Inguinal fat pad weight plotted versus body weight as a method of genotype identification in 16-day-old Zucker rats

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Summary By plotting the weights of inguinal fat pad versus body weights in littermates from *fa/fa* × *Fa/fa* crosses, we observed that the data distributed along two widely separated regression lines as of 16 days of age. This procedure enabled us to determine unequivocally the genotype of every pup in seven litters. By its rapidity, its simplicity, and reliability, this method of genotype identification may be useful to many investigators.—**Lavau, M., and R. Bazin.** Inguinal fat pad weight plotted versus body weight as a method of genotype identification in 16-day-old Zucker rats. *J. Lipid Res.* 1982. **23:** 941-943.

Supplementary key words obesity

The possible relevance of the genetically obese Zucker rat to some forms of human obesity has generated great interest in this model. Hyperphagia, fat cell hyperplasia, hyperinsulinemia, and hypertriglyceridemia are traits of the obese rat that are also shared by certain obese patients. The unraveling of the sequence of events that leads to the full syndrome has been hampered by the fact that the pups bearing the pathological genotype cannot be visually distinguished from their lean littermates until they develop manifest obesity (around 5–6 weeks of age). Therefore the identification of the *fa/fa* genotype in young Zucker littermates has been a challenge to investigators. Pertinent techniques have been devised in recent years. Identification of preobese rats was made by Godbole, York, and Bloxham (1) on the basis of a low rectal temperature on 3 consecutive days (from day 16 onwards). Individual rats were labeled as either *fa/fa* or non-*fa/fa* at 18 days of age on the basis of their oxygen consumption at 25°C by Kaplan (2). The comparison of the frequency distribution curves of cell diameters of collagenase-isolated fat cells allowed Boulange, Planche, and de Gasquet (3) to predict *fa/fa* genotype in pups from the same litter and same sex, by 7 days of age. Obese and lean rats were correctly identified by determining total body water with tritiated water at 2 weeks of age, although precise prediction was not possible (4). Most of these methods involve delicate and time-consuming measures.

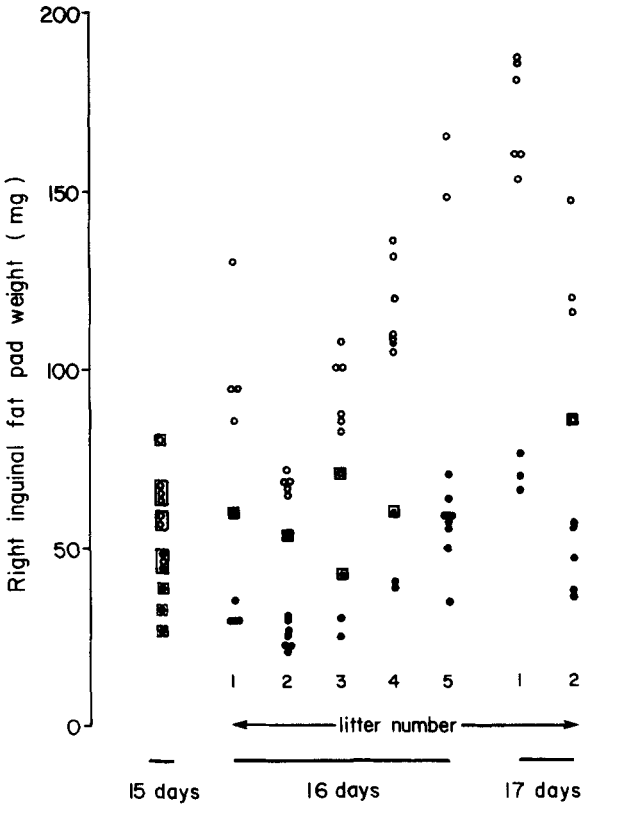

Fig. 1. Determination of genotype using individual data of inguinal fat pad weight in lean (*Fa/fa*) and obese (*fa/fa*) littermates aged 15, 16, or 17 days. The inguinal fat pad was removed surgically and the genotype was tentatively predicted. Actual genotypes were determined at 60 days of age. Black dots, lean pups; white dots, obese pups; dots in a square, data that did not allow prediction of the genotype of the pups.

In a recent developmental study of inguinal fat pad weights in suckling Zucker pups, we found an overlapping of fat pad weights between the two genotypes as late as 14 days of age (5). In contrast, by 17 days of age the fat pad weights clustered in two widely separated groups within each litter. This observation prompted us to examine whether genotype diagnosis could be based on the weight of the inguinal fat pad. The study presented here demonstrates that the weight of the inguinal fat pad plotted against body weight affords 100% reliability in the identification of *fa/fa* genotype as of 16 days of age.

MATERIALS AND METHODS

All rats used in this study were bred in our laboratory from breeding pairs originally provided by the Harriet G. Bird Memorial Laboratory, Stowe, MA. 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. The dams and pups had free access to stock diet (U.A.R., 91360 Epinay sur Orge, France). Eight litters numbering between 9 and 13 pups were used. Surgery was performed between 9 and 11 AM. The pups were lightly anesthetized with ether and a small incision was made over the right inguinal depot. The whole fat pad was carefully dissected from the skin and from the abdominal wall, starting from the ventral part and proceeding to the dorsal region, using forceps and scissors to cut the connective tissues. There was nearly no bleeding and therefore it was not necessary to make any blood vessel ligation. The skin was sutured with four or five stitches (5 or 6/0 silk). The pads were collected in saline, blotted, and weighed. The body weight of the pups was also recorded. The pups were marked by clipping toes and returned to their mothers. They were kept alive until 60 days of age when phenotype was clearly apparent and could then be compared to our blind prediction. Linear regression analyses were performed by the method of least squares (6).

RESULTS

Fig. 1 shows that the inguinal fat pad weights in the litter of 15-day old pups ranged from 26 to 80 mg with values spread evenly over this interval. No prediction of genotype could be made at this age. As expected, the smallest and biggest fat pad were from the lean and obese pups, respectively although an overlap of values between the two genotypes occurred.

In litters of 16-day old pups, except for litter 2, the weights of fat pads were scattered over a wide range of values. In each of the five litters, there was a clear separation between the low and high range values, strongly suggesting the presence of two populations. However, in four of the five litters, there were fat pad weights that fell between the two clustered groups. The genotype of these pups could not be predicted by examination of these diagrams only.

The data from the 17-day-old pups were similar to those obtained from the pups aged 16 days and there was still one unidentifiable rat.

Fig. 2 shows that the uncertainties in the identification

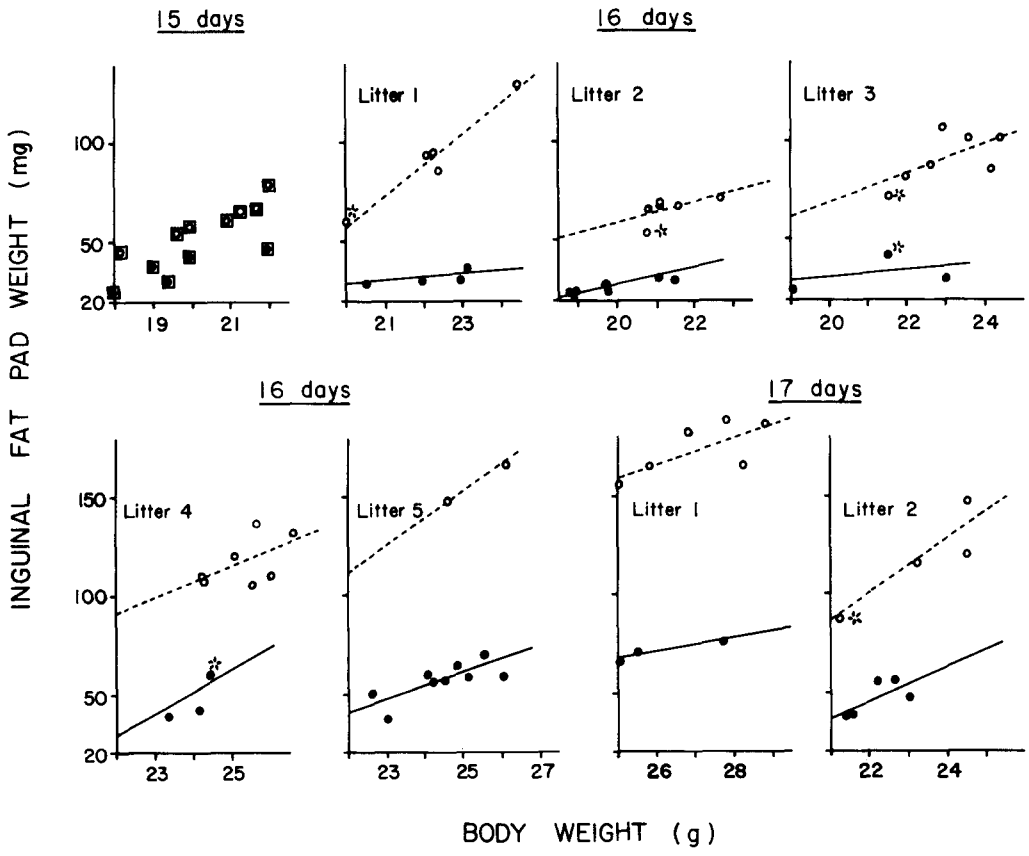

Fig. 2. Determination of genotype using inguinal fat pad weights (mg) plotted versus body weights (g) in lean and obese Zucker littermates aged 15, 16, or 17 days. Black dots, lean pups; white dots, obese pups; dots in a square, data that did not allow prediction of the genotype of the pups; *, pups whose genotype was uncertain in Fig. 1. Regression lines were fitted by the method of least squares.

TABLE 1. Regression coefficients for fat pad weights versus body weights

Age	Litter	Lean	Obese
16 Days	1	$y = 28 + 1.8 (x - 20)$; $r = 0.71$	$y = 59 + 15 (x - 20)$; $r = 0.98$*
	2	$y = 23 + 2.6 (x - 18.5)$; $r = 0.89$*	$y = 52 + 5 (x - 18.5)$; $r = 0.62$
	3	$y = 28 + 2 (x - 19)$; $r = 0.47$	$y = 63 + 7 (x - 19)$; $r = 0.62$
	4	$y = 19 + 14 (x - 22)$; $r = 0.70$	$y = 91 + 8 (x - 22)$; $r = 0.53$
	5	$y = 41 + 6.5 (x - 22)$; $r = 0.76$*	$y = 112 + 13 (x - 22)$; r = not determined
	All data combined	$y = 18 + 6 (x - 18.5)$; $r = 0.87$**	$y = 36 + 13 (x - 18.5)$; $r = 0.87$**
17 Days	1	$y = 66 + 3.7 (x - 25)$; $r = 0.96$	$y = 160 + 6.4 (x - 25)$; $r = 0.70$
	2	$y = 37 + 8.6 (x - 21)$; $r = 0.70$	$y = 84 + 6.4 (x - 21)$; $r = 0.89$

y, Fat pad weight (mg); x, body weight (g); r, correlation coefficient.
* or **, r significant at the level $P < 0.025$ or $P < 0.001$, respectively.

of genotypes in 16- and 17-day-old pups could be resolved by plotting the weight of inguinal fat pad versus body weight. All the data fell on two straight lines (whose regression coefficients are tabulated in Table 1) which differed very significantly by their intercepts. The population in which the unidentified pups belonged then became clear. These plots enabled us to correctly predict the genotype of every pup in the seven litters (71 pups). However, this method did not clarify the results obtained with 15-day-old pups.

Table 1 shows that, when all data were combined in order to have a reasonable number of data, a very significant correlation between fat pad weight and body weight could be pointed out in 16-day-old pups. The regression line equations differed very significantly between the two genotypes in both slope and intercept. In most of the groups the relationship could not reach the level of statistical significance due to too small numbers of data.

DISCUSSION

This work clearly demonstrates that the weight of inguinal fat pad may be used as a marker of *fa/fa* genotype in littermates from *fa/fa* × *Fa/fa* crosses as of 16 days of age. The identification of the genotype of the pups may be determined when the animals are killed; it may also be done through surgical lipectomy, which is easy to perform with some training, so that the pups may be kept.

This new method of preobese rat identification may be of use to many investigators. It presents significant advantages over the techniques described in the literature (1–4) in terms of simplicity and rapidity. Moreover it allows an unequivocal determination of the genotype of each pup in a litter. This is particularly important in view of the fact that the metabolic differences, if any, between genotypes are likely to be very small at the early phases of obese development. Therefore any misidentification or exclusion might greatly bias the conclusions.

This technique, however, was not applicable before 16 days of age, at least under the conditions of this study where large litters were used. It is possible that, with a reduced number of pups per litter, this method might be applicable a few days earlier.

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