subnormal protein synthesis underlies the development of obesity is inconsistent with the characteristics of the female obese Zucker rat. The obese female Zucker rat attains normal or above-normal lean body mass (Radcliffe and Webster 1976, 1978, see discussion Experiment 2). This evidence suggests that either female obese Zucker rats are not subject to an inhibition of protein synthesis or that they are able to compensate for subnormal protein synthesis, thereby maintaining normal lean body mass.

Urinary 3-MH excretion is widely used as an indicator of muscle protein breakdown (Young and Munro 1978, Ward and Buttery 1980). Both male and female adult obese Zucker rats excrete above-normal amounts of 3-MH (Dunn and Hartsook 1980, see results, Experiments 1 and 2). However, the genotypic difference in 3-MH excretion is greater in female than in male Zucker rats. This evidence suggests that changes in rates of muscle protein degradation, as well as protein synthesis, may contribute to the characteristic differences in lean body mass of male and female obese Zucker rats.

The purpose of this experiment was to determine if young obese female Zucker rats exhibit an early inhibition of protein synthesis similar to that of obese male Zucker rats and to determine the effects of obesity on 3-MH excretion in young female Zucker rats.

## Methods

All animals were obtained from the animal colony of the Food and Nutrition Department of Iowa State University. Female lean and obese weanling rats were selected by appearance, at 21 days of age and fed a 3-MH-free diet (Table 1) for 3 days. During the post-weaning period, rats were housed individually in metabolism cages under the conditions described in experiment 1. A second group of female lean and obese rats were maintained within the colony on standard pelleted rat ration (Teklab) until 10 weeks of age. All rats were then housed in metabolism cages under the conditions described above and fed a 3-MH-free diet for three days.

A 24 hour urine sample was collected on all rats, with the collection period ending on the day rats were killed. Metabolism funnels and screens were washed with distilled water and urine was filtered, measured and placed in a tightly capped vial and stored at 0 C. All rats were weighed on an Ohaus balance model 700. Carcass composition was determined as described in Experiment 1.

Determination of Radioactivity in Tissues. Using a modification of the technique reported by Garlick et al (1980), rats were intravenouly injected, via the tail vein, with L-[4-$^{3}$H]phenylalanine solution (1.0 ml/ 100g body weight). For this solution, L-[4-$^{3}$H]phenylalanine (Amersham) was combined with a 150mM unlabled phenylalanine (Sigma) aqueous solution to give 50 uCi/ml.

In the experiment using 10 week old female rats, the dose given to the obese animals was adjusted because of the large difference in body weight and body fat when obese animals are compared to lean animals of the same age. Prior to the experiment, several lean and obese 10 week old female rats were decapitated and percent carcass fat was determined to be approximately 10% and 40% respectively. The formula used for dose correction was as follows: body weight - (body weight x 0.3) / 100 = amount injected (ml).

After injection, the rats were allowed to metabolize the dose for a known amount of time, then decapitated. Liver and hind limb muscle samples were quickly removed, weighed and frozen in liquid nitrogen. Each tissue sample was homogenized with a glass on glass tissue homogenizer in 10% trichloroacetic acid (Fisher) to make a 20% homogenate. The homogenate was washed three times in 10% TCA and three times in 95% ethanol. The remaining protein precipitate was lyophilized using a Virtis Unitrap II freeze drier and weighed on a Mettler H6T balance. The protein precipitate was transferred to a glass scintillation vial and 1.0 ml of NCS tissue solubilizer (Amersham) was added. The vials were incubated at 50 degrees C. for 48 hours to digest the protein. Samples were counted in a Beckman Scintillation Counter using a scintillation solution consisting of 10 ml toluene containing 0.006 percent 2,5-diphenyloxazole and 0.0075 percent p-bis-2'(5'-phenyloxozolyl)benzene (Amersham) plus 5 ml ethanol. Correction for efficiency was made using external standard mode.

Determination of Urinary 3-Methylhistidine. Urinary 3-MH was determined as described in Experiment 2.

## Results

### Body weight and body composition

At 25 days of age, lean and obese female Zucker rats were similar in body weight, carcass weight and lean carcass weight (Figure 9). Percent carcass fat of obese rats was significantly elevated.

At 10 weeks of age, obese female Zucker rats had significantly higher body weight and carcass weight than lean ones (Figure 10), although lean carcass weight was still similar in both genotypes. Percent carcass fat of obese female rats was significantly above normal, comprising nearly half of carcass weight.

### Incorporation of $^{3}$H-phenylalanine

In both lean and obese weanling (25 days old) rats, incorporation of $^{3}$H-phenylalanine into liver protein was approximately 6 times as rapid as for muscle. Lean weanling rats incorporated significantly more $^{3}$H-phenylalanine into muscle protein than obese weanling rats did (Figure 11); the rate of $^{3}$H-phenylalanine incorporation into muscle of obese rats was only 70% of that of lean rats ($p<0.05$). Lean weanling rats also incorporated significantly more $^{3}$H-phenylalanine into liver protein than did the obese ones (Figure 12); the rate of $^{3}$H-phenylalanine incorporation into liver protein of obese rats was only 78% of that of lean rats ($p<0.05$).

In adult (10 week old) rats, as in weanling rats, incorporation of $^{3}$H-phenylalanine into liver protein was approximately 7 times as rapid as for muscle protein in both lean and obese rats. Lean and obese rats incorporated similar amounts of $^{3}$H-phenylalanine into proteins of both muscle (Figure 13) and liver (Figure 14).

Urinary 3-methylhistidine

Obese weanling rats excreted approximately 70% as much 3-methylhistidine (3-MH) as lean rats did (Table 10). The change in 3-MH excretion between 25 days and 10 weeks of age was significantly ($p<0.05$) greater in the lean than obese rats, suggesting that the obese rats had an abnormally low rate of muscle protein catabolism which was normalized by 10 weeks of age.

At 10 weeks of age, lean and obese female Zucker rats excreted almost identical amounts of 3-MH (Table 10). Ten week old lean and obese rats excreted approximately half the 3-MH that their weanling counterparts did.

## Discussion

Body composition

The obese weanling female rats of this experiment deposited above-normal carcass fat (Figure 9). This is consistent with the findings that obese male Zucker rats deposit above-normal body fat by 16 days of age (Bell and Stern 1977). Lean carcass weight was similar in both lean and obese female Zucker rats (Figure 9). In contrast, obese male Zucker

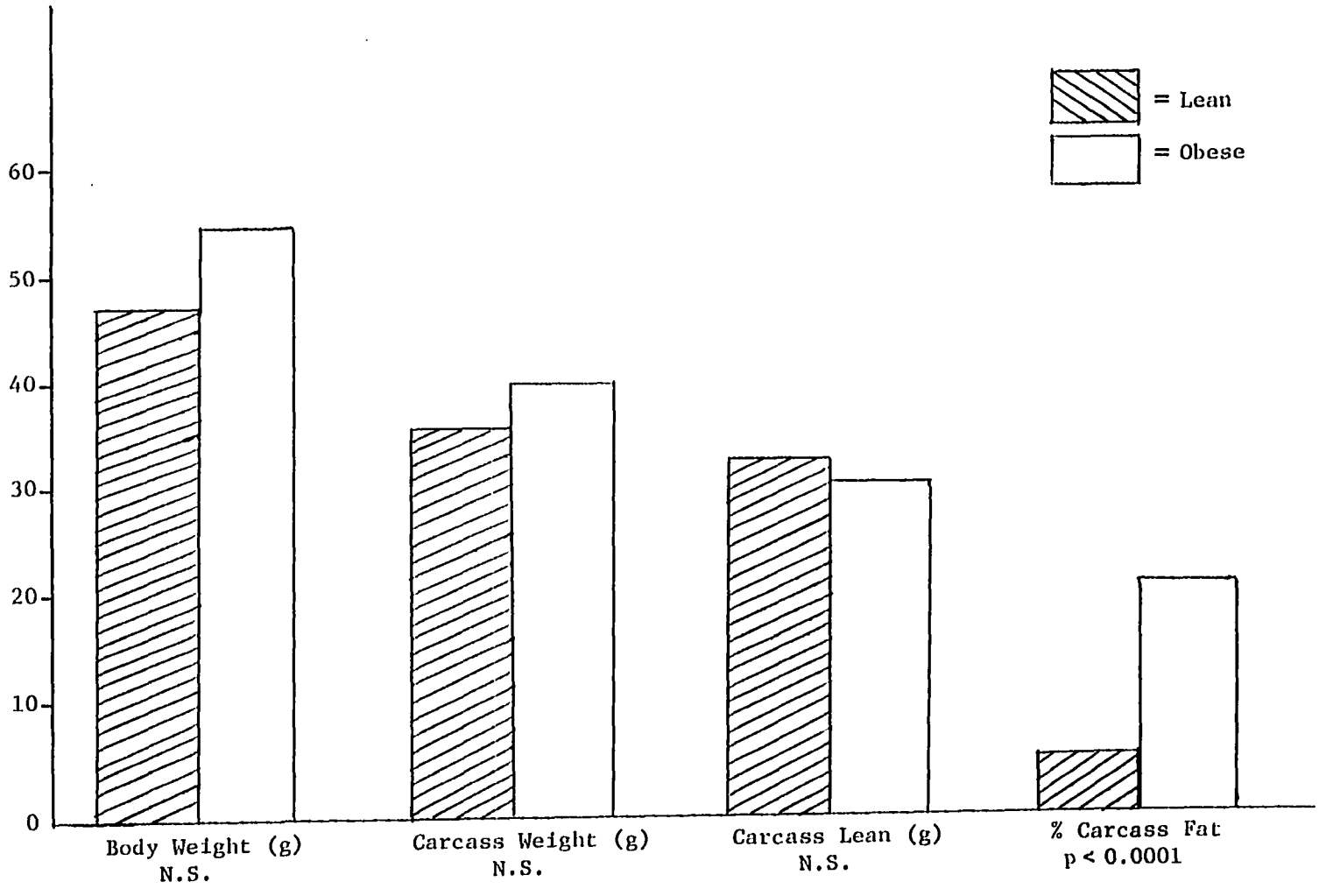


FIGURE 9. Body composition of 25-day-old female Zucker rats

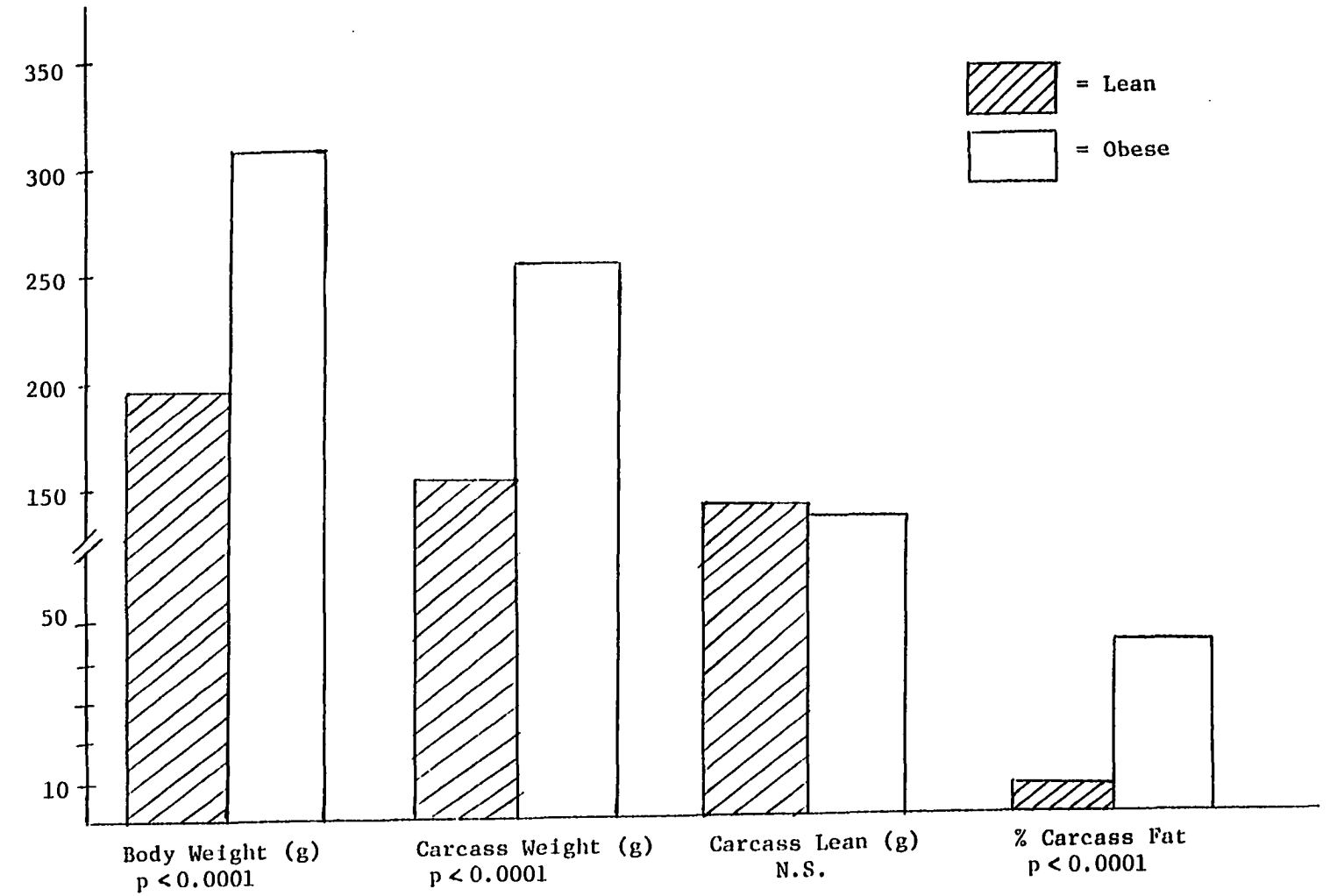


FIGURE 10. Body composition of 10-week-old female Zucker rats

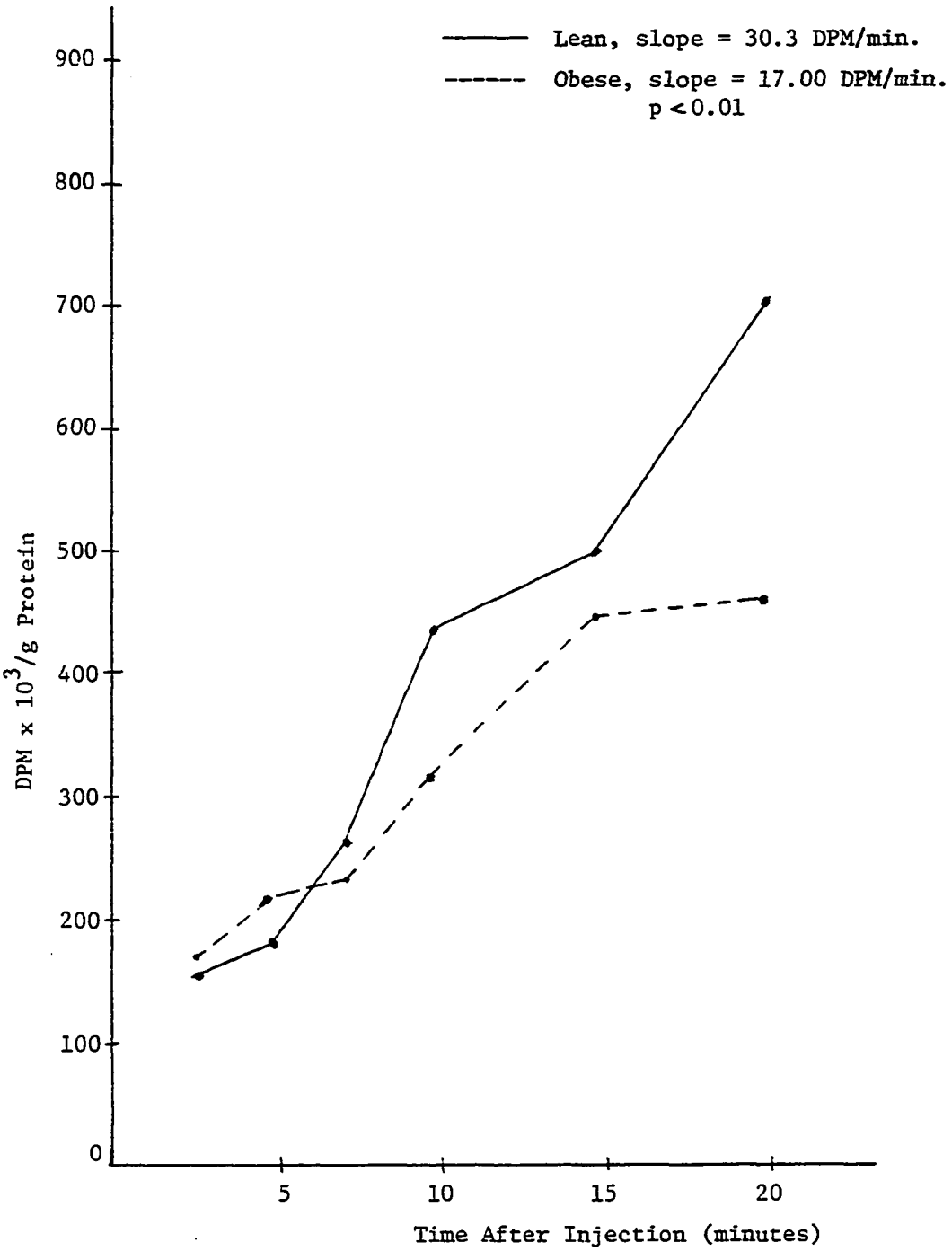


FIGURE 11. Uptake of $^{3}$H-phenylalanine into muscle protein of 25-day-old female Zucker rats

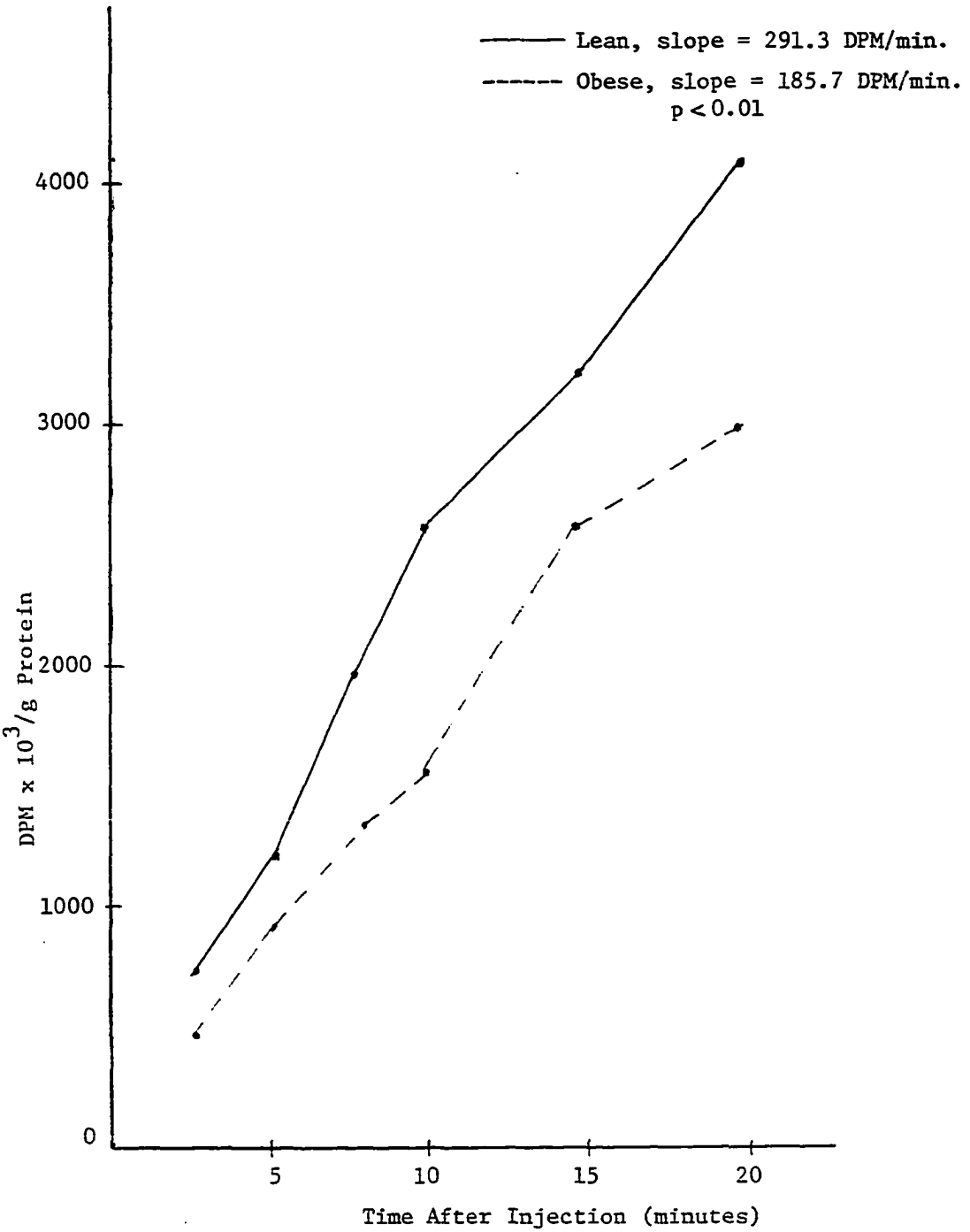


FIGURE 12. Uptake of $^{3}$H-phenylalanine into liver protein of 25-day-old female Zucker rats

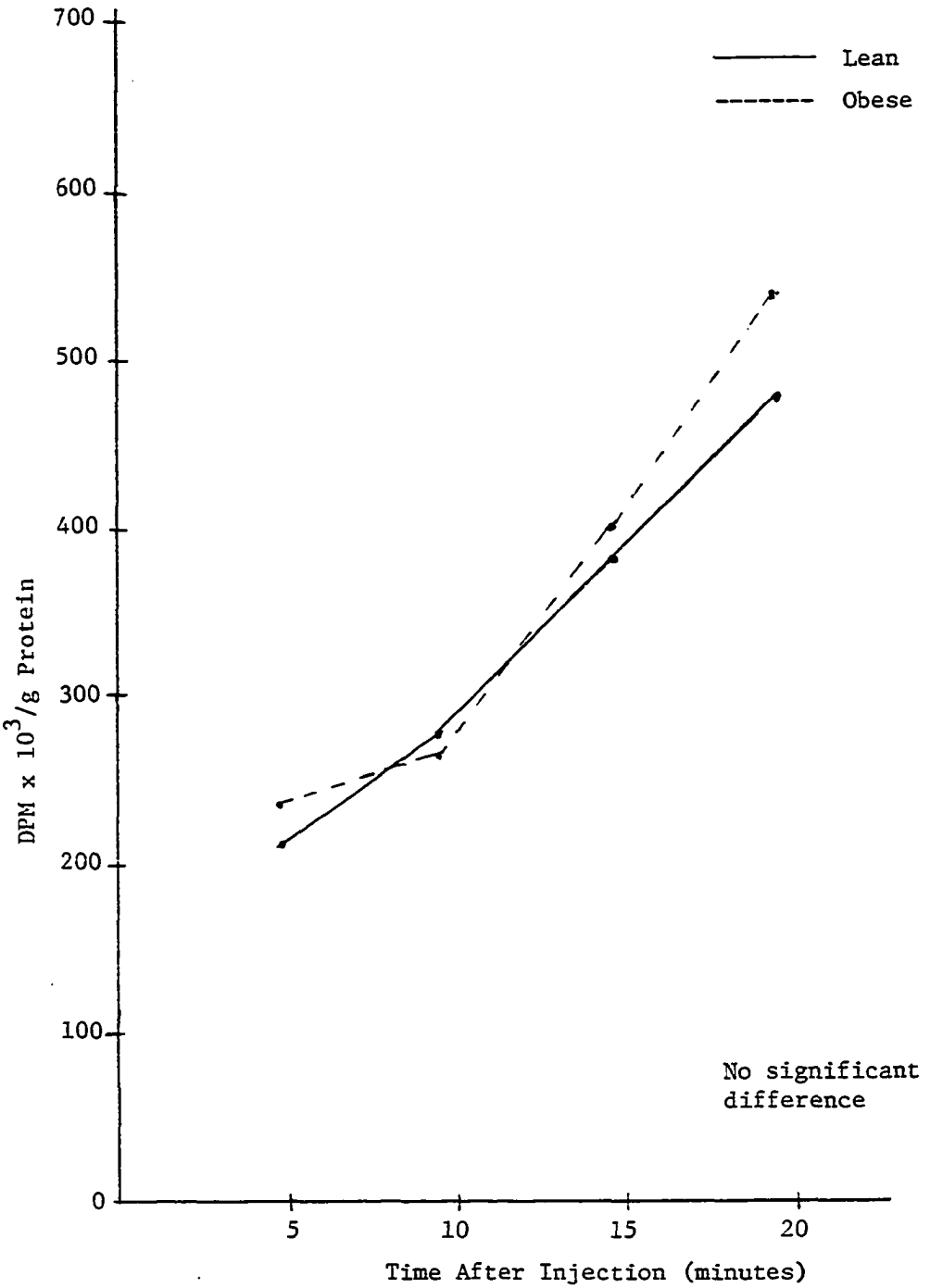


FIGURE 13. Uptake of $^3$H-phenylalanine into muscle protein of 10-week-old female Zucker rats

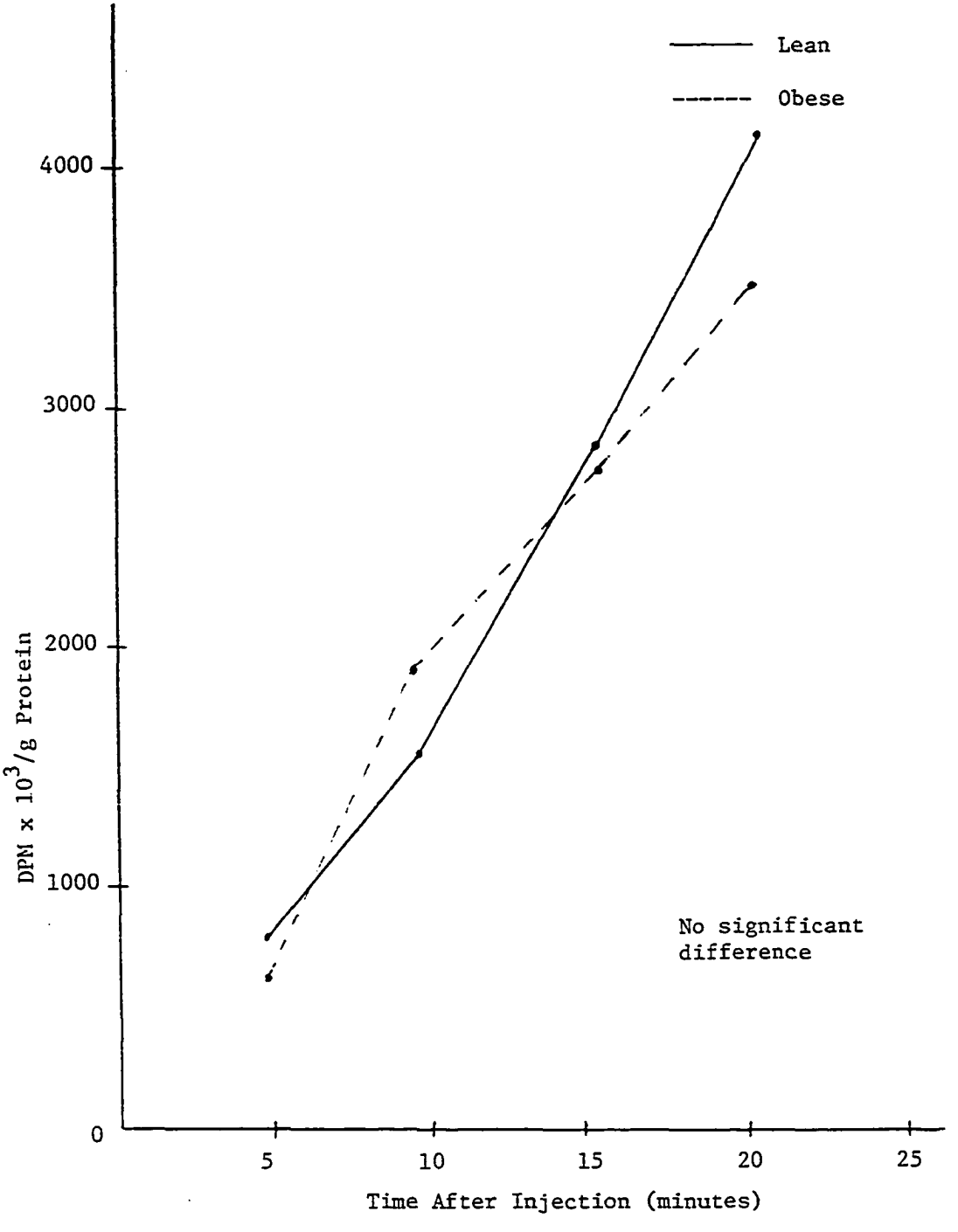


FIGURE 14. Uptake of $^3$H-phenylalanine into liver protein of 10-week-old female Zucker rats

TABLE 10. Urinary 3-MH excretion of female Zucker rats

| Genotype[a] | Urinary 3-MH nM/g Carcass Lean | | Δ |
|---|---|---|---|
| | 25 days of age | 10 weeks of age | |
| Lean | 122 | 45 | 87[A] |
| Obese | 87 | 50 | 37[B] |
| L.S.D.[b] | 37.8 | 14.9 | 26.2 |

[a] n=10 for all group.

[b] Least Significant Difference.

[A,B] Different superscripts within colum indicates significant difference ($p < 0.05$).

rats had subnormal body protein content as early as 23 days of age (Reeds et al. 1982). Therefore, the established pattern of subnormal lean body mass of adult male obese rats and normal or above-normal lean body mass of adult female obese rats is also apparent at weaning.

As expected, 10 week old obese female Zucker rats markedly increased carcass fat deposition compared to that of lean rats (Figure 10). Lean carcass weights of 10 week old lean and obese female rats were similar (Figure 10). These results support earlier reports of normal or above-normal lean body mass in adult obese female Zucker rats (Radcliffe and Webster 1976, 1979). The results of this experiment might appear to conflict with the findings of above-normal lean carcass weight of 16 week old obese female Zucker rats and the proposed relationship to subnormal estrogen status (see discussion, Experiment 2). However, at 10 weeks of age female rats have only recently achieved sexual maturity. The effects associated with subnormal estrogen status may be cumulative with age and therefore may not be apparent soon after sexual maturity.

Incorporation of $^{3}$H-phenylalanine

In obese weanling female Zucker rats, incorporation of $^{3}$H-phenylalanine into both muscle and liver proteins was approximately 70% of that of lean counterparts (Figures 10 and 11). Reeds et al. (1982) found a similar inhibition of muscle protein synthesis in obese weanling male Zucker rats. However, incorporation of $^{3}$H-phenylalinine into liver

protein was similar in both lean and obese weanling male Zucker rats. One reason for the lack of difference in the liver of male rats might be the use of intraperitoneal injection instead of the recommended procedure of intravenous injection (Garlick et al. 1980). Intraperitoneal injection of the labelled amino acid might delay attainment of equilibrium between blood and tissue, thus altering the linear decline of tissue label levels on which the accuracy of this method depends. Also, the possibility of adsorption of the label onto the liver surface, even after rinsing the sample, cannot be discounted. Despite differences in methodology, apparently obese female weanling Zucker rats exhibited an inhibition of protein synthesis similar to that of obese male weanling Zucker rats.

At 10 weeks of age, lean and obese female Zucker rats incorporated similar amounts of $^{3}$H-phenylalanine into muscle proteins (Figure 13). These data are consistent with results of studies of adult male and female Zucker rats in which fractional rates of protein deposition were similar in both genotypes (Pullar and Webster 1974, Radcliffe and Webster 1976, 1979). In contrast, the gastrocnemius muscles of 8 week old obese female rats contained less protein (mg/g muscle) than that of lean counterparts (Lanza-Jacoby and Kaplan 1984). Although these results apparently conflict with my results, it should be emphasized that the protein synthesis rate reflects the immediate protein metabolism, whereas the protein content reflects the earlier protein metabolism of the muscle. Furthermore, the analysis of one muscle

cannot be presumed to accurately represent whole body muscle protein metabolism.

The similar rates of $^{3}$H-phenylalanine incorporation into liver proteins in lean and obese female rats (Figure 14) are consistent with the findings of liver protein content in female Zucker rats. The livers of both male and female adult obese Zucker rats contain more total protein than that of their lean counterparts, primarily because of above-normal liver size (Kaminski et al. 1984, Chanussot et al. 1984). However, mg protein/g liver was decreased in obese male Zucker rats compared to lean rats (Chanussot et al. 1984). In contrast, mg protein/g liver was similar in lean and obese female Zucker rats (Kaminski et al. 1984). Therefore, compared to the obese male Zucker rat, the obese female rat has a greater ability to defend liver protein content as well as lean body mass.

Urinary 3-methylhistidine excretion

Obese weanling female Zucker rats exhibited 3-MH excretion of 75% of that of lean rats (Table 10). In contrast, lean and obese weanling male Zucker rats have similar calculated rates of protein degradation (Reeds et al. 1982). This evidence suggests that unlike their male counterparts, obese weanling female Zucker rats decrease muscle protein degradation to adapt to lower rates of protein synthesis and thereby maintain normal body protein deposition.

At 10 weeks of age, lean and obese female Zucker rats excreted similar amounts of 3-MH (Table 10). This is consistent with other

findings in this study in which lean carcass weights and $^{3}H$-phenylalanine incorporation rates were similar in both genotypes at 10 weeks of age. Therefore, 10 week old obese female Zucker rats apparently adapt to normal protein synthesis rates with a slower decrease in muscle protein degradation with age.

In summary, obese weanling female Zucker rats exhibit an inhibition of protein synthesis similar to that of obese male weanling Zucker rats. However, unlike the obese male rat, the obese female rat apparently maintains lean body mass by decreasing muscle protein degradation to a similar degree. This evidence supports the hypothesis that an early inhibition of protein synthesis may increase the energy available for fat storage.

At 10 weeks of age, lean and obese female Zucker rats synthesize protein and excrete 3-MH at similar rates. The findings of studies on male Zucker rats suggest that lean and obese rats synthesize protein at similar rates at this age (Radcliffe and Webster 1979, Reeds et al. 1982). However, 3-MH excretion of 10 week old male Zucker rats has not been reported. Reeds et al. (1982) suggest that after the early phase of protein synthesis inhibition, obese rats synthesize protein at the normal rate, but cannot further increase protein synthesis to achieve normal lean body mass. The inability of exercise to increase the lean carcass weight of obese female Zucker rats supports the concept that the ability of adult obese Zucker rats to synthesize protein is limited (see discussion, Experiment 1). Therefore, the results of this study support

the hypothesis that protein synthesis inhibition of weanling obese rats is followed by a period of normal protein synthesis in adult obese rats. Furthermore, the ability to decrease muscle protein breakdown allows the obese female Zucker rat to achieve normal lean body mass despite an early period of protein synthesis inhibition.

# GENERAL DISCUSSION

The suggestion that subnormal protein synthesis and adrenal gland malfunction are underlying causes of obesity in the Zucker rat appears to be consistent with the characteristics of the obese male Zucker rat, but not with those of the obese female rat. The characteristics of the obese female Zucker rat which are not consistent with this explanation have been commonly overlooked in the literature. The major question is, if decreased protein synthesis and subnormal lean body mass are underlying factors in the obesity, then how does the female Zucker rat maintain normal or above-normal lean body mass in the presence of obesity? The experimental results of this dissertation provide some explanations for this inconsistency.

The failure of exercise to increase lean carcass weight or alter 3-MH excreton in obese female Zucker rats suggests defective regulation of protein metabolism (see discussion, Experiment 1). Furthermore, the response of obese female Zucker rats to exercise was similar to that of exercised ovariectomized rats, thus suggesting subnormal estrogen status.

The results of Experiment 2 show that subnormal estrogen status may be responsible for the above-normal lean body mass of obese female Zucker rats. Also, the ability of EE feeding to decrease the lean carcass weight in female Zucker rats is not directly mediated through a decrease in muscle protein catabolism.

The results of experiment 3 suggest that obese weanling female Zucker rats experience the same inhibition of protein synthesis that has been demonstrated in obese weanling male rats (Reeds et al. 1982). However, unlike the male rat, the obese weanling female Zucker rat decreased muscle protein breakdown, thereby maintaining normal lean body mass. At 10 weeks of age, obese female rats have normal rates of protein synthesis and 3-MH excretion as well as normal lean body mass (see discussion, Experiment 3). Although the mechanism is unknown, the ability of obese female rats to alter muscle protein breakdown appears to be related to the maintenance of lean body mass.

In contrast to younger rats, 16 week old female Zucker rats have above-normal lean carcass weight despite elevated 3-MH excretion, and presumably elevated muscle protein degradation (see discussion, Experiments 1 and 2). The above-normal 3-MH excretion of obese female rats is consistent with the increased muscle protein degradation found in corticosterone treated normal rats (Millward et al. 1983, Tomas et al. 1984b). However, if both muscle protein breakdown and lean carcass weight are above-normal, then apparently muscle protein synthesis is also elevated in the obese female Zucker rat. This implied elevated protein synthesis rate is inconsistent with the ability of high plasma corticosterone levels to depress protein synthesis in normal rats (Millward 1975).

The subnormal estrogen status of the obese female Zucker rat may be a factor in achieving above-normal lean body mass, despite high plasma

corticosterone levels. Although estrogen feeding decreased lean carcass weight in both genotypes, estrogen feeding had no effect on 3-MH excretion in either lean or obese female Zucker rats (see discussion, Experiment 2). These results suggest that the above-normal lean carcass weight of obese female rats is not a consequence of decreased muscle protein degradation. An alternative explanation is that the above normal lean body mass of obese female rats is achieved through increased protein synthesis. However, this explanation is also inconsistent with the effect of corticosterone on protein synthesis. Therefore, apparently an antagonistic relationship exists between the effects of subnormal estrogen status and high corticosterone levels.

A relationship between subnormal estrogen status and adrenal gland function has been demonstrated. Ovariectomized normal rats increase body weight and food intake. However, the weight gain after castration can be prevented by adrenalectomy (Mook et al. 1972). This effect was postulated to be caused by removal of progesterone which is primarily secreted by the adrenals in the rat. It has been established that estrogen and progesterone have opposite effects on weight gain and food intake in the rat (Wade and Gray 1979). Although the mechanism of action is unknown, it is suggested that the effects of ovariectomy are a consequence of an abnormal estrogen/progesterone ratio. Adrenalectomy of ovariectomized rats apparently corrects this ratio.

Lean female Zucker rats responded normally to ovariectomy and adrenalectomy (Yukimura and Bray 1978). However, ovariectomy of obese

female Zucker rats did not result in increased body weight gain, suggesting that subnormal estrogen status was already present. Furthermore, adrenalectomy of ovariectomized obese female rats markedly reduced food intake and weight gain to below the levels of intact obese female rats. Therefore, the response to ovariectomy and adrenalectomy is different in lean and obese rats. Removal of corticosterone, as well as progesterone, is probably responsible for the more marked effects of adrenalectomy in obese female Zucker rats.

Although lean body mass was not reported in the previously discussed studies, above-normal lean body mass is a consequence of ovariectomy in normal rats (Dohm and Beecher 1981, Harris et al. 1984, Shaw et al. 1983). Therefore, altered estrogen/progesterone ratios may be a factor in the above-normal lean body mass of obese female Zucker rats. As discussed previously, the above-normal lean body mass and 3-MH excretion of obese female rats suggests elevated rates of protein synthesis. Perhaps an altered estrogen/progesterone ratio could overcome the effects of high corticosterone levels in the obese female rat, thus increasing both protein synthesis and degradation.

The cause of subnormal estrogen status in the obese Zucker rat is unknown (Gray and Greenwood 1984). However, the symptoms of subnormal estrogen status develop even when food intake and weight gain are restricted (see review of literature). Furthermore, it has been suggested that the underlying metabolic defect of the Zucker rat may also be responsible for subnormal estrogen status (Gray and Greenwood 1984).

Adrenal gland malfunction as the cause of obesity could be consistent with the development of subnormal estrogen status. As previously discussed, high plasma corticosterone levels decrease protein synthesis in normal rats and may also decrease the synthesis of insulin receptors in the Zucker rat (Czech et al. 1978). Subnormal estrogen status in obese female Zucker rats could be explained by decreased number or function of estrogen receptors, which may be caused by elevated corticosterone levels.

As an alternative to corticosterone and estrogen having a cause and effect relationship, elevated corticosterone levels and subnormal estrogen status may both be independent symptoms of the underlying genetic defect in the Zucker rat. As discussed previously, elevated corticosterone levels results in high body fat in forms of obesity other than that of the Zucker rat (Sclafani 1984). Also, adrenalectomy can prevent obesity even if adrenal gland malfunction is not the priamry cause of obesity, as is the case in VMH lesioned rats (Bruce et al. 1982). It may be that elevated corticosterone levels are another symptom, rather than the cause, of obesity in the Zucker rat. This suggestion is supported by the finding that hypophysectomy also corrects many symptoms of obesity in the Zucker rat (Powley and Morton 1976). It is possible that a receptor mechanism in the hypothalamus is defective, thereby causing widely diversified alterations in metabolism through hormonal changes (Shaw et al. 1983, Bray and Fisler 1985). These changes could include elevated corticosterone levels (via defective ACTH

secretion) as well as subnormal estrogen status (via defective gonadatropin secretion). Therefore, a defective receptor mechanism in the hypothalamus could explain both high corticosterone levels and subnormal estrogen status.

In conclusion, the ability of the obese female Zucker rat to maintain normal lean body mass is apparently related to the ability of the rat to decrease 3-MH excretion early in life (Figure 15). After sexual maturity, the above-normal lean body mass of the obese female Zucker rat is apparently a consequence of subnormal estrogen status, which may result in increased protein synthesis. Furthermore, the characteristics of the obese female Zucker rat are consistent with the hypothesis that defective regulation of metabolism is an underlying factor in the obesity of the Zucker rat.

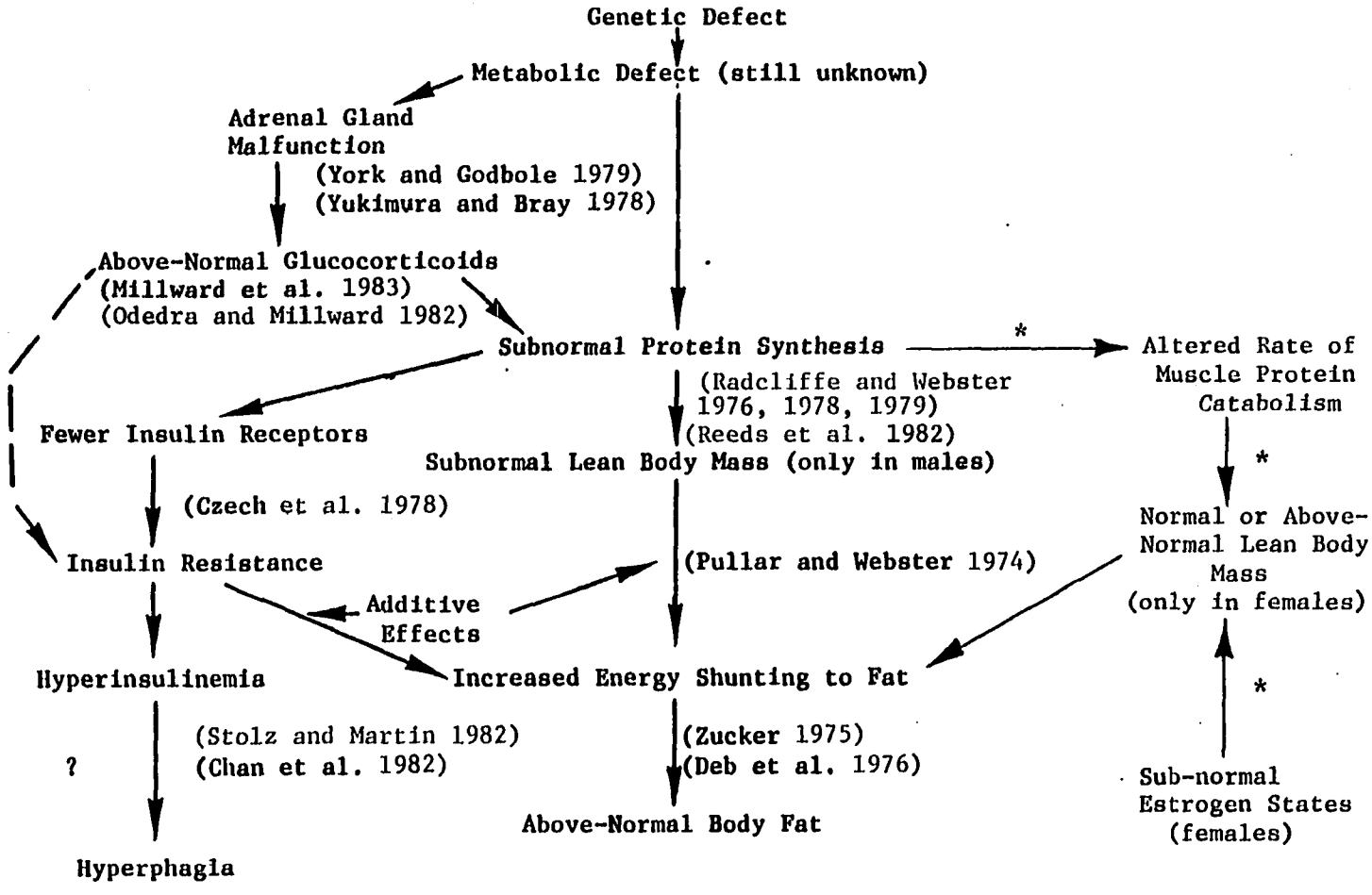


FIGURE 15. Relationships of some characteristics of the Zucker rat to the development of obesity (* relationships suggested by dissertation research)

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