Obesity: Prediction of Preobesity Among Progeny from Crosses of ob/+ Mice[1] (37442)

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The obvious ethical limitations in studying metabolic and hormonal adaptations in human obesity at the organ and tissue levels have mandated the use of experimental animal models, such as the genetically obese hyperglycemic mouse. Similar to obese humans (1), the obese mouse exhibits hyperglycemia and hyperinsulinemia along with insulin resistance by adipose tissue (4, 5, 17, 18) and muscle (4, 17, 18). In addition, activities of some key lipogenic enzymes in liver and adipose tissue are elevated, including alpha-glycerophosphate dehydrogenase (9), glucose-6-phosphate dehydrogenase (9), citrate lyase (16), acetyl-Co-A carboxylase (2), and fatty acid synthetase (2) concomitant with increased rates of hepatic (6, 13) and adipose tissue lipogenesis (6).

All past investigations in genetic obesity have dealt with endocrinological and metabolic differences well after the onset of apparent obesity. Many of the noted differences are probably secondary to the obesity itself. To improve our understanding of obesity and to prevent its occurrence, it is crucial to differentiate between those characteristics preceding obesity and those which are secondary to its development. Unfortunately, the obese mice (ob/ob) have a very high incidence of sterility (12), making the availability of 100% ob/ob progeny from ob/ob × ob/ob crosses only remotely possible. Therefore, ob/ob individuals are usually generated from ob/+ × ob/+ crosses, resulting in a mixture of +/+, ob/+, and ob/ob individuals, all appearing identical with no detectable difference in body weight prior to 4–5 weeks of age (12). To detect and study ob/ob mice prior to the accumulation of appreciable amounts of adipose tissue, a simple and fairly reliable detection method is needed. Metabolic studies at this early developmental stage have been prevented by the absence of such a test.

Fried and Antopol (8) measured oxygen consumption in progeny from ob/+ × ob/+ crosses five times over a period of 15–28 days of age and observed which animals eventually became obese at 5–6 weeks of age. Retrospectively, they observed that the oxygen consumption was considerably lower among those animals which eventually became obese and suggested that the value of 2000 μl/hr/g of body weight might be used to predict future obese and thin mice.

In the present study, we were interested in ascertaining the reliability of using the suggested value of Fried and Antopol (8) for the detection of ob/ob mice among the progeny of heterozygote crosses, prior to the phenotypic expression of obesity.

Materials and Methods. Mice, known to be heterozygote for the obese gene (ob/+), were obtained from the Jackson Laboratory at Bar Harbor, Maine. Animals were maintained in a temperature-controlled room at 70–80°F. They were fed *ad libitum* with Wayne Lab-Blox (Allied Mills, Inc., Chicago, IL), had free access to water, and were housed in shoe-box type cages with heat-treated wood chips as bedding. Heterozygote pairs were housed together continuously while being used for reproduction. The progeny were numbered by clipping toes at 15 days of

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age and were weaned at 21 days of age.

Oxygen consumption was determined by differential constant pressure manometry which measures volume directly. A reference flask and a sample flask were fastened on opposite sides of the same manometer, eliminating the necessity of a separate thermobarometer measurement, since the sample flask changes only with respect to the reference flask. A single animal was placed into a specially designed Warburg flask as described by Fried *et al.* (10) with two side arms containing 2 ml of 20% KOH and a folded strip of filter paper. The reference flask contained 4 ml of 20% KOH to equalize vapor pressure on both sides of the manometer. Both the reference and sample flasks were attached to a Gilson all-glass differential manometer fitted with a 2-ml Gilmont micrometer syringe at the top. The entire apparatus was fastened to a specially designed plastic holder and immersed into a 20° water bath equipped with a heater-circulator pumping water at 12 liter/min. The apparatus and the barometric pressure and temperature of room air were recorded. The stopcocks were then closed and the decrease in volume over a three minute period was recorded and corrected for standard temperature and pressure. The calculations for each day were based on duplicate 3-min determinations. This method of determining oxygen consumption is both simpler and faster than the open-train method used for mice in the past (15), and allows for the determination of the oxygen consumption of individual animals.

Results. Prior to approximately 28–33 days of age there was generally no detectable difference in the body weight or obvious difference in the body shape of animals which eventually became either obese or thin. The body weights of the very young mice varied considerably from one litter to another due to variations in litter size.

After calculating the daily oxygen consumption, the values obtained at 17–22 days of age were averaged to obtain the preweaning value for each mouse. All the individual preweaning values calculated in this way were then grouped together to obtain the mean and SEM of all animals that eventually became either obese or thin. The postweaning values for 22–25 days of age were calculated in a similar manner. While the thin animals showed a 30% decrease in oxygen consumption after weaning, the obese mice exhibited a 50% decrease, resulting in larger differences in oxygen consumption between future obese and thin mice after weaning (Table I). These postweaning values were used to identify ob/ob mice on an individual basis prior to the phenotypic expression of obesity. Animals exhibiting an oxygen consumption below 2000 μl/g of B. W. were identified as ob/ob. On a group basis, the ob/ob exhibited lower oxygen consumptions than the thin animals over Days 17–20. However, these preweaning differences were not large enough for purposes of individual predictions.

At 5–6 weeks of age, well after the phenotypic expression of apparent obesity, the animals in the study were positively identified as either obese or thin. The number of correct and incorrect predictions, *i.e.*, identification of obese and thin mice made prior to the phenotypic expression of obesity (23 days old), were recorded (Table II). The

TABLE I. Oxygen Consumption Among Offspring from ob/$^+$ × ob/$^+$ Crosses.

		Preweaning	Postweaning	p
Thin	♂	3188 ± 103 (29)	2352 ± 70 (34)	<0.001
	♀	3493 ± 148 (34)	2491 ± 87 (28)	<0.001
Obese	♂	2769 ± 190 (10)	1404 ± 132 (12)[a]	<0.001
	♀	2590 ± 112 (15)[a]	1283 ± 80 (15)[a]	<0.001

[a] Significantly different from the appropriate thin values at the $p < 0.001$ level. Values are the means (μl/g of B.W./hr at STP) of the number of observations in parentheses ± SEM. The progeny were routinely weaned at 21 days of age. Preweaning values were determined at Days 17–20 while postweaning values were measured at Days 22–25.

TABLE II. Predictability of ob/ob Offspring from ob/+ × ob/+ Parents on the Basis of Oxygen Consumption.[a]

	Actual (no.)	Predicted (no.)	Error (no.)	Error (%)
Thin	68	68	3	4.4
Obese	28	28	3	10.7
Total	96	96	6	6.3

[a] Values above 2000 μl/hr/g of B.W. at STP were used to predict presumptive thin offspring while those below were used to predict presumptive obese offspring at 23 days of age. At 5–6 weeks of age the phenotype of each animal was positively identified and the errors in prediction were tabulated.

value of 2000 μl/hr/g of B. W. oxygen consumed has approximately 90% reliability in identifying ob/ob animals prior to the phenotypic expression of obesity. The observation that the number of actual and predicted obese and thin animals found are identical is an artifact due to a reciprocal number of errors in both groups.

Among the 14 litters studied, obesity was extremely variable within each litter (Fig. 1) and in litters numbered 5, 10, and 14 there were no obese mice at all. The 25% obese offspring that is expected from heterozygote crosses was not obtained until a large number of animals had been screened.

Discussion. The observation of early differences in oxygen consumption in the preobese phase of development indicates the existence of gross metabolic differences between genetically ob/ob and non-ob/ob mice in the period preceding the phenotypic expression of apparent obesity and the period of extremely rapid weight gain that begins at 4–6 weeks of age (12). At present, the cause of the lower oxygen consumption of the obese animals remains unknown. For present purposes, however, the obvious difference in energy metabolism between obese and thin siblings is useful for the early detection of ob/ob individuals. Danielsson *et al.* (7) have also attempted to detect genetically ob/ob mice prior to the phenotypic expression of obesity. These workers measured the presence or absence of a glucosuria one hour after an intraperitoneal injection of a glucose solution. Almost all the mice which eventually became obese exhibited glucosuria following the glucose injection while only one-half of those animals which eventually became thin exhibited a glucosuria. Indeed, there was a higher frequency of glucosuria in the ob/ob following the glucose load, but its rather high

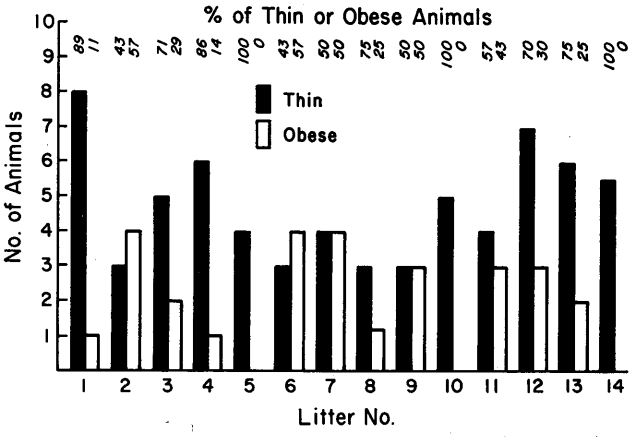

FIG. 1. Number of obese and thin offspring per litter from ob/+ × ob/+ crosses. The animals were positively identified obese or thin at 5–6 weeks of age. The numbers at the top of the bars represent the percentage of each phenotype per litter.

frequency in the very young thins excludes this as a useful diagnostic test on an individual basis. It was not until 29 days of age that these workers could clearly distinguish between future obese and thin animals when none of the thins exhibited glucosuria following the glucose injection. This is only one or two days prior to detection of obesity on the basis of body shape alone. By using oxygen consumption as an index for detecting obesity, future obese and thin animals were clearly distinguishable at 23 days of age.

When working with small numbers of animals, as usually is the situation in metabolic experiments, one cannot glibly assume that the offspring from ob/$^+$ $\times$ ob/$^+$ parents are necessarily 25% ob/ob individuals at the pre-obese stage of development. Genuth *et al.* (11) has investigated insulin sensitivity of muscle during the pre-obese phase by using offspring from heterozygote crosses, without knowing whether any of the animals in their group were really ob/ob. They assumed that nine out of 40 animals were ob/ob. Since they found no difference in insulin sensitivity among any of the animals, they cannot be assured that any of those young mice were, indeed, ob/ob individuals. Chlouverakis (3) found only three ob/ob mice out of 37 animals derived from five heterozygote crosses. This was ⅓ of the expected number of obese individuals. It is also possible, as indicated in this report, to obtain by chance no ob/ob individuals at all in three litters from heterozygote crosses. Therefore, a simple test, such as the one reported here, will enable workers to study pre-obese metabolism and the effects of dietary manipulation prior to the development of obesity and will permit an appraisal of "biochemical imprinting" on the lipogenic process.

Summary. The reliability of using oxygen consumption for the detection of ob/ob mice, among the progeny of heterozygote crosses, prior to the phenotypic expression of obesity was ascertained. Differences between obese and thin siblings that were great enough for predicting purposes were observed only after weaning. At 23 days of age ob/ob mice were identified on the basis of their individual oxygen consumption with 90% reliability. The ability to predict a pre-obese condition makes possible the differentiation of metabolic alterations preceding obesity from those that subsequently develop.

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