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Oxygen Consumption and Body Fat Content of Young Lean and Obese (OB/OB) Mice[1] (40063)

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It is now well established that genetically obese rodents have an increased ability to convert dietary energy to body energy relative to their lean counterparts (1). Alterations in energy metabolism, as indicated by decreased rates of oxygen consumption, are observable in obese (ob/ob) mice by 3 weeks of age (2–4); however, few studies have focused on younger obese mice. Marked changes in oxygen consumption of obese and lean mice have been reported at the time of weaning. Oxygen consumption per g body weight decreased by 25–50% within the first several days after weaning (3, 4). These studies and others (5–9) indicate that alterations in energy metabolism may appear very early in the life of obese mice.

The present study was designed to monitor oxygen consumption, an indirect indicator of energy expenditure, of lean and obese mice from birth to 112 days of age; major emphasis was focused on developmental changes prior to 21 days of age. Body fat content of lean and obese mice at 7 and 14 days of age was also determined.

Materials and methods. Heterozygote breeding mice (C57BL/6J ob/+)[2] were housed in solid-bottom cages with wood shavings for bedding. They were fed a stock diet[3] *ad libitum*. Ambient temperature was 25 ± 2°. Pregnant dams were removed from the breeding cages and placed in separate similar cages. Litter size was routinely standardized to six pups per litter; however, in a few instances litters with five or seven pups were utilized. Pups were weaned at 21 days of age unless indicated otherwise.

In the first experiment pups were individually identified at 1 day of age. From 1 to 19 days of age daily oxygen consumption measurements and body weights were obtained on individual pups. Litters were also killed at 7 and 14 days of age. Food residue was removed from the stomach and the carcasses were homogenized in an equal weight of water. Carcass fat was extracted with chloroform/methanol (2:1) and quantitated gravimetrically.

Oxygen consumption measurements and body weights of pups on days 18–20 and again on days 22–24 of age were obtained in the second experiment. Only lean mice were used. In one treatment pups remained with the dam; in the other treatments pups were weaned to a high-carbohydrate or a high-fat diet at 21 days of age. The high-fat diet was formulated to approximately simulate mouse milk on a protein-energy basis (10) and contained per 100 g diet: 43.5 g casein, 32.0 g tallow, 5.0 g corn oil, 9.6 g glucose, 5.0 g cellulose, 4.0 g mineral mix (11), 0.4 g vitamin mix (11), 0.2 g choline chloride and 0.3 g methionine. The high-carbohydrate diet was formulated by replacing the tallow with glucose on an equal-energy basis.

In the last experiment oxygen consumption measurements and body weights were recorded weekly from 3 weeks of age to 16 weeks of age. Lean and obese male and female mice were utilized.

The apparatus described by Watts and Gourley (12) was utilized to obtain estimates of oxygen consumption in the mice. Food was available to the mice until the oxygen consumption measurements commenced. Mice were placed in a flask immersed in a water bath maintained at 30°. After 10 min, five consecutive readings of oxygen consumption were obtained during the next 10 min. Oxygen consumption was then calculated at STP and expressed as ml oxygen consumed

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[2] Jackson Laboratory, Bar Harbor, Maine.

[3] Wayne Lab-Blox, Allied Mills, Inc., Chicago, Illinois.

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per g body weight, per g body weight to the 0.75 power (13) and per animal. Relative treatment effects were very similar whether the results were calculated per g body weight or per g body weight to the 0.75 power, thus only results per g body weight are presented.

Results. Body weight and oxygen consumption of pups from 1 to 19 days of age are presented in Fig. 1. The obese (ob/ob) and lean (ob/+ or +/+) pups were identified retrospectively after the obesity became obvious by visual inspection. Differences in body weight were observed as early as the 6th day after birth. Body weights of the obese mice remained higher than that observed for the lean mice during the remainder of the 19-day study. Obese mice consumed less oxygen per g body weight than lean mice by the 5th day after birth. Statistical differences in oxygen consumption per metabolic body size (g body weight to the 0.75 power) between lean and obese mice were also observed by the 5th day after birth; however, the magnitude of this difference was somewhat less than observed per g body weight. In both obese and lean mice the oxygen consumption per g body weight decreased during the second week of life and then remained lower than values observed during the first week of life. When oxygen consumption of the pups was expressed per animal, obese mice tended to consume less oxygen than the lean mice from the 5th day after birth but the differences were significant only on days 5, 12–15 and 18–19. Both obese and lean mice exhibited a five- to sixfold increase in oxygen consumption per animal during the first 19 days after birth.

Lean and obese mice were identified at 7 and 14 days of age, on the basis of lower oxygen consumption per g body weight in obese mice, and the body fat content of these mice was determined (Table I). Thirty-one and 28 percent of the mice screened at 7 and 14 days of age, respectively, were classified as obese. This closely corresponds with the expected ratio of one obese to three lean from heterozygote crosses. Obese mice weighed more and consumed less oxygen per g body weight than did lean mice at both 1 and 2 weeks after birth. As early as 7 days after birth obese mice had 38% more fat than did lean mice; this difference increased to 53% at

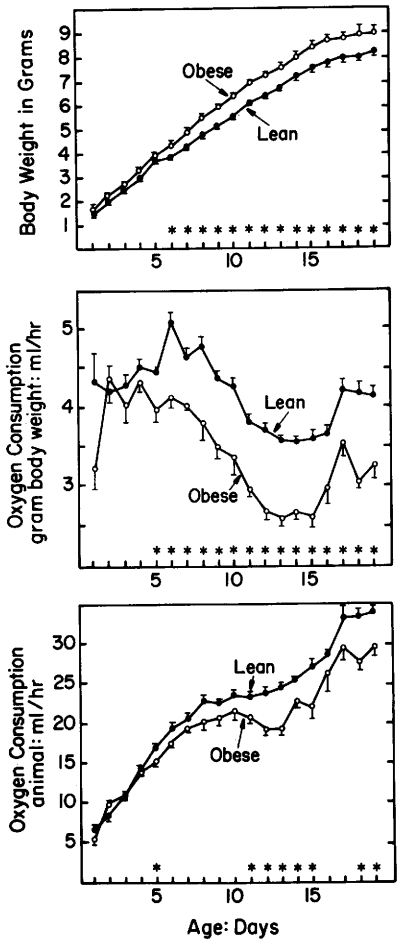

FIG. 1. Body weight and oxygen consumption of lean and obese mice from 1 to 19 days of age. Each point represents the mean ± SEM for 24–55 lean and for 8–27 obese mice, approximately equal numbers of males and females are represented. The asterisks indicate significant ($P < 0.05$) differences between lean and obese mice.

14 days of age. At 21 days of age obese mice contain 100% more fat than lean mice (9).

A 25–50% decrease in oxygen consumption per g body weight has been reported in mice at the time of weaning (3, 4). Table II presents the oxygen consumption of mice several days before and after 21 days of age. Regardless of whether the pups remained with their mother or were weaned to a high-fat or a high-carbohydrate diet, oxygen consumption per g body weight decreased approximately 17% between days 18 and 20 and days 22 and 24 of age. However, oxygen consumption per animal was not changed during this period

TABLE I. BODY WEIGHT, OXYGEN CONSUMPTION AND BODY FAT CONTENT OF ONE AND TWO WEEK OLD LEAN AND OBESE MICE.[a]

	Age			
	7 days		14 days	
Parameter	Lean	Obese	Lean	Obese
Body weight:g	3.62 ± 0.10	4.34 ± 0.13[c]	7.09 ± 0.15	7.72 ± 0.16[c]
Oxygen consumption:ml/g/hr[b]	4.12 ± 0.08	3.30 ± 0.06[c]	3.52 ± 0.09	2.56 ± 0.05[c]
Body fat:%	6.4 ± 0.2	8.8 ± 0.2[c]	13.5 ± 0.4	20.7 ± 0.8[c]

[a] Mean ± SEM for 31 lean and 14 obese pups at 7 days of age and for 28 lean and 11 obese pups at 14 days of age, except body fat was determined on only 20 lean pups at 7 days of age. Approximately equal numbers of males and females were represented in each group.

[b] Oxygen consumption was determined on days 5–7 and on days 12–14; averages for the 3-day periods are presented.

[c] Significantly different from the lean value, $P < 0.05$.

TABLE II. OXYGEN CONSUMPTION OF LEAN MICE SEVERAL DAYS BEFORE AND AFTER 21 DAYS OF AGE.[a]

		Oxygen consumption	
Treatment	Parameter	Preweaned	Postweaned
Pups remained with mother	Body wt.-g	6.7 ± 0.05	7.8 ± 0.1[b]
	O_2-ml/g/hr	6.08 ± 0.18	5.09 ± 0.16[b]
	O_2-ml/animal/hr	40.53 ± 1.24	39.47 ± 1.30
Pups weaned to high-fat diet	Body wt.-g	6.8 ± 0.2	8.0 ± 0.2[b]
	O_2-ml/g/hr	5.98 ± 0.25	4.64 ± 0.18[b]
	O_2-ml/animal/hr	40.59 ± 2.00	36.22 ± 1.43
Pups weaned to high-carbohydrate diet	Body wt.-g	6.9 ± 0.1	8.4 ± 0.1[b]
	O_2-ml/g/hr	5.78 ± 0.25	4.97 ± 0.17[b]
	O_2-ml/animal/hr	39.88 ± 1.92	41.69 ± 1.63

[a] Values are means ± SEM for 15–24 mice. There were five to six pups per litter. Pups were removed from the mother on day 21, except in the one treatment where pups remained with the mother throughout the study. Observations were made on days 18–20 and again on days 22–24. Approximately equal numbers of males and females were represented in each group.

[b] Significantly different from preweaned value, $P < 0.05$.

because body weight increased proportionately.

In the last experiment oxygen consumption rates of lean and obese mice were obtained from weaning to maturity (Fig. 2). Body weights of the obese mice were heavier than that of the lean mice; however, because of the variation within groups the differences were not statistically significant the first several weeks after weaning. Obese mice have been reported to be stressed more by weaning than are lean mice (9), which may have contributed to the variation in body weights during the first several weeks after weaning. Prior to weaning, body weights of the lean and obese mice were very uniform, this allowed small differences in body weight between the two groups to be statistically significant. Body weights of the obese mice continued to increase whereas body weights of the lean mice tended to plateau by 7–8 weeks of age. Oxygen consumption per g body weight of the obese mice was lower than that of the lean mice throughout the 13-week period, but the differences were not statistically significant at two of the points for male mice. Similar results were obtained when the rates were expressed per g to the 0.75 power. Oxygen consumption per animal increased two- to threefold for all animals. After 10 weeks of age obese mice tended to consume more oxygen per animal than did lean mice; the differences were statistically significant for the female mice.

Discussion. The results of this study clearly demonstrate that there are differences between lean and obese mice in body composition and energy metabolism as early as 1 week after birth. Previously, body fat content of obese mice had been reported to be elevated by 17–21 days of age (7, 9) and fat cell size of the obese mice to be increased above

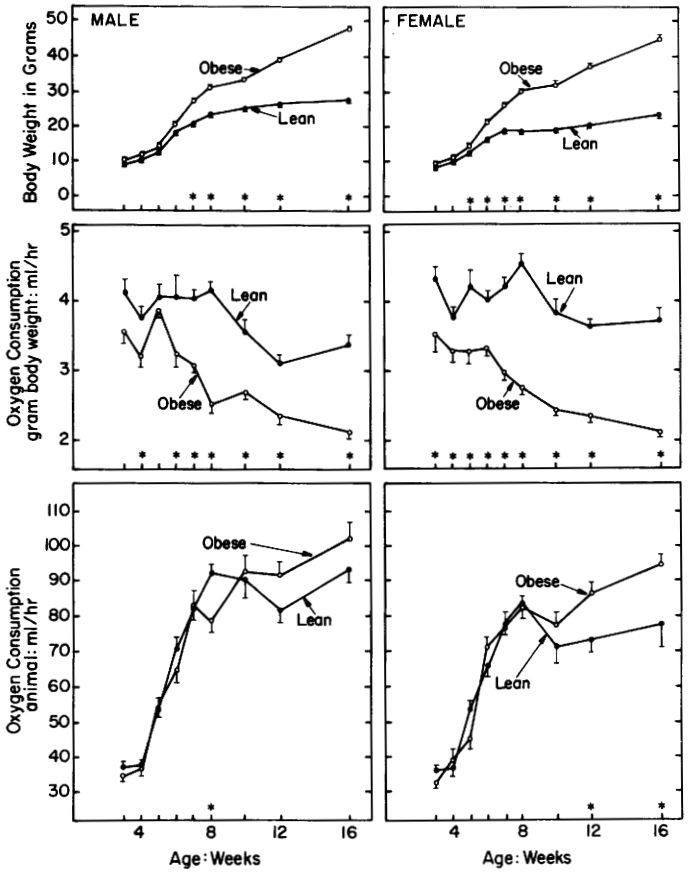

FIG. 2. Body weight and oxygen consumption of lean and obese male and female mice from 3 to 16 weeks of age. Each point represents the mean ± SEM for 10–26 lean and 11–16 obese mice. The asterisks indicate significant ($P < 0.05$) differences between lean and obese mice.

the levels observed in lean animals by 12–14 days of age (5). Because of the magnitude of the difference in body fat between lean and obese pups at 7 days of age it is probable that differences in body fat already exist within a few days after birth. Hyperphagia does not appear to be a factor in the increased accumulation of body fat in 1- to 3-week old obese mice (9).

The lower rectal temperature of obese pups by 10 days of age (6) and the altered response of the obese pups to cold by 12 days of age (8) suggested that metabolic rates might be lowered in these obese mice prior to the previously observed depressions at 17–20 days of age (2–4). In the present study differences between lean and obese mice in oxygen consumption per g body weight were observed by the 5th day of age. Several days later the obese mice exhibited an increased body fat content.

All oxygen consumption measurements were recorded at 30° in the present study; this temperature is probably below the thermoneutral zone for the very young mice (14). The changes in oxygen consumption per g body weight from 1 to 19 days of age may reflect in part a change in the thermoneutral zone of these young mice with development. It has been reported that differences in oxygen consumption between young obese and lean mice were easier to detect as the temperature of the determination was increased from 20° to 25° (3, 4). Whether a shift in temperature would allow demonstration of differences in oxygen consumption between lean and obese mice prior to 5 days of age was not determined.

Oxygen consumption of lean mice per g body weight decreased approximately 17% between days 18 and 20 and days 22 and 24 of age regardless of the treatment imposed. These measurements were conducted at 30°. The decrease in oxygen consumption was approximately 24% at 25° (4) and 28% at 20° (3). The greater drop in oxygen consumption observed at the lower ambient temperatures may reflect a lowering of the thermoneutral zone of these mice as they develop (14). A decrease in energy intake decreases metabolic rate, at least in certain species (13, 15). Energy intake of these mice has been suggested to be decreased for several days immediately after weaning (9). Mice left with their mother may also have undergone similar changes in food intake because milk production decreases markedly after the 20th day of lactation (9). These factors together with the general decrease in oxygen consumption per unit weight as body weight increases (16) probably explain the previously observed changes in oxygen consumption (3, 4) at the time of weaning.

Beyond 3 weeks of age oxygen consumption of obese mice, expressed per animal, was very similar to that observed in lean mice even though obese mice were heavier and consumed more food than lean mice (1, 9). The mechanism(s) responsible for the increased energy efficiency of the obese mice remains to be established.

Summary. Rates of oxygen consumption were determined daily from birth to 19 days of age and weekly thereafter until 16 weeks of age in lean and obese mice. As early as 5 days after birth obese mice consumed less oxygen than lean mice. Obese mice weighed more than lean mice by 6 days of age and contained 38% more fat than lean mice at 7 days of age. At 14 days of age obese mice contained 53% more fat than lean mice. Beyond 3 weeks of age oxygen consumption of obese mice was less than observed in lean mice when the results were expressed per g body weight, but the values for obese and lean mice were similar when expressed per animal. These results demonstrate that alterations in energy metabolism occur very early in the life of obese mice.

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