Phenotypic variability in unweaned 3-week-old Zucker rats

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Summary. At 3 weeks of age, Zucker rats could not be classified as normal or obese according to body or liver weight. On the other hand, determination of subcutaneous adipose tissue weight per 100 grams of body weight permitted both phenotypes to be distinguished, provided that the growth variations particular to each litter were taken into account. Rats whose future obesity is predictable using this method, have significantly elevated serum levels of triacylglycerols, total cholesterol and phospholipids. However, further analysis of these values indicated that in fact there were two subgroups of both obese and non-obese rats, i.e. those which had elevated serum lipid levels and those which did not.

Introduction.

The Zucker rat, homozygous for the recessive gene for obesity, fa/fa, becomes manifestly obese by 4-5 weeks of age (Zucker and Zucker, 1961). Before that, the preobese pup is undistinguishable by its body weight or shape from its lean counterpart (Fa/fa or Fa/Fa). As the adult fatty rat is infertile and the pups issue from heterozygous parents, the litters consist of mixed populations of both phenotypes.

Taking advantage of the fact that some abnormalities of the Zucker syndrome appear early in postnatal life, several authors have recently proposed methods for identifying the preobese pup at an early age ; these methods are based on decreased rectal temperature (Godbole et al., 1978), decreased O_2 consumption (Kaplan et al., 1980) or increased adipose cell size (Boulangé et al., 1979). The latter authors have suggested that fat cell hypertrophy is accompanied by an increase in the weight of inguinal adipose tissue as early as 7 days of age. Therefore, in a study on the influence of age on various lipid parameters in the Zucker rat, we thought that we should be able to identify preobese pups by their inguinal fat pad weight. However, as we observed and the present study shows, in only a limited number of cases can 3-week old pups be phenotypically distinguished solely by their inguinal adipose tissue weight. Moreover, that type of determination is not facilitated by taking into account serum lipid levels. We therefore propose a procedure that keeps subjective interpretation to a minimum.

Material and methods.

Animals. — Our laboratory obtained 17 female Zucker rats (CSEAL ; Orléans-La Source, 45045 Orléans cedex, France, with their offspring (8 to 11 days old). As both parents were heterozygous (Fa/fa), there were two phenotypes, obese (fa/fa) and non-obese (Fa/fa or Fa/Fa), in the litters. We used a total of 84 young males, divided into groups of 3 (2 litters), 4(6), 5(5), 6(2), 8(1) and 9(1) rats each. Each litter was housed separately with the dam in a metal cage. Laboratory chow (A04 from UAR, 91360 Epinay-sur-Orge, France) and acidulated tap water was furnished *ad libitum*. At the age of 21-22 days, the unfasted male pups, left with the mother until sacrifice, were decapitated between 08:30 and 11:00 h. The blood was collected into a cold centrifuge tube using a funnel. It was then immediately centrifuged at 2 500 × g for 5 min to obtain the serum. In addition, the liver and subcutaneous inguinal adipose tissue (two pads) were removed and weighed.

Plasma determination. — In each serum the following lipids were determined : triacylglycerols (triglyceride reagent, Dow Diagnostics), cholesterol (Cholesterin Enzymatish, Merckotest) and phospholipids (Phospholipid Beta-Test, Wako Biochemicals).

Statistical study. — The results were expressed as the mean ± SEM. The means were compared using Student's *t*-test with a significance limit of 5 p. 100. A test (χ^2 test : Schwartz, 1963 or Rankits test : Bliss, 1967) for normality of the distribution was carried out on the values obtained for each parameter.

Results and discussion.

Distribution of adipose tissue weight. — Table 1 shows the mean values obtained in the 84 rats for the different parameters studied. The SEM were low ; the different end values were very dissimilar. Body weight (X) and liver weight (Y), which were normally distributed, correlated well with each other (Y = 0.050 8 X — 0.049 8 ; r = 0.932 3 ; P < 0.001). The other parameters did not follow a normal distribution pattern. As suggested by the distribution of adipose tissue weight/body weight (fig. 1), this pattern could result from the use of two different populations, one of obese (genotype fa/fa) and the other of non-obese (Fa/-) rats. However, the proximity of those two populations prevented any identification of the animals whose adipose tissue weight ratios were in the overlap zone, i.e. 1.4-1.8 g/100 g.

Variation in adipose tissue weight (fig. 1) did not take into account any eventual intra or interlitter differences in animal development. Individual pups were then examined according to their litter of origin in an attempt to identify as many as possible. Three types of litter were found (fig. 2).

1) In the first litter type, the pups could be clearly segregated into two distinct groups. In litters 3, 7, 8, 9, 11 and 12 the mean pad weight per 100 g of body weight of the two groups differed by a factor of 2, being around 1 g/100 g in the thinner and 2 g/100 g in the fatter group. These groups were therefore assumed to represent Fa/- and fa/fa phenotypes, respectively.

TABLE 1

Overall results

	Body weight (g)	Liver weight		Adipose tissue weight (2 pads)		Serum lipids		
		(g)	(g/100 g body weight)	(g)	(g/100 g body weight)	Triacyl-glycerols (mg/ml)	Total cholesterol (mg/ml)	Phospho-lipids (mg/ml)
X ± SEM (n = 84)	43.2 ± 0.8	1.70 ± 0.05	3.89 ± 0.04	0.50 ± 0.03	1.14 ± 0.06	1.33 ± 0.06	1.35 ± 0.05	2.20 ± 0.08
Lower limit.........	27.0	0.90	3.16	0.05	0.18	0.45	0.33	1.20
Upper limit.........	59.4	2.80	5.01	1.19	2.90	2.73	2.52	3.79
Test of normal distribution	(N)	(N)	(N)					

Triacylglycerol and phospholipid levels were calculated using average molecular weights of 885 and 801, respectively. (N) The distribution seems to be normal.

TABLE 2

Characteristics of obese and non-obese 3-week old Zucker rats

Rats	Body weight (g)	Liver weight		Adipose tissue weight (2 pads)		Serum lipids		
		(g)	(g/100 g body weight)	(g)	(g/100 g body weight)	Triacyl-glycerols (mg/ml)	Total cholesterol (mg/ml)	Phospho-lipids (mg/ml)
Fa/-(non-obese) (n = 58)	43.6 ± 1.0 (N)	1.74 ± 0.06 (N)	3.96 ± 0.05 (N)	0.37 ± 0.02 (N)	0.82 ± 0.03 (N)	1.13 ± 0.06	1.20 ± 0.05	1.90 ± 0.07
fa/fa (obese) (n = 20)	44.1 ± 1.4 (N)	1.72 ± 0.09 (N)	3.86 ± 0.10	0.89 ± 0.04 * (N)	2.02 ± 0.07 * (N)	1.87 ± 0.12 * (N)	1.82 ± 0.09 * (N)	3.04 ± 0.14 *

Triacylglycerol and phospholipid levels were calculated using average molecular weights of 885 and 801, respectively. The results are expressed as : X ± SEM. The differences were analyzed by Student's t-test. * Significant difference, $p < 0.05$. (N) Population distribution seems normal.
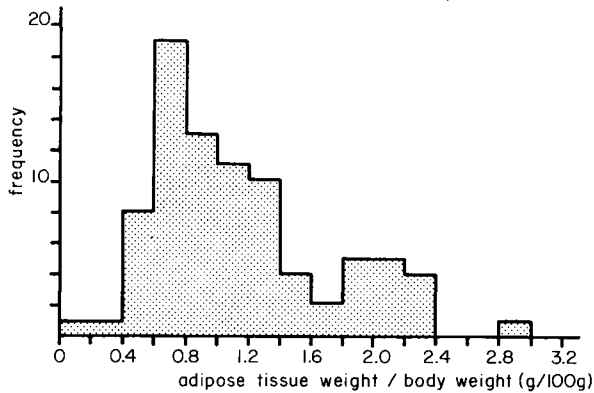
FIG. 1. — *Distribution of adipose tissue weights.*

2) In the second litter type, the relative adipose tissue weights of all the littermate clustered closely together around the values of either 1 or 2 g/100 g of body weight. By analogy with what was done above, the pups in those litters were identified as Fa/- (litters 1, 4, 5, 6, 10, 13 and 16) and fa/fa (litter 14), respectively.

3) In the third litter type, the adipose tissue weight/body weight ratio of each of the three remaining litters (2, 15 and 17) was distributed over a wide range of values, making it difficult to identify all the pups. Pups in litters 2 and 15 were among the smallest in the experiment, being similar in body weight to pups of litters 1 and 13. The adipose tissue weights of the lean would be expected to be comparable in the four litters. This was the case only for rat 10 in litter 2 and rats 67, 68, 69, 74 and 75 in litter 15, which were therefore identified as Fa/-. Applying the criteria established above, we assumed that pups 9 (litter 2) and 71, 72, 73 were fa/fa. This procedure left 4 pups (6, 7, 8 lean ? and 70 obese ?) unidentified. Litter 17 consisted of 4 pups of average body weight. Pups 82 and 83 were assumed to be fa/fa because their adiposity was comparable to that of the already identified obese pups. Consequently, considering that adipose tissue weight between phenotypes should differ by a factor of 2, pup 81 was tentatively designated as Fa/-. Pup 84 could not be identified ; however, as the adipose tissue weight/100 g of pup 84 was close to that of the previously identified obese pup of comparable body weight (pups 28, 33, 54, 66), the possibility that it also was obese had to be considered. If that was the case, the identification of pup 81 as lean was doubtful. Therefore, we did not consider the phenotype of either pup 81 or 84 as firmly established.

Characteristics of obese and non-obese rats. — If we excluded those rats which were difficult to classify (6, 7, 8, 70, 81 and 84), the remaining ones could be catagorized as obese or non-obese. The characteristics of these groups are shown in table 2. Appa_rently, excluding 6 out of 84 pups did not introduce a bias in the results which were not significantly altered when pups 6, 7, 8, 81 and 84 were added to the lean group and pup 70 to the obese group. Finally, we should like to note that, as the proportion of obese rats was equal to 20/(58 + 20) or 26.6 p. 100, the Mendelian theory was satisfied.
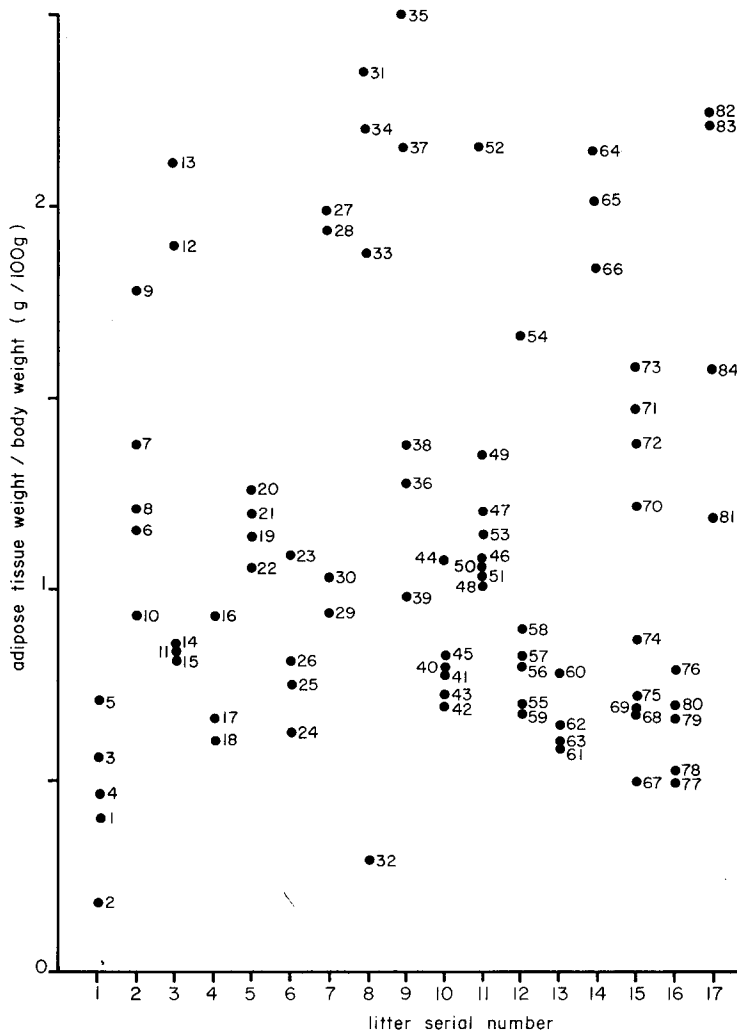
FIG. 2. — *Rat adipose tissue weight in the different litters.*
Abscissa : litter serial numbers ; Each point represents a rat.

At 3 weeks we could not distinguish obese from non-obese rats by body weight. Zucker rat obesity does not really become manifest until the age of 4 to 5 weeks, even if some authors (Powley and Morton, 1976 ; Stern and Johnson, 1977) consider that 3-week old fa/fa rats already weigh significantly more than Fa/-rats. In addition, hepatomegaly has not yet set in. At 3 weeks of age, liver weight per 100 g of body weight was identical in both groups of animals. The adipose tissue weight of the obese rats increased on the average by 140 p. 100 (absolute weight) and by 146 p. 100 (relative weight) as compared to lean rats.

TABLE 3

Characteristics of the different groups of 3-week old Zucker rats

	Body weight (g)	Liver weight		Adipose tissue weight (2 pads)		Serum lipids		
		(g)	(g/100 g body weight)	(g)	(g/100 g body weight)	Triacyl-glycerols (mg/ml)	Total cholesterol (mg/ml)	Phospho-lipids (mg/ml)
Fa/-(non obese) :								
Group 1. Elevated serum lipids (n = 5)	33.7 ± 1.8 (2, 3, 4) (N)	1.16 ± 0.07 (2, 3, 4) (N)	3.43 ± 0.11 (2, 4) (N)	0.28 ± 0.03 (3, 4) (N)	0.83 ± 0.07 (3, 4) (N)	1.99 ± 0.19 (2, 4) (N)	1.97 ± 0.08 (2, 4) (N)	3.12 ± 0.07 (2, 3, 4) (N)
Group 2. Low serum li-pids (n = 53)	44.6 ± 1.0 (1) (N)	1.79 ± 0.06 (1) (N)	4.00 ± 0.05 (1, 3) (N)	0.38 ± 0.02 (3, 4) (N)	0.81 ± 0.04 (3, 4) (N)	1.04 ± 0.04 (1, 3, 4) (N)	1.13 ± 0.04 (1, 3, 4) (N)	1.78 ± 0.06 (1, 3, 4) (N)
fa/fa (obese) :								
Group 3. Elevated serum lipids (n = 13)	41.8 ± 1.6 (1, 4) (N)	1.51 ± 0.06 (1, 4) (N)	3.62 ± 0.06 (2, 4) (N)	0.86 ± 0.06 (1, 2) (N)	2.04 ± 0.11 (1, 2) (N)	2.19 ± 0.09 (2, 4) (N)	2.07 ± 0.07 (2, 4) (N)	3.47 ± 0.06 (1, 2, 4) (N)
Group 4. Low serum li-pids (n = 7)	48.0 ± 2.0 (1, 3) (N)	2.07 ± 0.16 (1, 3) (N)	4.28 ± 0.17 (1, 3) (N)	0.95 ± 0.05 (1, 2) (N)	1.99 ± 0.07 (1, 2) (N)	1.32 ± 0.14 (1, 2, 3) (N)	1.39 ± 0.05 (1, 2, 3) (N)	2.30 ± 0.08 (1, 2, 3) (N)

Triacylglycerol and phospholipid levels were calculated using average molecular weights of 885 and 801, respectively.
The results are expressed as $X \pm$ SEM. The differences have been calculated by Student's t-test. Number in parenthesis indicates groups with which there is a significant difference.
(N) Population distribution seems normal.

Table 2 shows that obese 3-week old rats had hyperlipemia, the serum levels of triacylglycerols, total cholesterol and phospholipids being significantly higher ($+$ 65, $+$ 52 and $+$ 60 p. 100, respectively) than those found in the lean rats. Boulangé (1977) reported that the hypertriacylglycerolemia of fa/fa rats, caused by a defect in plasma clearance, is already present in the second week of life, whereas Jamdar (1979) found no change in that parameter until 32 days after birth or 11 days after weaning. For Zucker and Zucker (1962), hyperlipemia was clearly evident at the age of 39 days. We recently found (to be published) that all serum lipid fractions are higher in 35-day old fa/fa rats than in lean littermates. From 3 to 5 weeks of age, however, there was a decrease in all the lipid levels for both the fa/fa and the Fa/- rats, a decrease undoubatedly caused by changes in the type of diet.

Classification of rats according to serum lipid level. — The analysis of the distribution of the values of the different parameters (table 2) showed that while classifying the rats into two groups helped to normalize adipose tissue weight distribution (in fact, that was the aim of the operation), it did not normalize the distribution of all serum lipid concentrations. Indeed, each group of rats included animals with both normal and high serum lipid levels, the cut-off point being arbitrarily fixed at around 1.6 mg/ml for the triacylglycerols, 1.7 mg/ml for total cholesterol and 2.7 mg/ml for the phospholipids.

Each group was thus divided into two subgroups with the same adipose tissue development but with different serum lipid levels (table 3). Most of the parameters studied were normally distributed, even though the test for normality with such a limited sampling must be interpreted with caution.

It is interesting to note that this classification, based on the serum lipid levels, produced selection according to animal liver and body weights.

Is this arbitrary distinction between the two subgroups valid, or is it just a question of chance ? In each group of rats (fa/fa or Fa/-), the heavier animals had the lowest serum lipid levels and inversely. Can this be explained by a difference in triacylglycerol clearance form the blood ? In suckling rats this clearance capacity depends principally on muscle lipoprotein lipase (Planche *et al.*, 1980) whose level increases as muscle volume augments ; this latter increase is reflected indirectly by body weight. Another explanation of this distinction between the two subgroups could be the difference in the developmental stage of the animals which would cause divergencies in the time of spontaneous weaning ; it is known that this latter event is accompanied by a fall in serum lipid levels (Cryer and Jones, 1978).

Résumé.   A trois semaines d'âge, ni le poids corporel, ni le poids du foie ne permettent de classer les rats Zucker en normaux et en obèses. Par contre, l'analyse du poids du tissu adipeux sous-cutané rapporté à 100 g de poids corporel, permet chez la majorité de ces

animaux de définir le phénotype, à la condition de tenir compte des caractéristiques propres de la croissance des rats de chaque portée. Les futurs obèses ainsi sélectionnés, présentent des teneurs sériques en triacylglycérols, en cholestérol total et en phospholipides significativement augmentées. Cependant, une analyse plus approfondie de ces valeurs indique chez les obèses, comme chez les non obèses, l'existence de deux sous-groupes dont l'un est caractérisé par des valeurs sériques de lipides élevées et l'autre, par des valeurs plus basses.

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