PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 164, 530–533 (1980)

Hepatic Lipogenesis in the Preobese Zucker Rat (40910)[1]

IRIS J. TURKENKOPF,[2,3] JANET L. OLSEN, L. MORAY, M. R. C. GREENWOOD, AND P. R. JOHNSON

Department of Biology, Vassar College, Poughkeepsie, New York 12601, and Department of Biology, Mount Saint Mary College, Newburgh, New York 12550

Abstract. Hepatic lipogenesis was examined during the preweaning and early postweaning period in Zucker *fafa* and lean rat pups by measuring total *in vivo* lipogenesis and the activity of two rate-limiting lipogenic enzymes, acetyl-CoA carboxylase (ACC) and fatty acid synthetase (FAS). No significant differences in *in vivo* lipogenesis, ACC, and FAS activity were present during the preweaning period. After weaning, total lipogenesis and the two lipogenic enzyme activities, ACC and FAS, were significantly elevated in *fafa* rats compared to lean rats. Thus, early overproduction of lipids by the liver *does not* appear to be a primary effect of the *fa* gene.

The genetically obese Zucker rat (*fafa*) is considered a useful model for human juvenile-onset obesity because of a number of similarities in its obese profile and that of those individuals who become obese during childhood and puberty (1–3). These characteristics include fat cell hypertrophy and hyperplasia (4), hyperlipidemia (5), hyperinsulinemia (6), and essentially normal plasma glucose levels (7).

The etiology of the condition remains unknown, but several organs have been implicated in early changes during development of the obese condition. For example, the earliest postnatal differences between *fafa* rats and their lean littermates have been noted in the adipose tissue, which by definition is abnormally enlarged in obesity. Gruen *et al.* (8) observed both fat cell hypertrophy and elevated adipose tissue lipoprotein lipase (LPL) activity by 12–13 days in Zucker *fafa* rats. Furthermore, Bell and Stern (9) measured total body lipid in litters from homozygous recessive (*fafa*) and heterozygous lean (*Fafa*) matings in which 50% of the pups are expected to show the obese phenotype. A bimodal distribution of total body lipid was detected at 13 days of age with significant differences between the two groups. While it has also been proposed that an early modification in the brain could alter feeding behavior and lead to obesity, Cleary *et al.* (10) have shown that hyperphagia is not necessary for obesity to develop fully. In addition, Boulange *et al.* (11) reported that 5- to 7-day-old Zucker *fafa* rats have enlarged fat cells in their inguinal depots when carefully compared to lean littermates of the same age, sex, and body weight. They also report no evidence of hyperphagia in the 1-week-old *fafa* pups. Another potential site for early expression of the genetic lesion is the pancreas, since Zucker *fafa* rats are hyperinsulinemic and insulin stimulates lipogenesis in liver and adipose tissue and promotes hyperphagia. Elevated serum immunoreactive insulin (IRI) has been shown in *fafa* rats at the end of the suckling period (6), but has not yet been carefully documented in individual fatty pups before that age.

The liver could also contribute to the development of Zucker rat obesity since hepatic lipogenesis is a source of substrate for fat cell enlargement. Godbole *et al.* (12) demonstrated elevated rates of *in vivo* hepatic lipogenesis in the *fafa* rats compared to lean littermates as early as 23 days of age and showed that while fatty acid synthesis in adipose tissue of Zucker *fafa* rats de-

[1] This investigation was supported in part by NIH Grants HD12637 and AM 19382 and a grant from the Weight Watchers Foundation.

[2] The work reported in this manuscript comprises a portion of the Ph.D. dissertation to be submitted by I. J. Turkenkopf to Columbia University.

[3] Iris J. Turkenkopf holds an NSF Faculty Development award.

0037-9727/80/080530-04$01.00/0

creased between 5 and 13 weeks of age, hepatic lipogenesis increased twofold during the same period. Furthermore, hepatectomy prevented the accumulation in adipose tissue of 80–90% of newly synthesized fatty acids. These findings suggest that the liver might well be a tissue in which the genetic lesion is expressed early in development and leads to other manifestations of obesity.

Therefore, we chose to examine hepatic lipogenesis during the preweaning and early postweaning periods in Zucker *fafa* and lean rat pups by measuring total *in vivo* lipogenesis and the activity of two rate-limiting lipogenic enzymes, acetyl-CoA carboxylase and fatty acid synthetase.

Methods and materials. Homozygous obese (*fafa*) and heterozygous lean (*Fafa*) pups were obtained from mating *fafa* males with heterozygous females according to the method of Hemmes *et al.* (13). Hemmes *et al.* (13) have demonstrated that the occurrence of the obese phenotype from such matings is 45%, rather than the predicted 50%. Homozygous lean (*FaFa*) pups were obtained by mating homozygous lean males and females.

Because it is not possible to identify the preobese rat with certainty before the third postnatal week, we partially hepatectomized young rats from 5 to 48 days of age to obtain tissue samples for *in vivo* lipogenesis and lipogenic enzyme activity determinations. When the hepatectomized rats had reached 6 weeks of age, they were identified as obese or lean.

In the first experiment, rates of *in vivo* lipogenesis were determined for both male and female pups of all those genotypes by measuring the incorporation of ^3H_2O into total liver lipid in 7-, 10-, 15-, and 28-day-old pups. All suckling pups were separated from their mothers for 2 hr prior to injection of 4 mCi ^3H_2O. Following injection, pups were returned to their mothers and allowed to suckle for 1 hr after which partial hepatectomies were performed and liver lipids were extracted with chloroform:methanol (2:1). The amount of ^3H_2O incorporated into liver lipid was determined using a Packard Tri-Carb liquid scintillation spectrometer and expressed as counts per minute per milligram of liver tissue.

In the second experiment, liver samples for lipogenic enzyme activity determinations were collected by partial hepatectomy for pups sired by *fafa* males. Surgery was performed on both male and female pups at 5, 7, 15, 22, 28, and 48 days of age. The 22-day-old pups were hepatectomized 24 hr after weaning on Day 21. The hepatectomized rats which were still of suckling age (5, 7, 15 days) were returned to their mothers postoperatively. Tissue samples to be used for enzyme assays and protein determinations were immediately chilled in a cold homogenization medium consisting of 0.15 *M* KCl and 0.004 *M* $MgCl_2$ at pH 7.6. Samples were blotted, weighed, and homogenized in 3 vol of homogenization medium and centrifuged for 60 min at 40,000 rpm in an IEC Model B-20 using the No. 870 rotor. The supernatant fraction was used for all enzyme determinations.

For the measurement of acetyl-CoA carboxylase (ACC) (14) an aliquot of supernatant was preincubated for 30 min in 87.5 m*M* Tris, pH 7.5, 35 m*M* $MgCl_2$, 1.75 m*M* glutathione, and 1.375 g/liter bovine serum albumin. The reaction was initiated by the addition of 0.3 ml of a mixture containing 40 m*M* ATP, 4 m*M* acetyl-CoA, 20 m*M* citrate, and 10 m*M* $NaHCO_3$ and run for 10 min. ACC activity was determined by the incorporation of $NaH^{14}CO_3$ into malonyl-CoA. Fatty acid synthetase (FAS) activity was determined by the incorporation of [1-^{14}C]acetyl-CoA into lipid according to the method of Hsu *et al.* (15). Protein content of tissue samples was measured using the method of Lowry *et al.* (16).

Results. In vivo lipogenesis was not significantly different among the three groups until after the animals had been weaned. At 28 days of age, lipogenesis in the *fafa* rats was significantly elevated compared to both of the lean groups (Fig. 1).

The pattern of lipogenic enzyme activities, in general, reflected the pattern of total *in vivo* lipogenic activity. In liver tissue derived from lean rats ACC activity expressed as counts per minute per milligram of protein decreased significantly between 5

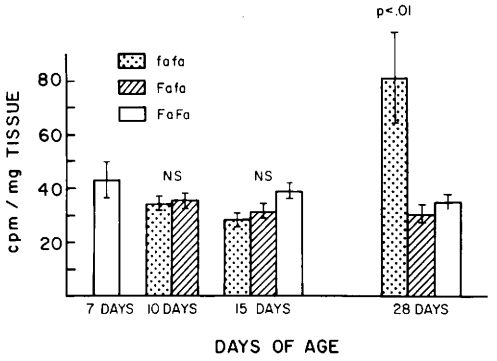

FIG. 1. *In vivo* hepatic lipogenesis in developing lean and obese Zucker rats determined by the incorporation of ^3H_2O into total lipids. Results are expressed as mean ± SEM.

and 22 days of age and then began to increase after weaning (Fig. 2). ACC activity in liver tissue derived from preobese rats showed no significant changes between 5 and 22 days of age. There was no significant elevation of hepatic ACC activity in preobese pups compared to lean pups at 5, 7, 15, and 22 days of age. In fact, the lean rats show a slightly higher activity of this enzyme than do the preobese rats at the early ages. After weaning, by 28 days of age, *fafa*-derived liver exhibited significantly elevated ACC activity compared to that derived from lean littermates. There were no significant differences between obese and lean rats in FAS activity until the rats were weaned (Fig. 3). There was a significant fourfold elevation in FAS activity in *fafa* rats at 22 days (24 hr postweaning) when compared to lean rats.

Discussion. The data from these experiments establish that the hepatic hyperlipogenesis seen in the postweaning obese Zucker rat is not present during the suckling period. Consequently, one cannot suggest that the primary effect of the *fa* gene is attributable to early overproduction of lipids by the liver. Our findings are consistent with those of Godbole *et al.* (12), who reported no significant differences in hepatic fatty acid synthesis in preobese *fafa* and lean Zucker rats at 18 days of age, but a significant elevation in *fafa* versus lean tissues 72 hr after weaning (23 days of age).

The developmental pattern of *in vivo* liver lipogenesis and hepatic ACC and FAS enzyme activity obtained in this study of Zucker rats is similar to that reported by other investigators (17–19) for other rat strains and appears to be influenced by nutritional factors. In general, there is a de-

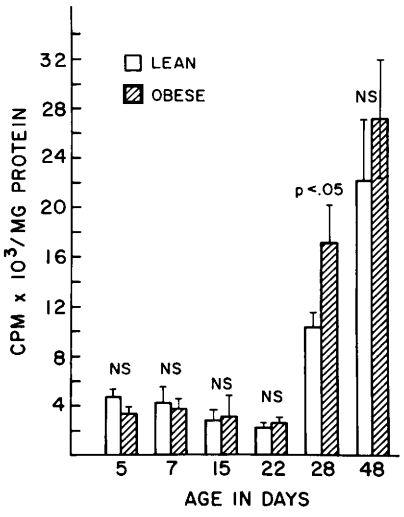

FIG. 2. Acetyl-CoA carboxylase activity in liver tissue of lean and obese Zucker rats measured pre- and postweaning. Results are expressed as mean ± SEM.

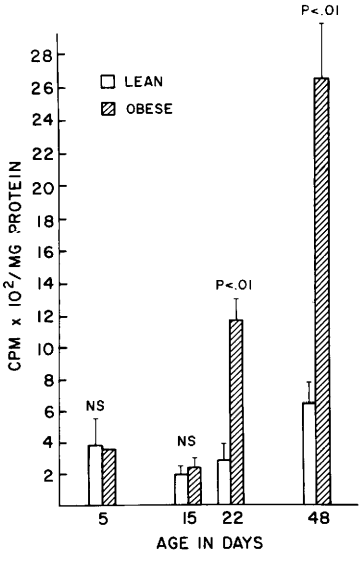

FIG. 3. Fatty acid synthetase activity in liver tissue of lean and obese Zucker rats measured pre- and postweaning. Results are expressed as mean ± SEM.

crease in hepatic lipogenesis after birth, the level remaining depressed during the suckling period, followed by a rise shortly after weaning (17). Over this period in development, nutritional changes occur which may be responsible for this pattern. In the first instance, the rat pups are shifted at birth from a predominantly carbohydrate diet which they receive via the placental circulation (17) to a milk diet which is much higher in fat content. They primarily consume this high-fat diet until they are weaned onto standard laboratory chows which are low in fat (4.5–5%) and much higher in carbohydrate content (57%). Therefore, as the diet shifts from low fat to high fat and back to low fat, one observes corresponding shifts in hepatic lipogenesis and the rate-limiting lipogenic enzymes such as ACC, FAS, and ATP-citrate lyase (17).

It is thus possible that dietary suppression of lipogenesis during the suckling period obscures a developing capacity for enhanced lipogenesis in the fatty rat. However, recent findings from our laboratory in which total lipogenesis was measured in primary cultures of fetal hepatocytes derived from fetuses with or without the fatty gene suggest that fatty liver cells have a *diminished* capacity for lipid synthesis in the fetal stage of development (20). Thus, we must conclude that some compensatory shift occurs in the liver's capacity for lipid synthesis over this period of early development in the fatty rat, such that the marked expression of hyperlipogenesis is manifest shortly after weaning. The findings of both Cleary *et al.* (10) and Boulange and co-workers (11) apparently rule out the possibility that hyperphagia is responsible for increased availability of substrate for storage in adipose tissue during this early period. Thus, it appears necessary to understand both the liver's role in providing lipid for storage in enlarging adipocytes and the factors acting in adipocytes per se which lead to their early enlargement in order to understand the development of Zucker rat obesity.

The authors gratefully acknowledge the technical assistance of Stella Tseng and Virginia Thompson and the assistance in preparation of the manuscript by Evelyn Buzzy.

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Received January 28, 1980. P.S.E.B.M. 1980, Vol. 164.