THE EFFECT OF EARLY NUTRITION AND ACTIVITY LEVELS ON THE DEVELOPMENT OF OBESITY IN RATS

by

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INTRODUCTION

The study of obesity is currently the subject of many investigations, particularly in view of the fact that about three million young people in the United States between the ages of 12 and 19 suffer from this disease (1). Add to this, the number of adults and children of other age groups also suffering from obesity and the magnitude of this problem becomes strikingly apparent. In addition there is a larger group of overweight individuals who would not be diagnosed as obese.[1] There are also individuals suffering from overfatness whose actual weight does not vary significantly from the ideal weight for a person of that height and frame, but who nevertheless possess excessive adipose depots.

Life insurance surveys report a greater proportion of our society than ever before are suffering from obesity and related health problems (3,4). The health problems associated with obesity are numerous. Obesity is closely associated with respiratory problems, greater susceptibility to disease, hypertension - the cause of one sixth of all deaths, cardio-vascular dysfunction and over all increased mortality. The close association of obesity with many health problems has resulted in the medical recognition of obesity as a disease of the living organism. Unfortunately our society considers obesity more in terms of aesthetics than as a disease of major importance. In an effort to eliminate this

[1] Obese refers to those individuals who weigh 20% or more than their ideal weight whereas overweight refers to those who weigh more than 10% but less than 20% of their ideal weight.

disease, scientists are developing and testing many theories concerning the etiology of obesity. The etiological factors which contribute to the development of this disease and the mechanisms by which they contribute to the development of excessive adipose depots have not yet been clearly defined. Environmental, psychological and hereditary influences are the three primary aspects of obesity which are examined by research personnel.

It was the purpose of this experiment to examine the effects of litter size, diet and activity on body weight gain, the degree of adiposity, energy utilization and serum growth hormone levels.

REVIEW OF LITERATURE

A definition of obesity is prerequisite to any study of this subject. Obesity may be defined as the accumulation of excessive adipose tissue resulting from insufficient caloric expenditure relative to caloric intake. In view of the complexity of this disease, such a definition is greatly oversimplified. Obesity has also been classified as either metabolic or regulatory obesity. Metabolic obesity is manifest in abnormalities of fat and carbohydrate metabolism (2). Regulatory obesity refers to an impairment of the central mechanism regulating food intake (2). The primary components of this mechanism are the hypothalamus and related structures of the central nervous system. Experimental data continues to provide additional evidence that these structures do function in appetite control (2-6) and are mediated by the maintenance of the proper level of exercise (2,3,4,6). Destruction of the hypothalamus either by surgery or goldthioglucose has been found to be an effective method of experimentally producing obesity in both rats and mice (7-11). Such animals exhibit gross abnormalities in eating behavior and lipid metabolism (9-11). These abnormalities include increased free fatty acid (FFA) levels, hypertriglyceridemia, hyperinsulinemia, and increased adipose tissue glucose oxidation with subsequent conversion to lipid but decreased fatty acid oxidation. Camus and Rousy found hypothalamic lesions in obese cadavers (4). More recently Schachter (5) found that obese subjects were not capable of discerning whether or not they were actually hungry.

The Heritability of Obesity

Genetic and environmental factors play major roles in the development of obesity. In spite of the viewpoint of many that heredity is the main etiological agent of this disease, there is at the same time recognition that the environment exerts considerable influence on the development of obesity. Mayer, Sheldon and others reported data that provides indirect support for genetically determined obesity (4,8). The 1949 study by Angel revealed that two-thirds of the offspring of obese parents are obese (4). In another study involving 75 obese women, 82% had one or two obese parents while only 38% of a non-obese group had one obese parent (8). Johnson et al. (12) showed that 8 to 9 percent of children of normal weight parents were considered obese whereas 40% of the children having one obese parent were obese, and if both parents were obese, 80% of the children were obese. Withers (1964) reported that the weights of adopted children did not appear to have any relation to the weights of their adopted parents (7). Mayer emphasized the role of genetics in the development of obesity but he also noted that the environment requires consideration (4). For example, studies by Von Vershuer, Newman and others found the weight of identical twins to vary more from one another than any other anthropological measurements taken (4). Eid (13) showed that the rapidity of weight gain during infancy was a better indication of later susceptibility to obesity than the weight of the parents.

Human studies provide indirect support of genetic factors contributing to the development of obesity; however, animal husbandrymen have

been selecting within species for their degree of body fatness for centuries. Laboratory animals furnish direct support that genetic factors are involved in the development of obesity. At least three obese genes have been identified in the mouse and some in the rat, dog and chicken (3). Bray and York (7) conducted a thorough investigation of inherited obesity in rodents. They stated that afflicted animals cannot be identified at birth. Moreover, the earliest age at which any of the genetically obese subjects can be identified is at two weeks of age (7). Inheritance of genetically transmitted obesity may be either dominant as in the yellow obese mouse (A^{Y}, A^{VY}, A^{IY}) or recessive as in the obese mouse (ob/ob), adipose mouse (ad/ad), fatty rat (fa/fa) and the diabetes mouse (db/db) (7). Genetic investigations have not yet identified any single protein that is generally recognized as causing obesity (7). Increased weight gain in genetically obese rodents was first apparent at puberty and continued to increase with age; fat represented more than 90% of the weight increment and is the greatest when the animals were fed a high fat diet (7).

Like their hypothalamic lesioned counterparts, genetically obese rodents exhibited abnormalities in energy expenditure, food intake, glucose oxidation as well as in lipid and hormonal metabolism (7). Specifically, the animals tended to consume more energy and produced a greater weight gain per gram of food consumed (7,14). Higher rates of lipogenesis, decreased lipolysis, hypertriglyceridemia, hyperinsulinemia and increased levels of FFA were all characteristic of genetically obese rodents (7,8,14). Studies in several species of rodents indicated that

the hypothalamus may be the site of genetic impairment associated with these abnormalities (7).

Several studies showed other factors which determine weight in rodents and their degree of importance. Dadlani and Prabhu (15) stated that litter weight at birth, as litter size, was a permanent characteristic of mice, specific to a particular breed and to the stage of development. They added that the most important individual factor in determining weaning weights of litters was the post-natal maternal influence (15). According to Sabisch (1962), litter weight increased linearly with the weight of the dam indicating a genetic influence (15). In another article by Dadlani and Prabhu (16), they stated that studies by Carmon and Golley (1964) in mice, and Smith and Donald (1939) in pigs found hereditary differences in growth were more clearly expressed after weaning than before. Uterine environment also exerted a large influence on the growth of mice as well as the nursing ability of the dam (16).

Endocrine Aspects of Obesity

Optimum nutrition, and hence, the maintenance of the ideal body weight depends upon normal endocrine function as well as an adequate diet (17). Two hormones which have received a great deal of interest in relation to the obese condition are insulin and growth hormone. Insulin plays a major role in lipogenesis whereas growth hormone (GH), which has many important metabolic functions, operates in lipolysis. The relationship between these two hormones in lipid and carbohydrate metabolism is very complex. The investigators of an article on "the influence of growth hormone on the pathogenesis of obesity in children"

found evidence that the impairment of GH secretion after insulin induced hypoglycemia and arginine infusion represented an aggravating factor of obesity, although the impairment of such a lipolytic hormone could not be regarded as a cause of obesity (18). A direct relationship exists between obesity and the failure of the individual to raise plasma GH levels in response to certain stimuli (19). This relationship does not necessarily implicate GH as an etiological agent but poses the question as to whether or not the association between changes in insulin and growth hormone secretion and obesity is a causal or compensatory arrangement (19). Several studies showed that there is a return to normal insulin and GH levels with the correction of the obese condition (19). In view of the presence of impaired adreno-cortico function in obese children which can be corrected by weight loss, Steiner (20) suggested that emotional deprivation in children could alter the hypothalamic-hypophyseal axis resulting in reduced GH levels and hence, subsequent growth deprivation characteristic of a hypopituitary syndrome. Although GH and insulin are probably not etiological agents of obesity, their relationship to the obese condition requires examination.

Growth Hormone

Administration of growth hormone produces tremendous changes in carbohydrate, fat, protein and mineral metabolism (21,22,23). Specifically GH administration decreases urinary and fecal nitrogen excretion and therefore favors nitrogen retention. GH also stimulates amino acid uptake and protein synthesis (23). Growth hormone appears to enhance lipolysis and increases oxygen consumption (24). Raiti and Blizzard (7)

reported that GH inhibits glucose utilization and uptake (partly because of its inhibitory action on insulin); and that GH also enhanced FFA uptake by muscle tissue and FFA release from adipose tissue. Thus, it becomes essential to recognize the potential impact of GH abnormalities in the body related to lipid metabolism and obesity.

Impaired release of growth hormone and subnormal serum GH levels are characteristic of the obese condition (7,8,18,19,20,23,24,25,26,27,28,29,30). Lessof et al. and Greenwood found that the impaired release of GH tends to maintain the obese condition (8). Bray and York (7) state that experimental reports suggest that the impairment of GH release in obese rats and mice might be found in the hypothalamus in association with hyperphagia and decreased activity. Studies of the response of normal and obese children to the oral administration of glucose have found impaired hypothalamo-pituitary function in the obese subjects (25,26,27). The obese children produced significantly lower GH levels in response to oral glucose administration whereas the non-obese children did respond. Theodoridis et al. (25) hypothesized that because obese children secrete more insulin than normal children, and because insulin and growth hormone were anabolic in nature, that GH requirements were not as great in obese children. It was felt that these findings explained the low levels of FFA's in obesity since GH, which was suppressed, was partly responsible for the lipolysis of fat into free fatty acids.

Studies on the response of GH serum levels to oral glucose and arginine infusion in normal and obese subjects led to the investigation

of diurnal variation in serum GH levels over a 24 hour period. These studies showed that the central nervous system (CNS) monitors the secretion of GH and that the activation of the pituitary gland by the CNS has major physiological implications (28). CNS regulation was manifest by a major peak in GH level during sleep in all of the subjects studied (23,28,29,30). A wide range of adaptive responses were found to vary with nutritional status (28). For example, overnutrition as manifest in the obese condition corresponded to attenuated responses to all stimuli of growth hormone release (28). These results were confirmed by studies of Quabbe et al.(29).

The neurological and nutritional factors regulating GH secretion require further examination in order to ascertain the influence of this hormone upon the development of obesity (31,32,33,34). Several neurochemical compounds have been identified which affect GH levels. The administration of the monoamines, noradrenalin and dopamine, in rats decreased GH levels; the alpha-adrenergic receptor blocker, propranolol did not change GH levels; and serotoin stimulated GH release (31). Epinephrine increased GH levels in baboons (32). Various anesthetics affect GH levels: ether depressed base line GH levels in mice (33), whereas in rats pentobarbitol increased GH levels while urethane did not change GH levels over a two hour period (34). Studies in both animals and man indicated that GH levels exert a negative feedback or autoregulatory effect on further GH release (35).

Growth hormone levels are also controlled by nutritional status. The effects of oral glucose and arginine infusion have previously been

mentioned. The administration of 60 grams corn oil (Lipomul) three hours prior to testing followed by IV administration of heparin at the time of insulin or arginine infusion, suppressed GH levels in a group of human female subjects (36). The authors felt there was a possible regulatory role for plasma FFA's in the regulation of human GH secretion (36).

Other factors have been examined which are related to the effect of growth hormone upon the development of obesity. A study on the relationship between body composition and insulin and GH response in obese adolescents showed that the obese child responds differently than adults to stimulatory factors of GH and insulin release (37). Schultz and Parra (37) concluded from this study that the nature of the mechanism which blunts GH response in adults is not yet fully operative in the obese adolescent; the need for GH for growth might account for this difference in adolescents and adults. Regardless of age, the overweight condition does affect normal hormonal control in the body.

Exercise has been discovered to be associated with the regulation of GH secretion and the development of obesity. During muscular exercise in normal adults GH secretion appeared to be responsible for the initiation and maintenance of fat mobilization in response to energy demands when exogenous carbohydrate was not available (38). The effect of heavy daytime exercise was not found to augment peak GH levels in sleep in another study in adults (39). Subnormal GH response to exercise appeared in overweight men but not in normal weight or muscular overweight men (40). Subnormal responses have been found in juvenile

diabetics which can be corrected by stringent metabolic control with insulin and diet (41).

Insulin

Insulin probably has been studied more than any other hormone in relation to the obese condition. Diabetes, the most common of all endocrine diseases, is associated with the incidence of adult obesity in 80% of all individuals with the diabetic condition. Insulin resistance and hyperinsulinism are metabolic abnormalities which occur frequently in obese subjects (8,42,43,44,45,46,47). Most investigators feel that the hyperinsulinism in obesity does not indicate that insulin is an etiological agent of this disease (19,20,44,45,47,48). The reason for this feeling was that abnormal blood levels of both insulin and growth hormone returned to normal in obese subjects with normal glucose tolerance who undergo appropriate weight reduction.

The effect of the hyperinsulinemic condition of obesity is, nevertheless, still important. Merimee (47) reported that the cause of the hyperinsulinism has generally been considered to be the result of compensatory adjustment of the pancreatic beta cells to the antagonism of peripheral tissue to insulin. Merimee (47) added that the overall composition of diet and not just merely caloric intake is important in producing the hormonal aberrations of obesity. Several studies showed that changes in carbohydrate intake are responsible for the hyperinsulinism (43,48). Stern et al. (43) proposed that if dietary carbohydrate played a primary role in the genesis of hyperinsulinism in obesity, one would expect that total body fat would best be correlated with plasma

immunoreactive insulin (IRI). The results of their study showed that adipose cell size correlated best with plasma immunoreactive insulin (43). El-Khodary et al. (44) found a significant correlation of plasma insulin with body fat (r=0.861) and with body weight (r=0.702) in the obese and thinned obese non-diabetics, but not in the obese with overt diabetes; they felt the hyperinsulinemia was clearly related to the increase in total body fat. Grey and Kipnis (48) provided irrefutable evidence that the composition of the diet does affect plasma insulin levels. Obese female subjects exhibited significantly reduced basal plasma insulin levels on a low (25%) carbohydrate diet whereas despite continued weight loss, refeeding of a high (62%) carbohydrate diet resulted in markedly increased basal plasma insulin in the same subjects (48). A dietary history of these subjects on admission revealed that the obese individual consumes more carbohydrates than the normal weight person both in terms of absolute amount and in terms of the percentage of total calories consumed (48). The same report also indicated that proteins as well as carbohydrates are insulinogenic (48). Felig et al. (49) reported that hyperaminoacidemia may also stimulate the hyperinsulinemia of obesity.

Studies on the cellularity of obesity provide a possible hypothesis as to the cause of the insulin resistance in adipose tissue of obese subjects. Enlarged adipocytes from obese subjects were found to be relatively insensitive to the action of insulin in terms of glucose uptake and utilization (50). The larger the diameter of the adipose cells, the greater was the insensitivity to insulin (50). In addition, insulin

sensitivity was restored by weight reduction and concommittant atrophy of adipose cell size (50). Other reports indicate that the effect of insulin upon glucose oxidation to CO_2 is a function of adipose cell size and number (43,51,52). An attempt to explain the mechanism by which the insulin insensitivity develops with increasing cell diameter was made in 1971 Nutrition Reviews (51). The explanation was based on the assumption that as an animal becomes older, and as the cell increased in size there was no increase in the number of insulin receptor sites on the cell membrane (51). Eventually the cell surface reaches such dimensions that the number of insulin molecules on receptor sites is not sufficient to effectively enhance glucose utilization (51). Bjorntorp et al. (52) provided some support of this concept. They found that the fat cell size and lipid content of cells increased with age in men (52). Of the factors analyzed, fat cell diameter had the greatest correlation ($r=0.51$) with insulin secretion (52).

There may be evidence forthcoming that the role of insulin in DNA synthesis may be related to the increase in adipose cell number in obesity. Insulin was found to be associated with the enhancement of DNA synthesis (thymidine incorporation) and that hyperinsulinism was found to nearly double the fat cell content of an adipocyte (53). Vost and Hollenberg (53) suggested that high levels of insulin early in life could affect the rate of proliferation of primordial fat cells.

The Assessment of Obesity

The determination of what criteria are suitable for the assessment of the obese condition represents a subject of utmost concern in

interpreting experimental results. Body weight alone is an inadequate method of defining obesity (55). The inadequacy of body weight as a measurement of obesity is illustrated by Kalkoff and Ferrou (40). A comparison of metabolic differences between obese overweight, muscular overweight and normal weight men of similar height and ages showed that metabolic changes in the overweight are more closely related to degrees of adipose tissue accumulation than to deviations from the ideal body weight (40). The use of body composition as a means of assessing the degree of adiposity is restricted primarily to laboratory animals. Densiometric methods, counting ^{40}K, a natural isotope, and ^{3}H labeled water have been used in animals and men as a means of assessing adiposity. The determination of cell size and number has been a more recent means of assessment. The use of calipers to measure skinfold thickness, an indirect measure of subcutaneous adipose tissue thickness, has become the most practical method to supplement the evaluation of human adiposity (3).

The study of obesity requires the observation of subjects exhibiting this condition either as a result of metabolic or functional abnormalities. Bray and York (7) cited four general methods of producing regulatory obesity in rodents. These methods consisted of lesions in the hypothalamus via electrolytic destruction, chemical agents such as goldthioglucose or by microsurgical techniques; endocrine manipulation; dietary manipulation; and the restriction of activity. Dietary manipulation produced obesity in certain strains of mice and rats by feeding the experimental subjects a high fat diet (56,57,58). The production of

obesity in some species but not in others via dietary manipulation is considered to be an index of hereditary-environmental interaction (7). Fenton and Dowling (55) showed that some strains of mice became obese on a high fat diet while others did not (55). Shcemmel, Mickelson and Gill found the same to be true in rats (56).

Schemmel, Mickelson and Fisher (59) found that the composition of diet and the time at which the diet was introduced to rats was important in the determination of ultimate body weight and adipose tissue accumulation. They found that the nature of the diet consumed after weaning was far more important in determining body size than the dietary regimen of the dam during lactation in both male and female pups (59). Rats were heavier when fed a high-fat diet following weaning than those fed a grain ration (59). The diet fed the dams had no significant effect on the body weights of their offspring other than at the time of weaning (59). The composition of diet fed the dam during lactation did not have as great an effect on the weaning weight of the pups as did marked differences in litter size (59). The composition of the diet fed after weaning appeared to be more important than the diet during lactation in relation to the ultimate body weight obtained (59). In addition, the high-fat diet produced a greater percentage of body fat ($P < 0.01$) in all rats fed this diet (59) regardless of the age at which the high fat diet was fed.

The Role of Exercise in Obesity

Exercise is of major importance in the development of obesity. Many studies have demonstrated that caloric intake does not vary

significantly between obese subjects and their normal weight counterparts (1,60,61,62,63). In contrast, some of these same studies showed the activity of obese subjects to be much less than control groups of normal weight individuals (1,60,61). Some studies, however, revealed no significant differences in activity in their subjects (2,63). Mayer (4) reported that "exercise is the great variable in energy expenditure" and " [that] exercise does not necessarily increase food intake." Mayer (4) stated that many attitudes concerning the role of exercise in weight control, such as exercise expends little energy, and appetite increases with exercise, were gross misconceptions. Rats in experimental studies, exercised one to two hours daily, actually ate less than unexercised rats (4). Appetites in obese boys did not increase as much as non-obese boys following physical activity (4). A central thesis of Mayer's theory of appetite control was that exercise is essential to the proper maintenance of this mechanism.

Ahrens (64) showed that the fat:protein ratio of weight gain in moderately exercised animals was below that of other animals. Forced exercise in other studies not only influenced weight control, but also changed body composition by decreasing the fat:protein ratio (65,66). Rats on a voluntary exercise regimen fed 70% of an ad libitum group's intake exhibited significantly lower serum cholesterol levels ($P < 0.05$), lower serum insulin levels ($P < 0.01$), a lower fat:protein rate of gain and six times the level of activity ($P < 0.01$) - i.e. 3674 rev/day vs 370 rev/day (67).

Exercised rodents via treadmill running or swimming possess a significant leaner body mass than their sedentary counterparts (68).

Female rats increased their food intake (+46%) above sedentary controls in response to prolonged exercise (6 hours swimming per day), whereas male rats did not increase their feed intake and exhibited lower body weights in comparison to their controls (68). The differences in these responses were not accounted for. Oscai et al. (68) found no hypertrophy of the forelimbs or of the girdle muscles in these female rats, although the heart, lungs, kidneys and various other tissues exhibited a significantly greater lean tissue mass than the sedentary controls. These results indicated that the type of exercise program in experimental studies should be examined. An exercise program should affect muscular development as well as benefit cardiac-respiratory efficiency.

Ahrens et al. (69) studied the effect of source of dietary carbohydrate on rats which were force exercised via treadmill. All rats were fed a high fat diet in which carbohydrates provided 12% of the total calories; however, cornstarch was the only source of carbohydrate for one group while the carbohydrate portion of the diet in the other group consisted of a mixture of starch and sugars equivalent in composition to the U. S. "market basket" diet (69). The exercised animals fed the carbohydrate mixture tended to gain more weight and lose less body fat than those fed the cornstarch diet (69). Exercise significantly reduced food intake, body weight gain, liver weight, the concentration of lipid and cholesterol in the liver, serum insulin levels and the fat content of the carcass (69).

A study by Hebert and Lopez-S (70) in which male rats were allowed food and exercise ad libitum demonstrated that the exercised rats had

greater food consumption (+7%), lower weight gain (-5%), larger adrenal glands (+15%), lower serum cholesterol (-3%), lower serum triglycerides (-30%), lower liver glucose-6-phosphate dehydrogenase activity (-20%), and higher alpha glycerophosphate dehydrogenase activity (+15%0 than a group of sedentary controls. The study also found that the differences between the two groups because of exercise completely disappeared after 5 weeks (70).

An experiment with forced exercised rats found that the epididymal fat pad adipocytes exhibited significantly greater glucose lipogenic activity after 60 minutes under basal (+56%) and insulin stimulated (+77%) conditions than a group of ad libitum controls (71). These differences were significant only when compared as a function of surface area (71). Another study found that 13 weeks of intensive exercise training via treadmill increased the potential of epididymal adipose tissue release of FFA in response to epinephrine stimulation (72). The results of a previous study by Askew et al. (73) support this finding. They also found that exhaustion caused an increase in epinephrine stimulated activity of similar magnitude in untrained animals (73). The results of this study were compatible with the concept that physical training increases lipolysis in adipose tissue and lipoprotein lipase mediated triglyceride FA uptake by muscular tissues (73).

EXPERIMENTAL

Animals, Management, Diet and Design

Two experiments using 21 day old male weanling rats from Charles River (CD strain)[1] were used in this study. The rats were placed on a four day adjustment period during which they were given food (Purina Chow)[2] and water ad libitum. At the end of the adjustment period, the rats were ranked by weight in outcome groups of four each. The rats of each outcome group were randomly distributed into the following four treatments: Group I - high fat diet, spontaneous exercise; Group II - high fat diet, restricted exercise; Group III - Purina Chow diet, spontaneous exercise; and Group IV - Purina Chow diet, restricted exercise. During the experimental period all of the rats were given food and water ad libitum. Feed intake was measured daily and body weights were taken every other day. The first experimental group of subjects (A) consisted of 60 rats obtained directly from Charles River. The second group of 36 rats (B) came from litters of four each in which all of the dams had been fed ad libitum during gestation and lactation the same high fat diet used during the experimental period.

The high fat diet consisted of (by weight) 60% fat (Crisco), 22% vitamin-free casein[3], 10% cornstarch[3], 4% Jones-Foster mineral mix[3], 2% vitamin fortification mix[3], and 2% non-nutritive fiber (Alphacel)[3].

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[1]Charles River Breeding Laboratories, Wilmington, Mass.

[2]Purina Laboratory Chow, Ralston Purina Company, St. Louis, Mo.

[3]Nutritional Biochemical Corp., Cleveland, Ohio.

The high fat diet had an estimated available energy content of 6.42 kcal per gram and the chow diet 3.26 kcal per gram.

Feeding periods of 12 hours duration were established. The feeding period began at 6:00 p.m. at which time the lights were turned off and ended the following morning at 6:00 a.m. at which time the lights came on.

The rats in the restricted exercise groups were individually housed in portable, polypropylene mouse cages, 13x18x28 cm (504 cm^2; 6,552 cm^3), whereas the spontaneous exercise groups were individually housed in portable, polypropylene rat cages, 16x24x54 cm (1,296 cm^2, 20,736 cm^3). In addition each exercise group was alternated in activity cages (Wahman Mfg.)[4] for 24 hour exercise periods. The number of revolutions was recorded at the end of each exercise period and converted to meters run. One revolution was equal to 1.12 meters.

Methods and Analytical Procedures

Blood samples were collected via the venous plexus localized in the orbit behind the eyeball and by heart puncture following ether anesthetization two hours after food had been withheld. Blood was drawn on the initial day of the experimental period and every two weeks following thereafter for a six week period. Blood samples were collected, labeled and centrifuged at 12,000 rpm for 25 minutes at 4°. Serum from the samples was frozen at -20° until the day of analysis.

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[4]Wahmann Manufacturing Co., Baltimore, Maryland.

Animals were sacrificed by ether anesthetization and were frozen immediately. The carcasses were held at -20° until analyzed.

Sample preparation for total body fat determination was accomplished by freeze-drying the exposed surface of the intact carcass. Each carcass was weighed after lyophilization. The carcass was then chopped into small pieces and homogenized in a Waring blender. The hide of each animal was scraped in an effort to remove all remaining epidermal tissue layers. Total body fat was determined by using the procedure of T. C. Campbell and I. Friedman (74) as summarized below. Total body fat was determined in group A.

Total Body Fat Determination:

Twelve milliliters of distilled water was added to each 0.5 gram carcass sample. Ten milliliters of a 1% acid alcohol solution was added. This was followed by 25 milliliters of ethyl ether and 25 milliliters of petroleum ether. Each flask was shaken for 30 seconds after each addition of ether. A 25 milliliter aliquot of the ether phase was transferred to a tared aluminum pan and the ether was evaporated off. A 25 milliliter aliquot of a 1:1 petroleum and ethyl ether mixture was added to the flask and shaken for 30 seconds. Another 25 milliliter aliquot was taken from the flask and added to the aluminum pan. The ether phase was evaporated off. The pan was then placed in a dessicator and later weighed to a constant weight.

Calculations: the weight of residue in the aluminum pan represents the ether extractable fraction of lipid content.

Per cent lipid = (residue weight x 1.33 / dry sample wt.) x 100
(dry basis)

Per cent lipid = (lyophilized carcass wt / wet carcass wt.) x %lipid (dry) (wet basis)

The calculation assumes 100% extraction of lipids in the two extractions. The procedure was found to result in 99.6% extraction of the total extractable material (74). The factor 1.33 represents the fact that 50% of the total lipid content was transferred to the evaporation dish in the first extraction, and an additional 25% of the total was transferred in the second extraction. 100%/75% = 1.33.

Adipocyte Number Determination:

Estimation of adipose tissue lipid and DNA analysis was conducted by the method of Hubbard and Matthew (75). Isolated fat cells were prepared by a modification of the procedure of Rodbell (76). Both epididymal fat pads from each rat were excised, blotted and weighed. The entire left epididymal fat pad (pad L) of each rat was taken for total neutral lipid and DNA determinations, and the right pad (pad R) was used to prepare isolated fat cells. Pad L was stored in a 50 milliliter plastic centrifuge tube containing methanol at 5° until the following day for analysis while pad R was stored at 5° in 5 milliliters of 0.4% saline solution until the following day for analysis.

Pad L Preparation: The methanol was evaporated under nitrogen at 70°. The fat pad was then removed from the centrifuge tube and hand homogenized in 10 milliliters of 2% perchloric acid. The homogenized pad was incubated at 90° with sitrring every 3-5 minutes. After hydrolysis, the homogenized sample was brought to 30-40°. Ten milliliters of hexane was added. The contents were mixed and transferred to the original 50 milliliter centrifuge tube. The homogenizer was washed by the

addition of 10 milliliters hexane and 5 milliliters 2% perchloric acid. The wash was also added to the centrifuge tube. After 5 minutes of mechanical shaking, the phases were separated by centrifugation. The hexane phase was transferred to a preweighed plastic tube containing a boiling chip. The homogenizer was washed with an additional 10 milliliters hexane and the process repeated. After evaporation at 70° under nitrogen, the slightly discolored lipids were dried to constant weight in a dessicator.

Two milliliter aliquots of the perchloric acid solution were taken and DNA was determined colorimetrically after reaction with diphenylamine according to Burton (77). The stock standard solution was prepared with salmon testes DNA[5] at 0.8 g/ml in 5 mM NaOH. Working standard solutions were made by mixing 2 milliliters of stock solution with an equal volume of IN perchloric acid and heating at 70° for 15 minutes.

The diphenylamine reagent was made by mixing 1.5 g diphenylamine, 100 milliliters redistilled acetic acid, 1.5 milliliters concentrated sulfuric acid and 0.5 milliliter aqueous acetaldehyde for each 25 samples. Four milliliters of this reagent was added to each 2 milliliter aliquot of the perchloric acid solution. Tubes containing from 6.67 to 33.33 ug/ml DNA and a blank containing no DNA were also prepared. Color development occurred by incubation at 30° for 16-20 hours. Absorbance was measured at 600nm.

Pad R Preparation: On the day of analysis, the saline rinsed R pad was placed in a siliconized flask containing 3 milliliters of 0.4%

[5]Worthington Biochemical Corporation, Freehold, New Jersey.

albumen buffer containing 0.54 mg of glucose per milliliter and centrifuged for 1 minute at 400 x g. The washing was repeated three times. Each sample of isolated fat cells was diluted to 20 milliliters with albumen buffer. A 10 milliliter aliquot was removed following stirring on a Vortex mixer and placed in a separate 50 milliliter plastic centrifuge tube. After the fat cells floated to the surface, the suspension medium was aspirated away and 1 milliliter of stock DNA was added as an internal standard. The DNA suspension was then brought to a volume of 10 milliliters with 2% perchloric acid and 10 milliliters of hexane was added. The tubes were capped, shaken mechanically and centrifuged for 1 minute at 400 x g. The hexane phase was transferred to a preweighed tube and the process repeated. The combined hexane phases were evaporated at 70° under nitrogen and dried to a constant weight in an evacuation dessicator. The 2% perchloric phase was heated for 20 minutes at 90° with stirring every 3-5 minutes on a Vortex mixer. Two milliliter aliquots were taken for DNA determination by Burton's method (77) described above. The above procedures yield the following data: wet weight of epididymal fat pads L and R, total DNA to wet weight ratio of pad L, DNA to lipid ratio of pad R of each rat.

Calculations:

$$\text{Total cells} = \text{ug DNA in pad L} + \frac{\text{ug DNA}}{\text{g wet wt in pad L}} \times \text{g wet wt pad R} \times \frac{\text{cells}}{\text{ug DNA}}$$

$$\text{Total adipocytes} = \text{g lipid in pad L} + \frac{\text{g lipid}}{\text{g wet wt in pad L}} \times \text{g wet wt pad R} \times \frac{\text{ug DNA}}{\text{g lipid in R}} \times \frac{\text{cells}}{\text{ug DNA}}$$

These calculations (75) are based upon the following assumptions and values:

1. The percentage of epididymal fat pad wet weight as lipid is the same for both pads.
2. The total DNA to lipid ratio is the same for both epididymal fat pads.
3. The calculation of cells per ug of DNA was based on the commonly accepted value of 7×10^{-6} of DNA per cell.
4. The average cell neutral lipid has a molecular weight of 809.
5. The recovery of DNA was 55%.

Lee Index:

The Lee index was determined for each animal in an attempt to find a rapid and sensitive means of comparing the degree of adiposity of one treatment with that of another. The Lee index was calculated after a modification of the formula by Bernardis and Patterson (78) except for adjustments to account for our measurements in cm instead of mm.

$$\text{Lee index} = \frac{\sqrt[3]{\text{Body weight (g)}}}{\text{naso-anal length (cm)}} \times 1000$$

RESULTS AND DISCUSSION

Weight Gain and Food Intake

The pups raised by the dams fed the high-fat ration ad libitum during gestation and lactation (Study B) were heavier at weaning than the pups of the dams fed a chow diet ad libitum during gestation and lactation (Study A) - 73 and 55 g, respectively (Figures 1 and 2 and Tables 1 and 2). At the end of the experimental period, the pups from Study B tended to maintain heavier weights than the pups from Study A (285.3 g vs 273.6 g). A comparison of the individual treatments in these two experiments tends to support this trend; however, the pups in treatment 1 exhibited the reverse trend. The pups in Study B attained a final group body weight of 246.25 ± 8.66 g whereas the pups in Study A attained a final group average body weight of 263.60 ± 10.31 g. With the exception of treatment 1 these data would indicate that the composition of the diet fed during gestation and lactation can be an important factor in determining the adult body weight.

A comparison between the pups in our study and those by Schemmel et al. (59) are somewhat limited since the Osborne-Mendel rat appears to be heavier than the Charles River (CD strain) rat. The grain fed rat (GG) in the above study had the lowest body weight which was also true of my chow fed rat with restricted activity in TB1. However, the chow fed group in activity cages were significantly heavier than any other treatment group in experiment Study A $P < 0.05$ (Table I), and in experiment Study B $P < 0.05$ (Table II). In spite of greater body weights, the

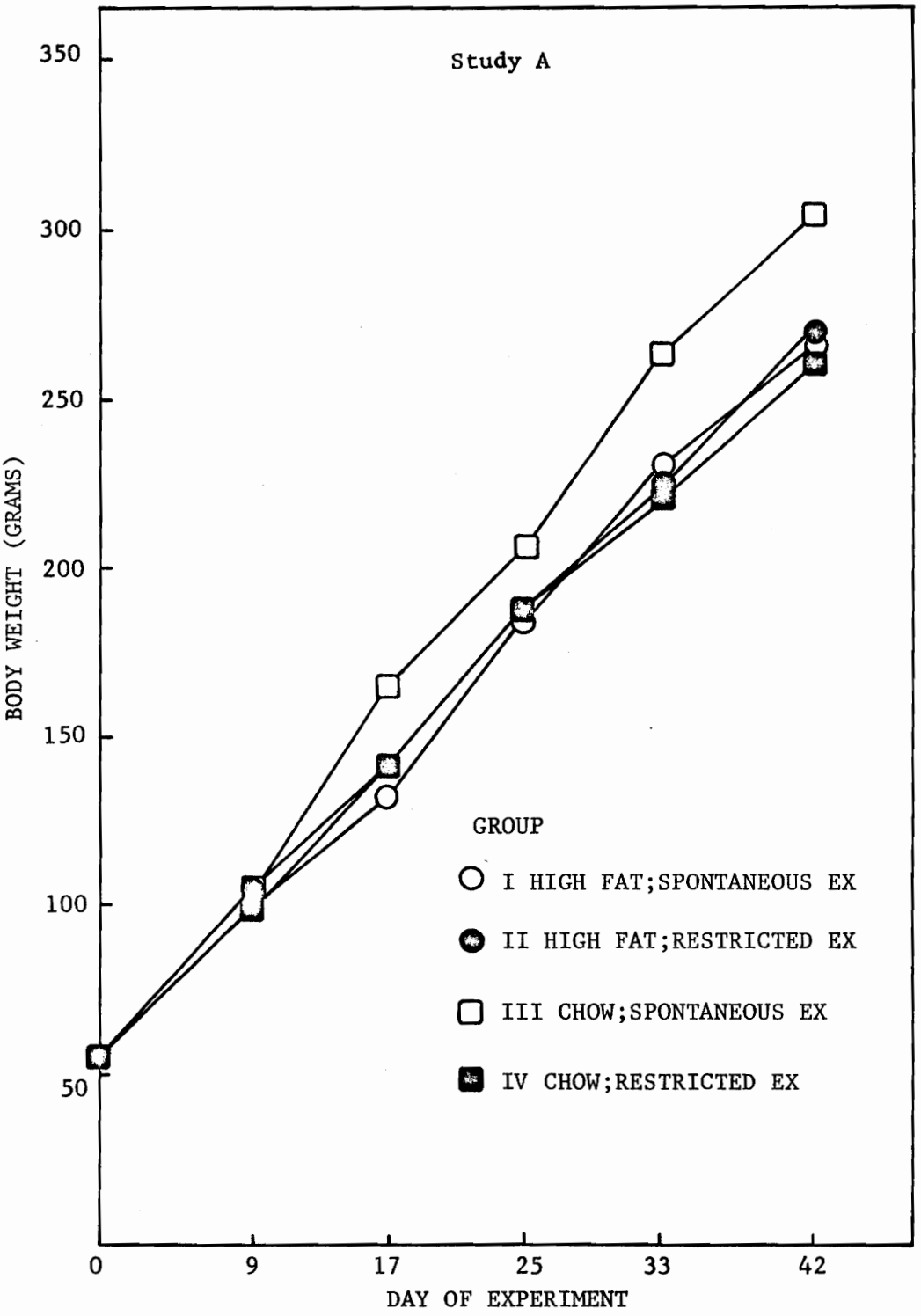

FIGURE 1 - AVERAGE BODY WEIGHT PER GROUP

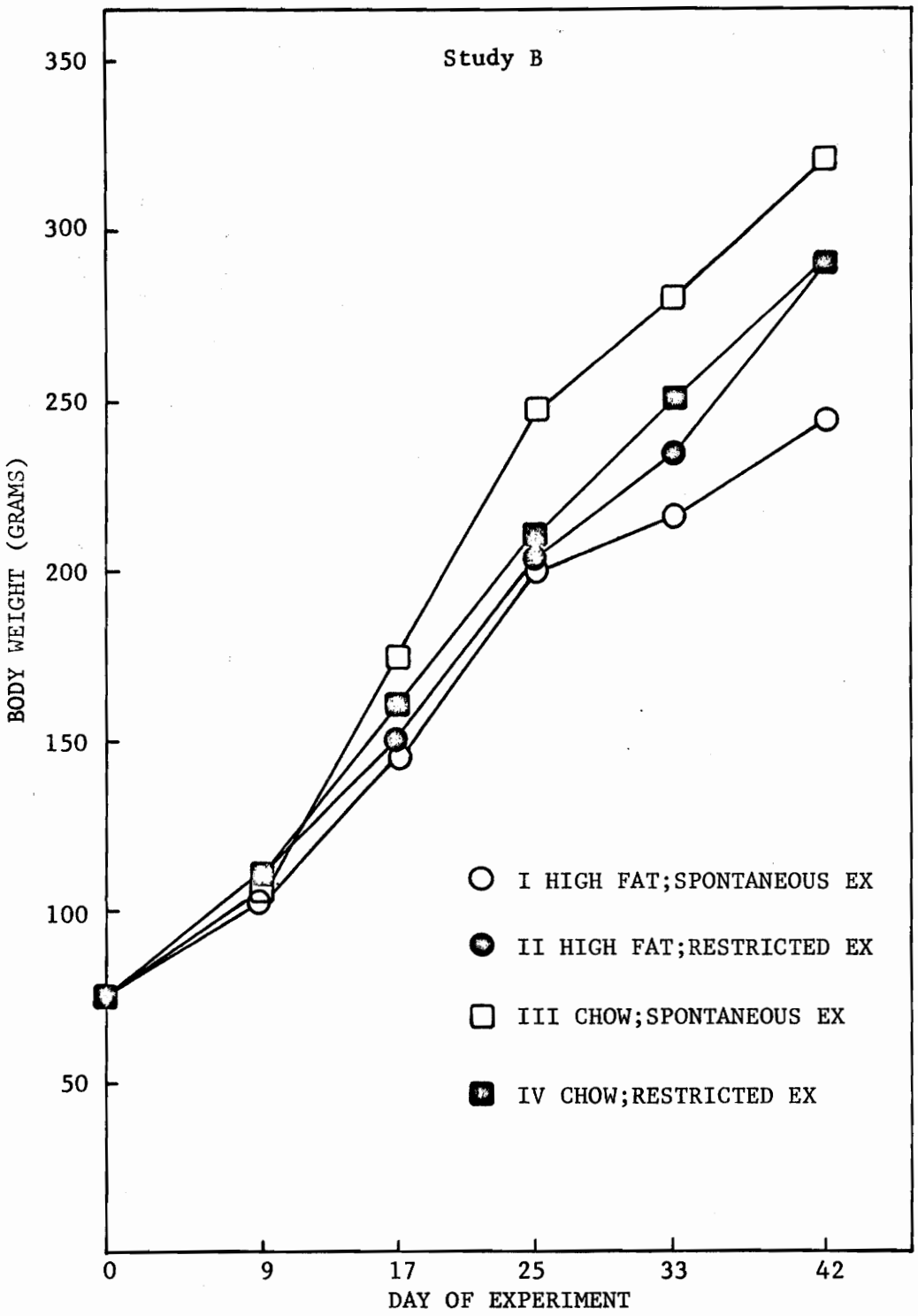

FIGURE 2 - AVERAGE BODY WEIGHT PER GROUP

TABLE I

EFFECT OF DIET AND ACTIVITY LEVEL ON FOOD CONSUMPTION, WEIGHT GAIN AND FINAL WEIGHT

Study A	Group[1]	I	II	III	IV
	n	13	13	15	13
Initial Weight g		54.53 ± 0.30[a]	55.40 ± 0.30[a]	54.87 ± 0.30[a]	54.40 ± 0.30[a]
Final Weight g[2]		263.60 ± 10.31[a]	268.38 ± 10.31[a]	298.47 ± 9.39[b]	263.98 ± 10.31[a]
Average Total Wt. Gain g		206.98 ± 9.49[a]	209.60 ± 9.49[a]	235.27 ± 8.64[b]	203.60 ± 9.49[a]
Average Daily Wt. Gain g		4.92 ± 0.22[a]	4.98 ± 0.22[ab]	5.59 ± 0.20[b]	4.84 ± 0.22[a]
Kcal Consumed[2] Per day		62.74 ± 2.55[a]	55.98 ± 2.55[ab]	61.19 ± 2.32[ab]	51.84 ± 2.55[b]
Kcal Consumed Per Gram Wt. Gain[3]		12.86 ± 0.36[a]	11.30 ± 0.36[b]	10.89 ± 0.33[b]	10.80 ± 0.36[b]

1 - Identification of treatments: I - high-fat, spontaneous exercise; II - high-fat restricted exercise; III - chow, spontaneous exercise; IV - chow, spontaneous exercise.
2 - Statistically significant by F test at $P < 0.05$.
3 - Statistically significant by F test at $P < 0.01$.
ab - Row with common superscript are not significantly different (P 0.05)

TABLE II

EFFECT OF DIET AND ACTIVITY LEVEL ON FOOD CONSUMPTION, WEIGHT GAIN AND FINAL WEIGHT

Study B	Group[1]	I	II	III	IV
	n	6	4	7	7
Initial Wt. g		73.00 ± 0.91[a]	74.56 ± 0.91[a]	72.67 ± 0.91[a]	72.44 ± 0.91[a]
Final Wt. g[2]		246.25 ± 8.66[a]	287.65 ± 10.95[b]	320.84 ± 7.61[c]	286.45 ± 7.90[b]
Average Total Wt. Gain[2]		170.77 ± 8.46[a]	211.48 ± 10.69[b]	244.83 ± 7.43[c]	211.31 ± 7.71[b]
Average Daily Wt. Gain[2]		4.10 ± 0.22[a]	5.02 ± 0.25[b]	5.84 ± 0.18[c]	5.04 ± 0.18[b]
Kcal Consumed Per Day		72.00 ± 5.21[a]	67.11 ± 6.59[ab]	62.23 ± 4.58[ab]	53.32 ± 4.76[b]
Kcal Consumed Per[2] Gram Wt. Gain		17.36 ± 1.09[a]	13.42 ± 1.38[b]	10.63 ± 0.96[b]	10.65 ± 1.00[b]

1 - Identification of treatments: I - high-fat, spontaneous exercise; II - high-fat, restricted exercise; III - chow, spontaneous exercise; IV - chow, spontaneous exercise.

2 - Statistically significant by F test at $P < 0.05$).

abc - Row with common superscripts are not significantly different ($P < 0.05$).

carcasses of the pups in Study A exhibited the lowest percentage body fat and the average epididymal pad weight in Study B was lower for the chow fed rats than for the 60%-fat-fed rats (Table IV and Table V).

In Study A there was no statistically significant difference in final body weight attained or in the average total weight gain during the experiment between groups I, II and IV. The final body weight attained (298 ± 9 g) and the average total weight gain (235 ± 9 g) for group III (chow; spontaneous exercise) was statistically different from the other three treatments ($P < 0.05$).

In Study B, the pups on treatment III were significantly higher ($P < 0.05$) than the other three groups in terms of final body weight attained (321 ± 8 g) and total weight gain (245 ± 7 g). The pups on treatment I (high-fat diet; spontaneous exercise) were also significantly lower ($P < 0.05$) from the other three groups in relation to the final body weight attained (246 ± 8 g).

To evaluate these data, the composition of the high-fat diet must be considered. The caloric density or available energy was 6.42 kcal per gram for the high-fat diet and 3.26 kcal per gram for the Purina Chow diet. If the protein in the high-fat diet was not adequate, then there should have been differences in real growth rates between the animals fed the high-fat ration and those fed the chow ration. The final body lengths and the final body weights of the rats in treatments I, II and IV in Study A were virtually the same. These parameters were also the same for treatments II and IV in Study B. On the basis of these results, the chow diet did not provide growth advantages for those pups

in the restricted exercise treatments, however, based on body weight, the caloric density of the high-fat diet may have been too great for the spontaneously exercised animals in TB1 and TBS1. This may be due to a combination of factors. The nutritional status of the dams fed the high-fat diet during gestation and lactation may have in some way altered the physiological ability of their pups to grow at a rate comparable to the other groups due to the additional stress of exercise. If the nutritional status of the dams was reflected as a result of the stress of exercise, then the nutritional component most likely involved was an adequate source of protein. The vitamin and mineral content of the high-fat diet fed to the dams may have been insufficient to meet the increased nutritional demands for milk synthesis during lactation. The increased activity and subsequent energy expenditure may have resulted in reduced efficiency of the pups in question to adequately utilize the high caloric density of the 60%-fat diet. On the other hand, these results may simply be a manifestation of an attempt by the hypothalamus and associated structures of the CNS to regulate energy intake. A pilot study was conducted in our laboratory in which rats were given a diet which was 50% fat (Crisco) and 30% protein (vitamin-free casein) in order to ascertain whether or not the protein component of the 60% fat diet was growth restricting. The weight gains of these animals was not different from those animals fed the 60% fat diet. This matter still requires further investigation.

The number of kcal consumed per day was significantly different ($P < 0.05$) among groups I and IV in Studies A and B. The number of kcal consumed per gram of weight gain during the experimental period was

significantly higher in the high-fat; spontaneous exercise treatment. This relationship held true for both experiments. Pups in Study B consistently consumed more energy than their Study A counterparts whose dams were fed the chow diet during gestation and lactation. This difference between experiments appears to be much greater for the pups given the 60%-fat ration. These results may most likely be attributed to the fact that the pups in Study B were generally larger rats throughout the study. It is also possible that the available kcal in the chow diet was underestimated. Data comparing the metabolic turnover rate of these diets in muscle, adipose and liver tissue could substantially enhance the understanding of this effect. Pups fed the high-fat diet in both experiments consumed more energy per each gram of weight gained, but was significantly greater only for treatment I. This difference was more dramatic in Study B than in Study A. The animals on a high-fat diet in the study by Schemmel et al. (59) consumed about 27% more energy than the animals fed the grain ration.

The relationship of exercise to feed intake in my study does not complement the work done by Ahrens et al. (69). They studied the effect of forced exercise and source of dietary carbohydrate (12% of the diet) in rats fed a high-fat diet (about 28% fat) and found exercise significantly reduced food intake, body weight gain and the fat content of the carcasses. In my study the exercised animals fed the 60%-fat, 10%-cornstarch diet had a higher caloric intake than animals with restricted exercise. An explanation of this result in my experiments would involve increased energy intake to balance increased energy expenditure. The

weight gain was not different ($P < 0.05$) between the spontaneously exercised rats and the restricted exercised rats in experiment TB1 fed the chow diet during gestation and lactation.

Our body composition data (Tables IV and V) does support the results of Ahrens study (69). The spontaneously exercised rats exhibited a lower percentage of carcass fat than their restricted exercised counterparts. The study by Hebert and Lopez-S (70) found that male rats allowed food and exercise ad libitum exhibited greater food consumption (+7%) and lower weight gain (-5%) than sedentary controls. The food consumption data of their experiment (70) agrees with the findings for treatment I in Study B. The differences from one study to another with regard to results and experimental design indicate a need for a more comprehensive study.

Exercise

The diet fed the pups in this experiment appeared to have an effect on the distance run in the activity cages although this effect was not statistically significant (Table III). The 60%-fat-fed pups ran on the average about 1000 meters more than their chow fed counterparts during each exercise period. This difference increased to an average of over 3000 meters per exercise period more for the 60%-fat-fed pups in Study B. A possible explanation of these trends would involve the greater energy consumption to balance the greater energy expenditure. This explanation is supported by the greater kcal per day consumed (Tables I and II) by the pups fed the high-fat ration than the pups fed the chow ration. Recall that the pups in Study B consumed a greater number of kcal than

TABLE III

THE EFFECT OF DIET ON ACTIVITY LEVELS

	Expt - Study A		Expt - Study B	
Group[1]	I	III	I	III
n	13	15	6	7
Total number of meters run	47,338 ± 9371	34,530 ± 8194	91,228 ± 19,495	47,092 ± 17,048
Number of meters run/ exercise period	3.683 ± 716	2,697 ± 626	6,516 ± 1,392	3,364 ± 1,218

1 - Identification of treatments: I - high-fat, spontaneous exercise; II - high-fat, restricted exercise; III - chow, spontaneous exercise; IV - chow, spontaneous exercise.

their counterparts in Study A. The fact that the feed consumption was not statistically different may partially explain why the differences in the distances run were also not statistically different. The relationship of energy consumption to weight gain could possibly be better defined if the energy expenditure from running was measured. Ahrens et al. (69) found; however, that their older force-exercised rats consumed fewer kcal which accounted for the negative nitrogen balance observed in these animals. The ad libitum-fed, force-exercised weanling rats gained more body protein per kcal than body fat stored per kcal than their sedentary counterparts; these animals exhibited a lower caloric intake (69). In another study, Ahrens et al. (67) observed that 100 day-old Sprague-Dawley rats increased their voluntary running ($P < 0.01$) when fed 70% of the ad libitum intake of another group on a diet containing a mixture of carbohydrates representing the U. S. "market basket" diet. Another group of rats in this study fed 70% of the ad libitum intake of a fourth treatment group fed a diet in which starch represented the only source of carbohydrates, exhibited a lower but, insignificant amount of running (67). Protein contributed 38% of the calories in this diet whereas fat and carbohydrate contributed 50% and 12% of the calories in this diet respectively (67).

The choice of the type of exercise to be examined in animal experiments needs to be carefully studied. The relevance of different exercise regimes to the development of obesity also requires consideration. For example, forced exercise regimes have been found to sufficiently stress animals such that alterations in enzyme activity, hormonal secretions

and glandular size have been observed (4,40,73). Mayer has indicated that swimming animals 1 hour per day for 30 days was not sufficient to result in changes in body composition (4). Oscai et al. found no hypertrophy of the forelimbs used by rats swimming as much as 6 hours per day, 5 days a week. The results of Oscai et al. (68) seem to indicate that the effort involved and the energy expended in swimming was not any greater than that of control animals left in their own cages. The hair of a rat gives such buoyancy that the animal practically floats; there is only a momentary movement of the forehands so that it may have been possible that animals left in their own cages were more active. Swimming for long periods of time may also pose unnecessary stress on rats since by nature they are nibblers and would not be able to eat while swimming. Consideration of these factors resulted in my choice of a voluntary exercise program of running in activity cages. My experiment would have been improved if the animals in the spontaneous exercise treatments could have had more than the 14 twenty-four hour exercise periods they were given.

Assessment of Obesity

According to Bernardis and Patterson (78), a fast method of determining obesity has been described by several teams of investigators, Heatherington, Szentagothai et al. and Bernardis and Skelton. This method consists of dividing the cube root of the body weight by the naso-anal length (cm). The resulting figure is known as the nutritive ratio or the Lee index. Multiplication of this number by 100 gives a figure of for example 300 instead of 3.00. A figure of about 300 is

TABLE IV

EFFECT OF DIET AND ACTIVITY LEVEL
ON THE DEGREE OF ADIPOSITY

Study A	Group[1]	I	II	III	IV
	n	13	13	15	13
% Body Fat[2] (wet basis)		13.35 ± 0.89[a]	21.34 ± 0.99[b]	6.64 ± 1.43[a]	13.92 ± 0.99[a]
% Body Fat[2] (dry basis)		34.71 ± 1.44[a]	47.90 ± 1.60[b]	18.47 ± 1.31[c]	29.38 ± 1.44[d]
Lee Index[2] (wet basis)		277.29 ± 1.97[a]	276.34 ± 1.97[a]	254.41 ± 1.79[b]	274.86 ± 1.97[a]
Lee Index[3] (dry basis)		200.90 ± 2.34[ab]	208.43 ± 2.63[a]	193.98 ± 3.78[b]	200.29 ± 2.80[ab]
Final Body Length[2] cm		23.08 ± 0.18[a]	23.29 ± 0.28[a]	24.33 ± 0.25[b]	23.02 ± 0.28[a]
Final Body Width[2] cm		7.84 ± 0.18[a]	8.64 ± 0.18[b]	8.47 ± 0.17[c]	7.77 ± 0.18[a]

1 - Identification of treatments: I - high-fat, spontaneous exercise; II - high-fat, restricted exercise; III - chow, spontaneous exercise; IV - chow, spontaneous exercise.
2 - Statistically significant by F test at $P < 0.01$.
3 - Statistically significant by F test at $P < 0.05$.
abcd - Row with common superscript are not significantly different ($P < 0.05$).

TABLE V

EFFECT OF DIET AND ACTIVITY LEVEL
ON THE DEGREE OF ADIPOSITY

Study B	Group[1]	I	II	III	IV
	n	6	4	7	7
Lee Index (wet basis)		278.51 ± 2.24[a]	280.12 ± 2.84[a]	275.84 ± 1.97[a]	280.62 ± 2.05[a]
Final Body Length[2] cm		22.51 ± 0.19[a]	23.56 ± 0.24[b]	24.81 ± 0.17[c]	23.46 ± 0.18[b]
Final Body Width[2] cm		7.85 ± 0.14[a]	8.36 ± 0.18[b]	8.71 ± 0.17[b]	8.47 ± 0.13[b]
Average Pad Wet Wt. g.		0.76 ± 0.22[a]	0.72 ± 0.22[a]	0.63 ± 0.28[ab]	0.59 ± 0.03[b]
Total Cells (x 10^6)[2]		88.51 ± 11.56[a]	43.01 ± 17.02[ab]	82.47 ± 11.41[ab]	32.88 ± 10.49[b]
Total Adipocytes[2] (x 10^6)		215.35 ± 18.98[a]	68.79 ± 27.94[b]	156.42 ± 18.73[a]	14.41 ± 11.23[c]
Total Adipocyte DNA[2]		656.29 ± 71.14[a]	195.25 ± 104.70[b]	509.59 ± 70.20[a]	137.00 ± 64.56[c]

1 - Identification of treatments: I - high-fat, spontaneous exercise; II - high-fat, restricted exercise; III - chow, spontaneous exercise; IV - chow, spontaneous exercise.
2 - Statistically significant by F test at $P < 0.01$.
abc - Row with common superscripts are not significantly different ($P < 0.05$)

considered normal (78). Bernardis and Patterson (78) found a significant correlation between the Lee index and carcass fat content when the index was referred to either wet body weight per cent fat determinations or dry body weight per cent fat determinations. The Lee index value for their animals was approximately 300 or greater. They found the Lee index to increase over 300 with increasing per cent fat.

The Lee index values obtained in this study were all considerably below 300 (Tables IV and V). My Lee index values were not statistically different even though the per cent fat was significantly different ($P < 0.01$) between the four treatments in Study A. The Lee index values tended to increase with increasing fat percentage however. A possible explanation for the discrepancy in these results is that the hypothalamic lesions introduced by Bernardis and Patterson in their rats stunted growth due to the destruction of neural structures of the hypothalamus involved in the release of growth hormone. Impaired release of growth hormone would eliminate the lipolytic actions of this hormone throughout the body and thus the hypothalamic lesioned animals would have exhibited a tremendous tendency to become obese. Destruction of the ventromedial nuclei could also have destroyed the appetite control mechanisms which would also have resulted in obese animals. Differences between the two species of rats considered in these investigations may also have been involved and thus partially account for these discrepancies. On a dry weight basis the weanling VMN lesioned rats and the weanling control rats were 45.8 and 23.3 per cent fat respectively at the end of the 21 day experimental period whereas the per cent fat of

the carcasses in my experiment ranged in value from 18.47 per cent for treatment III to 47.91 per cent fat for treatment II. The length, final body weights and Lee index of treatment III was statistically different ($P < 0.05$) from the other three treatments with no real differences in the other treatments. These data support one another; however, they do not correlate well with the per cent fat values obtained for the four treatments. On the basis of these data it does not appear that the Lee index was an accurate index of obesity in rats without hypothalamic lesions.

The per cent body fat was determined according to the method of Campbell and Friedman (74). The per cent error in duplicates ranged from 0.00 to 7.16 per cent error. The mean per cent error was 2.44 per cent. On a wet basis, only the pups on the 60%-fat ration; restricted exercise treatment were different from other treatments ($P < 0.05$) in Study A (Table IV). On a dry weight basis all four treatments were different ($P < 0.05$).

Exercise and the composition of the diet both appeared to be important factors in determining the per cent carcass fat. The effect of spontaneous exercise in the activity cages appeared to have a tremendous effect since the pups with this treatment effect exhibited the lowest percentage carcass fat on a wet weight basis. The animals in treatment I were 30% less fat than the animals in treatment II; the animals in treatment III were 60% less fat than the animals in treatment IV. The animals in treatment I were 90% more fat than the animals in treatment III; the animals in treatment II were 60% more fat than the animals in treatment IV.

On the basis of these comparisons it would appear that the effect of the composition of the diet was of greater importance than the effect of the exercise treatments. The per cent moisture was greater in the pups in treatment I (62.13%) than for the pups in treatment IV (47.20%) which accounts for the change in the order of fatness in these two groups when compared on dry and wet weight basis.

Body composition was not determined on any pups at 21 days of age due to a lack of subjects. A study by Schemmel, Mickelson and Fisher (59) in which the pre-weaning treatments of the dams was similar, reports that the carcasses of pups of dams fed a 60%-fat diet were 16.5% fat (wet basis) vs 5.2% fat for pups of dams fed a 3% fat ration.

The importance of body composition analysis cannot be emphasized enough. The body composition data provides an entirely different picture of the effects of diet and exercise on the development of obesity than the weight gain and body weight information. One might assume that the animals with the greatest weight gains and the heaviest body weight would be the most obese; however, the results of the body composition analysis indicate that this is an erroneous assumption. The animals in treatment III were not only the heaviest but were also significantly more lean than the animals of any other treatment. In Study B the rats in the chow-fed, spontaneous exercise treatment had smaller epididymal fat pads than the rats in the high-fat,spontaneous exercise tratment.

An attempt to explain these results would be rather futile in the absence of serum insulin and growth hormone levels and no measure of serum FFA's, blood triglycerides and cholesterol levels. A greater

understanding of the changes in body composition reflected by the differences in per cent carcass fat could possibly be obtained by gas exchange experiments with various substrates such as glucose and specific fatty acids in in vitro adipose, muscle and liver preparations. Information on the assimilation of ^{14}C-labelled glucose and FA's would probably also increase the understanding of the body composition changes due to these effects.

The results obtained from our body composition studies are supported by the results of other investigators. Fenton and Dowling (55) showed that some strains of mice become more obese than others on a high-fat diet. Schemmel, Mickelson and Gill (59) found that the composition of the diet and the time at which it was introduced was important in the determination of adipose tissue accumulation. They found the high-fat diet produced a greater percentage of body fat ($P < 0.01$) in all rats fed this diet regardless of the age at which the diet was fed (59). Exercised rodents via treadmill running or swimming have been found to exhibit a lesser degree of adiposity than sedentary counterparts (65,66.67,68,69).

The results of our attempt to determine cell size and number in the rats in Study B are inconclusive since certain parts of the data are irrational. The results are incongruous since we observed a greater number of total adipocytes than total cells in three of the four treatments. On the other hand our data may reflect the short comings of this procedure. Hirsch and Gallian (79) report that the DNA method of determination is a less desirable method of determining cell size and number than determinations done with a Coulter Counter. The shortcomings of

the DNA method are: (1) DNA determination is an inexact estimate of adipose tissue cellularity since blood vessels, fibrous tissue, mast cells, and macrophages contribute unknown amounts of DNA to the total; and (2) collagenase treatment usually causes a great deal of cell breakage and thus, the final suspension may not give a representative or valid sample of original cell numbers. Lemmonier (80) uses histological slide preparations in his determinations of cell size and number. Despite the incongruencies between our determinations for total cells and total adipocytes there are some interesting trends present in the data. In consideration of the fact that all samples were treated the same, it would seem fair to consider variations between treatments for each analysis. A comparison of the total cell determinations shows that there were a greater number of total cells in both of the spontaneous exercise treatments, i.e. treatments I and III, than total cells in the restricted exercise treatments, i.e. treatments II and IV (see Table V). The greatest number of total adipocytes was observed in treatment I. The next greatest number of total adipocytes was observed in treatment III and the smallest number of total adipocytes was observed in treatment IV. One might assume that the increase in adipose depots could be atttributed primarily to an increase in cell size in treatments II and IV rather than to increases in cell number since the pad weights of the four treatments were similar. On the other hand, the increase in the adiposity of the epididymal fat pad in treatments I and III was due more to an increase in cell number than to an increase in cell size. If my assumptions are correct and if they do reflect the actual physiological

changes that occurred during the experimental period, then these data support the findings in studies by Knittle and Hirsch which indicate that increases in adiposity tend to occur primarily by increases in cell size than by increases in cell number. Our exercise treatments may support the findings of Lemmonier (80) who found that increases in adiposity occur more by an increase in cell number than by an increase in cell size. If this data could be reproduced with confidence in the cell size and number determinations, the data would be of considerable importance in light of the present conflicting studies reported by Knittle and Hirsch and those reported by Lemmonier.

The only data in which we have confidence is the average pad wet weight. On the basis of these data it appears that the animals given the high-fat diet attained greater adipose depots than their chow fed counterparts. This data would indicate that the composition of diet is very important in determining the degree of adiposity in the growing rat.

SUMMARY AND CONCLUSIONS

This investigation was undertaken to study the effects of feeding two levels of caloric density factorially with two levels of activity on the growth rate and body composition of male weanling pups from 25 to 67 days of age. The effects of ad libitum feeding the dams of these pups the same two diets during gestation and lactation on the growth rates and activity levels of these pups were also examined. Following weaning, the pups in both Studies A and B, were placed by weight in outcome groups of four each. The pups in each outcome group were then randomly assigned to one of the following four treatments: I - high-fat diet, spontaneous exercise; II - high-fat diet, restricted exercise; III - chow diet, spontaneous exercise; IV - chow diet, restricted exercise.

The pups whose dams were given the 60% fat ration during gestation and lactation (B) attained a higher weaning weight (73 g) than the pups of the dams fed a 4.5% fat ration (Purina Chow) during gestation and lactation - A (55 g). The final body dimensions (length and width) did not appear to be different (Tables IV and V). Pups in Study B tended to run considerably more meters per day than the pups in Study A although the differences were uncertain because of the wide variation. The final body weights attained were greater for the pups in Study B than the pups in Study A except for treatment I. Except for treatment I, the order of the weight gains within treatments among experiments was comparable. It appeared that the differences in final body weight attained could be

attributed to the initial differences present at weaning which reflected the dietary treatments of the dams during gestation and lactation. The difference of treatment I in Study B from the trends exhibited by the other three treatments, was manifest by the lower final body weight attained (246.25 ± 9 g) and the lower total weight gain (170.77 ± 8 g) of the pups in comparison to the final body weight attained (263 ± 10 g) and total weight gain (206.98 ± 10 g) of the pups in treatment I of Study A. The animals in Study B all consumed a greater number of kcal per day than the animals in the corresponding treatments in Study A. The differences in feed consumption between the two experiments appeared to be significant only for those animals receiving the 60%-fat diet.

Study A

Pups fed the chow diet and involved in the spontaneous exercise regimen gained the most weight and exhibited the lowest per cent carcass fat of any of the treatments. They attained the greatest final body weight and the longest body length. The final weight attained, average total weight gain and final body weight were not statistically different between the other three treatments. The per cent body fat was statistically different ($P < 0.05$) for all treatments on a dry carcass weight basis. Treatment IV pups consumed the smallest number of kcal per day (52 ± 3) which was statistically different only from treatment I pups (63 ± 3).

Study B

The final body weight, the total weight gain and final body length were statistically different from one another for all treatments except

treatments II and IV. The chow fed, spontaneously exercised pups gained the most weight, were the longest in length and exhibited the greatest weight gain during the experimental period. The pups in the high-fat, spontaneous exercise treatment gained the least weight, exhibited the lowest weight gain and were the shortest in length. In addition, the pups in this treatment consumed the greatest amount of energy. The average wet weight of the epididymal pad of the pups on the chow diet was lower than the average weight of the epididymal fat pad of the pups on the high-fat diet. The data concerning the cell size and number analysis were inconclusive.

In conclusion, the effects of diet and exercise appear to be involved in the degree of carcass fat and epididymal fat pad tissue accumulation in the rat. Exercise reduced the percentage of carcass fat and epididymal fat pad weight while a diet of high caloric density had the opposite effect on both parameters with or without exercise.

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VITA

Terry Lee Bazzarre was born in Beckley, West Virginia on May 19, 1949. The son of an Air Force officer, Terry attended many public schools as well as Armed Forces Dependents Schools throughout this country and in Germany. Terry entered Virginia Polytechnic Institute in September 1967 and completed the B.S. degree in March 1971. On April 10 of the same year he married the former Christine Lee Hunter, a December graduate of Virginia Polytechnic Institute and State University in Human Nutrition and Foods. From April to July, Terry did forrestry reclamation work in southern West Virginia. Terry and Chris returned to Virginia Polytechnic Institute and State University in July 1971 as graduate students in the department of Human Nutrition and Foods. Terry should complete the requirements for the M.S. degree during the Fall quarter of 1973 after which he plans to continue his education under a combined Ph.D. program between the Human Nutrition and Foods department of Virginia Polytechnic Institute and State University and the department of Pediatrics at the University of Virginia.

Terry Lee Bazzarre

THE EFFECT OF EARLY NUTRITION AND ACTIVITY LEVELS ON THE DEVELOPMENT OF OBESITY IN RATS

by

Terry Lee Bazzarre

(ABSTRACT)

The effects of early nutrition and activity on growth rate and adiposity of the rat were examined in two experiments. Dams of pups in experiment TB1 were given Purina Chow (4.5% fat) during gestation and lactation whereas dams of pups in experiment TBS1 were fed a diet containing 60% fat. At weaning, pups were assigned by weight to outcome groups of four each and randomly assigned to the following four treatments: I - high-fat, spontaneous exercise; II - high-fat, restricted exercise; III - chow diet, spontaneous exercise; IV - chow diet, restricted exercise.

Weaning weights of pups in TBS1 (73 g) were greater than weaning weights of pups in TB1 (55 g). This difference in weight was maintained throughout the experiments by the pups in treatments II, III and IV in TBS1 over the same treatments in TB1. The pups in treatment III in both experiments achieved the greatest final body weight, body length and total weight gain. Pups fed the high-fat diet exhibited greater spontaneous activity than the chow fed pups in both experiments although these differences were not statistically significant due to the wide variation within treatments. The carcass fat (dry weight basis) was 43.71 ± 1.44% for treatmentI, 47.91 ± 1.60% for treatment II, 18.47 ± 1.31% for treatment III and 29.38 ± 1.44% for treatment IV, all of which were statistically different from one another ($P < 0.05$). The average

epidymal fat pad weight was greater for pups on the 60% fat diet than pups fed the chow diet.