EFFECTS OF OVERFEEDING SUCKLING RATS ON FAT ORGAN CELLULARITY

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY
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1976

M-009

ABSTRACT

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By

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Obesity is associated with two processes, an increase in the number (hyperplasia) and size (hypertrophy) of adipocytes. Human obesity exists in both forms (Björntorp and Sjöström 1971, Brook et al. 1972, Salans et al. 1973).

The methods used to successfully induce hyperplastic obesity in animals include: feeding a high fat diet (Herberg et al. 1974, Lemonnier 1972, Lemonnier and Alexiu 1974) and possibly tube feeding (Narins and Hirsch 1974) and reducing litter size (Knittle and Hirsch 1968). The genetic obesity of the Zucker rat has been classified as hyperplastic (Johnson et al. 1971, Lemonnier 1971, Lemonnier and Alexiu 1974) as has been the obese hyperglycemic mouse (Herberg et al. 1970b, Johnson and Hirsch 1972) both relative to their lean controls.

Little or no work has been done to determine the fat cell differences between strains of the same species. However, weight gain, when rats are fed a high fat diet, differs among strains (Lemonnier et al. 1971, Mickelsen et al. 1955

Schemmel et al. 1970a).

For these studies two strains of rats, one an obesity-susceptible strain when fed a high fat diet, the Osborne Mendel, and the other an obesity-resistant strain, the S 5B/Pl (Schemmel et al. 1970a), were used. At birth, 40 rats of each strain were suckled by dams fed a high fat diet. The other 40 were suckled by dams fed a low fat diet. Half of each of the four groups of 40 were nursed in litters of six while the other half were nursed in litters of three. Finally, half of the rats were supplemented, twice daily via tube feedings, while the other half were not. At weaning (24 days of age) 5 animals from each group of sixteen were sacrificed and the remaining 5 animals were raised until 15 weeks of age at which time they were sacrificed. Fat depots were removed, weighed and rapidly frozen for subsequent analysis of cell size and number.

Adipocyte size and number were determined on a sample of all inguinal tissue and samples of testicular and perirenal tissues from 40 animals representing the extreme dietary treatments (high fat diet, supplemented, three per letter versus low fat diet, non-supplemented, six per litter) for each strain and age. Sizing of fat cells was done microscopically on cells isolated by a modification of Rodbell's (1964) procedure. Cells were stained with 2% cresyl violet and fixed with a glutaraldehyde solution prior to sizing. The diameter of cells from each depot were measured. From the resulting data cell size (weight of lipid, in ng., of

the average fat cell) and cell number were calculated.

Osborne Mendel rats had about two times the number of fat cells in the entire left inguinal fat pad as did the similarly treated S 5B/Pl strain at 24 days of age. Both strains had increases of cell number between 24 days and 105 days of age. For the Osborne Mendel strain this was a two to three fold increase while for the S 5B/Pl it was a three to four fold increase. This resulted in all 105 day old animals having similar numbers of inguinal adipocytes, except for the Osborne Mendel strain fed the high fat diet. This group of animals had three times the number of cells as the other groups. Therefore, at 15 weeks of age, the obesity-susceptible Osborne Mendel strain of rat fed the high fat diet had hyperplasia.

The obesity-susceptible male Osborne Mendel strain always had larger cells than the S 5B/Pl strain. This difference was significant in half of the groups when similar ages were compared. The Osborne Mendel strain had larger cells than the S 5B/Pl in inguinal tissue when first evaluated at 24 days of age. When this was coupled with their greater increase in size with age (three fold versus two fold) they completed the 15 weeks of the study with cells $2\frac{1}{2}$ times larger than comparably raised male S 5B/Pl animals. This was on an absolute scale.

Similar effects of strain, age and diet on fat cell size and number as those already described in the inguinal depot were also true for testicular and perirenal fat depots.

In testicular and perirenal tissue the Osborne Mendel rat had fat cells twice as large as the S 5B/P1 rat at 105 days of age. The largest fat cells were located in inguinal and testicular tissues.

The number of testicular cells in the whole left depot was two times greater in the high fat fed 105 day old Osborne Mendel rat than in all other 105 day old groups. For perirenal tissue this was almost a four fold increase.

High values for adipocyte number were found in 15 week old Osborne Mendel rats fed a high fat diet in each tissue studied. Feeding of a high fat diet versus a low fat diet had not significantly affected the size of the adipocytes by 15 weeks of age. Neither altering litter size from six to three pups nor supplementing neonatal pups twice daily significantlly affected the eventual depot cell size or cell number in inguinal fat depots.

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Food Science
and Human Nutrition

1976

To my daughter

Lindora

and my husband

Norman

ACKNOWLEDGEMENTS

My appreciation is extended to the following persons who contributed to this study:

My academic advisor, Dr. Rachel Schemmel, and Dr. Dorice Narins, Dr. Robert Merkel and Dr. Duane Ullrey, my committee; for their time, assistance and guildance.

Dr. Vance Sanger and assistants for help in finding a method to microscopically distinguish lipid droplets from adipocytes.

The department of pathology for use of the Leitz Ortholux microscope used in making photomicrographs.

Dr. Dale Romsos for suggestions on the use of collagenase and for clarification of calculations.

Dr. Roger McFeeters for writing the program for the Wang calculator to expedite calculations.

Dr. Kenneth Stevenson for use of the stage lamp and eyepiece and stage micrometers for microscopic work.

Marianne Stone, Janet Grommet and Anna McKay for assistance with dissection of the animals.

Cathy Nolan for help with calculations.

Mary Andrews for statistical help and computer programing.

My neglected family, Norman and Lindora.

The National Science Foundation which partially supports the Michigan State University computing facility.

This project was supported in part by the Weight Watchers Foundation, Inc.

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REVIEW OF LITERATURE

Obesity--Implications for Human Health

Risk Factor

Data have been obtained to show the positive relationship between obesity and many diseases and physical impairments. Micklejohn (1955) divided the complications of obesity into two categories--metabolic and mechanical. The metabolic complications included diabetis, hypertension and disorders of cholesterol metabolism. The mechanical problems were further classified by Sinclair (1955) to include postural changes, clumsiness and the direct effects from carrying extra weight. Postural changes led to respiratory and circulatory insufficiency; clumsiness to more accidents; and, since extra weight must be supported physically by the joints and metabolically by the heart and lungs, increased incidence of arthritis, varicose veins, hernias and pneumonia. In addition, increased incidence of high blood pressure, kidney disorders, gallstones (Eden 1975) influenza and digestive tract disorders (Wilkinson 1975) have been reported in the obese person. As a result, obesity has been associated with reduced life expectancy (Eden 1975, Micklejohn 1955, Sinclair 1955, Wilkinson 1975).

Defining Obesity and Overweight

While obesity connotes an excess storage and depotsiton of fat, Knittle (1972b) does not believe overweight directly implies fatness. Physiologically, according to Salans et al. (1973), obesity is characterized by an excess of adipose tissue mass. A person has been considered to be overweight between ten and twenty percent heavier for height than expected (Eid 1970, Shukla et al. 1972) from Scott's (1961) London County Council or Tanner et al. (1966) standards, respectively. Obese was twenty percent or more above weight for height (Brook et al. 1972, Brook and Lloyd 1973, Eid 1970, Shukla et al. 1972) both for given sex and age of subject. Asher (1966) has considered an individual obese when the weight for height was above the ninety-seventh percentile. Obesity was defined by Johnson et al. (1956) to be in or above the channel A_4 of Wetzel's grid (1941). The obese person, Knittle (1972b) has stipulated, has at least 40% of total body weight as fat.

The Cellular Classification of Obesity

There are three possible routes by which fat organ enlargement could occur; hyperplasia, hypertrophy, or a combination of the two. Hyperplastic obesity is characterized by an increase in the number of adipocytes. Hypertrophy is an enlargement of fat cells. The number of adipocytes is not increased in hypertrophic obesity relative to a

non-obese subject of similar age, sex and species. Finally, obesity could result from the combined effects of both hypertrophy and hyperplasia.

Incidence of Human Obesity

Thomas and Mayer (1973) have reported 50% of the middle aged Americans as being over-weight based on height weight tables. The Ten State Nutrition Survey (1968-1970) found 40% of adults and 39% of adolescents to be obese by weight. Johnson et al. (1956) reported ten percent of Boston area school children as obese in 1952. Shukla et al. (1972) cited 16.7% of three hundred infants obese and an additional 27.7% as overweight. The percentages of obese persons as obtained by Eid (1970), for children aged six through eight years having shown excess weight gains at six months, closly agree with the percentages of Shukla et al. (1972).

Adult Obesity

Cellular Classification

Salans et al. (1973), Brook et al. (1972) and Björntorp and Sjöström (1971) have associated the character of human obesity with the age at its onset. Hyperplastic obesity has been related to early onset, generally through the age of five years and between nine and thirteen years (Salans et al. 1973). Robertson and Lowrey (1964) reported that at least 26% of the subjects (aged four through sixteen years)

attending a clinic for overweight children had become overweight between the age of two and five years and 45% were overweight before they were six. Thus fat babies tended to become fat children (Eid 1970) and a high proportion of fat children became fat adults (Brook et al. 1972, Knittle 1972b). Hypertrophic obesity developed, according to Salans et al. (1973), later in life. This is consistent with the finding of Björntorp and Sjöström (1971), who found that the type of obesity (hyperplastic or hypertrophic) was associated with the degree of adult obesity. The moderately obese tended to be of the hypertrophic type whereas the most obese exhibited hyperplastic obesity.

Prognosis for Weight Reduction

Hirsch et al. (1966) was the first investigator to report that cell size, and not cell number, varied with adult weight gain and loss. This has been subsequently verified by many other investigators (Björntorp and Sjöström 1971, Bray 1970, Brook and Lloyd, Knittle 1972b, Salans et al. 1971). While weight reduction for hypertrophic obesity is not easy, it is apparently even more difficult for the individual with the hyperplastic form (Knittle 1972b).

Childhood Obesity

Since prognosis for hyperplastic, child-onset, obesity in the adult is poor, the cure lies in prevention. Cell number has been thought to become fixed in the late teens

or as young adults (Hirsch et al. 1966). Prevention, therefore, must focus through this age. Most of the obese students in the study of Johnson et al. (1956) had been persistently obese throughout their school years. Some and become obese during grades seven through nine, and a few had fluctuated between the obese and non-obese states. In the non-obese subject, the ealiest age at which adult cell number was achieved was twelve (Knittle 1972b). In the obese children studied, adult cell number was achieved in many by eleven years of age and in some as early as six years. In addition, the rapid increase in cell number, which normally began from nine to twelve years of age, was observed to have begun from five to seven years in some obese children.

Once obese successful weight reduction was rare (Asher 1966, Eid 1970, Robertson and Lowrey 1964). Rather than actually producing a weight loss the recommended treatment for obese children has consisted of maintaining stable weight while linear growth continues (Knittle 1972b, Wolff 1955). Little, however, is known about the cellular effects of maintaining obese children on a diet (Brook et al. 1972).

Obesity and birthweight in humans seem to be unrelated. Wolff (1955) failed to find this relationship while Asher (1966) failed to show a significant relationship.

Overweight in later years was more related to rapid infant weight gain than to the fact the child had one or two obese parents according to Eid (1970). In contrast, several other researchers (Tanner 1955, Tepperman 1958, Thomas and Mayer 1973) found that heredity was more important than

environment in determining the eventual weight of an individual.

That rapid increase in infant weights, reflected by recent trends toward shortened birthweight doubling is advantageous is now being questioned (Narins and Hirsch 1974, Wilkinson 1975). As early as 1960 Widdowson and McCance questioned the benefits from making children grow faster than they had previously.

Early nutrition has been implicated in the determination of later development of obesity. Some reports have suggested that early weaning and introduction of solid foods was causal (Shukla et al. 1972, Wilkinson 1975). Others (Beal 1957, Fomon 1969, Guthrie 1966) have disputed this due to poor infant acceptance and low caloric density of the solid foods relative to milk. Fomon (1974) speculated that early introduction of solid foods might encourage overeating and establish unsound eating habits. Shukla et al. (1972) found that milk consumption of the infant did not initially decrease when solid foods were added to infant diets. The average daily intake for all infants up to three months of age was 775 Kcal. while for infants in the same age range and receiving only milk was 590 Kcal--a substantial difference. Guthrie (1966) reported that infants receiving solid food had about the same caloric intake as those receiving none. Many babies have been receiving solid food while very young (Beal 1957, Guthrie 1966, Shukla et al. 1972). Forty percent of the three hundred infants studied

by Shukla et al. (1972) had received solids by four weeks. This resulted in an increased intake of protein, carbohydrate, fat and calories compared to the intake of unweaned babies. According to the study of Beal (1957), introduction of fruit and cereal occurred at an average of one month of age. Guthrie (1966) found eight percent of the fifty infants participating in her study to have been receiving solid foods at five weeks and all by nine weeks.

The relationship of bottle feeding and breast feeding to obesity is also controversial. Eid (1970) reported no difference between the two methods of feeding and the incidence of obesity. Bottle fed babies gained weight more rapidly than did breast fed babies, according to Fomon (1974), and he speculated the reason may have been overfeeding at the bottle. Shukla et al. (1972) found a small percentage of mothers began breast feeding and that there was a high drop out rate: only 6.3% of the population continuing at twelve weeks. There was a tendency, in this study, for bottle fed infants to receive solid foods earlier than those fed at breast.

Animal Studies

Many factors effect the size of the fat cell and cellularity of the fat organ. Among them are: species, anatomical site, location within the adipose organ, sex, diet and strain. In addition, single gene mutation resulting in obesity and obesity produced by lesions of the hypothalamus

provide information to further our understanding of the whole concept of obesity.

Growth and Development

Non-Adipose Tissue

Early work on cellular growth included information on organs of the Sprague-Dawley rat, but due to technical difficulties excluded adipose tissue (Winick and Nobel 1966, Winick and Nobel 1967). Growth was altered by over and under nourishment achieved by altering litter sizes. Parkes (1926) observed differences in body weight gains of mice reared in different sized litters; those from small litters gained weight more rapidly than those from large litters.

Reducing rat litter size to three pups resulted in a persistent increase in body size throughout the whole life of the animal compared to rats from large litters of eighteen (McCance and Widdowson 1966). Winick and Nobel (1967) reduced litter sizes of Sprague-Dawley rats to three and six and found many parameters increased, including: body weight, organ protein, RNA, DNA and the number of cells per organ. The size of the cells, however, were similar in both over-nourished and control rats. They also (1966) deprived rat pups by increasing litter size to eighteen and restricting neonatal intake at various ages. They concluded that the cellular response to deprivation depended on the tissue in question, the age at onset, and the duration of deprivation.

Adipose Tissue

Work on the developmental pattern of adipose tissue, too, has utilized the Sprague-Dawley rat. Peckham *et al*. (1962) used DNA to assess adipose tissue expansion and concluded that the epididymal site retained the capacity to generate adipocytes through thirty-four weeks. A similar conclusion was reached by Zingg *et al*. (1962) using Wistar rats. Enesco and Leblond (1962) found that epididymal tissue expansion occurred, in the Sherman rat, by both hyperplasia and hypertrophy as evidenced by DNA analysis.

Hirsch and Han (1968), using the technique of Hirsch and Gallian (1968) found that growth by hyperplasia ceased in the epididymal site at twelve weeks and in the retroperitoneal site at fifteen weeks. Beyond these ages depot enlargement in the chow fed Sprague-Dawley rat was by hypertrophy alone. This has been confirmed by Greenwood and Hirsch (1975) using a pulse-dose sequence of tritiated thymidine. These reports need not be in conflict since Rodbell (1964) showed that a large proportion of the DNA in adipose tissue is present, not in adipocytes, but in their supporting matrix. Thus, while the adipocytes themselves may not have been expanding in number past twelve weeks, the supporting matrix may have continued to expand.

Over and Under Nutrition

Many investigators have altered the size of rat (Heggness *et al*. 1961, Johnson *et al*. 1973, Knittle and

Hirsch 1968) and mouse (Lemonnier and Alexiu 1974, Lemonnier et al. 1973) litters in an effort to achieve either under or over nutrition with respect to adipose tissue growth. Tube feeding (Dymza et al. 1964, Narins and Hirsch 1974), alteration of diets of lactating dams (Emery et al. 1971, Schemmel et al. 1973, Knittle 1972a) and starvation (Hirsch and Han 1969) have also been used.

Heggness et al. (1961) rotated two litters of six Rochester rat pups each between three dams on an eight hour schedule such that one group always went to a dam that had not been suckled the preceeding eight hours. The second group, then, always went to the dam suckled the previous eight hours. In addition, regular litters of six, twelve and eighteen pups were raised. The animals from twelve and eighteen per litter gained weight less rapidly while having equally as efficient utilization. They were presumed to have been eating less. Group one was concluded to have gained weight at a maximum rate because group two compared well to the animals raised regularly six to the litter. Therefore, there must have been enough milk left after group one had suckled to adequately nourish group two. All body constituents were elevated by preweaning overnutrition but this was especially true of fat. Group one exhibited a growth spurt when weaned to the calorically more dense high fat diet.

Knittle and Hirsch (1968) altered litter sizes to either four or twenty-two pups per litter at birth. Animals from the small litters were heavier and had more adipocytes in the epididymal fat organ than those raised in large

litters. Beginning at ten weeks of age the animals from the small litters were significantly heavier and had significantly larger cells. Their epididymal fat depots were both absolutely and relatively heavier than those of their siblings. In addition, the pattern of adipose tissue growth had been altered in animals from small litters because hyperplasia continued through the twenty weeks of the study. Female mice raised in litters of four on a control diet had more and larger adipocytes at 4.5 months of age and an increase in body fat when compared to animals raised nine to the litter (Lemonnier and Alexiu 1974).

Pups tube fed by Dymza et al. (1964) on basal plus five percent rats milk had significantly more carcass fat and less moisture than controls. Narins and Hirsch (1974) supplemented neonatal rats and increased adipocyte size over controls. Although there was no significant difference in cell number between the supplemented and non-supplemented groups, both had elevated cell numbers compared to the animals of Knittle and Hirsch (1968) raised simultaneously.

A form of supplementation, giving lactating dams a high fat diet, was found by Emery et al. (1971) to increase milk fat two fold. Schemmel et al. (1973) reported that lactating Osborne Mendel rats showed this difference at eighteen days post-partum when fed grain or 60% fat (w/w) diets. Another method of altering rat milk supply was used by Knittle (1972a). He deprived pups by altering maternal protein and calorie intake. He found that pups from calorie

restricted dams could catch up to controls in growth while those from protein and calorie restricted dams could not.

Hirsch and Han (1969) reported that starving six week old Sprague-Dawley rats for one week resulted in a reduction of both cell size and number. Both parameters returned to control levels with refeeding. Prolonged semi-starvation from fifteen through twenty-six weeks of age resulted in a reduction in cell size only.

Hollenberg et al. (1970) have confirmed this in the lumbar and epididymal sites using animals previously injected with a dose of tritiated thymidine. More recently, Greenwood and Hirsch (1974) have used tritiated thymidine to determine the developmental pattern of Sprague-Dawley rat epididymal tissue. Mature adipocytes have gone through three characteristic developmental stages, those being: proliferation, differentiation and maturation. During proliferation the cells are thought to be produced at varying rates and times and be influenced by environmental conditions such as: hormones, temperature, age, and dietary intake. Cells leave the proliferative stage and enter the differentiating stage where they stay again for varying periods depending upon external stimuli. Finally, cells enter the mature population and enlarge or are lost from it. Several feedback loops are thought to influence the whole process.

In epididymal adipose tissue of the nine day old Sprague-Dawley rat the entire process was uninhibited, and proliferation occurred rapidly (Greenwood and Hirsch 1974).

Between two and four weeks of age proliferation was rapid but differentiation was slow. From five to seven weeks proliferation slowed, but time spent differentiating again decreased. Beyond this age, the adipocytes were filling with the lipids. Thus the pattern was hyperplasia and hypertrophy early, maturing to hypertrophy alone.

Species

Expansion of adipose tissue mass varies from species to species. In male Wistar rats and male Golden hamsters postnatal depot enlargement was primarily by hypertrophy while in male albino guinea pigs hyperplasia was causal (Di Girolamo et al. 1969, Di Girolamo and Mendlinger 1971).

Depot Location

The location of the fat organ within the animal affects both the number and size of cells that are established. In adult male rats (Di Girolamo et al. 1969, Di Girolamo et al. 1974, Hellman et al. 1962, Johnson et al. 1971) and mice (Lemonnier 1972) the perirenal and retroperitoneal depots have the largest cells, the epididymal depot the next largest and the subcutaneous depot the smallest. The order was different in genetically obese mice, however, being subcutaneous >epididymal>perirenal (Johnson and Hirsch 1972). A species difference existed also. In New Zealand rabbits subcutaneous adipocytes were the largest while those from the epididymal site were smallest (Di Girolamo et al. 1974).

Most fat cells are generally found in the male subcutaneous depot, the next largest number in the epididymal site with fewest in the perirenal or retroperitoneal for rats 26 weeks of age (Johnson et al. 1971) and mice 32 weeks of age (Lemonnier 1972) both raised on control diets.

In addition, different depots are affected differently by dietary alterations. Lemonnier and Alexiu (1974) and Lemonnier (1972) reported hyperplasia in the perirenal and perigenital sites while hypertrophy was evident in the abdominal subcutaneous location at thirty-two weeks of age as a result of feeding a high fat diet to mice since birth. When the high fat diet was given to male Swiss mice hyperplasia appeared in the perirenal depot too.

Work on adult human subjects has also revealed variations in cell size. Salans et al. (1971) found site to site variations which were defined further as gluteal>preperitoneal>abdomial>mesentary>tricepts>omentum (Salans et al. 1973). In general they found most cells in the omental site and fewest in the gluteal location.

Brook (1971) found cells from deep human sites in children aged 0.8-11.4 years to be smaller than those from subcutaneous sites. It has also been shown that cells along lobule borders are smaller than those centrally located (Smith 1971). Goldrick (1967) has observed variation of cell size from the periphery to the center of the lobule, which he described as a sectioning artifact.

Sex

Lemonnier (1971) studying ten week old genetically obese Zucker rats fed standard diet, observed that females had a 2.3 fold increase in cell number while males had significantly fewer cells than their lean littermates in the perigenital location at 2.5 months of age. Depot weight increased 9.7 times for females and 3.2 times for males. Cell size increased about four times in both sexes. These differences in sex and obesity disappeared by twelve months of age but were replaced by hyperplasia in the perirenal and sub-cutaneous depots of obese animals. Further evidence of sex difference was reported by Lemonnier and Alexiu (1974). Female control rats exhibited a two fold increase in cell number between the ages of 2.5 and 12 months while males did not. Sex differences were also apparent in adult mice with respect to cell size and number in perigenital, perirenal and abdominal subcutaneous locations (Lemonnier 1972).

Johnson and Hirsch (1972) have reported the most striking hyperplasia in six strains of genetically obese mice as occurring in the female obese hyperglycemic mouse.

Similar results to those found in female versus male rats (Lemonnier and Alexiu 1974) and mice (Lemonnier 1972, Johnson and Hirsch 1972) were also found in women by Björntorp and Sjöström (1971). Women tended to have more fat and more adipocytes than men.

Diet

Diets high in various types of lipids have been related to increased rat weight gain. Barboriak et al. (1958) reported solid fats such as Crisco were more effective in promoting growth than oils.

Osborne Mendel rats, fed a high fat (60% w/w) diet from weaning, had fat depots two to three times heavier than grain fed controls (Schemmel et al. 1970a). In rats such as the Osborne Mendel strain, which readily became obese when offered a ration high in fat; the diet after weaning had an equally important effect on the eventual weight of the animal as did the diet during the preweaning period (Schemmel et al. 1973, Schemmel and Mickelsen 1974). Peckham et al. (1962) also fed a ration high in fat. Using DNA as the indicator of cell proliferation, they determined that weight gain in the epididymal site of Sprague-Dawley rats was accompanied by increase in cell number. Switching animals from the high fat (57% w/w) to a calorically less dense pellet diet at 34 weeks of age resulted in a lower DNA value at 65 weeks than animals fed a high fat diet since weaning.

When NMRI mice were fed a high fat diet, cell size and cell number increased in epididymal and scapular subcutaneous locations (Herberg et al. 1974). Hypertrophy was the result in genetically obese obob mice (Lemonnier et al. 1971). Thirty-two week old Swiss mice, fed a high fat diet since birth, showed hypertrophy in abdominal subcutaneous

and perigenital depots with hyperplasia in the perirenal site.

Lemonnier and Alexiu (1974) reported hypertrophy in the epididymal site when either corn oil or hydrogenated palm oil was given as the energy source to weanling rats. Corn oil was associated with hypertrophy and hydrogenated palm oil with hyperplasia in the perirenal site. Genetically obese adult male Zucker rats fed a high fat diet from five through twelve months showed hyperplasia in perirenal and subcutaneous sites. In spite of the fact that the cell number of the lean animals had stabilized, when a high fat diet was fed to adult Zucker rats hyperplasia was observed in the perirenal site.

Strain

Different strains of the same species show marked differences in ability to gain weight on the same diet (Hammond 1955, Schemmel 1970a). Lemonnier et al. (1971) noted that Swiss albino mice were more prone to become obese when a high fat diet was consumed than were Bar-Harbor mice. Osborne Mendel rats gained the most weight and S 5B/Pl rats least when seven strains were fed a high fat diet (Schemmel et al. 1972, Thiel et al. 1972). Osborne Mendel rats were more efficient than the S5B/Pl strain in utilizing food calories for deposition within the body regardless of whether eating a high fat or grain ration. This was especially true of the high fat diet (Schemmel et al. 1972). Age and strain

did affect weight, but had no effect on the percentage of triglyceride or on fatty acid composition within the depot (Shier and Schemmel 1975).

Genetic Obesity

Studies have been conducted on genetically obese animals in order to define the character of obesities caused by a single gene mutation. Johnson and Hirsch (1972) found that the obesity of most varieties of mice exhibiting this characteristic was of hypertrophic origin. Exceptions included the obese-hyperglycemic mouse (Herberg et al. 1970b, Johnson and Hirsch 1972) and the New Zealand Obese mouse which exhibited hyperplasia as well. The latter strain, however, is not caused by a single gene mutation and thus has no lean control. When compared to NCS/R it exhibited hyperplasia (Johnson and Hirsch 1972) but Herberg et al. (1970b) classified it as hypertrophic.

The only rat which carries the trait for genetic obesity the Zucker strain, has been investigated. Bray (1969) noted that hypertrophy caused enlargement of the epididymal and parametrial depots of the genetically obese animal. Johnson et al. (1971) concluded from their developmental study that post-weaning growth of the lean Zucker rat was similar to the Sprague-Dawley. Tissue expansion was the result of combined hypertrophy and hyperplasia through fourteen weeks beyond which enlargement took place by hypertrophy alone. The exception was the scapular subcutaneous site which showed

continued hyperplasia through 26 weeks. The developmental pattern in the obob varied from his lean littermate. The fatty tended to be born with fewer cells but then caught up and, in fact, surpassed the control. Rate of proliferation exceeded that of the control, continuing through 26 weeks. Cell size was equivalent in all locations unlike the normal rat where subcutaneous7epididymal7retroperitoneal. Hyperplasia was observed in all adipose depots of the obese Zucker and, in addition to his reduced skeletal size, the animal had a greater percentage of total body fat.

Johnson et al. (1973) reported the effect of raising Zucker rats in litters of three or four and twelve to nineteen pups per litter in order to determine if genotype or nutritional effects predominated. During the first thirty days the nutritional effect was the predominant influence on weight gain while beyond twelve weeks genotypic difference became more important. Lemonnier (1971) reported hyperplastic obesities in female genetically obese Zucker rats.

Hypothalamic Obesity

Lesions in the ventromedial nucleus of the hypothalamus (VMH) are a method of inducing obesity in an animal. Johnson et al. (1971) studied VMH lesioned lean Zucker rats. They found that the cellular obesity was similar to that of the obese Zucker in the epididymal site, cell sizes being much larger than the controls. The retroperitoneal site of the VMH animal exhibited hypertrophic obesity with respect

to the control. In the scapular subcutaneous site the VMH lesioned Zucker had fewer cells than the obese Zucker and larger cells than the controls. Reduced skeletal size was observed in these rats.

Han et al. (1965) lesioned Sprague-Dawley rats and noted that thirty percent became obese when pair fed with controls. Lesioning caused an increased percentage of body fat but nose to occipital length, femur length and stomach weight decreased. The VMH animals gained equivalent amounts of weight while growing less rapidly. Han and Lui (1966) produced lesions in weanling Sprague-Dawley rats and then force fed them to control both quantity and pattern of intake. They concluded that the induced obesity was not caused by hyperphagia. Thomas and Mayer (1973) reported VMH obesity was caused by hyperphagia. A two to three fold increase in initial intake was reported. Kennedy (1955) reported VMH rats as having an increased drive to work for food. It seems, then, that they also have altered metabolic pathways allowing them to become obese when pair fed.

Hirsch and Han (1969) lesioned Sprague-Dawley rats at 7.5 and 13 weeks. They concluded that the induced obesity was hypertrophic. Han and Lui (1966) lesioned rats at 21 days. This resulted in increased body weight and reduced bone growth. They speculated that this might be due to close proximity between eating centers and growth hormone releasing factor centers in the hypothalamus.

INTRODUCTION

Human obesity exists in both the hypertrophic and hyperplastic forms (Björntorp and Sjöström 1971, Brook et al. 1972, Salans et al. 1973). The former has been associated with adult onset obesity. The hyperplastic form, which according to Knittle (1972b) is more difficult to correct because of the increased number of adipocytes, has been associated with onset in childhood (Björntorp and Sjöström 1971, Brook et al. 1972, Salans et al. 1973).

In contrast to working with human subjects, laboratory animals, such as rats and mice, permit rigid dietary and environmental control. In addition, genetic differences, which are important in influencing the build of an individual (Tanner 1955, Tepperman 1958, Thomas and Mayer 1973), can be controlled when animals are used.

Most obese laboratory rats and mice have been reported to have hypertrophic obesity (Lemonnier 1971). Few animal models exist for the hyperplastic form. Genetic obesities (Johnson and Hirsch 1972, Johnson et al. 1971, Lemonnier 1971, Herberg 1970b), feeding a high fat diet (Lemonnier 1972, Lemonnier and Alexiu 1974, Herberg et al. 1974) and possibly tube feeding (Narins and Hirsch 1974) or reducing litter size (Knittle and Hirsch 1968) have been associated

in some situations with hyperplasia. When observed it is most often in perirenal tissue (Lemonnier and Alexiu 1974). Females seem to have a greater susceptibility to hyperplasia than do males (Björntorp and Sjöström 1971, Johnson and Hirsch 1972, Lemonnier 1971). Little work has been done on differences in strain, although differences in ability to gain weight on a given nutritional plane have been documented (Hammond 1955, Lemonnier et al. 1971, Schemmel et al. 1970a).

This study was undertaken to determine the cellular effect of a combination of obesity inducing agents in two rat strains. Male Osborne Mendel rats served as obesity susceptible and male S 5B/Pl rats as obesity-resistant models (Schemmel et al. 1970a). Induction of obesity was attempted through the following manipulations: reducing litter size from the control level of six (normal for the S 5B/Pl strain) to three; feeding a high fat (44% w/w) diet to post-partum dams, their nursing litters, and their weanlings; and supplementing the intake of neonatal pups with tube feedings.

The primary purpose of this study was to evaluate how obesity-resistant and susceptible rat strains would respond from the viewpoint of adipose tissue cellularity, to different methods of overfeeding. Procedures known to have been associated with the production of obesity between birth and weaning as well as after weaning were used. Which dietary manipulations were associated with the production of hyperplastic obesity and which with hypertrophic

obesity were also of interest. In addition, the relationship, if any, of the number or size of adipocytes to body weight at sacrifice or percent carcass lipid was of interest. The correlation between body weight and body fat was also assessed.

Two primary methods exist for the assessment of adipocyte cell size and number. The technique of Hirsch and Gallian (1968) used osmium tetroxide to fix adipocytes which were then sized in a Coulter counter. This method has been widely used in recent years (Brook 1971, Brook and Lloyd 1973, Brook et al. 1973, Hirsch et al. 1966, Hirsch and Han 1969, Johnson et al. 1971, Johnson et al. 1973, Johnson and Hirsch 1972, Knittle 1972a, Knittle and Hirsch 1968, Narins and Hirsch 1974, Salans et al. 1968, Salans et al. 1971, Salans et al. 1972) but has two main disadvantages. One is cost, since both the osmium tetroxide and Coulter counter are expensive. The other is size limitation since the method does not assess adipocytes smaller than 25 μm. in diameter. This makes the technique of Hirsch and Gallian (1968) especially poor for young animals which have a higher proportion of small cells.

The second technique involves microscopic assessment of adipocyte diameters. This has been done on fixed sectioned whole tissue (Björntorp and Sjöström 1971, Hellman 1962, Lemonnier 1971, Lemonnier 1972, Lemonnier et al. 1971, Sjöström et al. 1971, Zingg et al. 1962) and on cells isolated from their supporting matrix by the method of Rodbell (1964).

Unstained isolated cells (Bray 1969, Bray 1970, Kovanen et al. 1975, Zinder et al. 1967) have been manually measured using an eyepiece micrometer in a microscope. Unstained cells have been mechanically sized by a Zeiss particle size analyzer using photomicrographs (Hirsch and Gallian 1968, Smith 1971) and on non-isolated cells (Goldrick 1968). Isolated cells have also been stained with methylene blue prior to manual sizing (Booth et al. 1974, Herberg et al. 1970b, Di Girolamo et al. 1971, Di Girolamo and Mendlinger 1971). The disadvantage of the sectioning procedure is that the cells are not spherical, as they are when isolated, making calculations of size (such as volume, surface area or weight) less accurate. Investigators using no stain or methylene blue may have been sizing lipid droplets in addition to adipocytes since the two are indistinguishable. The latter argument is true for sizing done by the Zeiss particle size analyzer as well. For these reasons a new method of microscopically identifying isolated adipocytes has been developed and described in this thesis.

METHODS

Experimental Design

Ninety-six Osborne Mendel (OM) and ninety-six S 5B/Pl[1] (S) male rat pups were assigned in groups of three or six, on the day following birth, to dams of their respective strains. For a complete representation of the experimental design see figure 1. Half of the dams assigned each litter size were offered a high fat and half a low fat diet. These diets are detailed in table 1. In addition, half of the animals from each strain, diet and litter size were given two supplemental tube feedings each day until weaned. The composition of the supplemental feedings were 24% and 18% corn oil for OM and similarly 20% and 15% for S. Milk was prepared with 15 gm of dry fat free milk solids, the above listed percentages of corn oil and distilled water to a volume of 100cc. This was mixed in a blender. Later groups were randomly reduced in number so that there were ten animals for each group. Five of each group were sacrificed at 24 days and the remainder at 105 days.

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[1]The pedigreed S 5B/Pl Cr breeding stock was secured from Samuel M. Poiley, Head, Mammalian Genetics and Animal Production Section DR and D, Chemotherapy, National Cancer Institute, DHEW.

The diets were designed so that for each Kcal. consumed, the animals would receive equivalent amounts of protein, vitamins, and minerals. The Kcal.:protein ration as calculated from Kcal. consumed/gm of dietary protein eaten was 17.3 for both diets. The diets differed in the source of the primary energy component. The low fat diet provided 66% of the calories from the carbohydrate cerelose. This diet contained 3.8 Kcal. per gram based on 4, 4 and 9 Kcal. per gram of protein, carbohydrate and fat respectively. Using the same basis the high fat ration contained 5.7 Kcal. per gram. It contained 44% fat in the form of hydrogenated vegetable shortening..

At weaning (24 days) animals were assigned to one of two groups. One group was sacrificed immediately and the second group was maintained on the same diet their mothers had been given until 105 days of age. At this age the remaining animals were sacrificed. Adipose tissue from the inguinal region for all rats and from the testicular and perirenal regions of animals representing the extreme experimental conditions for both ages and strains (supplemented, high fat diet, three per litter; and non-supplemented, low fat diet, six per litter) were assessed for adipocyte cell size and number. The percentage of lipid in each depot was also determined.

Experimental Conditions

All animals were housed in a ventilated room where the temperature remained at 23± 1°C. Twelve hours of light were permitted each day. Activity was neither promoted nor restrained.

Dams, having previously cast one litter, were mated four to a male. When pregnant, as determined by weight gain, they were transferred to individual plastic cages of the dimensions 33 x 28 x 13 cm. The top of the enclosure was wire to permit air circulation. Wood shavings were placed at the base of each cage and were changed at three day intervals. Water and pellets were offered ad libitum. On day one, litters were reduced numerically to rearrange appropriate numbers as previously described. The dams were changed from the pelleted diet to either the high or low fat diet which was available ad libitum in porcelain cups. The pups, so designated, began receiving supplemental feedings.

The pups were supplemented daily on days one through twenty-four. Supplementation occurred between 8 and 9 AM and between 4 and 5 PM ± 1 hour. Pups were fed up to one milliliter per feeding depending on their size and the fullness of their stomachs. They were fed with a bulbous syringe inserted into the stomach via the esophagus. As the pups grew older they were allowed to drink and eat the food given their dams.

At twenty-four days half of the pups were sacrificed, while the others were individually housed in wire screened

bottom metal cages. The dimensions were 18 x 18 x 25 cm. Each rat, in a porcelain cup, had a diet of the same compositon of his mother. Food and water were available ad libitum.

Procedure for Sacrificing Rats and Removing Fat Depots

On the day of sacrifice each experimental animal was lightly anesthetized with ether and decapitated. Inguinal, testicular and perirenal adipose depots were excised. In the weanling animals this involved both depots, while in the older animals only the left depot was removed. An incision was made down the midline of the ventral side of the rat. The inguinal depot(s) was removed using the ventral midline, lowest rib and urogenital organs as boundaries. The tissue was cooled rapidly then weighed to the nearest milligram. The testicular and perirenal fat organs were subsequently removed and treated in the same manner. Immediatly after weighing, all depots were individually wrapped in aluminum foil, labeled, placed in self-sealing plastic bags, frozen on dry ice and subsequently transferred to a chest-type freezer (-20 ± 2^{o}C.).

Depot Lipids

Weanling Animals

Using forceps, approximately 0.3 gm. of frozen adipose tissue was transferred into a tarred Roese-Goettlieb

flask[2]. The flask plus tissue were weighed and the difference yielded the wet weight of tissue to be analyzed. A correction was made for any moisture lost during storage according to the following formulae:

$$\text{Moisture lost per gm sacrifice weight (gm)} = \frac{\text{tissue sacrifice weight (gm)} - \text{tissue weight at analysis (gm)}}{\text{tissue sacrifice weight (gm)}}$$

$$\text{Corrected weight of tissue sample (gm)} = \text{gm moisture lost per gm sacrifice weight} \times \text{weight of tissue used (gm)} + \text{weight of tissue used (gm)}$$

Duplicate samples were analyzed whenever quantity permitted.

Ten ml. of diluted 37% HCl (4 parts acid to one part deionized water) were added to each flask. The contents were boiled for thirty minutes in a water bath in the hood. All samples were cooled for at least one hour but never longer than twenty-hour hours. When left in excess of two hours, flasks were stoppered. Deionized water was added until the level of liquid in the flask reached the base of the flask neck. Blanks of diluted hydrochloric acid and water only were treated similarly and handled the same as samples throughout the rest of the analysis.

Twenty-five ml. of anhydrous ethyl ether were added and each flask was covered with a rubber stopper. (The same cork and flask remained together throughout the experiment and the part of the cork in contact with the flask contents was not touched). Flasks were shaken sixty times, the stoppers

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[2]Purchased from Mejonnier Brothers Company, Chicago, Illinois.

removed and 25 ml. of petroleum ether were added. Each flask was subsequently shaken ninety times and the corks were again removed. Each flask stood thirty minutes while the layers separated. The upper ether layer was decanted into tarred fat flasks[3]. Ethyl and petroleum ethers were added then decanted twice more as previously described, however 15 ml. of each ether rather than 25 ml. were used. Prior to the third extraction, deionized water was added to raise the level of the water layer to the top of the neck of the Roise-Goettlieb flask.

Ether was evaporated until no fumes were discernable. The flasks containing fat were placed in a 70° C. oven for 15 minutes, cooled in a dessicator for 30 minutes and weighed on an analytical balance to an accuracy of four decimal places. Weight of lipid in the flask was calculated by difference. Percent depot lipid was calculated as follows:

$$\text{Percent of lipid in wet tissue} = \frac{\text{weight of lipid in sample (gm)}}{\text{corrected wet weight of tissue used (gm)}} \times 100$$

$$\text{Weight of lipid in depot} = \text{percent of lipid in wet tissue} \times \text{depot weight at sacrifice}$$

One Hundred and Five Day Animals

Approximately one gram samples of fat depot were minced while frozen. They were then transferred to tarred, labeled

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[3]The fat flasks had been prepared previously by heating overnight in a 70° C. oven, cooling for thirty minutes in a dessicator then weighing to the fourth place.

aluminum pans. Duplicate samples were taken whenever possible.

The pans plus minced fat depot were immediatly weighed to the fourth place and the weight of depot in each sample was calculated by difference. All samples were placed in a 70°C. oven for 48 hours. They were then moved to a vaccume oven also at 70°C. where they remained an additional 48 hours. The samples were weighed daily thereafter until constant weight (to the third place) was reached. Before each weighing samples were cooled for 30 minutes in the dessicator. Once dry, the samples were stored in dessicators until the lipids were extracted on the Goldfisch fat extraction apparatus. Extraction with ethyl ether was allowed to proceed for $6\frac{1}{2}$ hours. This length of time was found sufficient to remove all lipids as evidenced by no additional lipid following re-extraction. Extraction flasks had been previously weighed to the fourth place after drying overnight in a 70°C. oven 15 minutes and cooled in a dessicator for 30 minutes. Following extraction of the lipid, flasks were dried in a 70°C. oven 15 minutes, cooled in a dessicator for 30 minutes and weighed to the fourth place. The weight of lipid was calculated by difference. Calculations to determine depot lipid after correction for moisture losses were made as previously described for 24 day old animals.

Assessment of Adipocyte Number and Size

Isolation

Adipocytes were isolated using a modification of Rodbell's (1964) procedure. Frozen adipose depots were thinly sliced, using a microtome blade. Forty to 100 mg. of tissue were used per sample. Frozen minced tissue was quickly added to clear plastic test tubes. The tubes were tapped to facilitate transfer of the tissue to the bottom of the tube. One ml. of Krebs-Ringer phosphate buffer with bovine serum albumin and collagenase (figure 2) at pH 7.4 was added to each tube. This amount was found to cover the tissue and to contain a sufficient amount of enzyme to release adipocytes. Samples were incubated at 37°C. in a shaking water bath for thirty minutes.

Fixing and Staining

Tubes were removed from the water bath and two drops of 2% cresyl violet were added using a Pasteur pipette. Tubes were gently agitated to facilitate stain diffusion. One drop of the glutaraldehyde fixing solution (figure 3)[4] was added with a Pasteur pipette. The tubes were again gently agitated.

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[4]The fixing solution stopped enzyme activity making washing to remove the enzyme unnecessary. This was helpful in maintaining the integrity of the fragile cells.

Identification

All glass coming in contact with the isolated cells was first coated with silicon[5]. This included microscope slides and pasteur pipettes.

Suspensions were prepared the day of measurement to avoid bias from rupture of fragile cells. Tubes were lightly agitated prior to sampling. Small drops, of the stained fixed cell suspension were transferred to the treated slides using a Pasteur pipette. No cover slips were used. The diameters of 400$\pm$ 12 adipocytes per depot were optically measured. This was accomplished using an eyepices micrometer which had been previously calibrated. Calibration checks were made at intervals throughout the study. The magnification of the binocular microscope was 150 power. The eyepiece was 15 power and the objective 10 power. Illumination was directly from a stage lamp. The microscope had slide stage adjustment controls so that movement of the slide proceeded smoothly along the stage.

Isolated adipocytes were round and identified by their rough appearance (basketball-like) and a dark ring around their circumference. In addition most cells had picked up the violet hue of the stain. Cresyl violet stain is specific for protein and does not complex with lipid. Lipid droplets were smooth, the rims were not dark, and never picked up

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[5]Dri-coat, purchased from Fischer Scientific Company, Livonia, Michigan.

the stain (figure 4). Membranes and cellular debris will, however, pick up the stain--but will not be round. Whenever too much debris was present, as evidenced by purple colored irregular shapes, the sample was discarded and reisolated from the frozen tissue.

Measurement began at the top of the microscopic field for each drop of cell suspension. Assessment then proceeded from left to right, then back to left, etc. until measurement was completed at the bottom of the droplet. Each cell was aligned with a micrometer grid using the stage slide adjustors. The grids between the grid already aligned and the opposite side of the cell were counted (see figure 5).

Tabulation

A tape recorder was placed adjacent to the microscope. Conveniently, it had a pause arrangement, allowing the person measuring cells to actually record, by the flick of a switch, only after three to four cells had been measured. This made tabulation from the tape to paper more expedient as it cut out all pauses while actual measurement was being made. Cells were measured and their diameters recorded without looking away from the microscope field. Four hundred plus or minus twelve cell diameters were thus measured for each depot. When assessment was approximately complete as judged by the counter on the tape recorder, the tape was played back and the number of cells from each diameter division were tallied. The sum of cells of each diameter was then

recorded for future calculation of cell size and cell number (see figure 6).

Calculations

The volume of cells from each depot was computed using the following formula:

$$\text{volume } (\mu m^3) = (\pi/6) \quad (3\sigma^2 + \bar{x}^2) \bar{x}$$

where the terms $\bar{x}$ (mean) and σ (standard error) are computed from the diameters of the 400 adipocytes measured from each tissue. Computation was done in this manner to overcome the skewedness that would result if volume (x^3) were computed in the usual manner. These calculations were done on the Wang calculator, grouped mean program.

The lipid content of the "average" cell in each depot was calculated in the following manner:

$$\text{Mean adipocyte lipid content } (\mu g.) = \text{mean volume } (\mu m^3) \times \text{triglyceride density}$$

The units of triglyceride density are $g./(10^6)(\mu m^3)$ as determined by conversion of density in gm/cc to $\mu g/cc$ and by calculation of the number of $\mu m/cc$. (Measurement was in μm.). The density of 0.918 was used throughout the study.

Cell number was calculated by dividing the weight of the "average" cell into the tissue lipid content.

$$\text{Cell number} = \frac{\text{tissue lipid content}}{\text{mean adipocyte lipid content}}$$

Photography

Photomicrographs were made representing many of the tissues and treatments. Both a Leitz 4"x5" and a 35 mm camera were used on a Leitz ortholux microscope[6]. The former used black and white Ektapan film[7] and the latter used 35 mm colored film[8]. Both types of pictures were taken at the same magnification, which was determined by the following Leitz magnification formula:

$$\text{occular} \times \text{objective} \times 1.25 \times \frac{\text{bellows extention}}{25}$$

or: 10 x 10 x 1.25 x (28/25) = X140. Prints of the 4"x5" were made directly from the negative without enlargement. Prints of 35mm slides, however, were enlarged (see figure 4).

Statistical Analysis of Data

A five way multivariate analysis of variance and univariate analysis of variance were used to identify the differences in the data. This was accomplished on the Michigan State University computer and included inguinal cell size in µg lipid; cell number in left depot and percent depot fat.

While two variables remained fixed, differences between

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[6] 4"x5" Leitz automatic camera and Leitz Wetzlar ortholux microscope: Distributed by Donald Main and Co., Scientific Instruments, 510 North Dearborn Street, Chicago, Il. 60610.

[7] Ektapan film: 4"x5", 4162 thick, Eastman Kodac Co., Rochester, NY. 14650.

[8] 35 mm film: Eastman Kodac Co., Rochester, NY. 14650.

the third variable were analyzed by the Students T test. The three groups included age, strain and treatment. The two treatments were control fed (low fat diet, non-supplemented, 6/litter) and overfed (high fat diet, supplemented, 3/litter). Data on the forty animals for which inguinal, testicular and perirenal tissue had been analyzed were used. The Michigan State University computer performed these computations as well.

Students T tests (Chou 1963) for individual group differences were mechanically performed to decipher differences in cell size and number between the three tissues.

Pearsons correlation coefficients were computed on the Michigan State University computer. Cell size and cell number for inguinal fat pads were compared with percent body fat and sacrifice weight in 105 day old animals.

RESULTS AND DISCUSSION

Inguinal Cell Number

Data are summarized in Tables 2 and 3 and Figures 7, 8, 9 and 10. Table 2 gives mean cell number values and standard errors about the mean for each group of five animals. Table 3 lists situations where each variable resulted in a significant ($\alpha \leq 0.05$) alteration in cell number. The mean cell number as affected by the various treatments is graphically shown in Figures 7, 8, 9 and 10. Each treatment variable, as litter size, supplementation, ration, strain and age and its influence on cell number is treated separately.

Age

In general fat cell number in whole left inguinal fat depots significantly increased between 24 and 105 days of age. However, there were three exceptions. These exceptions were: OM, high fat fed, supplemented, 3/litter; OM, low fat fed, non-supplemented, 6/litter; and OM low fat fed, non-supplemented, 3/litter. The number of cell in whole left depots generally doubled with age in similarly treated groups.

Strain

Even though S rats are very resistant to obesity (Schemmel et al. 1970a) it was very suprising to note that they had approximately the same number of adipocytes in the left inguinal fat depot as did the OM rats when they were fed the low fat ration.

Twenty-four day old, low fat fed, OM rats which had been supplemented and raised three to the litter (cell number in left depots; OM 5.55 $\pm$ 1.14, S 3.30 $\pm$ 0.52, all values for cell number are X 10^6) as well as those non-supplemented and raised three to the litter (cell number in left depots; OM 7.42 $\pm$ 1.88 X 10^6, S 4.85 $\pm$ 1.14 X 10^6) had significantly more cells than similarly treated S rats.

When fed the high fat diet there were at least three fold differences in cell number between the two strains whether assessed at 24 or 105 days of age. More specifically, rats fed the high fat ration and which had been supplemented and raised in litters of three had 12.07 $\pm$ 3.92 X 10^6 versus 6.23 $\pm$ 1.67 X 10^6 cells for S rats; if supplemented and raised in litters of six OM rats had 15.07 $\pm$ 4.24 X 10^6 and S rats 5.00 $\pm$ 1.12 X 10^6 cells; and if non-supplemented and raised six to the litter the OM had 12.38 $\pm$ 2.04 X 10^6 cells whereas S rats had 5.96 $\pm$ 1.47 X 10^6 cells, all in whole left depots. The latter two sets of OM rats compared, also had more cells in whole left depots than the S strain at 105 days of age, OM 60.08 $\pm$ 25.88 X 10^6, S 24.48 $\pm$ 7.35 X 10^6; OM 54.79 $\pm$ 18.71 X 10^6, S 11.41 $\pm$ 3.23 X 10^6, respectively. Finally

high fat fed 105 day OM rats, non-supplemented and raised in litters of three had significnntly more cells (in whole left depots) 39.55 $\pm$ 12.38 X 10^6 than similarly treated S rats, 13.26 $\pm$ 5.46 X 10^6.

Ration

The most meaningful increase in adipocyte number was caused by feeding OM rats a high fat diet. Other less meaningful differences related to ration were observed. Significant diet differences were shown in both strains of rats at 24 days of age when supplemented and raised in litters of six. For OM rats this was a three fold increase. Cell number for OM rats fed the high fat diet was 15.05 $\pm$ 4.24 X 10^6 and if fed the low fat diet, 5.55 $\pm$ 1.14 X 10^6, in whole left depots. These differences were even more pronounced in OM rats at 105 days of age because rats fed the high fat diet had 60.08 $\pm$ 25.88 X 10^6 cells and those fed the low fat diet had 15.60 $\pm$ 2.64 X 10^6 cells. Although differences in cell number were significant for S rats in the one case, the fact that it failed to occur in the other three situations makes this observation less meaningful. For the 24 day old S strain cell number for the whole left inguinal fat depots were 5.00 $\pm$ 1.12 X 10^6 for rats fed the high fat diet and 3.30 $\pm$ 0.52 X 10^6 for rats fed the low fat diet. Cell numbers in whole left depots at 105 days of age were 24.48 $\pm$ 7.35 X 10^6 and 12.97 $\pm$ 5.30 X 10^6 for rats fed high and low fat diets, respectively.

Supplementation

Dietary supplementation by directing a bulbous syringe into the stomach had little effect on the number of adipose cytes. Although there were significant differences in two situations, both of which were observed in the S 5B/Pl strain, the fact that one caused an increase in cell number and the other a decrease tends to minimize the effect of supplementation, in general. In the 24 day old animals the only difference between supplementation groups was in low fat fed S rats raised in litters of six. The non-supplemented rats had more cells ($5.46 \pm 1.59 \times 10^6$ versus $3.30 \pm 0.52 \times 10^6$) than did the supplemented animals in whole left depots. In 105 day old animals, however, supplemented S rats had more cells when raised in litters of six and fed a high fat diet. The supplemented animals had $24.48 \pm 7.35 \times 10^6$ cells and the non-supplemented rats 11.41 ± 3.53 cells for the total left inguinal fat depot.

Litter Size

Generally, adipocyte number was not affected by raising rats in litters of 3 or 6. However, there was one exception. This was in 24 day old rats of the S strain, fed the low fat ration and supplemented. Those raised in litters of three had 4.58 ± 0.83 cells while those raised in litters of six had 3.30 ± 0.52 cells in whole left depots. Since a significant difference did not persist to 105 days of age,

altering litter size did not have a permanent effect on the number of inguinal adipocytes.

Summary, Inguinal Fat Cell Number

Inguinal fat cell number was significantly increased by feeding OM rats a high fat diet but but this was not true for S rats. Furthermore, in general, there was an increase in fat cell number between 24 and 105 days of age for both strains of rat regardless of diet, supplementation or litter size. At 24 days, S rats had 1/3 to 1/2 as many adipocytes in the whole left inguinal fat depot as OM rats. This difference in strains was also true for high fat fed animals at 105 days of age but not for low fat fed rats at 105 days of age. Suprisingly, at this age both OM and S rats had a similar number of fat cells when fed a low fat diet.

Adipocyte Size in the Inguinal Fat Depot

Mean cell size has been expressed as weight in ng. of lipid per cell in Table 6 and the significance of the treatments, that is, the effect of age, strain, ration, supplementation and litter size are presented in Table 7. Graphic presentations of these effects are represented in Figures 7, 8, 9 and 10. Meaningful effects of each treatment will be discussed separately.

Age

In eleven out of sixteen possible treatments, 105 day old rats had significantly larger fat cells in the inguinal fat depot than 24 day old rats when strain, ration, supplementation and litter size were held constant. For the OM strain, age caused a significant increase in the quantity of lipid per cell in every case except one. The exception was the group of rats which was fed a low fat diet, non-supplemented and raised 3/litter.

In genreal, S rats approximately doubled the weight of lipid in the fat cells of the inguinal fat depot between 24 and 105 days of age. For $\frac{1}{2}$ of the gruups the increase was significant. Three of these were fed the high fat diet. Further breakdown included: supplemented 3/litter (162 ± 42 for 105 day old rats, 81 ± 8 for 24 day old rats--all cell size values are mean cell lipid weight in ng.); supplemented 3/litter (160 ± 39 for 105 day rats, 83 ± 16 for 24 day old rats); and non-supplemented 6/litter (118 ± 34 for 105 day old rats, 70 ± 17 for 24 day old rats). Only one low fat fed S group of rats showed a significant increase; 90 ± 22 for 105 day old rats, 46 ± 12 for 24 day old rats, in cell size with age. This was in the group that had been non-supplemented and raised 6/litter.

Strain

The quantity of lipid per cell was related to the strain of the rat, with OM rats having from two to four times as much lipid in their cells as S rats when the other four variables were held constant (Table 6). From among the sixteen treatment groups, eight were significantly different and these differences were more likely to occur among 105 day rather than 24 day old rats. In general, large standard errors tended to minimize the significance of the other groups despite a two to four fold difference in mean value.

More specifically, 24 day old OM rats fed a low fat ration, supplemented and raised 6/litter had significantly more fat in their fat cells (137 $\pm$ 35)than did S rats (84 $\pm$ 10). There was nearly a three fold difference by 105 days of age (405 $\pm$ 145 in the OM versus 143 $\pm$ 61 in the S rats) which also was significant. Twenty-four day old low fat fed, non-supplemented OM rats raised 3/litter had significantly more fat in their inguinal depot adipocytes than did S rats (114 $\pm$ 34 versus 65 $\pm$ 19). By 105 days of age the mean weight of fat in the inguinal fat depot adipocytes of OM rats held constant for diet, supplementation and litter size was approximately $2\frac{1}{2}$ times as much as S rats. Groups 105 days of age where strain significantly affected cell size included: high fat fed animals, supplemented and raised in litters of six (OM 373 $\pm$ 70, S 162 $\pm$ 42); non-supplemented, and raised in either size litter (6/litter, OM 285 $\pm$ 55,S 118 $\pm$ 34; 3/litter, OM 346 $\pm$ 43, S 138 $\pm$ 36); low fat fed animals,

non-supplemented and raised in litters six (OM 350 ± 110, S 91 ± 22). Thus, in animals fed the high fat diet and raised to 105 days of age, the OM strain always had larger inguinal cells than the S strain when similar groups were compared. In the low fat fed 105 day old animals, those OM rats raised in litters of six had larger cells than similarly treated S rats regardless of whether or not they were supplemented.

Litter Size, Supplementation and Ration

The size of the litter in which the rat was reared made a significant difference ($\alpha < 0.05$) only in OM, high fat fed, supplemented rats 24 days old. For this group, rats raised in litters of three had larger adipocytes in the inguinal depot than rats raised in litters of six (148 ± 27 versus 107 ± 37). Feeding supplementary milk by tube resulted in a significant increase in lipid weight per cell only for 24 day old, S low fat fed rats raised 6/litter. The supplemented group had a mean cell weight of lipid of 84 ± 10 and the non-supplemented group had a mean cell lipid weight of 46 ± 12 ng. per cell. Neither of the above differences persisted into later (105 day) life. Variation in litter size from three to six rats and supplementary feeding of the pups from 1-24 days of age therefore contributed very little to the eventual enlargement of inguinal adipocytes of either strain of rat when the conditions were controlled as in this study. Diet, high versus low fat, was not important either since it never resulted in a significant difference in cell size.

Summary, Fat Cell Size in the Inguinal Fat Depot

Age and strain were the two most effective variables which significantly increased the size of inguinal adipocytss. When strains were compared, significant differences were most often observed in older animals; when age was compared differences were most often in OM animals. This was confirmed by the two way interaction observed when the five was analysis of variance was run. This graphically depicted in Figure 15.

Relation of Results to Body Weight and Body Fat

At 105 days of age inguinal cell number was highly related (r=0.85) to percent body fat. The number of inguinal cells was related to body weight (r=0.70) and percent inguinal fat (r=0.59).

Inguinal cell size was related (r=0.74) to body weight at sacrifice and to a lesser degree (r=0.63) to percent body fat.

Body fat was also highly correlated (r=0.88) to body weight at sacrifice.

Cause of Osborne Mendel Inguinal Obesity

The OM strain of rat had larger cells at 24 and 105 days of age regardless of diet, supplementation, or litter size than did the S strain. The S strain resists obesity (Schemmel et al. 1970a) and this study showed that the size of their adipocytes had not increased to the size of the OM

strain. Thus the OM strain, in gereral, had larger fat cells in the inguinal depot than the S strain, when similar ages were compared, through fifteen weeks of age. The OM strain also had more adipocytes in inguinal tissue at 24 days of age, when similar diets were compared, than did the S strain. Both strains showed an increase in the number of inguinal adipocytes with age, but the high fat fed OM strain had three times the number of adipocytes as did all other 105 day old animals. Therefore, when the OM, obesity-susceptible, strain of rat was fed a high fat diet from birth, at 15 weeks of age they gave evidence of hyperplastic obesity.

In 105 day animals, when the number of fat cells was expressed on a per gram basis, the S strain had at least twice as many cells as the OM strain regardless of diet. For all low fat fed animals the S rats had 7.02×10^6 cells per gram of inguinal tissue while the OM had 2.49×10^6 cells. In animals fed the high fat diet values were 5.78×10^6 and 2.72×10^6 for S and OM strains, respectively.

Comparison of Number and Size of Adipocytes in Three Fat Depots

In order to compare adipocyte number and size among fat depots, the testicular and perirenal fat depots were also analyzed for cell size and number in four groups of rats at both 24 and 105 days of age. The four groups selected were: both the Osborne Mendel and S 5B/P1 strains fed the low fat ration, non-supplemented, and raised in litters of six; and

both the Osborne Mendel and S 5B/Pl strains when fed the high fat ration, supplemented, and raised in litters of three. This allowed for comparisons to be made in relation to strain and age for adipocyte number and size as well as the extremes insofar as dietary treatments were concerned.

Adipocyte Number

Inguinal Tissue

Comparisons of age and strain have already been made for inguinal tissue. Age, 105 days versus 24 days, and strain, OM versus S 5B/Pl, made significant differences in the number of inguinal adipocytes. Direct comparisons between the two treatments, that is high fat diet, supplemented, 3/litter and low fat diet, non-supplemented, 6/litter were not previously made. They were not found to significantly different (Table 7 and 8 and figure 11).

Testicular Tissue

Between 24 and 105 days of age fat cell number in the testicular fat pad showed six to thirteen fold increases (Tables 6 and 7 and Figure 12). If these were considered altogether, cell number increased from 1.68×10^6 at 24 days of age to 15.83×10^6 cells at 105 days of age in the whole left depot. At 105 days of age, the Osborne Mendel, low fat fed and the two groups of S 5B/Pl rats had comparable numbers of fat cells in the testicular fat depot whereas Osborne Mendel rats fed the high fat diet, raised 3/litter

and supplemented had at least twice as many cells as the others. This is an extention of earlier data from our laboratory which indicated that weight of this tissue was substantially increased between 24 and 100 days of age when these rats were fed a high fat ration (Schemmel et al. 1970b).

Perirenal Tissue

At 24 days of age, the Osborne Mendel rats fed the high fat diet, supplemented and nursed 3/litter had twice as many cells in the entire depot as did the three other groups of rats. Between 24 and 105 days of age there was a seven to seventeen fold increase in the number of cells in this whole left depot. The greatest increase was shown by the Osborne Mendel rats fed the high fat diet, supplemented and raised in litters of three. This was from 5.29×10^6 at 24 days of age to 85.77×10^6 at 105 days of age, both for whole left tissues. Even at 105 days of age, the other three groups of rats had no more than a two fold difference in the number of fat cells. The Osborne Mendel rats fed the low fat diet had even more cells than the S 5B/Pl rats fed the high fat diet in whole left depots (Table 6 and 7 and Figure 13).

Comparison of the Number of Cells in the Three Depots

At 105 days of age, Osborne Mendel rats, regardless of diet, had $1\frac{1}{2}$ to 2 times as many fat cells in perirenal tissue as they did in inguinal tissue. For OM rats, the number of cells in the testicular tissues were comparable to those in

the inguinal tissue when evaluated on the entire weight of the tissue. For S 5B/Pl rats at 105 days of age, the three fat depots tended to contain the same numbers of cells (Table 6). The trend in adipocyte proliferation seemed to be that the inguinal depot proliferated early in life, followed by proliferation in fat cell number in the testicular and perirenal depots.

Fat Cell Size

Adipocytes always significantly increased in size with age regardless of anatomical site of the fat depot (Table 8 and 9 and Figures 11, 12 and 13). In general, the increase in cell size was two fold for inguinal tissues but three to eight fold for testicular and three to four fold for perirenal tissues.

Inguinal Tissue

Results for age and strain differences with respect to inguinal fat cell size have already been discussed. Treatment extremes, that is high fat diet, supplemented, 3/litter versus low fat diet, non-supplemented 6/litter have not been previously compared. They were found significantly different when subgroups, except 105 day old Osborne Mendel rats were compared. All other inguinal groups, that is 24 day old S and OM as well as 105 day old S showed treatment differences at the 0.02 level of confidence.

Comparison with Other Data

The inguinal tissues, mean of all 105 day OM rats, had larger cells (349 ng./cell) than 10 week (57 ng./cell) or 20 week (136 ng./cell) old Charles River rats in the scapular subcutaneous site (Salans et al. 1972). These Charles River rats had been raised 6/litter on chow-type diet + 5% glucose water. The S strain (mean of all 105 day rats) had inguinal cells (138 ng./cell) similar in size to 20 week old Charles River rats in the scapular subcutaneous site.

The size of the S inguinal adipocytes at 15 weeks was comparable to the size of the non-obese male Zucker scapular cells at 26 weeks of age (162 ng./cell) while the OM had cells about twice as large (349 ng./cell)(Johnson et al. 1971). The Zucker rats, both non-obese and genetically obese, were raised 8/litter on a chow-type ration. The size of the obese Zucker rat scapular cells were much larger than either the non-obese Zucker, S or OM animals. The cells of the obese were 1263 ng./cell as compared to 162 ng./cell, 138 ng./cell and 349 ng./cell, respectively. Twenty-six week old male Zucker rats overfed by raising in litters of four and offering a high fat paste in addition to the chow had cells 212 ng./cell and 1160 ng./cell for non-obese and obese rats, respectively (Johnson et al. 1973).

Testicular Tissue

In this study testicular fat cell size was similar to inguinal fat cell size when age, strain and dietary treatments were held constant. Levels of significance changed only

slightly between the two tissues. Older animals had larger cells (285 ng./cell) than younger animals (63 ng./cell) when compared over all treatment and strains. This was significant at α=0.000 and for subgroups at α<0.024. The OM strain had two to three times larger cells (242 ng./cell) than the S strain (106 ng./cell), significant at α=0.013. The above means are representative of all four groups of rats of each strain (Tables 8 and 9). For individual groups the OM strain had significantly larger cells at the 0.024 level of confidence, except 24 day old, low fat fed, non-supplemented rats raised in litters of six where no significant difference was noted (Figure 12).

In general, the testicular fat depot had smaller fat cells if rats were fed the low fat diet, non-supplemented and raised 6/litter than if rats were fed the high fat diet, supplemented and raised 3/litter (α< 0.01). The exception was the group of Osborne Mendel rats fed the high fat diet, supplemented and raised 3/litter at 105 days of age where cell size was comparable to the group of rats matched for strain and age.

Comparison with Other Data

Testicular tissue of the male OM rat had cells slightly larger (438 ng./cell in the high fat diet group and 394 ng./cell in the low fat group) at 15 weeks than the Sprague-Dawley at 12 weeks of age (330 ng./cell) by comparison with Knittle's (1972a) data (for male rats raised 12/litter on a semipurified ration). This was less clear at 15 weeks

according to the data of Hirsch and Han (1969) who reported values of (330 ng./cell and of Narins and Hirsch (1974) whether supplemented (348 ng./cell) or not supplemented (267 ng./cell).[1] Knittle and Hirsch (1969) reported 15 week old male Sprague-Dawley rats, fed chow-type ration, as having 537 and 281 ng.lipid/cell for rats raised in litters of four or twenty-two, respectively. By 20 weeks of age the cells had expanded to 639 and 375 ng./cell in the Knittle and Hirsch study (1969). In any case, the S male rats have smaller fat cells in testicular tissue, 118 and 190 ng./ cell than any of the previously mentioned sizes listed for Sprague-Dawley rats. In fact, the size of fat cells in S rats is comparable 157 ng./cell) to the 40 day old male cell size of supplemented rats raised by Narins and Hirsch (1974). Their Sprague-Dawley rats had 169 ng. lipid per cell.

The Charles River strain at 10 weeks of age had cells about the same size as the S strain (110 ng./cell for Charles River and 157 ng./cell for S) at 15 weeks (Salans et al. 1971). Male OM rats had larger testicular adipose cells at 15 weeks of age (438 ng./cell in the high fat fed and 395 ng. /cell in the low fat fed) than the Charles River strain at 20 weeks (302 ng./cell, on a chow type diet + 5% glucose water)(Salans et al. 1971).

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[1] Hirsch and Han (1969) raised their male rats on chow-type diets; Narins and Hirsch (1974) reared their male pups, beginning with 12/litter but systematically reducing by 2 weeks of age to 6/litter, on a semipurified ration.

Johnson et al. (1971 and 1973) have reported non-obese male Zucker rats fed chow as having cells containing 344 ng./cell at 26 weeks of age. This is less than the OM and more than the S both at 15 weeks of age. The obese Zucker rat, however, had larger cells than either the OM or S strain. Male Zucker rats overfed by offering a high fat paste in addition to chow and rearing in litters of four had 1250 ng./cell and 440 ng./cell in genetically obese and non-obese rats, respectively (Johnson et al. 1973). The latter is similar to the size of 15 week old OM rats fed the high fat diet. Male genetically obese Zucker rats were reported by Bray (1969) as having a mean cell size of 1350 ng./cell, by Johnson et al. (1971 and 1973) of 1225 ng./cell at 26 weeks, and by Lemonnier (1971) of 1275 ng./cell at 10 weeks of age (all reared on chow type diets).

Perirenal Tissue

Between 24 and 105 days of age there was a four fold increase in the size of perirenal fat cells for male Osborne Mendel rats fed the low fat diet. It was less ($2\frac{1}{2}$ fold) for OM rats fed the high fat diet (Tables 8 and 9 and Figure 13). However, there had been a large increase in the number of fat cells between these two ages for this latter group of rats and it would suggest that the perirenal tissue had not yet reached its full capacity for growth in rats 105 days of of age. Previous data from our laboratory indicated that this tissue, in OM rats fed a high fat diet, extensively increases in weight after 105 days of age (Schemmel et al.

1970b) which further suggests incompleteness of development. In general, the fat cells in the perirenal depot were not as large as fat cells of the inguinal or testicular depot for male OM rats regardless of dietary treatment.

Likewise, between 24 and 105 days of age, fat cell size in the perirenal depot for S rats increased three fold. However, unlike the OM rats, perirenal fat cell size was similar to the cell size of inguinal and testicular fat depots.

Comparison with Other Data

Hirsch and Han (1969) have reported Sprague-Dawley perirenal adipocytes as being larger than the S or OM cells in perirenal tissue. At 15 weeks of age the chow-fed Sprague-Dawley rats had cells which were 270 ng./cell while the S and OM strains had 111 and 227 ng./cell, respectively, at the same age. By 26 weeks of age the cells in Sprague-Dawley rat retroperitoneal tissue had expanded further to 550 ng./cell.

Johnson et al. (1971 and 1973) have found both obese (15 weeks 1500 ng./cell, 26 weeks 1180 ng./cell) and non-obese (15 weeks 400 ng./cell, 26 weeks 490 ng./cell) male Zucker rats raised 8/litter on chow type rations to have larger perirenal fat cells than either the OM (227 ng./cell) or the S strain(111 ng./cell) at 15 weeks of age. This was even more pronounced when genetically obese or non-obese Zucker rats were overfed by rearing in litters of four and

offering a high fat paste in addition to the chow diet (894 ng./cell and 553 ng./cell, respectively)(Johnson et al. 1973).

Strain and Adipocyte Density

At 15 weeks of age the S strain consistently had more cells per gram of adipose tissue than did the OM strain. This was true regardless of tissue or diet. Collectively utilizing all data on 105 day old rats fed the high fat diet the S male rats had 5.78×10^6 while the OM had 2.73×10^6 cells per gram of wet tissue in the inguinal fat depot (depot weights 3.01 gm. and 18.20 gm., respecitvely). When fed the low fat diet values were 7.02×10^6 and 2.49×10^6 for S and OM strains, respectively (depot weights 2.24 gm. and 6.31 gm.). Results in testicular tissue in similar order were: high fat 5.81×10^6 and 1.81×10^6 (depot weights 1.09 gm. and 11.21 gm.); low fat 7.49×10^6 and 2.67×10^6 (depot weights 1.49 gm. and 5.77 gm.). In perirenal tissue values in similar order were 7.50×10^6 and 5.46×10^6 for high fat fed rats (depot weights 2.29 gm. and 14.82 gm.) and 10.17×10^6 and 3.68×10^6 for low fat fed rats (depot weights 2.06 gm. and 6.75 gm.). All calculations of cellular density were carried out on 15 week old animals. It is interesting that the S rat, which is resistant to obesity even when fed a high fat diet (Schemmel et al. 1970a) has a greater complement of fat cells than the obesity- susceptible OM strain when expressed per gram wet tissue. Some control mechanism must prevent the S strain adipocytes from expanding,

thus maintaining the more cellularly dense, yet smaller cells and fat depots. Furthermore, a high fat diet did not cause hyperplasia in the S strain of rat.

Implications for Human Obesity

The difference between the OM and S strains of rat provide a model for human obesity. Some humans are resistant to obesity as are the S strain of rat. Other less fortunate humans are susceptible to obesity of hypertrophic, hyperplastic, or their combined origin. The OM strain of rat provides a model for the investigation of obesity for such people. The obesity of the OM strain at 15 weeks of age has been associated with hypertrophy when fed a low fat diet from birth and with hyperplasia and possibly also hypertrophy when fed a high fat diet over the same period relative to the S strain. This makes the OM strain excellent as a model for human obesity. The cellulaiity of the S strain, too, invites further investigation as to the control of the non-obese state.

TABLE 1.--Diet Composition.

Ingredients	gm./67 gm. diet high fat	gm./100 gm. diet low fat
protein[a]	22.0	22.0
salt mix[b]	4.0	4.0
fiber[c]	2.0	2.0
vitamin mix[d]	1.0	1.0
DL-methionine[e]	0.1	0.1
liver mix[f]	1.5	1.5
fat: crisco	29.51	---
corn oil	---	3.0
carbohydrate[g]	7.0	66.39
Total Kcal.[h]	381	381
Kcal./gm.[h]	5.7	3.8

[a]Casein: General Biochemicals, Chargin Falls, Ohio.

[b]Rogers and Harper salt mix: General Biochemicals, Chargin Falls, Ohio.

[c]Cellulose type: General Biochemicals, Chargin Falls, Ohio.

[d]A.O.A.C. Vitamin mix: General Biochemicals, Chargin Falls, Ohio. Supplied the following (gm./kgm. diet): p-aminobenzoic acid, 0.10; B_{12}, (0.1% in mannitol), 0.03; biotin, 0.0004; calcium pantothenate, 0.04; choline, free base, 2.0; folic acid, 0.002; l-inositol, 0.10; menadione, 0.005; niacin, 0.04; pyrodoxine HCl, 0.04, riboflavin, 0.008, thiamin HCl, 0.005; dextrose, anhydrous, q.s.; (units/kgm) Vitamin A, 20,000.00; Vitamin D_2, 2,000.00; Vitamin E acetate, 100.00.

[e]Purchased from General Biochemicals, Chargin Falls, Ohio.

[f]Purchased from General Biochemicals, Chargin Falls, Ohio.

[g]Cerelose, purchased from Michigan State University Stores, East Lansing, Michigan.

[h]Values used for calculating Kcalories were 4, 4 and 9 for one gram of protein, carbohydrate and fat, respectively.

TABLE 2.--Mean fat cell number and standard error in the entire left inguinal fat depot for each group of animals (n=5).

STRAIN			OSBORNE MENDEL		S 5B/P1	
DIET FAT	SUPPL.[a]	LITTER SIZE	CELL NUMBER ($\times 10^6$)	STANDARD ERROR	CELL NUMBER ($\times 10^6$)	STANDARD ERROR
			105 DAY OLD			
LOW	YES	6	15.60	2.64	12.97	5.30
LOW	YES	3	14.39	3.83	16.55	8.69
LOW	NO	6	16.53	6.16	16.66	5.23
LOW	NO	3	14.79	5.99	14.13	4.07
HIGH	YES	6	60.08	25.88	24.48	7.35
HIGH	YES	3	40.19	24.05	19.41	3.20
HIGH	NO	6	54.79	18.71	11.41	3.23
HIGH	NO	3	39.55	12.38	13.26	5.46
			24 DAY OLD			
LOW	YES	6	5.55	1.14	3.30	0.52
LOW	YES	3	7.84	2.65	4.58	0.83
LOW	NO	6	8.00	4.61	5.46	1.59
LOW	NO	3	7.42	1.88	4.85	1.14
HIGH	YES	6	15.07	4.24	5.00	1.12
HIGH	YES	3	12.07	3.92	6.23	1.67
HIGH	NO	6	12.38	2.04	5.96	1.47
HIGH	NO	3	12.69	8.51	6.04	1.67

[a]supplementation; yes means supplemented and no means not supplemented.

TABLE 3.--List of significant differences ($\alpha < 0.05$) in left inguinal cell number between ages, strains, rations, supplementation groups and litter sizes when the other four variables are held constant.

VARIATION BY	AGE[a] (days)	STRAIN[b]	DIET[c] (fat content)	SUPPLEMENTED[d]	LITTER SIZE[e]
AGE[a]		S	low	no	three
		S	low	no	six
		S	low	yes	three
		S	low	yes	six
		S	high	no	three
		S	high	no	six
		S	high	yes	three
		OM	low	yes	three
		OM	low	yes	six
		OM	high	no	three
		OM	high	no	six
		OM	high	yes	six
STRAIN[b]	24		low	no	three
	24		low	yes	six
	24		high	no	six
	24		high	yes	three
	24		high	yes	six
	105		high	no	three
	105		high	no	six
	105		high	yes	six
RATION[c]	24	S		yes	six
	24	OM		yes	six
	105	S		yes	six
	105	OM		no	three
	105	OM		no	six
	105	OM		yes	six
SUPPLEMENTATION[d]	105	S	high		six
LITTER SIZE[e]	24	S	low	yes	

[a] 105 days versus 24 days.

[b] Osborne Mendel (OM) versus S 5B/Pl (S).

[c] High fat diet versus low fat diet.

[d] Supplemented versus non-supplemented.

[e] Three per litter versus six per litter.

TABLE 4.--Mean cell size and standard error, expressed as weight in ng., in left inguinal depots for each group of five animals.

STRAIN			OSBORNE MENDEL		S 5B/P1	
DIET FAT	SUPPL.[a]	LITTER SIZE	MEAN WEIGHT LIPID/CELL ng.	STANDARD ERROR	MEAN WEIGHT LIPID/CELL ng.	STANDARD ERROR
			105 DAY OLD			
LOW	YES	6	405	145	143	61
LOW	YES	3	361	141	177	92
LOW	NO	6	350	109	90	22
LOW	NO	3	279	143	116	28
HIGH	YES	6	373	72	162	42
HIGH	YES	3	391	153	160	39
HIGH	NO	6	285	55	118	34
HIGH	NO	3	346	43	138	36
			24 DAY OLD			
LOW	YES	6	137	35	84	10
LOW	YES	3	112	36	81	15
LOW	NO	6	83	30	46	12
LOW	NO	3	114	34	65	19
HIGH	YES	6	107	36	81	8
HIGH	YES	3	148	27	83	16
HIGH	NO	6	101	23	70	17
HIGH	NO	3	138	52	88	25

[a]Supplementation; yes means supplemented and no means not supplemented.

TABLE 5.--List of significant differences ($\alpha < 0.05$) in left inguinal cell size, expressed in ng., between ages, strains, rations, supplementation groups, and litter sizes when the other four variables are held constant.

VARIATION BY	AGE[a] (days)	STRAIN[b]	DIET[c] (fat content)	SUPPLEMENTED[d]	LITTER SIZE[e]
AGE[a]		S	low	no	six
		S	high	no	six
		S	high	yes	three
		S	high	yes	six
		OM	low	no	six
		OM	low	yes	three
		OM	low	yes	six
		OM	high	no	three
		OM	high	no	six
		OM	high	yes	three
		OM	high	yes	six
STRAIN[b]	24		low	no	three
	24		high	yes	three
	105		low	no	six
	105		low	yes	six
	105		high	no	three
	105		high	no	six
	105		high	yes	three
	105		high	yes	six
RATION[c]					
SUPPLEMENTATION[d]	24	S	low		six
LITTER SIZE[e]	24	OM	high	yes	

[a] 105 days versus 24 days.

[b] Osborne Mendel (OM) versus S 5B/Pl (S).

[c] High fat diet versus low fat diet.

[d] Supplemented versus non-supplemented.

[e] Three per litter versus six per litter.

TABLE 6.--The effect of age, diet and strain on mean adipocyte number in three selected depots of male rats. (n=5)

AGE			24 DAY OLD		105 DAY OLD	
DEPOT	STRAIN	TREATMENT	CELL NUMBER ($\times 10^6$)	STANDARD ERROR	CELL NUMBER ($\times 10^6$)	STANDARD ERROR
INGUINAL	OM[a]	C[b]	8.00	4.61	16.65	6.16
		O[c]	12.07	3.92	40.18	24.05
	S 5B/Pl	C	5.46	1.59	16.52	5.23
		O	6.22	1.67	19.41	3.20
TESTICULAR	OM	C	1.94	0.43	13.26	3.21
		O	2.11	0.84	26.94	8.98
	S 5B/Pl	C	1.32	0.43	11.19	1.93
		O	1.35	0.25	11.95	7.43
PERIRENAL	OM	C	1.65	0.38	24.98	9.39
		O	5.29	1.00	85.77	47.91
	S 5B/Pl	C	1.09	0.42	11.17	4.09
		O	2.39	1.52	16.65	7.37

[a]OM: Osborne Mendel strain.

[b]C: control (low fat diet, non-supplemented, 6/litter).

[c]O: overfed (high fat diet, supplemented, 3/litter).

TABLE 7.--Comparison of cell number X 10^6 in three left fat depots by three variables.

BY	DEPOT		INGUINAL			TESTICULAR			PERIRENAL		
	STRAIN	TREATMENT[a]	24 DAYS	105 DAYS	LEVEL OF SIGN.	24 DAYS	105 DAYS	LEVEL OF SIGN.	24 DAYS	105 DAYS	LEVEL OF SIGN.
AGE	S 5B/Pl	C[b]	5.46	16.65	0.0036	1.32	11.19	0.0000	1.09	11.17	0.0012
	S 5B/Pl	O[c]	6.22	19.41	0.0000	1.35	11.95	0.0250	2.39	16.65	0.0052
	OM[d]	C	8.00	16.52	NS[e]	1.94	13.26	0.0000	1.65	24.98	0.0010
	OM	O	12.07	40.18	NS	2.11	26.94	0.0006	5.29	85.77	0.0120
	COLLAPSED		7.94	23.19	0.0002	1.68	15.83	0.0000	2.59	34.65	0.0012
STRAIN	AGE	TREATMENT	S 5B/Pl	OM	LEVEL OF SIGN.	S 5B/Pl	OM	LEVEL OF SIGN.	S 5B/Pl	OM	LEVEL OF SIGN.
	24	C	5.46	8.00	NS	1.30	1.94	NS	1.09	1.65	NS
	24	O	6.22	12.07	0.0300	1.35	2.11	NS	2.39	5.23	0.0162
	105	C	16.65	16.52	NS	11.19	13.26	NS	11.17	24.98	0.0334
	105	O	19.41	40.18	NS	11.95	26.94	0.0412	16.65	85.77	0.0120
	COLLAPSED		11.94	19.19	NS	6.45	11.07	NS	7.83	29.41	NS
TREATa	AGE	STRAIN	C	O	LEVEL OF SIGN.	C	O	LEVEL OF SIGN.	C	O	LEVEL OF SIGN.
	24	S 5B/Pl	5.46	6.22	NS	1.30	1.35	NS	1.09	2.39	NS
	24	OM	8.00	12.07	NS	1.94	2.11	NS	1.65	5.23	0.0002
	105	S 5B/Pl	16.65	19.41	NS	11.19	11.95	NS	11.17	16.65	NS
	24	OM	16.62	40.18	NS	13.26	26.94	0.0248	24.98	85.77	0.0456
	COLLAPSED		11.66	19.47	NS	6.92	10.60	NS	9.72	27.51	NS

[a]Treatment: control (C) or overfed (O).

[b]C: control; low fat diet, non-supplemented, 6/litter.

[c]O: overfed; high fat diet, supplemented, 3/litter.

[d]OM: Osborne Mendel strain.

[e]NS: not significantly different.

TABLE 8.--The effect of age, diet and strain on mean fat cell size, expressed as weight in ng., in three fat depots of male rats. (n=5)

AGE			24 DAY OLD		105 DAY OLD	
DEPOT	STRAIN	TREAT-MENT	MEAN WEIGHT LIPID/CELL ng.	STANDARD ERROR	MEAN WEIGHT LIPID/CELL ng.	STANDARD ERROR
INGUINAL	OM[a]	C[b]	78	30	344	110
		O[c]	146	153	388	153
	S 5B/P1	C	42	12	86	22
		O	78	16	156	39
TESTICULAR	OM	C	46	16	438	167
		O	92	8	394	135
	S 5B/P1	C	42	10	118	21
		O	72	10	190	36
PERIRENAL	OM	C	58	15	268	82
		O	68	16	186	63
	S 5B/P1	C	26	12	76	19
		O	46	28	146	74

[a]OM: Osborne Mendel strain.

[b]C: control (low fat diet, non-supplemented, 6/litter).

[c]O: overfed (high fat diet, supplemented, 3/litter).

TABLE 9.--Comparison of cell size, expressed as weight in ng., in three left fat depots by three variables

BY	DEPOT		INGUINAL			TESTICULAR			PERIRENAL		
	STRAIN	TREAT-MENT[a]	24 DAYS	105 DAYS	LEVEL OF SIGN.	24 DAYS	105 DAYS	LEVEL OF SIGN.	24 DAYS	105 DAYS	LEVEL OF SIGN
AGE	S 5B/P1	C[b]	42	86	0.0096	42	118	0.0002	26	76	0.0030
	S 5B/P1	O[c]	78	156	0.0090	72	190	0.0002	46	146	0.0438
	OM[d]	C	78	344	0.0016	46	438	0.0016	58	268	0.0008
	OM	O	146	388	0.0164	92	394	0.0022	68	186	0.0072
	COLLAPSED		86	243	0.0002	63	285	0.0000	50	169	0.0000
STRAIN	AGE	TREAT-MENT	S 5B/P1	OM	LEVEL OF SIGN.	S 5B/P1	OM	LEVEL OF SIGN.	S 5B/P1	OM	LEVEL OF SIGN
	24	C	42	78	NS[e]	42	46	NS	26	58	0.0102
	24	O	78	146	0.0026	72	92	0.0108	46	68	NS
	105	C	86	344	0.0018	118	438	0.0058	76	268	0.0018
	105	O	156	388	0.0224	190	394	0.0238	146	186	NS
	COLLAPSED		91	239	0.0006	106	242	0.0126	73	145	0.0198
TREAT[a]	AGE	STRAIN	C	O	LEVEL OF SIGN.	C	O	LEVEL OF SIGN.	C	O	LEVEL OF SIGN
	24	S 5B/P1	42	78	0.0132	42	72	0.0050	26	46	NS
	24	OM	78	146	0.0096	46	92	0.0006	58	68	NS
	105	S 5B/P1	86	156	0.0196	118	190	0.0100	76	146	NS
	105	OM	344	388	NS	438	394	NS	268	186	NS
	COLLAPSED		137	192	NS	161	187	NS	107	111	NS

[a]Treatment: contorl (C) or overfed (O).

[b]C: control; low fat diet, non-supplemented, 6/litter.

[c]O: overfed; high fat diet, supplemented, 3/litter.

[d]OM: Osborne Mendel strain.

[e]NS: not significantly different.

	24 DAYS	105 DAYS
HIGH FAT	3 per litter, supplement (5) 6 per litter, supplement (5) 3 per litter, no supplement (5) 6 per litter, no supplement (5)	3 per litter, supplement (5) 6 per litter, supplement (5) 3 per litter, no supplement (5) 6 per litter, no supplement (5)
LOW FAT	3 per litter, supplement (5) 6 per litter, supplement (5) 3 per litter, no supplement (5) 6 per litter, no supplement (5)	3 per litter, supplement (5) 6 per litter, supplement (5) 3 per litter, no supplement (5) 6 per litter, no supplement (5)

Repeat for each strain
( )=number of rats

FIGURE 1.--Experimental Design.

Solution X: 27.8 gm. monobasic sodium phosphate[1] in 1000 ml. deionized water.

Solution Y: 53.65 gm. $Na_2HPO_4 \cdot 7H_2O$[1] OR 71.7 gm. of $Na_2PO_4 \cdot 12H_2O$; in 1000 ml. deionized water. (0.2M)

To achieve a pH of 7.4, 19 ml. of solution X and 81 ml. of solution Y are combined and diluted to 200 ml. with deionized water. The pH is again adjusted to 7.4.

For adipocyte isolation work this solution should contain 4% bovine serum albumin. Immediatly prior to use 3mg./ml. bacterial collagenase[2] is added. The purification of this enzyme varies so that the concentration is not exact and incubation time may require adjustment. The enzyme is not stable at room temperature. It should be stored frozen and used within three months of purchase.

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[1] Purchased from Fischer Scientific Company, Chemical Manufacturing Division, Fairlawn, N.J. 07410.

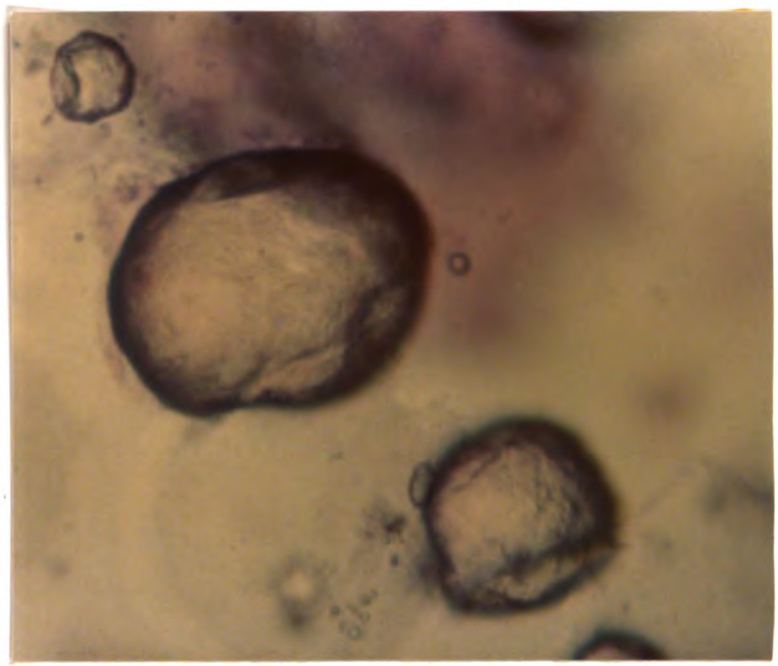
[2] Purchased from Worthington Biochemical, Freehold, N.J.

FIGURE 2.--Krebs-Ringer Phosphate Buffer.

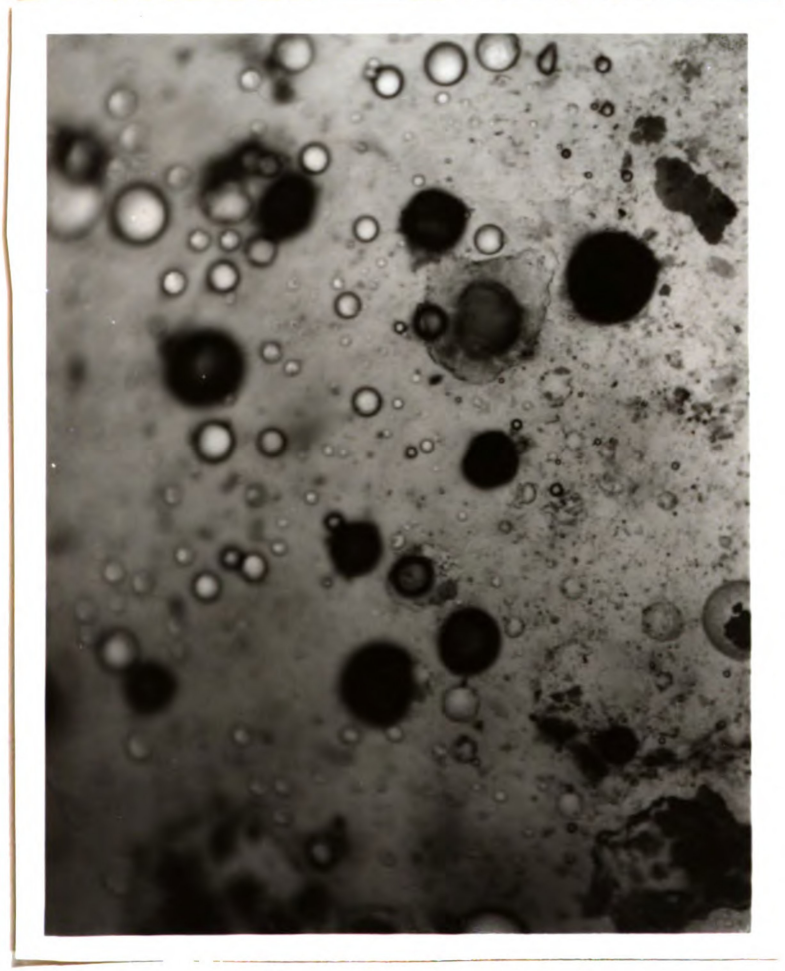
S-Collidine buffer: 4.65 gm. of 2,4,6 tri-methyl pyridine were weighed into a 100 ml. beaker. Approximately 70 ml. of deionized water were added. The pH was adjusted to 7.4 using a pH meter and 50% HCl. The solution, at pH 7.4, was transferred to a 100 ml. volumetric flask which was then filled.

Glutaraldehyde Solution: 30 ml. of 25% glutaraldehyde and 31.25 ml. of S-Collidine buffer (above) were diluted to 250 ml. in a volumetric flask. The pH was again adjusted to 7.4.

FIGURE 3.--Glutaraldehyde Solution.



Adopocytes as they appear, stained, microscopically.



Adipocytes, dark circular shaped objects, and lipid droplets, clear round shapes. Magnification 140 power.

Figure 4.--Photomicrographs.

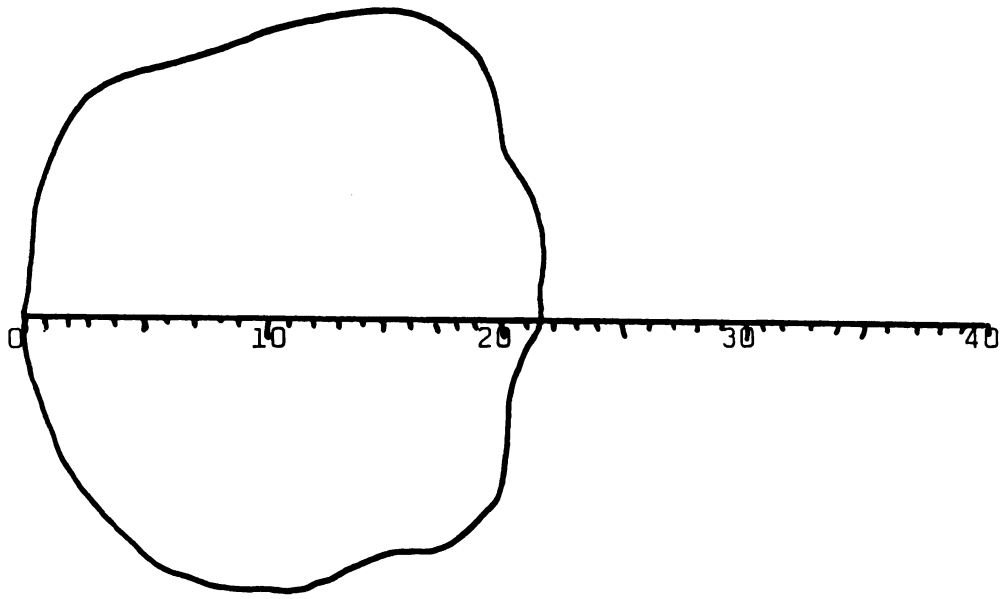
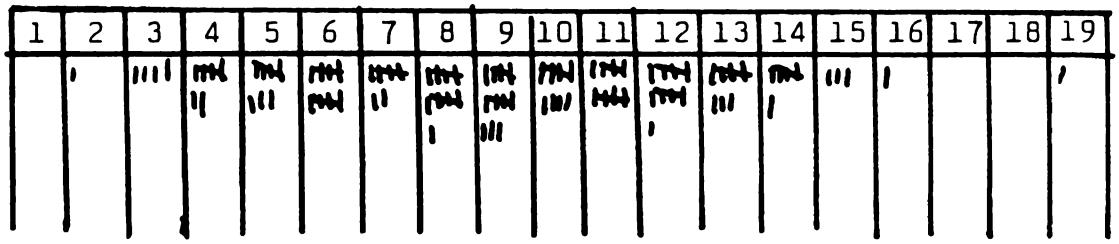


FIGURE 5.--Assessment of Adipocyte Diameter. The adipocyte is aligned, using stage adjustors, with the grid marked "0". The number of grids the adipocyte spans, in this case 22, is recorded.



Each cell was tallied in a column representing its specific width as determined in Figure 5. The total number of cells in each column was then recorded for further calculations of surface area, volume, weight, and cell number. For the above situation, this would be:

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
0	1	4	7	8	10	7	11	13	9	10	11	8	6	3	1	0	0	1

FIGURE 6.--Tallying of Sized Adipocytes.

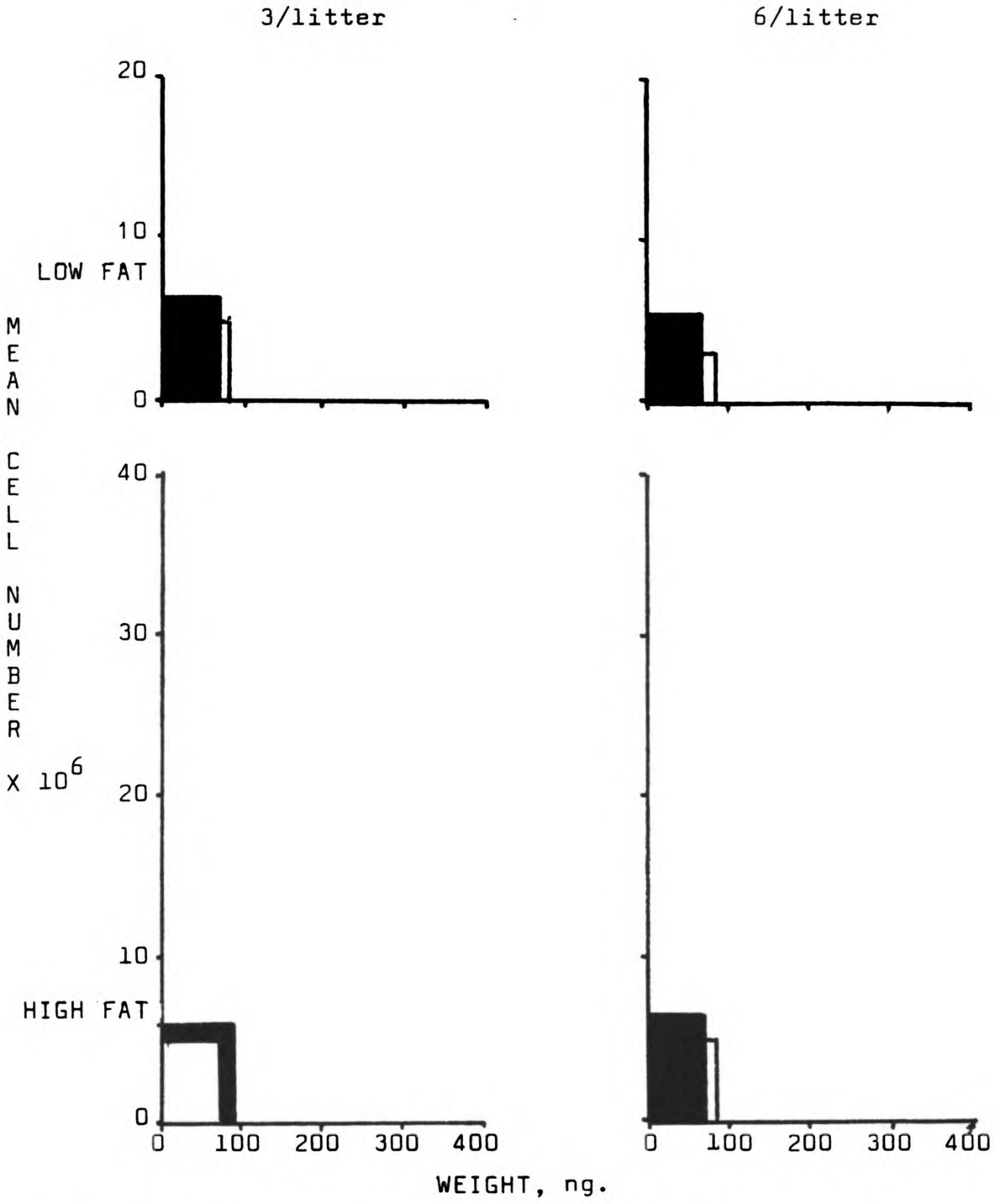


FIGURE 7.--Mean cell number X 10^6 versus mean cell size, expressed in ng. lipid, in left inguinal depots for twenty-four day old S 5B/Pl rats. High fat and low fat diets are represented in rows; litter sizes in columns; and supplemented □ or not supplemented ■ within each graph.

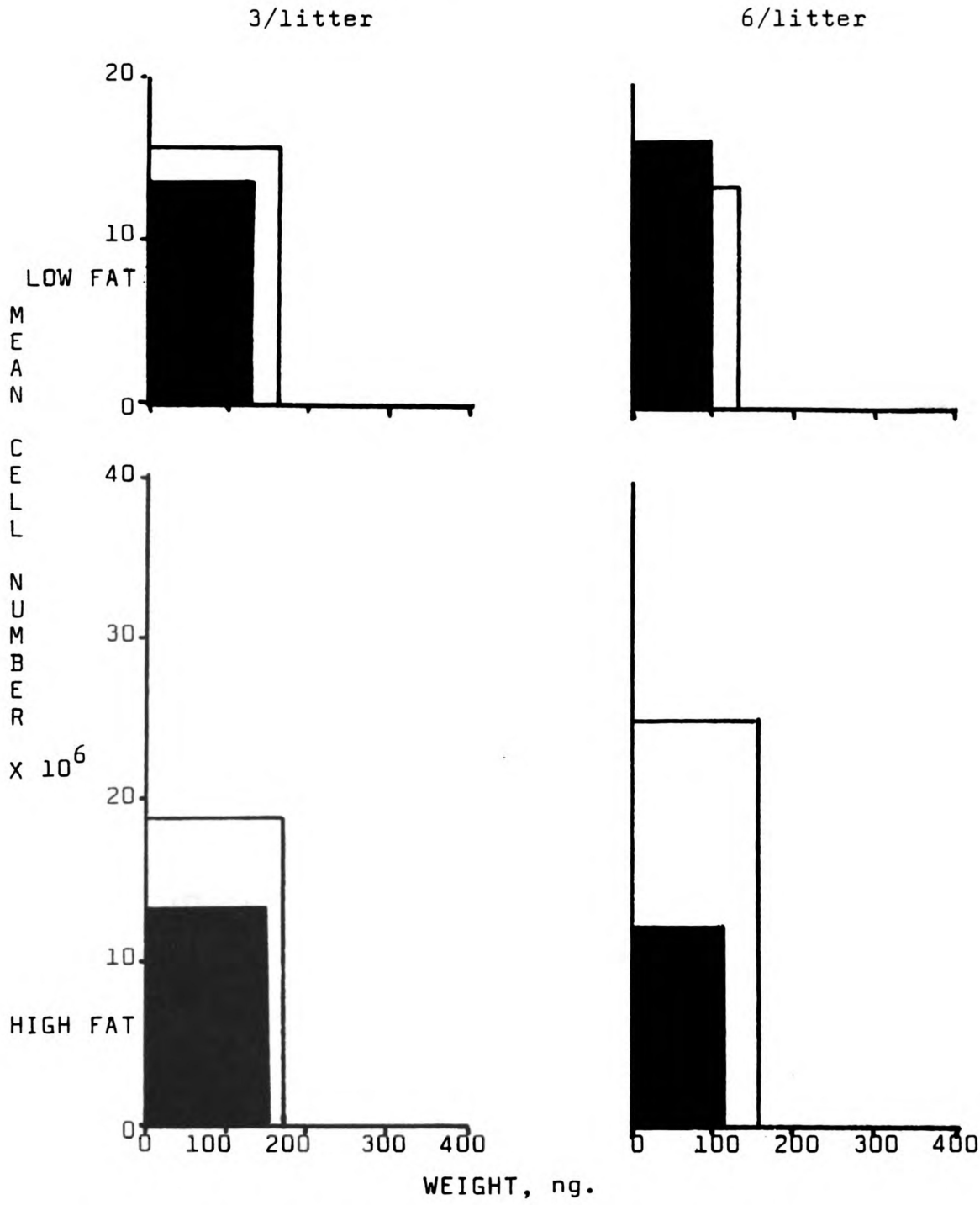


FIGURE 8.--Mean cell number X 10^6 versus mean cell size, expressed in ng. lipid, in left inguinal depots for one hundred and five day old S 5B/Pl rats. High fat and low fat diets are represented in rows; litter sizes in columns; and supplemented □ or not supplemented ■ within each graph.

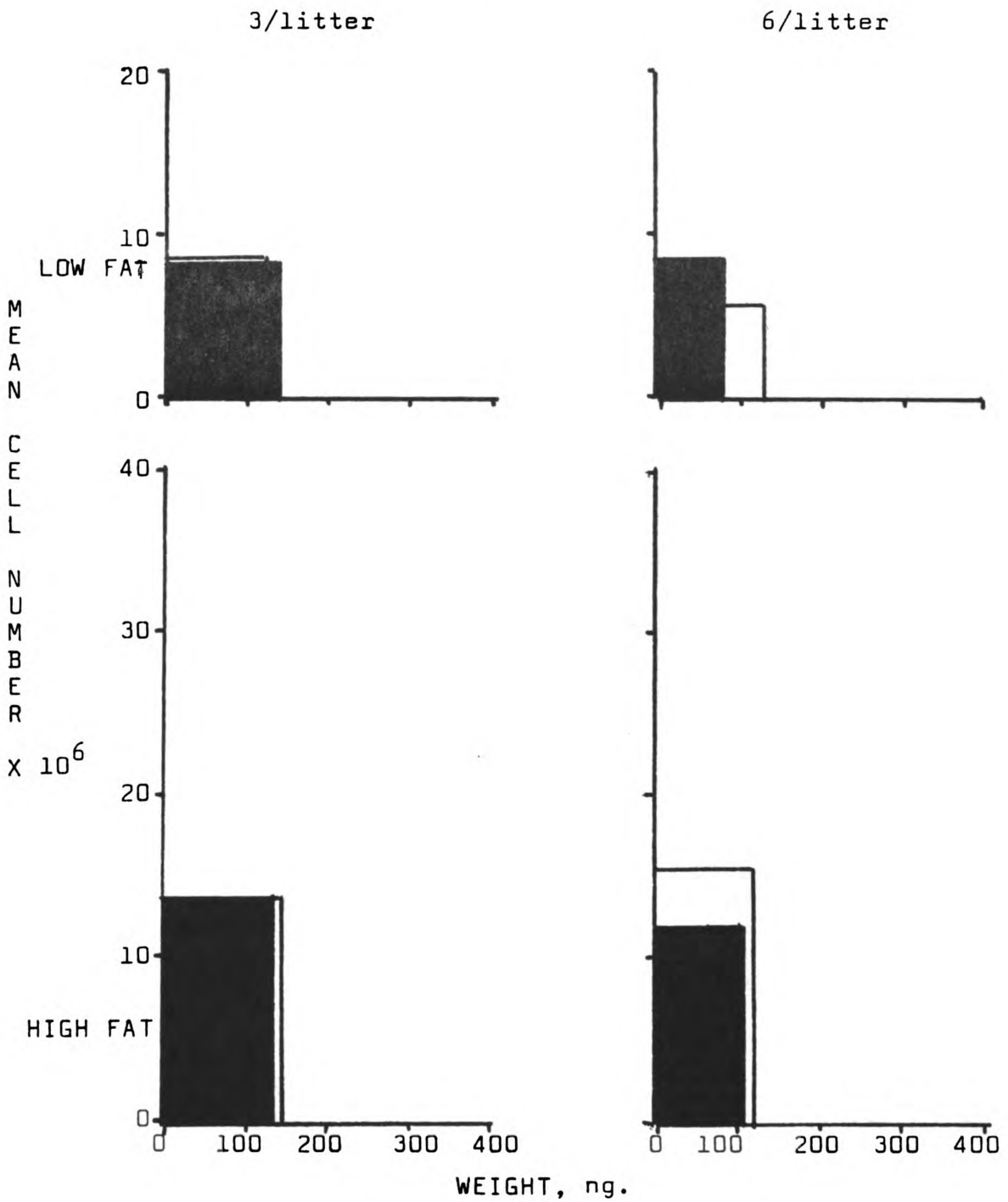


FIGURE 9.--Mean cell number X 10^6 versus mean cell size, expressed in ng. lipid, in left inguinal depots for twenty-four day old Osborne Mendel rats. High fat and low fat diets are represented in rows; litter sizes in columns; and supplemented □, or not supplemented ■ within each graph.

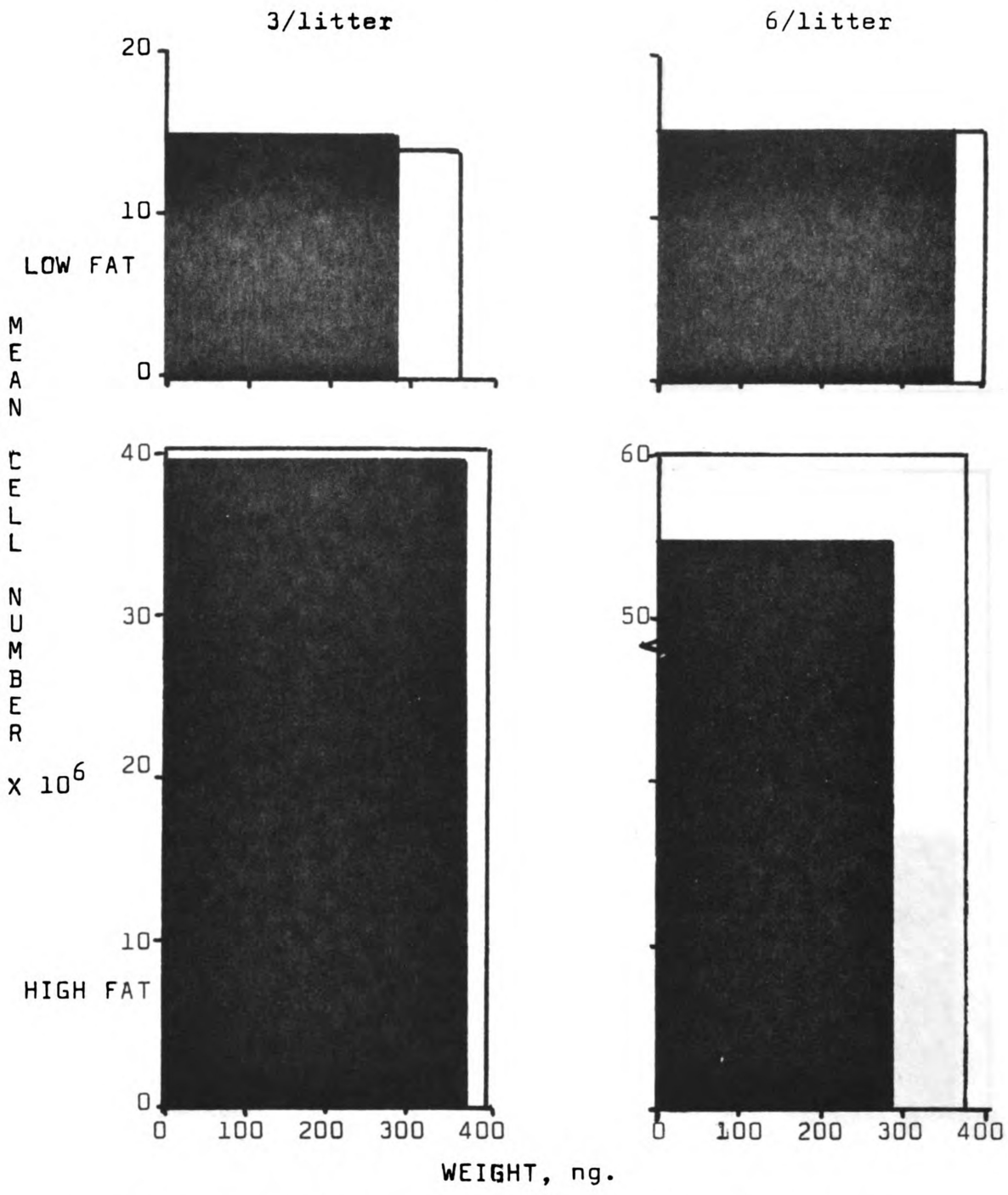


FIGURE 10.--Mean cell number X 10^6 versus mean cell size, expressed in ng. lipid, in left inguinal depots for one hundred and five day old Osborne Mendel rats. High fat and low fat diets are represented in rows; litter sizes in columns; and supplemented □, or not supplemented ■ within each graph.

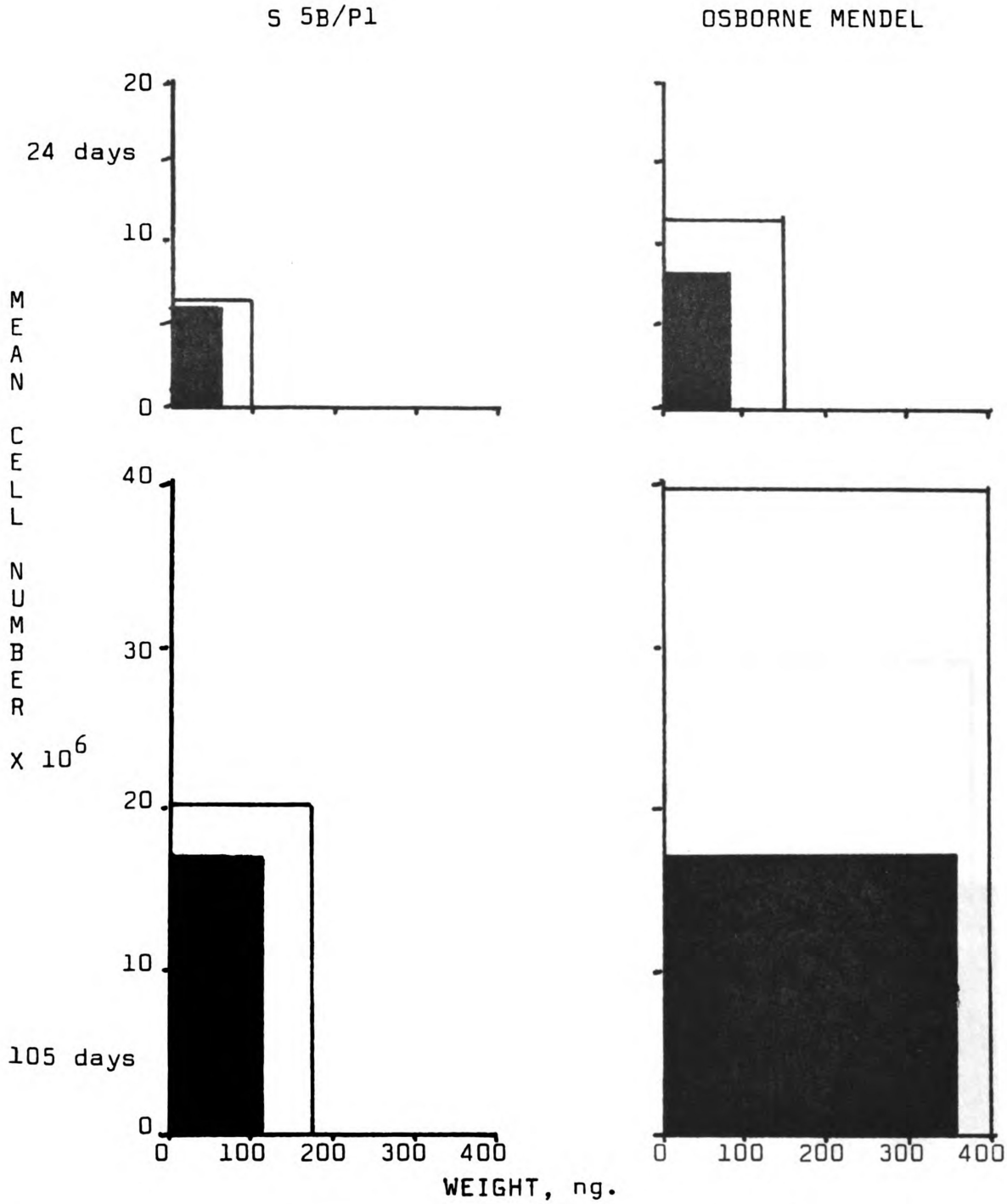


FIGURE 11.--Mean cell number X 10^6 versus mean cell size, expressed in ng. lipid, in left inguinal depots. Strains are represented in columns; ages are represented in rows; and treatments, control (low fat diet, non-supplemented, 6/litter) ■ , or overfed (high fat diet, supplemented, 3/litter) □ within each graph.

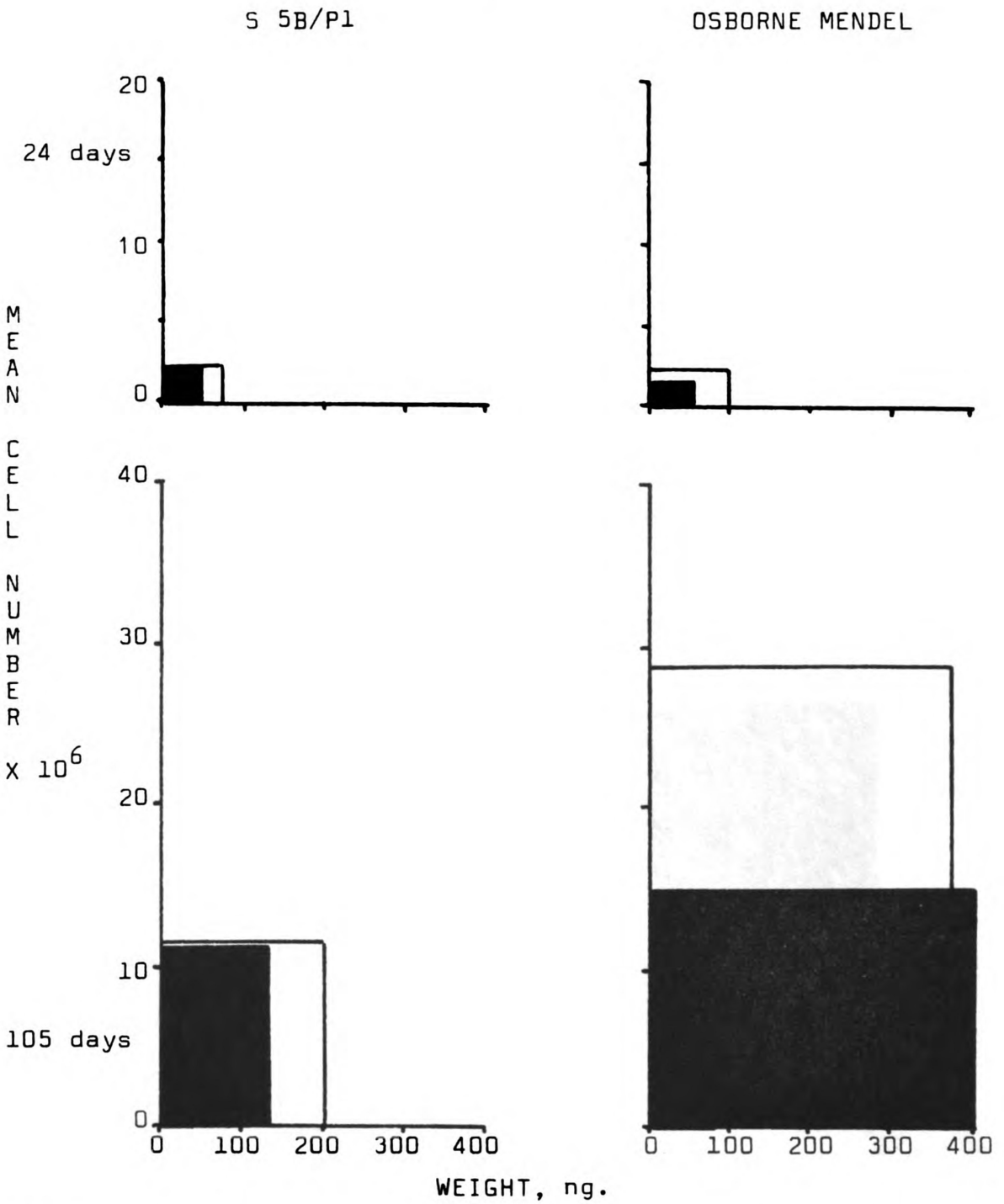


FIGURE 12.--Mean cell number X 10^6 versus mean cell size, expressed in ng. lipid, in left testicular dspots. Strains are represented in columns; ages are represented in rows; and treatments, control (low fat diet, non-supplemented, 6/litter) ■ , or overfed (high fat diet, supplemented, 3/litter) □ within each graph.

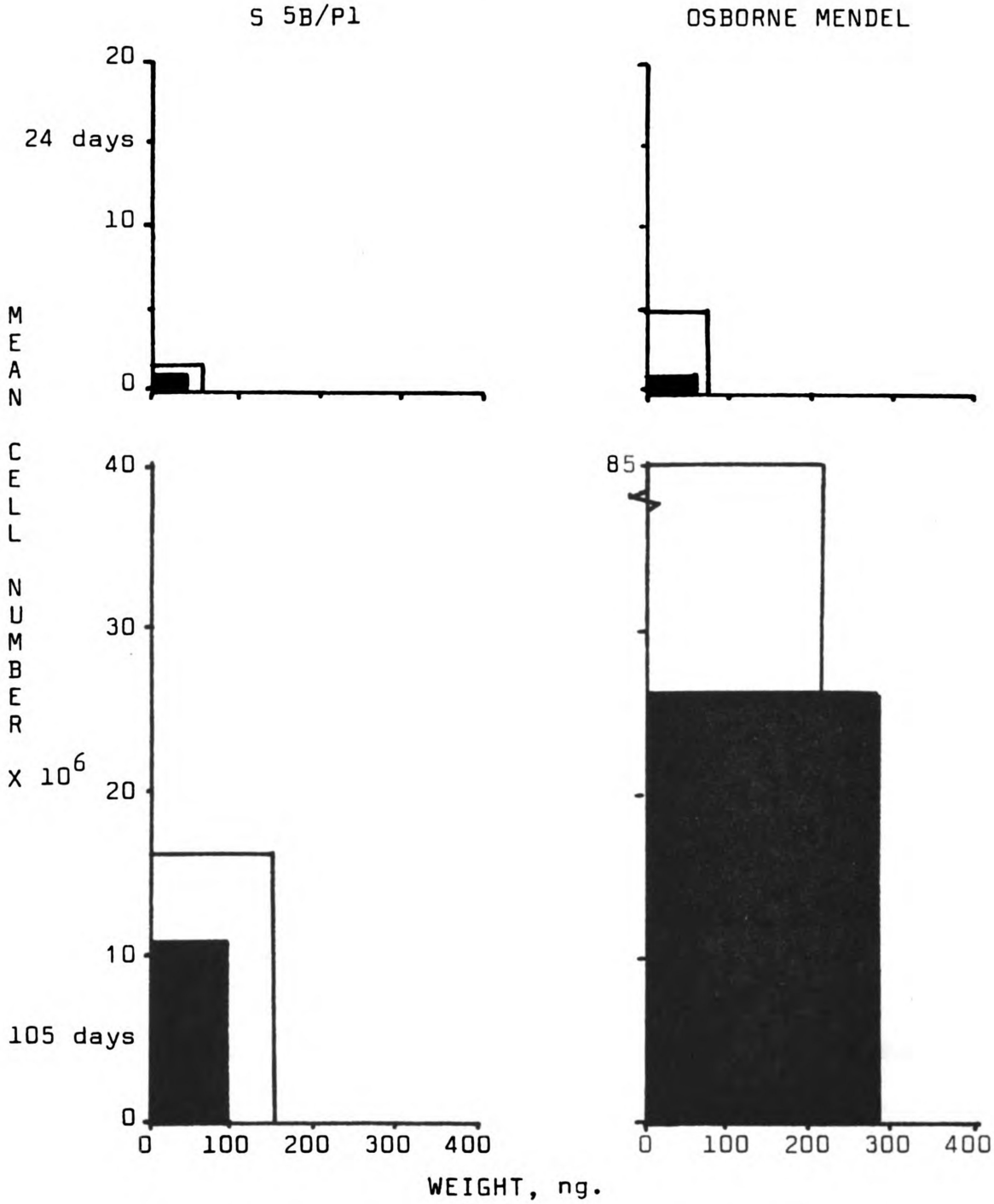


FIGURE 13.--Mean cell number X 10^6 versus mean cell size, expressed in ng. lipid, in left perirenal depots. Strains are represented in columns; ages are represented in rows; and treatments, control (low fat diet, non-supplemented, 6/litter) ■, or overfed (high fat diet, supplemented, 3/litter) □, within each graph.

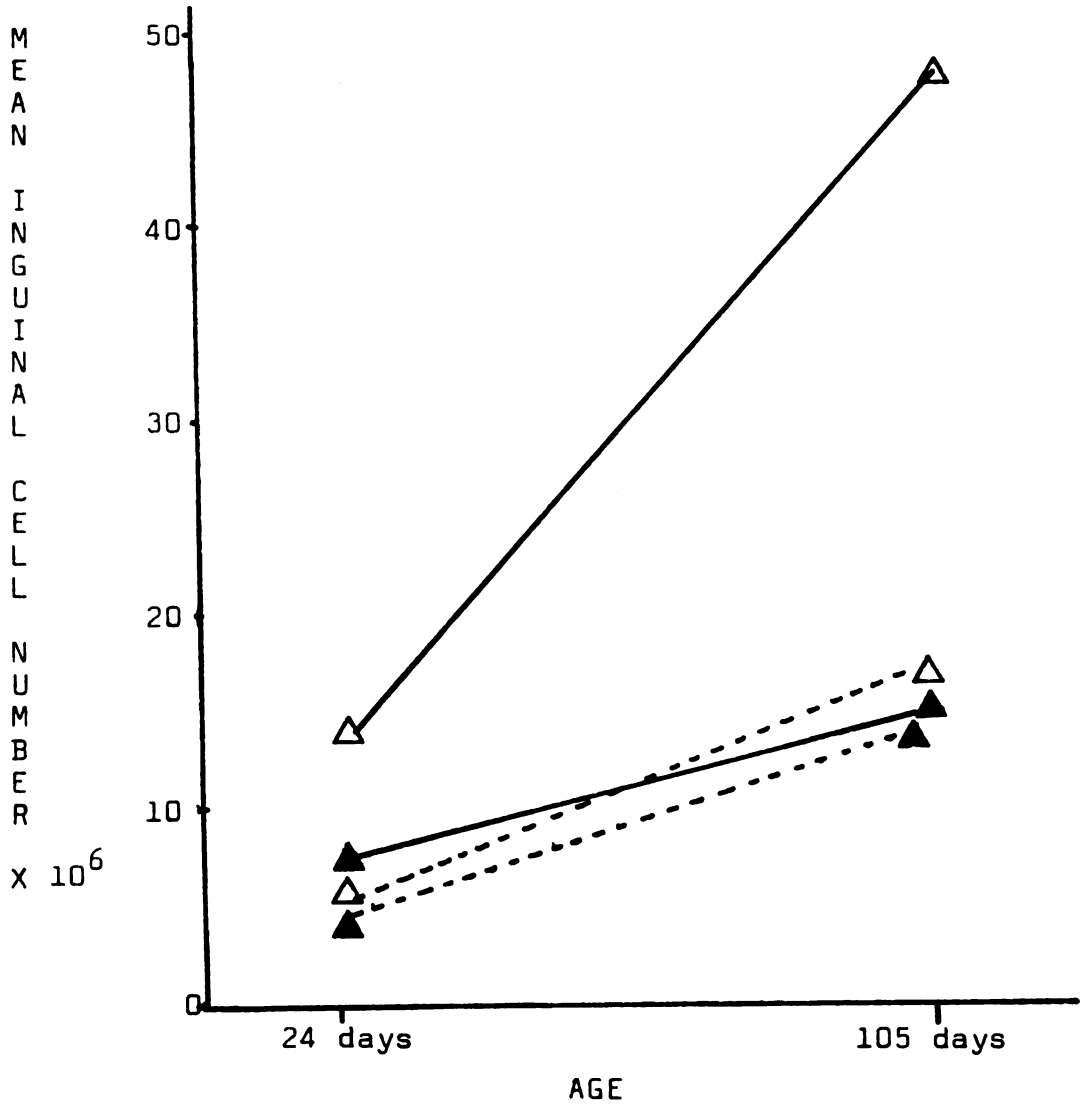


FIGURE 14.--Mean inguinal cell number X 10^6 versus age in left depots. The Osborne Mendel strain ——, S 5B/Pl strain ----, high fat diet △ and low fat diet ▲ are represented. The three way interaction between age, strain and diet on left inguinal cell number is significant ($p<0.001$).

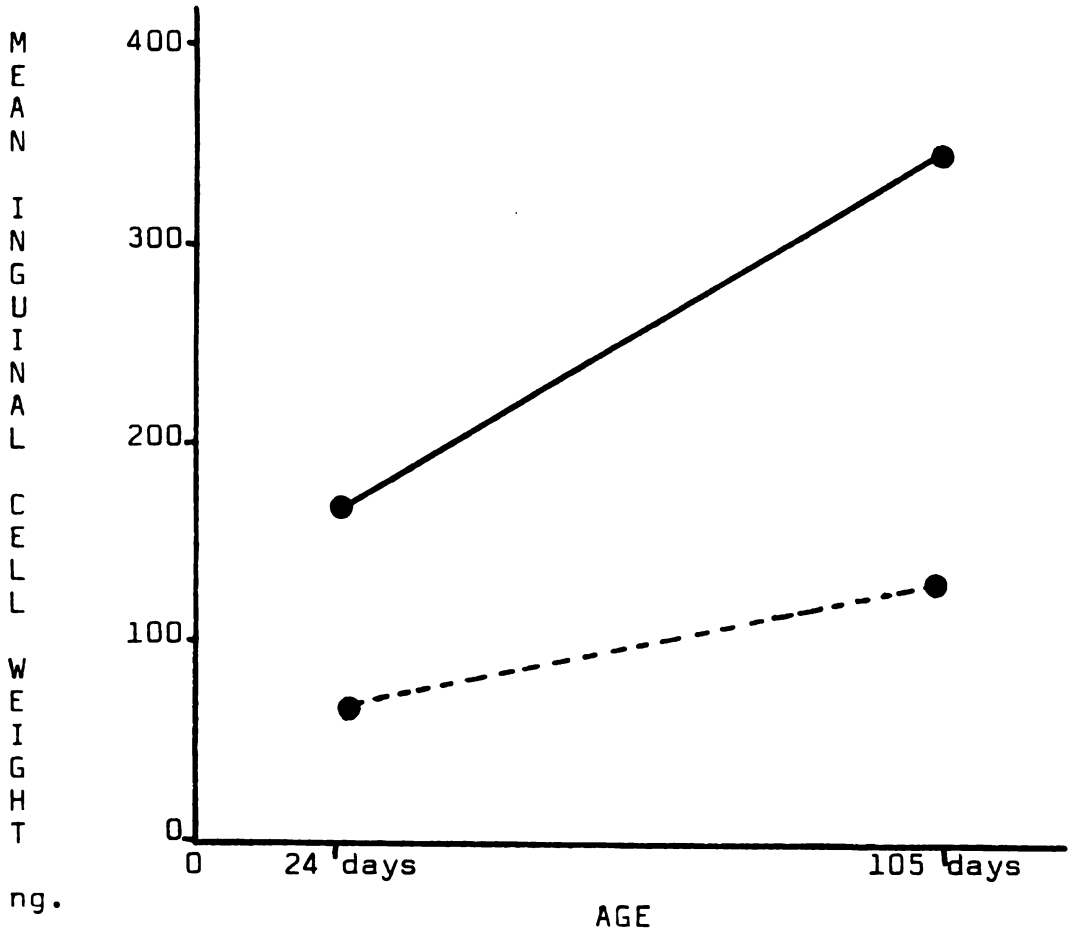


FIGURE 15.--Mean inguinal cell size, expressed in ng. lipid per cell, versus age in left depots. The Osborne Mendel ——, and S 5B/Pl strains --- are represented. Five way multivariate analysis of variance found the two way interaction between age and strain for cell size significant ($p<0.0001$).

CONCLUSIONS

Male Osborne Mendel rats have larger fat cells than do the S 5B/Pl rats. In 24 day old rats values for OM and S strains in inguinal tissue were 118 ng. lipid/cell and 75 ng. lipid/cell, respectively; in testicular tissue 74 ng. lipid/cell and 61 ng. lipid/cell; and in perirenal tissue 68 ng. lipid/cell and 40 ng. lipid/cell. One hundred and five day old animals had values of 349 ng. lipid/cell and 138 ng. lipid/cell in inguinal tissue; 419 ng. lipid/cell and 157 ng. lipid/cell in testicular tissue; and 232 ng. lipid/cell and 116 ng. lipid/cell in perirenal tissue, respectively. However, when the OM strain was given a high fat (44% w/w) diet from birth, at 15 weeks of age they had at least twice as many adipocytes in inguinal, testicular and perirenal tissues as did those fed a low fat diet. In the inguinal tissue the high fat fed OM rat had 48.65 $\times 10^6$ cells while 15.33 $\times 10^6$, 17.14 $\times 10^6$ and 15.08 $\times 10^6$ were values for the number of cells in low fat fed OM rats and for S rats in whole left depots, fed high and low fat diets, respectively. Testicular tissue had 26.95 $\times 10^6$ as compared to 13.27 $\times 10^6$, 11.95 $\times 10^6$ and 11.20 $\times 10^6$ cells while perirenal tissue had 85.78 $\times 10^6$ versus 24.98 $\times 10^6$, 16.66 $\times 10^6$ and 11.18 $\times 10^6$ for OM rats raised on

high fat diets versus OM rats raised on low fat diets, S rats raised on high and low fat diets, respectively (all cell number figures are in whole left depots). Thus offering a high fat diet from birth, used in conjunction with an obesity-susceptible strain of rat (Osborne Mendel), can induce hyperplastic obesity. This is not true of the obesity-resistant S 5B/Pl strain, however.

Although there were trends which may have been suggestive of some effects on fat cell number and size from raising rats in litters of three rather than six or tube feedings, these were not statistically significant. Aging, between 24 and 105 days, increased the size and number of adipocytes as did being of the OM rather than the S strain. In addition, the feeding of a high fat diet from birth was important in increasing the number of fat cells in the Osborne Mendel strain. It had not affected the number of cells in the S 5B/Pl strain by 15 weeks of age and had not affected the size of cells in either strain.

SUGGESTIONS FOR FURTHER STUDY

Control of Obesity

Why are animals of the S strain smaller in body weight, adipose cell size and adipocyte number than animals of the OM strain? Is there any way to alter this trend?

Since the pups of the S strain have smaller and fewer adipocytes at 24 days of age, are born in smaller litters, weigh less at birth, and are supplied with a naturally less calorically dense milk than OM pups it is likely that the former are undernourished both postpartum and in utero relative to the OM. To see if altering prenatal nourishemnt can affect the above, one horn of the S strain female uterus could be ligated. Fewer pups per pregnancy would result and they might therefore receive more nourishment. Investigation of the question of caloric density of milk could proceed by raising pups from each strain by mothers of the opposite strain from birth.

Given that animals of the S strain are smaller at weaning than animals of the OM strain, why do they continue to be smaller in size? Work in our laboratory has shown that the S strain eats less food and converts food calories to body energy less efficiently than the OM strain (Schemmel et al. 1972). Do they also have tighter appetite control or

regulation than the OM strain and why should this be the case? What controls the size of adult fat depot adipocytes? Research in our laboratory is underway concerning the quantity of fecal fat excreted in each strain. The S 5B/Pl strain may well be less able to absorb dietary fat than the OM strain. This would account, at least in part, for the reduced efficiency of the S relative to the OM to convert dietary calories to body energy. Biochemical studies might clarify why the S strain is a less efficient converter of dietary energy to body energy too. When fed the high fat diet the activity of lipoprotein lipase would give information concerning availability of circulating triglycerides to fat cell absorption. The activities of acetyl coenzyme A carboxalase and of the fatty acid synthetase complex would yield similar information for de novo synthesis of triglycerides.

Microscopic Versus Coulter Counter Sizing

The data collected on the 105 day OM strain raised on the high fat diet should be checked since such adipocytes are large and more susceptible to rupture by collagenase. This could be done by the method of Hirsch and Gallian (1969). An attempt was made to fix the cells with osmium tetroxide and size them microscopically. This was futile due to the irregular shape of the resulting cells.

Proliferation Pattern

How does the cellular pattern of adipose tissue development differ between the two strains? The S strain had fewer cells than comparably treated OM rats at 24 days of age. At 105 days of age all animals of both strains, except the OM rats fed the high fat diet, had approximately the same number of cells in their whole left inguinal depots.

Is the pattern of adipose tissue proliferation over time altered in overfed animals? Knittle and Hirsch (1968) have recorded data which indicate that proliferation of the number of adipocytes might continue beyond the normal fifteen week limit in epididymal tissue. Overfeeding was accomplished, in their study, by altering litter sizes. The length of time over which proliferation continues as well as the resulting final number of adipocytes formed would shed further light on the results of overfeeding the very young.

Sex Difference

Are females of either strain more susceptible to hyperplastic obesity than males? Lemonnier (1971) has observed that females are more susceptible to hyperplastic obesity than males. Work in our laboratory has shown that, in the Osborne Mendel strain, females accumulate more genital fat while males accumulate more inguinal fat (Schemmel et al. 1970b). How would the females of both strains react, on the cellular level in adipose tissue, to high and low fat diets?

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