Development of hepatic and adipose tissue lipogenic enzymes and insulinemia during suckling and weaning on to a high-fat diet in Zucker rats[1]

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Abstract This study was designed to monitor the developmental changes in insulinemia and lipogenic enzyme activities in both inguinal adipose tissue and liver during suckling (7, 9, 14, and 17 days of age) and weaning (22 and 30 days of age) on to either a low-fat or a high-fat diet in lean (*Fa/fa*) and obese (*fa/fa*) rats. Tissues were removed through surgery and genotypes were retrospectively determined. During suckling, there was no difference in liver enzyme activities between the two groups. In contrast, adipose tissue fatty acid synthetase was increased by 50% and citrate cleavage enzyme and malic enzyme by 30% by 9 days of age. By 17 days of age, there was a threefold elevation in these enzyme activities and 6-phosphogluconic dehydrogenase and a twofold increase in glucose-6-phosphate dehydrogenase per inguinal fat pad in *fa/fa* versus *Fa/fa*. Consistent with these results, fat pad weight was increased by 20%, 50%, and 100% at 9, 14, and 17 days of age, respectively, in obese as compared to lean pups. However only by 17 days of age could a slight but significant increase in insulin level be detected in obese pups. Enlargement of inguinal fat pad accelerated after weaning on to a low-fat diet and still more after weaning on to a high-fat diet. Weaning on to a low-fat diet elicited an induction of hepatic lipogenic enzymes two or three times greater in *fa/fa* than in lean pups, while weaning on to a high-fat diet blunted the differences between genotypes. The lipogenic enzyme activities displayed per total inguinal fat were three to ten times greater in obese than in lean pups, regardless of the diet. However, adipose tissue lipogenic enzyme activities were much lower after weaning on to a high-fat than on to a low-fat diet in obese pups. The high-fat diet was as effective as the low-fat diet in triggering hyperinsulinemia in obese pups. The increased adipose tissue capacity for lipogenesis, starting during the suckling period, could play an important etiologic role in the development and maintenance of obesity in the Zucker rat.—**Bazin, R., and M. Lavau.** Development of hepatic and adipose tissue lipogenic enzymes and insulinemia during suckling and weaning on to a high-fat diet in Zucker rats. *J. Lipid Res.* 1982. **23:** 839–849.

Supplementary key words obesity • inguinal fat pad • fatty acid synthetase • citrate cleavage enzyme • malic enzyme • glucose dehydrogenases

The genetic obesity in rats discovered by Zucker and Zucker (1) is due to a single recessive autosomal gene. Rats bearing the *fa/fa* genotype develop a massive obesity with hyperinsulinemia and hypertriglyceridemia. Although obese rats are markedly hyperphagic under free-feeding conditions, hyperphagia is not a prerequisite for the development of obesity. Thus when growing *fa/fa* rats are pair-fed to the intake of normal lean rats they still deposit three or four times as much total body fat as the lean controls at the expense of non-lipid depots (2–4). This implies a metabolic abnormality that forces nutrients into lipid synthesis in obese rats. Although the mechanism by which dietary substrates are preferentially shunted to body fat in the obese Zucker rat has not yet been elucidated, several hypotheses have been considered. One concept holds that the anabolic profile is initiated by early hyperinsulinemia (5). However, an increase in body fat has been observed by 2 weeks of age (6, 7) prior to the increase in serum insulin which was detectable only at 3 weeks of age (7). A "pull" mechanism wherein the major primary event is an increase in adipocyte lipoprotein lipase activity levels has been advocated by Cleary, Vasselli, and Greenwood (4). This is supported by experimental evidence that shows an increase in lipoprotein lipase in the hypertrophic fat cells of preobese pups within the first days of life (8, 9). Another possibility is that an elevated lipid synthetic activity in the liver and/or the adipose tissue dictates the channeling of nutrients into lipids. However, experiments by Godbole, York, and Bloxham (10) and Turkenkopf et al. (11) have suggested that lipogenic enzyme activities are increased in liver of *fa/fa* rats only with the emergence of hyperinsulinemia after weaning. Adipose tissue lipogenic enzymes, which have been shown to be six to ten times more active in obese than in lean rats per total adipose tissue (12, 13), have never been investigated in preobese Zucker pups. The purpose of this study was

Abbreviations: FAS, fatty acid synthetase; CCE, citrate cleavage enzyme; ME, malic enzyme; G6PDH, glucose-6-phosphate dehydrogenase; 6PGDH, 6-phosphogluconic dehydrogenase; LF, low-fat diet; HF, high-fat diet.

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to document during the earliest stages of obese development at the suckling and weanling periods any changes in lipogenic enzyme activities in both liver and adipose tissue in relation to changes in insulinemia. To gain further insight into the possible effects of the switch from a high- to a low-fat diet (mother's milk to lab chow) on the dramatic metabolic changes observed at weaning, the effects of weaning on to a high-fat diet were also examined.

MATERIALS AND METHODS

Animals and diets

The rats used were bred in our laboratory from pairs originally provided by the Harriet G. Bird Memorial Laboratory, Stowe, MA. They were fed stock diet (U.A.R., 91360 Epinay sur Orge, France) ad libitum. Known heterozygous (*Fa/fa*) lean females and obese males (*fa/fa*) were mated. From this mating, 50% of the litter is expected to be obese and 50% of the litter lean and of the heterozygous (*Fa/fa*) genotype. We selected litters numbering between 9 and 11 pups of both sexes. Redman and Sweney (14) have demonstrated that pups may begin to eat the mother's stock diet as early as 13 days of age. In order to avoid a switch from a high-fat diet to a low-fat diet before weaning by the pups, the dams were fed a high-fat diet[2] when the pups were 10 days old. The high-fat diet, which contained 70% of calories as fat and 10% of calories as carbohydrate as described previously (15), was very close to the composition of Zucker rat milk at 10 days of lactation (16). At 17 days of age, the pups were separated from the mother and weaned on to either stock diet (12% fat by calorie: i.e., low-fat diet: LF) or the high-fat diet (HF) (see above).

Methods

At least three litters were used for tissue studies at each of the following ages: 7, 9, 14, 17, 22, and 30 days. The rate of survival of the pups after surgery was 84% for pups 7 or 9 days old and 99% for older ones. The whole right inguinal fat pad and a liver sample (about 20 mg) were surgically removed under ether anesthesia between 9 and 11 AM. The tissues were placed in cold saline, blotted, and weighed. They were homogenized (all-glass homogenizer) in 0.5 ml of ice-cold 0.25 M sucrose containing 1 mM dithiothreitol and 1 mM EDTA at pH 7.4. The pads removed from 22- and 30-day old pups were homogenized in 2 ml or 5 ml of this medium. Homogenates were centrifuged, (Model L5-50, Ti rotor, Beckman Instruments, Palo Alto, CA) at 105,000 *g* at 0°C for 60 min. The clear supernatants were either used immediately for assays of fatty acid synthetase (17) and citrate cleavage enzyme (EC 4.1.38) (18) or frozen at −20°C and used on the following day for assays of glucose-6-phosphate dehydrogenase (EC 1.1.4.49), 6-phosphogluconate dehydrogenase (EC 1.1.1.44) (19), and malic enzyme (EC 1.1.1.40), which was measured by the method of Ochoa (20) but with 10 mM malate. All the enzymatic assays were run at 37°C in duplicate, using a double-beam spectrophotometer (Perkin-Elmer model 555, Uberlingen, Germany) equipped with a recorder; they were linear with respect to time and to sample concentration. Protein was determined in the supernatant by the method of Lowry et al. (21) with bovine serum albumin as the standard. All enzyme data are expressed as units of activity. One unit is defined as the amount of enzyme needed to catalyze the oxidation or reduction of one nanomole of NAD(P) or NAD(P)H per min.

At least two additional litters of pups were used at each age to determine plasma insulin levels in blood withdrawn from the jugular vein. Duplicate 50-μl plasma samples were used in a radioimmunoassay procedure (22) utilizing the kits provided by Sorin C.E.A. France with a rat insulin standard (Novo Laboratories, Denmark). All the pups were returned to their mothers and kept alive until 60 days of age when phenotype was clearly apparent. Results are expressed as mean ± SEM. The level of significance of the differences between groups was calculated either by Student's t test or by a two-way analysis of variance (unweighted means, negligible interaction) as described by Snedecor and Cochran (23).

RESULTS

Developmental changes during suckling

Body and fat pad weight. **Fig. 1** shows the developmental changes in body weight and fat pad weight in preobese *fa/fa* Zucker pups as compared to heterozygous *Fa/fa* lean pups. The *fa/fa* genotype had no detectable effect on body weight during suckling. In contrast, the growth of inguinal adipose tissue differed markedly between the two groups of pups (Fig. 1A). At 7 days of age we could not demonstrate any change in the size of inguinal fat pad in preobese pups as compared with lean pups whether data for males and females were pooled or separated. However a 25% increase ($0.05 < P < 0.1$) in the weight of inguinal fat pad was detectable in the preobese rats at day 9. By 14 days of age the difference

[2] The high-fat diet consisted of (g/100 g): casein, 29; lard, 44; corn oil, 1; wheat starch, 14; vitamin mixture, 3; salt mixture, 6; cellulose, 3.

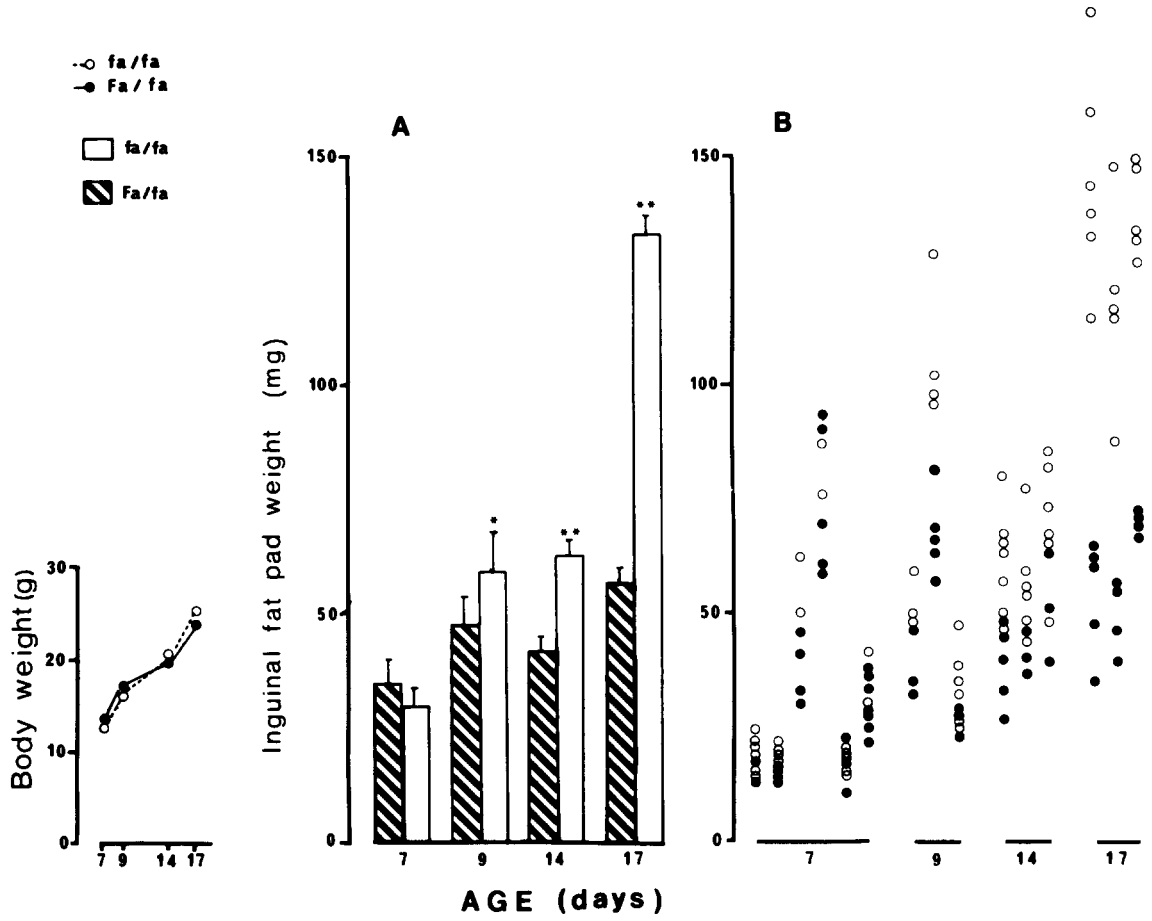

Fig 1. Body weight curves and inguinal pad weight (mg/pad) of lean and preobese suckling pups. Results are expressed as mean ± SEM except in B where individual data are reported. The number of lean pups were 25, 11, 12, and 13 versus 21, 13, 19, and 16 obese pups at 7, 9, 14, and 17 days, respectively. *, $0.05 < P < 0.1$; **, $P < 0.01$ as compared to lean pups the same age (two-way analysis of variance).

in fat pad weight between the two groups of pups was highly significant. From day 14 to day 17 the inguinal fat pad grew dramatically in preobese pups leading to a twofold weight increase in 3 days while the development of this fat depot was very moderate in lean pups. This resulted in a 100% difference in fat pad weight between the two groups of rats at age 17 days.

We observed (Fig. 1B) that the average fat pad weight for one genotype was very different from one litter to another at each age. The weight of inguinal fat pad also varied widely from pup to pup for a given genotype within the same litter. Fig. 1B clearly demonstrates that an overlapping of pad weights between the two genotypes occurred in nearly every litter as late as 14 days of age. In contrast, by 17 days of age, the values of fat pad weights clustered in two widely separated groups within each litter. These findings suggested fat pad weight as a possible means of identifying Zucker rat genotypes. Indeed we have shown that the weight of inguinal fat pad was a 100% reliable index of *fa/fa* genotype as of 16 days of age (24).

Lipogenic enzyme activities. **Fig. 2** shows the developmental changes in lipogenic enzyme activities in liver and adipose tissue of preobese *fa/fa* Zucker pups as compared to heterozygous *Fa/fa* lean pups. Five enzymes were studied: the key lipogenic enzyme fatty acid synthetase (FAS), which has been shown to be rate-limiting for overall lipogenesis under certain metabolic conditions (25), and four lipogenesis-related enzymes, citrate cleavage enzyme (CCE), malic enzyme (ME), glucose-6-phosphate dehydrogenase (G6PDH), and 6-phosphogluconate dehydrogenase (6PGDH) which respond in a coordinated fashion to multiple stimuli (26). Due to the minute amount of tissue available, the activity of acetyl CoA carboxylase could not be measured.

In lean Zucker pups liver FAS declined significantly between day 7 and day 9 ($P < 0.05$) and then stabilized at a low level over the entire suckling period. A similar developmental pattern was obtained for the activities of CCE and G6PDH. 6PGDH differed from this pattern by the significant rise ($P < 0.05$) in activity that occurred between 14 and 17 days of age. ME had already reached

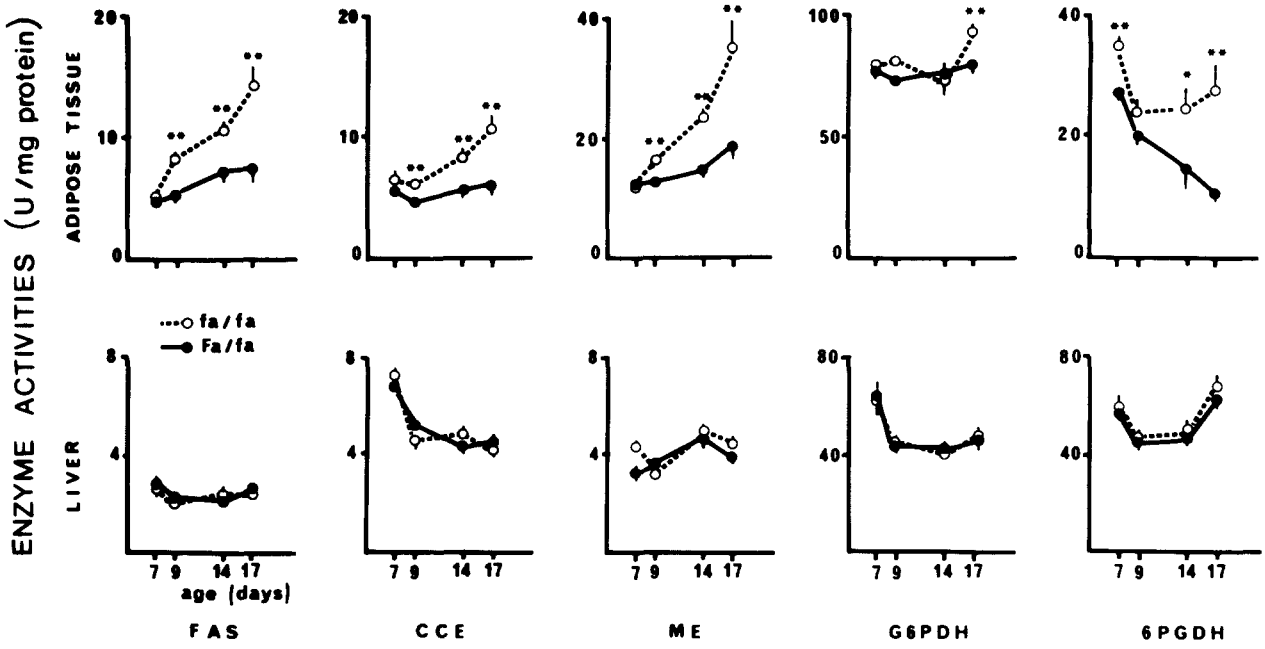

Fig. 2. Enzyme activities in U/mg protein in liver and adipose tissue from lean and obese pups. One unit is defined as the amount of enzyme needed to catalyze the oxidation or reduction of one nanomole of NAD(P) or NAD(P)H per min. Results are expressed as mean ± SEM. Lack of error bars indicates that SEM is smaller than symbol. The number of lean pups were 25, 11, 12, and 13 versus 21, 13, 19, and 16 obese pups at 7, 9, 14, and 17 days of age, respectively, * or **, $P < 0.05$ or $P < 0.01$ as compared to lean pups the same age (two-way analysis of variance).

the low-steady value by day 7. During the mid-suckling period (days 9 through 14), FAS was the least active of the five enzymes investigated, with a value approaching 2 U/mg protein which was half the value of CCE and ME.

The pattern of development of each lipogenic enzyme in the liver of preobese pups was strikingly similar to that observed in the liver of lean heterozygous pups in terms of both activity and developmental changes.

In adipose tissue of lean *Fa/fa* heterozygous pups, except for 6 PGDH which decreased steadily from day 7 to day 17, the activity of lipogenic enzymes studied underwent no significant changes over the time period investigated. This was in marked contrast to the developmental pattern of lipogenic enzyme activities exhibited by adipose tissue of *fa/fa* pups. There was no difference in FAS, CCE, and ME between the two groups of pups at day 7. However, beyond this age the activity of these enzymes rose rapidly in adipose tissue of preobese pups so that there was a highly significant increase over the lean values by 9 days of age. By 17 days of age, obese pups exhibited a twofold increase in those enzyme activities. The two dehydrogenases of glucose developed differently. G6PDH, which was several times more active than the other enzymes, remained at a steady level near that of lean pups from day 7 to day 14 but then increased, so that by 17 days of age it reached a value slightly but significantly higher than that observed in lean pups. 6PGDH was the only enzyme to be found more active in adipose tissue of preobese than in adipose tissue of lean pups by 7 days of age. As in lean pups, it fell sharply between day 7 and 9 but then remained at a steady level, thereby increasing progressively above the lean value.

When the results were expressed per fat pad, the differences in lipogenic enzyme activities between the two groups of pups were further increased (**Fig. 3**). By 14 days of age the inguinal adipose tissue of preobese rats was twice as active as the tissue of lean rats for all the enzymes studied except G6PDH. At day 17 there was a threefold increase in the activity per pad of all the enzymes studied (but for G6PDH which only doubled) in preobese pups as compared with lean pups. Increases in lipogenic enzyme activities per cell would be the same as increases per fat pad, since no significant differences in inguinal cell numbers were observed between obese and lean heterozygous (*Fa/fa*) pups during suckling (8, 9).

From Fig. 1A and Fig. 3 it can be seen that lipogenic enzyme activities were very significantly increased in preobese pups aged 9 days, i.e., at a time when fat pad weight hardly differed from controls. This lag of tissue development behind the onset of capacity for hyperlipogenesis suggests a cause and effect relationship between the increase in lipogenic enzyme activities and the fat accretion.

The inserts in Fig. 3 depict the increments in enzyme activities per total inguinal tissue (two pads) in pups bearing the obese genotype. These increments were very substantial if it is considered that the total liver content

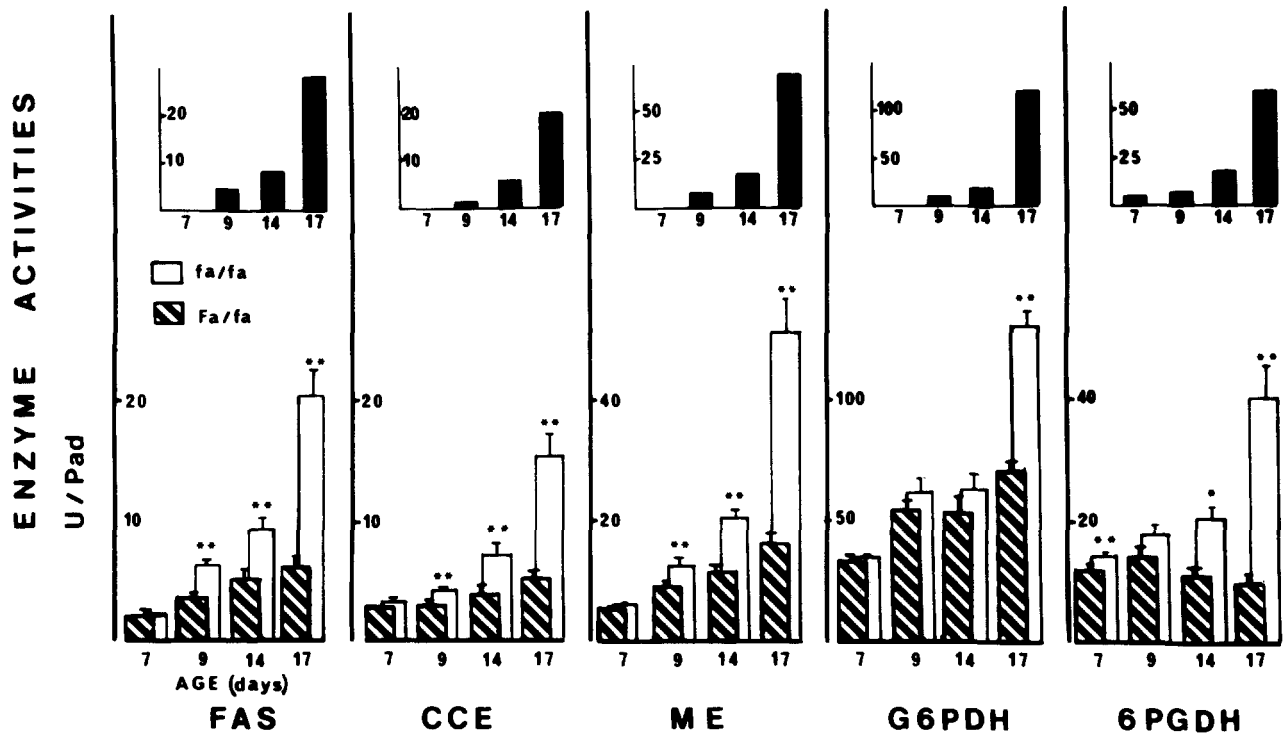

Fig. 3. Enzyme activities in one fat pad in adipose tissue from lean and obese suckling pups. One unit is defined as the amount of enzyme needed to catalyze the oxidation or reduction of one nanomole of NAD(P) or NAD(P)H per min. Results are expressed as mean ± SEM. The number of lean pups were 25, 11, 12, and 13 versus 21, 13, 19, and 16 obese pups at 7, 9, 14, and 17 days of age, respectively. * or **, $P < 0.05$ or $P < 0.01$ as compared to lean pups the same age (two-way analysis of variance). The insert for each enzyme represents the increment in activity per two pads.

of FAS, for example, was about 50 U at 9 days of age and 93 U at 17 days of age.

Insulinemia. The developmental changes in insulin levels in suckling Zucker rats are shown in **Fig. 4.** There were no detectable differences in hormonal status between lean and preobese pups as late as 14 days of age. By 17 days of age, insulin levels were significantly increased in the pups bearing the *fa/fa* genotype as compared to their lean littermates.

Developmental changes after weaning

Weaning on to a low-fat diet (lab chow); body and fat pad weight. **Fig. 5** shows that there was no difference in body weight between the two genotypes at 22 days of age. However by 30 days of age the preobese pups weighed 8 g more than their lean littermates. Fig. 5 also shows that the development of the obesity syndrome, as assessed by the enlargment of fat stores, accelerated after weaning. Thus the difference in fat pad weight between the two genotypes, which was 76 mg in 17-day-old pups, reached 420 mg 13 days after weaning. The rate of inguinal fat pad growth was nearly three times greater in obese than in lean pups.

Lipogenic enzyme activities. **Fig. 6** shows that weaning on to lab chow (LF) was a triggering signal for the liver of lean pups so that FAS, CCE, and ME rose sharply

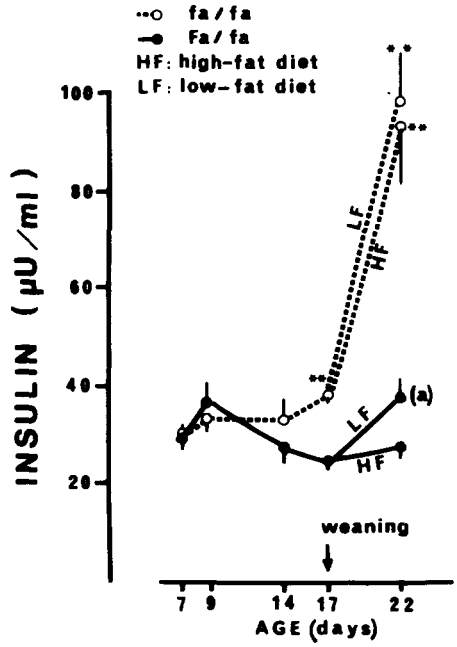

Fig. 4. Insulin levels in lean and obese pups during suckling (7, 9, 14, 17 days of age) and 5 days after weaning on to a low- (LF) or a high-fat (HF) diet. Results are expressed as mean ± SEM. The number of lean pups was 21, 10, 13, and 16 versus 14, 13, 20, and 20 obese pups at 7, 9, 14, and 17 days of age, respectively. The number of pups 22 days old was 10 lean, 16 obese weaned on to LF, and 10 lean, 18 obese on to HF. **, $P < 0.01$ as compared to lean pups the same age. a, $P < 0.01$ as compared to lean pups 17 days old.

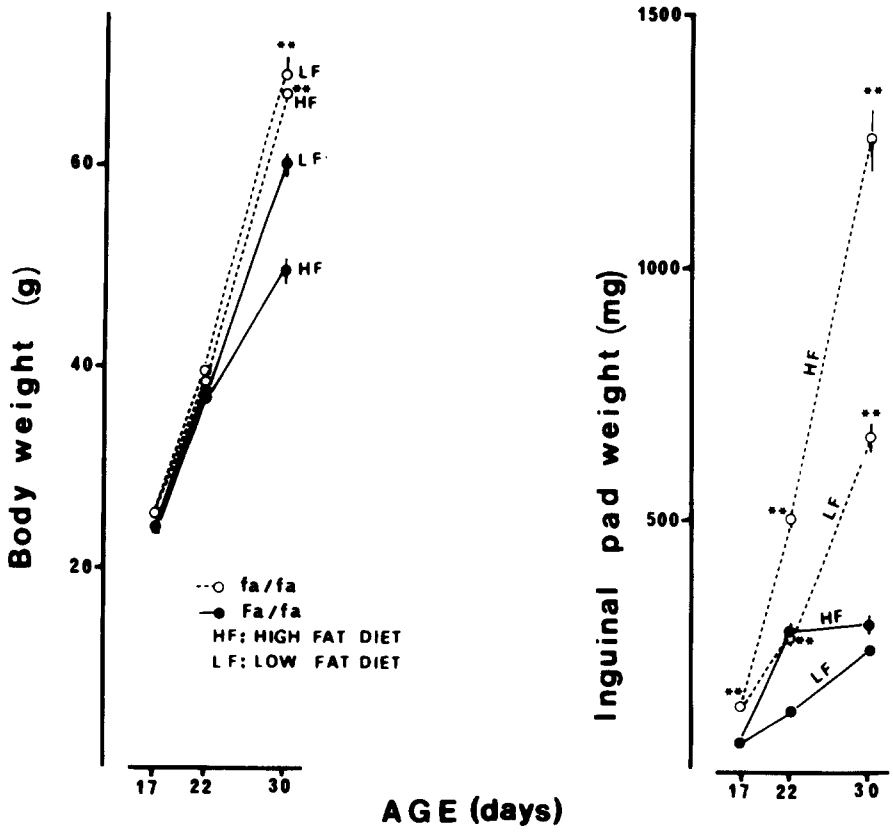

Fig. 5. Body weight curves and inguinal pad weight (mg/one pad) in lean and preobese pups after weaning on to a low- (LF) or a high-fat (HF) diet. Results are expressed as mean ± SEM. Lack of error bars indicates that SEM is smaller than symbol. Each data point represents 6–16 animals. Weaning was at 17 days of age.

from their steady, low preweaning level to reach six to tenfold higher values within 5 days. The dehydrogenases were much less responsive than FAS, CCE, and ME to the change of diet since they increased only by 120 to 150% as compared to their preweaning values. Between 22 and 30 days of age, FAS, ME, G6PDH, and 6PGDH activities underwent no significant changes whereas CCE declined significantly. In pups bearing the *fa/fa* genotype, weaning on to a low-fat diet elicited an induction of lipogenic enzyme activities two or three times greater than in liver of lean pups. Therefore, preobese pups that were indistinguishable from their lean littermates in terms of hepatic lipogenic enzyme activities during suckling differentiated sharply after weaning. By 22 days of age, livers of preobese pups displayed a twofold increase in FAS, CCE, G6PDH, and 6PGDH activities as compared with livers of lean rats. Malic enzyme was only increased by 30%. As depicted by Fig. 6, each one of these enzymes in livers of obese pups exhibited a developmental pattern from day 22 to day 30 similar to that found in lean pups.

In adipose tissue of lean rats, 5 days of weaning on to a low-fat diet increased the activities of FAS, CCE, ME, and 6PGDH to values, respectively, 11, 22, 21, and 7 times greater than the preweaning levels (Fig. 6). In contrast, G6PDH increased only by 50% during the same time. From 22 to 30 days of age all the enzyme activities seemed to stabilize in adipose tissue of lean rats since no significant changes were observed. Obese pups that already exhibited increased lipogenic enzyme activities in adipose tissue during suckling reacted to weaning by a large increase in all (except 6PGDH which only increased by 60%) enzyme activities within 5 days (FAS × 8; CCE × 15; ME × 12; 6PGDH × 4). From day 22 to 30 the developmental pattern in obese pups differed markedly from that obtained in lean pups in that all the enzyme activities kept rising sharply. By 30 days of age the lipogenic enzyme activities per mg of protein were three to fivefold higher in adipose tissue of obese than in adipose tissue of lean rats. Moreover postweaning obese pups had much larger fat pads than their lean littermates. Therefore the lipogenic enzyme activities displayed per total inguinal adipose tissue were three to eleven times greater in obese pups than in lean pups as shown in **Table 1.**

Insulinemia. Fig. 4 shows that weaning on to a low-fat diet was effective in raising insulin levels in both groups of rats. However the response of obese pups was far greater than that of lean pups.

Weaning on to a high-fat diet. We next considered the

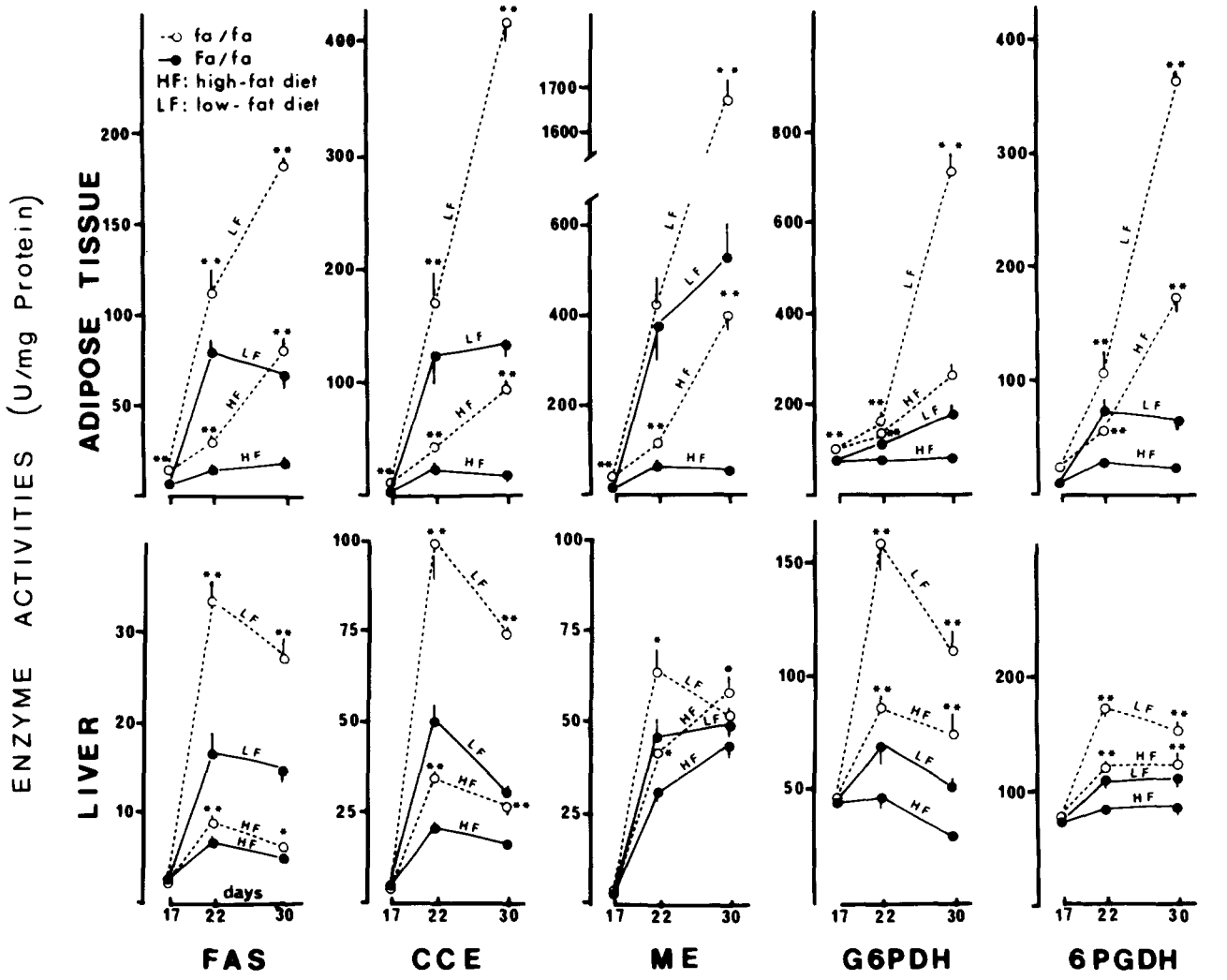

Fig. 6. Enzyme activities in U/mg protein in liver and adipose tissue from lean (*Fa/fa*) and obese (*fa/fa*) pups after weaning on to a low- (LF) or a high-fat diet (HF). Results are means of 6–16 animals at each data point. Vertical bars represent SEM. Weaning was at 17 days of age. One unit is defined as the amount of enzyme needed to catalyze the oxidation or reduction of one nanomole of NAD(P) or NAD(P)H per min. * or **, $P < 0.05$ or $P < 0.01$ as compared to lean pups the same age and on the same diet.

possibility that the previously observed dramatic effects of weaning on lipogenic enzyme activities and insulin levels in preobese rats were mediated by the high carbohydrate content of the diet. Therefore, we examined the effects of weaning lean and obese pups onto a high-fat diet. The results of these studies are illustrated in Figs. 4, 5, 6, and Table 1. Weaning on to a high-fat diet was not able to raise insulin above the suckling level in lean Zucker rats (Fig. 4). In contrast, in obese pups, the high-fat diet was as effective as the low-fat diet in triggering hyperinsulinemia, indicating that the amount of carbohydrate eaten, which was probably five to six times greater in LF-fed rats than in HF-fed rats, was not a determinant. Fig. 5 shows that the difference in body weight between the two genotypes was more marked when the pups were weaned on to a high-fat diet than on to stock diet. Fig. 5 shows also the dramatic growth of inguinal fat pads in obese pups weaned on to a high-fat diet. The burst in adiposity that occurred in the postweaning days with stock diet was aggravated by weaning on to a high-fat diet, which promoted the gain of nearly 0.1 g fat tissue per day in each inguinal depot.

Fig. 6 depicts the postweaning changes in lipogenic enzymes in adipose tissue and liver of the two genotypes. It shows that, in lean rats, G6PDH of both tissues and 6PGDH of liver were unresponsive to weaning on to a high-fat diet. Although the other enzymes were substantially increased over their preweaning levels, their responses to weaning on to a high-fat diet were markedly blunted as compared with their responses to low-fat diet except for hepatic ME. In obese pups also, weaning on to a high-fat diet was less effective than weaning on to a low-fat diet in raising lipogenic enzymes in both liver and adipose tissue. However, in obese pups, all the enzymes examined here were increased, including G6PDH and 6PGDH.

TABLE 1. Lipogenic enzyme activities per total inguinal adipose tissue in Zucker rats weaned at 17 days of age on to a low-fat (LF) diet or a high-fat (HF) diet

Diet	Age	Genotype	FAS	CCE	ME	G6PDH	6PGDH
	days		*units per two pads*[a]				
Low-fat	22	Fa/fa (9)	514 ± 72	758 ± 158	2,172 ± 544	792 ± 82	464 ± 64
		fa/fa (10)	974 ± 61**	1,406 ± 132**	3,462 ± 270*	1,396 ± 66**	954 ± 64**
	30	Fa/fa (12)	700 ± 104	1,478 ± 220	5,550 ± 910	948 ± 133	334 ± 54
		fa/fa (7)	3,546 ± 114**	8,382 ± 812**	16,383 ± 1,866**	7,053 ± 623**	3,578 ± 360**
High-fat	22	Fa/fa (14)	120 ± 12	216 ± 32	696 ± 159	714 ± 71	268 ± 32
		fa/fa (16)	304 ± 26**	448 ± 25**	1,272 ± 94**	1,416 ± 109**	644 ± 46**
	30	Fa/fa (6)	139 ± 15	126 ± 15	440 ± 72	696 ± 42	166 ± 85
		fa/fa (11)	1,118 ± 76**	1,326 ± 89**	5,324 ± 443**	3,634 ± 352**	2,422 ± 182**

[a] Results are mean ± SEM. Number of rats within parentheses. One unit is defined as the amount of enzyme needed to catalyze the oxidation or reduction of one nanomole of NAD(P) or NAD (P) H per min.

FAS, fatty acid synthetase; CCE, citrate cleavage enzyme; ME, malic enzyme; G6PDH, glucose-6-phosphate dehydrogenase; 6PGDH, 6 phosphogluconic dehydrogenase. * or **, $P < 0.05$ or $P < 0.01$ as compared to lean pups same age and same diet.

Fig. 6 shows that, although the genotypes were demonstrably different at 22 days of age on both diets, the differences in liver lipogenic enzyme activities between the two genotypes were reduced by weaning on to a high-fat diet as compared to weaning on to a stock diet. This was not the case in adipose tissue, as shown in Table 1. When weaned on to a high-fat diet, 30-day-old obese pups displayed five to fifteen times greater lipogenic enzyme activities per total inguinal adipose tissue than lean littermates.

DISCUSSION

The adult Zucker obese rat has been the subject of abundant investigation. Among other features, hyperinsulinemia and enhanced lipogenic capacity in both adipose tissue and liver have been well described (see review by Bray and York (27). In contrast, the abnormalities present at the onset of, or prior to, obesity are poorly documented. The scarcity of information arises because of the absence of genetic markers. Therefore the only protocols which can be used are those compatible with the survival of the pups for later identification of the genotype. This has considerably restricted the metabolic studies of tissues and virtually nothing is known about the earliest stages of obese development. In the present work we performed both a liver biopsy and the removal of an entire inguinal fat pad, which in our hands was followed by a high rate of survival of pups as of 7 days of age. This enabled us to monitor the developmental changes in lipogenic enzyme activities in liver and adipose tissue in an attempt to delineate the site and the age at which the capacity for hyperlipogenesis first occurs and to unravel the chronological order in the emergence of hyperinsulinemia and increased activity of lipogenic enzymes.

This study documents the growth of inguinal fat pads in lean and obese Zucker pups from 7 to 30 days of age. The inguinal depot was chosen because it develops in the first ten days of life when other fat depots are barely detectable. Our data show that *fa/fa* genotype as compared to *Fa/fa* had no effect on the weight of inguinal fat pad before 9 days of age, when a slight hypertrophy of the pad was detectable. The data reported here are in good agreement with those of Gruen, Hietanen, and Greenwood (9) who reported a significant 35% increase in inguinal pad weight in the obese compared to the heterozygous lean by 13 days of age. At variance with our results, an enlargement of the inguinal fat pad was observed at 7 days of age by Boulangé, Planche, and de Gasquet (8). The reason for this discrepancy may be the difference in the genotype of the lean control pups (*Fa/Fa + Fa/fa* in the work of Boulangé et al. (8), only *Fa/fa* in the present work) and the greater number of obese animals investigated here (21 vs. 8). Our data show that the rate of growth of inguinal fat tissue accelerates in fatties between 14 and 17 days when pups begin to have access to solid food. This study clearly demonstrates that the burst of adipose tissue mass that occurs at weaning in fatties is not due to a change in diet composition since weaning on to a high-fat diet, close to milk in composition, results in a still more dramatic enlargement of fat stores than weaning on to chow diet. This could be explained in part, by differential intakes of the two diets. It has been well shown that the obese pups weaned on to a low-fat diet present a marked hyperphagia (food intakes increased by more than 50%) as compared to their lean littermates (28). Weaning on to a high-fat diet might have led to a further increase in food intake in the obese rats, even though such a diet has been shown not to alter food intakes for the initial days postweaning in albino rats (29).

The monitoring of developmental changes in insulinemia performed in this work allowed us to detect the

time of onset of hyperinsulinemia in fatties. In agreement with previous observations of Zucker and Antoniades (7), who reported that there was no significant rise in 3-week-old fatties, we show here that a slight but significant hyperinsulinemia emerges in *fa/fa* pups by 17 days of age. Two sets of data clearly demonstrate that insulin level is triggered in fatties independently of a shift to a high carbohydrate diet: the emergence of hyperinsulinemia, which occurs when suckling pups begin to have access to solid food, here, a high-fat diet; and the dramatic insulin increase after weaning on to a high-fat diet. Our data suggest that the factor governing the hyperinsulinemia in obese pups may be the amount of food intake. During suckling, insulin is kept under control by the limited supply of milk. Hyperinsulinemia is detectable by 17 days of age, at the time when pups begin to show hyperphagia. In agreement with this, Stern and Johnson (30) have reported that an increase in intake of solid food in obese Zucker rats is detectable by 16 days of age. At weaning, under free-feeding conditions, the large quantities of nutrient secretagogues ingested cause a dramatic increase in insulin level.

The developmental patterns of hepatic lipogenic enzymes described here in suckling and postweaning lean Zucker rats are very similar to those reported in Wistar rats by Ballard and Hanson (31) and Taylor, Bailey, and Bartley (32). In addition, this study demonstrates that the rise in hepatic CCE, ME, and FAS after weaning on to a high-fat diet is not mediated through the rise in insulin since the hormone level in the Zucker lean is not changed after weaning on to this diet.

Our data, in good concordance with those of Godbole et al. (10) and Turkenkopf et al. (11), establish that lipogenic enzyme activities are not enhanced in the liver of suckling fatties even in the late suckling period when a slight hyperinsulinemia is present. Moreover this study makes it clear that weaning on to a high-fat diet, although it is much less efficient than weaning on to low-fat diet, does not abolish the expression of the *fa/fa* gene at the level of hepatic lipogenic enzymes, which are all substantially increased in activity over the values in lean pups weaned on to high-fat diet.

The present work is the first to document the developmental patterns of lipogenic enzymes in the adipose tissue of lean and obese Zucker rats. It shows that the specific activity of these enzymes in lean pups remains at a steady low level throughout suckling, except for 6PGDH which decreases. As in liver, weaning on to a low-fat diet is a powerful triggering signal for all these enzyme activities. In spite of the unchanged insulin level, weaning on to a high-fat diet increases the activity of lipogenic enzymes over the preweaning level, with the exception of G6PDH. The important finding of this study is the altered developmental pattern of lipogenic enzymes in adipose tissue of pups bearing the *fa/fa* genotype. A very significant 50% increase in these enzyme activities occurs in adipose tissue of fatties as compared with heterozygous lean pups as early as 9 days of age. By 17 days of age the inguinal fat pads of fatties are twice as big as those of lean rats and contain three times more lipogenic enzyme activities. Weaning brings the difference in lipogenic enzyme activities between genotypes to 10 to 1 regardless of diet composition; hyperphagia which occurs at weaning (28) is likely to play a major role in this induction.

In 30-day-old obese rats, the FAS activity exhibited by two inguinal fat pads is comparable to (weaning on to chow diet) or higher than (weaning on to high-fat diet) the FAS activity displayed by the whole liver. This result contrasts with the observation of Godbole and York (33) that hepatectomy reduces by 90% the endogenous fatty acid synthesis in adipose tissue of 5-week-old fatties. The low glucose level after hepatectomy, which might have limited the expression of the adipose tissue capacity for lipogenesis, could explain this discrepancy.

It is noteworthy that the capacity for hyperlipogenesis emerges in adipose tissue of suckling *fa/fa* pups in spite of the high-fat content of the diet, which normally suppresses lipogenic enzymes (15). As to whether these abnormal activities of lipogenic enzymes are a primary lesion of the *fa/fa* genotype or whether they are a secondary adjustment to other primary metabolic defects is open to speculation. However, the fact that the alterations of lipogenic enzymes are present only in adipose tissue and not in liver suggests that adipose tissue is a primary site of *fa/fa* genotype expression. Defects at the level of the adipocyte membrane, including hormone receptors and/or the glucose transport system, might be the early lesions. This would result, through increased glucose uptake, in increased levels of glycolytic metabolites within the adipocyte explaining the activation of both FAS, reported here, and LPL, reported by others (8, 9), since glycolytic intermediates are thought to regulate both these enzymes (34, 35).

The etiologic role of the early metabolic abnormality found in the inguinal fat pads of obese pups is not established in this study. The increased lipogenic enzyme activities exhibited by the adipose tissue of *fa/fa* pups are likely to enhance the channeling of substrates such as glucose, amino acids, or ketone bodies (which are abundant in suckling pups) into fatty acids eventually leading to hypertrophic fat pads. Such a view is supported by current studies in our laboratory showing that in vivo fatty acid synthesis is increased in adipose tissue of obese pups by 10 days of age.[3] However, in suckling

[3] de Waziers, I., E. Planche, and P. de Gasquet. Manuscript in preparation.

fatty pups, obesity is likely to result mainly from storage of dietary fat since an increased lipoprotein lipase (8, 9) is present to ensure the clearance of circulating triglycerides, the major suckling serum substrate.

The increment in capacity for lipogenesis exhibited by inguinal adipose tissue of suckling obese pups is not negligible with regard to the capacity displayed by liver. The total hepatic FAS activity would approximate 77 U in 14-day-old pups and 93 U in 17-day-old pups (assuming a liver weight of 700 mg and 800 mg, respectively). Therefore an increment of 8 U or 27 U in FAS activity, as exhibited by two inguinal fat pads of 14-day-old and 17-day-old obese pups, respectively, would represent an additional 10 or 30% liver. Moreover adipose tissue from other sites is also likely to exhibit increased lipogenic enzyme activities. If white adipose tissues are virtually non-existent in sites other than the inguinal, brown adipose tissue is well developed and could contribute significantly to the aberrant lipogenic capacity in obese pups. In current investigations we found a significant increment in FAS activity per total intrascapular brown adipose tissue in 14-day-old obese pups as compared to lean pups.[4] All together our data are rather in support of the "pull theory" of obesity, whose essential concept is an increased shunting of nutrients into adipose tissue leading to a compensatory hyperphagia. Thus, increased adipose tissue lipoprotein lipase has been advocated as the "metabolic pull" initiating the obesity syndrome of the Zucker rat by Greenwood et al. (4). Such a mechanism has been advanced also for hormonally induced obesity (36). With the results presented here, the following and admittedly speculative sequence of events can be suggested; the primary alteration in the fatty rat would be an increase, starting during the suckling period, in lipogenic enzyme activities in adipose tissue, channeling glucose, amino acids, and ketone bodies into fat stores. The removal from the circulation of these hunger-suppressor substrates would be a straightforward explanation of the food drive of the obese pups. Hyperphagia and a secondary hyperinsulinemia would develop leading to the emergence of hyperlipogenesis in the liver and a further increase in lipogenic enzyme activities in adipose tissues. However, in the absence of the appropriate data (*fa/fa* pups pair-fed to lean pups, same amount of food and same eating pattern), we cannot rule out an alternate etiology of hyperinsulinemia that would develop with age as a function of the genotype independently of hyperphagia.

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[4] Bazin, R., and M. Lavau. Unpublished results.

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