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Effect of a High Fat Diet on Body Composition, Cellularity, and Enzyme Levels during Early Development of the Rat (40874)[1]

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Abstract. A high fat (60%) and a low fat (5%) diet were fed to female rats during gestation and lactation and to their male and female offspring for 3 weeks postweaning. The cellular effects of feeding all combinations of the two diets during the three growth periods (gestation, lactation, and rapid growth) were determined in Wistar rats. "Nutritional imprinting" of earlier dietary regimes on enzyme profiles and adipose cell number was not observed after rats were fed a control diet to maturity. The high fat diet caused reduced fat deposition during gestation. During lactation, the high fat diet caused increased fat deposition. Postweaning rats had increased carcass fat, decreased protein, and lower carcass weight due to the high fat diet. Serum glucose was lower for fat-fed males during lactation and also for postweaning males and females. Fat feeding in lactation caused a decrease in adipose cell numbers which was not evident until rats were 6 weeks of age. The effect was only transient when rats were fed the chow diet until 21 weeks of age. Fat feeding for 3 weeks postweaning caused a significant decrease in liver DNA of 21-week-old male and female rats.

Many investigators have induced a nutritional form of obesity in rats and mice by feeding a high fat diet (40–60% fat, w/w). Rats have attained extremely high body weights when fed a high fat diet for extended periods of time (1). Schemmel *et al.* (2) demonstrated the ability of a high fat diet to induce varying degrees of obesity in seven strains of rats, which were otherwise unsusceptible to spontaneous obesity.

Another variable is the period or age at which the individual becomes most susceptible to diet-induced obesities (3, 4). Zargoza and Felber (5) fed a high fat diet for 3 to 5 weeks postweaning to determine the short-term effects of the diet. They did not find increased body weight and caloric intakes reported by workers using long-term studies. They did, however, find heavier epididymal fat pads, higher glucose levels, lower insulin levels, and reduced liver glycogen stores. Refeeding the control diet returned all values to normal except serum glucose, which remained high for 5 days. A longer lasting effect of feeding a high fat diet during early development was noted by Hahn and Kirby (6). Adult rats prematurely weaned on a high fat diet did not exhibit elevated cholesterol levels found in rats prematurely weaned on a carbohydrate diet.

The following study was undertaken to determine the effects of a high fat diet during the early stages of development of the rat. Three experimental periods of equal length were chosen in which the high fat diet could influence the developing rat, directly or indirectly: (a) gestation, (b) lactation, and (c) 3 weeks postweaning. A portion of the liver was stored frozen for DNA determination and the remainder was stored for enzyme analysis for another study.

At the end of lactation (3 weeks of age), one pup from each group within each litter was randomly selected for study, yielding four groups of six pups each. The four groups were designated by the dietary regimes they experienced during gestation

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and lactation (Chow–Chow; HF–Chow; Chow–HF; HF–HF).

Two of the four remaining pups in each group within litters were randomly selected and placed in group cages to be weaned on either the HF or Chow diet. The remaining two pups were housed individually and weaned in the same manner as the previous two pups. The pups could then be categorized according to gestational, lactational, and postweaning diet (Chow–Chow–Chow; HF–Chow–Chow; Chow–HF–Chow; HF–HF–Chow; Chow–Chow–HF; HF–Chow–HF; Chow–HF–HF; HF–HF–HF). Half of the pups (group cages) were fed *ad libitum* and sacrificed at 6 weeks of age. The other half (individual cages) was fed *ad libitum* with weekly body weights recorded for a period of 8 weeks postweaning. This group was then sacrificed at 3, 6, and 21 weeks of age. The total number of rats sacrificed at 3, 6, and 21 weeks of age, was 23, 48, and 46, respectively.

Blood samples were collected for serum glucose determination by the Glucostat Method (Worthington). The liver was removed and weighed. A small sample was used for DNA determination according to the method of Ceriotti (7) for rats at birth, 6 weeks, and 21 weeks. The liver sample of 3-week-old rats was used for glycogen analysis according to the method of Lo *et al.* (8). The remainder of the liver at all ages was used for enzyme analysis as described earlier (9).

Epididymal or parametrial fat pads were removed from 3-, 6-, and 21-week-old rats. A Stadie–Riggs hand microtome was used to prepare slices of the fat pad for cell number and size determination according to the method of Hirsch and Gallian (10). A Coulter Counter was used to determine the number of cells in the ranges of 25–50, 50–75, and 75–100 μm in diameter. Total cell number was determined by counting all cells greater than 25 μm. The remaining adipose tissue sample was used for enzyme analysis in another study.

The gastrointestinal tract was removed and after the contents were removed, it was flushed with saline and returned to the carcass. Carcasses were stored frozen until analysis according to the method of Hartsook and Hershberger (11). Dry matter was determined on duplicate samples. The remainder of the dry matter not accounted for by ash and fat content was considered to be protein.

Analysis of the above data was accomplished by combining the Chow–Chow and HF–Chow groups within each sex to obtain a group designated X–Chow. The X–Chow was compared with X–HF (grouped in the same manner as above). Similar groups were formed to compare Chow–X and HF–X. Finally the dietary combinations were tested (Chow–Chow; HF–Chow; Chow–HF; HF–HF). The data at 6 weeks and 21 weeks was handled in the same manner, using the two previous dietary regimes. The gestation diet was not included in these calculations because there was no significant carryover treatment effect. The primary influence on the variables measured at all ages was the most recent period in which the HF diet was fed.

Results. The HF diet had no effect on conception rate or litter size. Both groups averaged 11 pups per litter. The only detectable difference at time of birth was a lower litter weight in the HF group. This was reflected in an unexpected lowering of percent carcass fat (Table I). Genital fat pads were not evident at this age, so no values for weight and adipose cell number are given. At the end of lactation, no significant dietary effects were noted for carcass weight, or adipose cell number. There was a significant increase in carcass fat in the HF group at 3 and 6 weeks of age. These dietary effects were reversed by 21 weeks of age (Table I). A significant ($P < 0.05$) increase in adipose cell number was evident in HF female rats at 6 weeks of age only.

The feeding combination during lactation and 3 weeks postweaning had a significant effect on body weight, fat pad weight, and adipose cell number of rats at 6 weeks of age (Table II). These differences were not evident at 21 weeks of age. Feeding the HF diet during 3 weeks postweaning resulted in decreased body weights but an increase in fat pad weights. Feeding the high fat diet

TABLE I. EFFECTS OF FEEDING A HIGH FAT DIET DURING THREE STAGES OF EARLY DEVELOPMENT (GESTATION, LACTATION, OR 3 WEEKS POSTWEANING) ON CARCASS WEIGHTS, PERCENTAGE CARCASS FAT, AND GENITAL FAT ADIPOSE CELL NUMBERS OF MALE AND FEMALE WISTAR RATS

Sex	Age (weeks)	Carcass wt.[a] Chow (g)	Carcass wt.[a] High fat (g)	Carcass fat Chow (%)	Carcass fat High fat (%)	Adipose cell[b] Chow ($\times 10^6$)	Adipose cell[b] High fat ($\times 10^6$)
M and F	0[c]	5.2[d] ±0.2	4.7 ±0.1	1.2 ±0.2	0.5* ±0.1	—	—
M	3[e]	37 ±2	41 ±2	6.5 ±0.4	15.8** ±0.6	0.75 ±0.07	0.82 ±0.02
M	6[f]	136 ±2	110** ±3	6.5 ±0.4	11.1** ±0.6	4.6 ±0.7	5.5 ±0.4
M	21[f]	392 ±13	405 ±17	9.2 ±0.6	9.2 ±1.1	8.1 ±0.5	9.3 ±0.7
F	3[e]	38 ±2	44 ±3	8.4 ±0.6	16.7** ±0.5	0.62 ±0.09	0.70 ±0.03
F	6[f]	110 ±3	93** ±4	7.5 ±0.6	12.0** ±0.7	3.1 ±0.8	5.9* ±0.7
F	21[f]	225 ±7	244 ±8	11.5 ±1.1	12.2 ±0.7	6.0 ±0.9	7.3 ±0.5

[a] Exclusive of gastrointestinal contents, liver, and genital fat pads.
[b] Total number of adipose cells in both genital fat pads.
[c] Mean ± SEM.
[d] Rats grouped according to maternal diet during gestation.
[e] Rats grouped according to maternal diet during lactation.
[f] Rats grouped according to diet fed during 3 weeks postweaning. Significant differences between Chow and high fat means are indicated by * ($P < 0.05$) and ** ($P < 0.01$).

during lactation followed by a low fat diet caused rats to have the lowest fat cell number at 6 weeks of age.

Serum glucose levels were lowest in rats fed the high fat diet at 3 and 6 weeks of age; however, by 21 weeks of age, these differences were not significant (Fig. 1). However, at 21 weeks of age liver DNA was significantly lower in rats fed the HF diet during the postweaning period.

Liver enzymes involved in gluconeogenesis and amino acid catabolism are re-

TABLE II. BODY WEIGHTS, GENITAL FAT PAD WEIGHTS, AND GENITAL FAT PAD ADIPOSE CELL NUMBERS OF 6-WEEK-OLD MALE AND FEMALE WISTAR RATS AFTER FEEDING COMBINATIONS OF CHOW AND HIGH FAT DIETS DURING LACTATION AND 3 WEEKS POSTWEANING[a]

Dietary group	Body wt. Male (g)	Body wt. Female (g)	Fat pad wt.[b] (mg)	Adipose cell[b,c] ($\times 10^6$)
F F	126[d,e] (6) ± 8	102[e] (6) ± 3	877[e,f] ± 123	4.66[e] ± 0.31
C F	129[e] (8) ± 5	118[e,f] (4) ± 8	1139[e] ± 124	6.64[f] ± 0.39
F C	164[f] (6) ± 5	131[f] (6) ± 5	781[f] ± 40	1.78[g] ± 0.21
C C	159[f] (9) ± 3	127[f] (3) ± 3	663[f] ± 39	6.33[f] ± 0.36

[a] See text for explanation of dietary groups.
[b] Values for male and female rats were combined to calculate means, total combined (male + female) equals 12/group.
[c] Total number of adipose cells in both genital fat pads.
[d] Mean ± SEM.
[e,f,g] Column means with different superscripts are significantly different ($P < 0.05$).

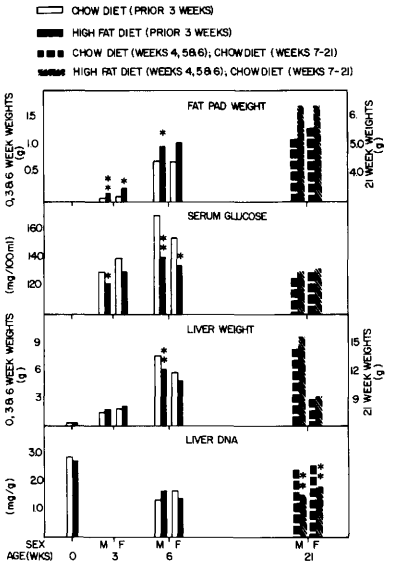

FIG. 1. Genital fat pad weight, serum glucose, liver weight, and liver DNA of male and female Wistar rats after feeding a high fat or Chow diet during three stages of early development (gestation, lactation, or 3 weeks postweaning). Serum glucose and genital fat pad weights were not determined at birth (0 weeks). Rats sacrificed at 21 weeks of age were grouped according to their postweaning diet. All 21-week-old rats were fed the Chow diet from 7 to 21 weeks of age. Significant differences between Chow and high fat means are indicated by *($P < 0.05$) and **($P < 0.01$).

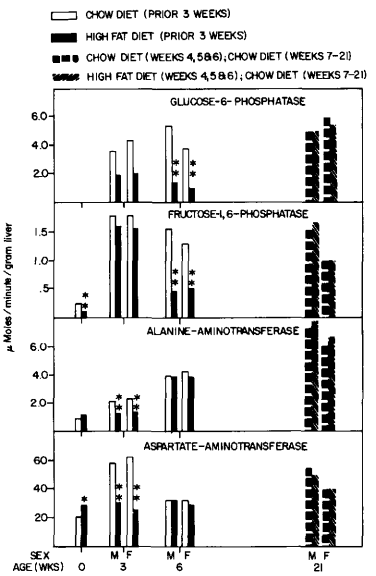

FIG. 2. Liver gluconeogenic enzymes of male and female Wistar rats after feeding a high fat or Chow diet during three stages of early development (gestation, lactation, or 3 weeks postweaning). Glucose-6-phosphatase activity was not determined at birth (0 weeks). Rats sacrificed at 21 weeks of age were grouped according to their postweaning diet. All 21-week-old rats were fed the Chow diet from 7 to 21 weeks of age. Significant differences between Chow and high fat means are indicated by *($P < 0.05$) and **($P < 0.01$).

ported in Fig. 2. Glucose 6-phosphatase and fructose 1,6-diphosphatase are significantly reduced by high fat diets at 6 weeks. Alanine aminotransferase and aspartate amino transferase were depressed by the high fat diet at 3 weeks. At birth, fructose 1,6-diphosphatase was depressed and aspartate aminotransferase was increased by the high fat diet. Feeding a HF diet lowered the activity of enzymes involved in supplying reducing equivalents for fatty acid synthesis at 6 weeks of age (Fig. 3). At 3 weeks of age, α-glycerol phosphate is decreased by HF diet. These differences in enzyme changes were not evident by 21 weeks of age.

Discussion. A high fat diet has been shown to have a variety of effects on the rat during the earliest stages of development. During gestation, the high fat diet had little effect on the fetus. This is probably due to the fact that maternal glucose was a primary energy source for the developing fetus. The lower fat deposition indicates that high fat feeding during pregnancy was of no value in increasing the rate of experimental obesity onset in the offspring. In fact, there was some decrease in a fetal liver enzyme involved in lipogenesis (6-phosphogluconate dehydrogenase) and an increase in an enzyme involved in esterification (glycerol phosphate dehydrogenase). Miguel and Abraham (12) found a reduction of *in vitro* [2-^{14}C]pyruvate incorporation into fatty acids by fetal liver slices from the treatment group receiving 15% corn oil diet. In the same study, tritiated water incorporation into fatty acids and fetal lipogenic

[4] Chemical composition (%) of diets are as follows for the chow and high fat diets, respectively: protein, 23.2, 24.9; fat, 4.6, 60.0; fiber, 3.7, 2.0; ash, 6.3, 5.0; carbohydrate, 52.7, 7.1; moisture, 9.5, 1.0.

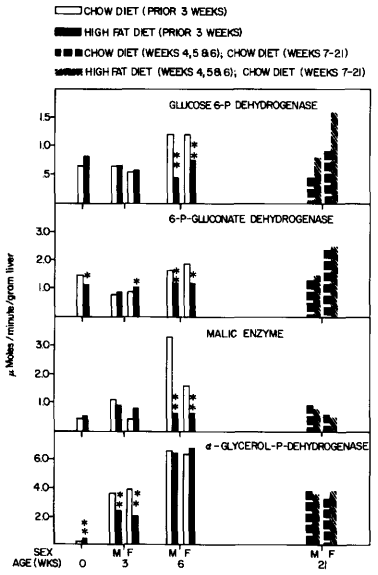

FIG. 3. Liver lipogenic enzymes of male and female Wistar rats after feeding a high fat or Chow diet during three stages of early development (gestation, lactation, and 3 weeks postweaning). Rats sacrificed at 21 weeks of age were grouped according to their postweaning diet. All 21-week-old rats were fed the Chow diet from 7 to 21 weeks of age. Significant differences between Chow and high fat means are indicated by *($P < 0.05$) and **($P < 0.01$).

enzymes were not effected by maternal diet. In a more recent study of fat feeding during pregnancy, it was found that the level of dietary fat had no effect on the amount of lipid accumulated in fetal tissues or fetal lipogenesis (13). The high fat diet does not appear to have any deleterious effects on conception rates or fetal survival.

During lactation, the pup received its nourishment via the mother's milk. At this state, the high fat diet can indirectly provide a positive response in body weight and fat deposition in the developing rat. Lemonnier (3) noted the beneficial effect of feeding a high fat diet to lactating mice on pup growth. He suggested that the diet causes changes in milk production or composition.

Knittle and Hirsch (14) have demonstrated that adipose cell numbers can be permanently affected in the normal rat during early development. The authors were successful in reducing adipose cell numbers by caloric and protein restriction during the suckling period.

When comparisons are made of feeding the high fat diet during lactation and 3 weeks postweaning, an apparent effect of feeding a fat diet during lactation is seen. It appears that those rats which were fed the high fat diet during lactation had decreased adipose cell numbers. This effect was not detected in 3-week-old rats. There may be several reasons why altered adipose cell numbers were not detected at the end of lactation. One possibility is that in the Hirsch method (10) for counting cells, cells less than 25 μm are not counted. Thus, the newly developed cells would not be counted if they had not reached 25 μm in diameter. Another possibility is that the diet triggers a slow reacting response which is not detectable in terms of cell number for several weeks.

It is proposed that the decrease in adipose cell proliferation caused by the lactational diet is the result of premature filling of adipocytes (differentiation). This early development initially results in decreased mitogenic activity. In more mature female rats, an apparent increase in adipocyte proliferation was induced by feeding a high fat diet (Table I). Adipose cell hyperplasia induced by feeding a high fat diet was not detected by Lemonnier (3) until 14 to 16 weeks. More recently, it has been shown that the high fat diets can stimulate thymidine incorporation into adipocytes of mature rats (15). In the present study, the effect of the high fat diet on adipose cell proliferation appeared to be temporary since by 21 weeks of age (after 15 weeks on a control diet) all apparent differences in adipocyte number were nonsignificant.

The high fat diet in this study has a consistent depressing effect on serum glucose and on gluconeogenic enzymes. Lower values of serum glucose occurred during lactation and postweaning when the high fat diet was fed. These results are contradictory to the higher values reported by Zargoza and Felber (5) and the unchanged values of Malaisse *et al.* (4). Part of the differences noted between this and other studies

may be due to the differences in the composition of the diets used. Another variable factor was the strain of rats used. Schemmel *et al.* (2) have provided information on the effects of a high fat diet on seven strains. The degree of response varied between strains. This variation could be the result of marked differences in energy metabolism.

Dietary-induced changes in enzyme activities are well documented (16–18). The present study demonstrates that a high fat diet induced changes in liver enzymes involved in amino acid catabolism, gluconeogenesis, and lipogenesis during early development, and that these changes were reversed when fed a control diet over an extended period of time. Other studies have suggested that dietary treatments imposed during early development in rats have long-range influences of metabolism even after the animals are switched to a control diet (19, 20). For the enzymes measured here, no apparent "dietary imprinting" occurred during perinatal development.

The three important findings in this study which deserve consideration are: (a) the decrease in adipose cell numbers caused by feeding the high fat diet during lactation; (b) the significant decrease in liver DNA of adult rats caused by feeding the high fat diet for only 3 weeks postweaning; and (c) reversibility of dietary-induced enzyme changes. Reduced DNA levels of the liver may be a permanent effect of feeding a high fat diet during early development, similar to the effect on cholesterol levels (6). Whereas, the dietary effect on adipose cell numbers does not appear to be permanent unless it is fed for long periods of time.

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1. Mickelsen, O., Takahashi, S., and Craig, C., J. Nutr. 57, 541 (1955).
2. Schemmel, R., Mickelsen, O., and Gill, J. L., J. Nutr. 100, 1041 (1970).
3. Lemonnier, D., J. Clin. Invest. 47, 2091 (1972).
4. Malaisse, W. J., Lemonnier, D., Malaisse-Lagae, F., and Mandelbaum, I. M., Horm. Metabol. Res. 1, 9 (1969).
5. Zargosa, N., and Felber, J. P., Horm. Metabol. Res. 2, 323 (1970).
6. Hahn, P., and Kirby, L., J. Nutr. 103, 690 (1973).
7. Ceriotti, G., J. Biol. Chem. 192, 297 (1952).
8. Lo, S., Russell, J. C., and Taylor, A. W., J. Appl. Physiol. 28, 234 (1970).
9. Martin, R. J., and Herbein, J. H., Proc. Soc. Exp. Biol. Med. 151, 231 (1976).
10. Hirsch, J., and Gallian, E., J. Lipid Res. 9, 110 (1968).
11. Hartsook, E. W., and Hershberger, T. V., Proc. Soc. Exp. Biol. Med. 113, 973 (1963).
12. Miguel, S. G., and Abraham, S., Biochem. Biophys. Acta 424, 213 (1976).
13. Platka-Bird, L., and Bennink, M. R., J. Nutr. 108, 1422 (1978).
14. Knittle, J. L., and Hirsch, J., J. Clin. Invest. 47, 2091 (1968).
15. Klyde, B. J., and Hirsch, J., J. Lipid Res. 20, 705 (1979).
16. Tepperman, J., and Tepperman, H. M., Fed. Proc. 29, 1284 (1970).
17. Leveille, G. A., J. Nutr. 91, 267 (1966).
18. Bartley, J. C., and Abraham, S., Biochem. Biophys. Acta 280, 258 (1972).
19. Reiser, R., Henderson, G. R., and O'Brien, B. C., J. Nutr. 107, 1131 (1977).
20. Berdanier, C. D., Amer. J. Clin. Nutr. 28, 1416 (1975).

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