Core temperature, $O_2$ consumption, and early detection of ob/ob genotype in mice

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KAPLAN, Murray L., and Gilbert A. Leveille. Core temperature, $O_2$ consumption, and early detection of ob/ob genotype in mice. Am. J. Physiol. 227(4): 912-915. 1974.—Oxygen consumption and core temperature of ob/ob mice and their thin littermates were measured at various ambient temperatures from 10 to 30°C. At every ambient temperature studied, adult obese mice exhibited lower $O_2$ consumptions and lower core temperatures than those of thin animals. Among the obese mice, changes in $O_2$ consumption with decreasing ambient temperature were minimal; among the thins, significant increases in $O_2$ consumption occurred with each 5°C decrease in ambient temperature. By raising the ambient temperature from 20 to 25°C, $O_2$ consumptions of preweaned ob/ob mice were markedly lower than +/+ mice, so that ob/ob individuals could be identified on day 18 with approximately 91% reliability prior to the phenotypic expression of obesity. This is a much earlier probosc identification than previously reported (Kaplan, M L., and G A. Leveille Proc. Soc. Exptl. Biol. Med. 143: 925-928, 1973). Grouped data of the $O_2$ consumption of these animals suggest the existence of a gradient, with ob/ob individuals at the low end of the gradient, ob/+ individuals in the middle, and +/+ individuals at the high end of the gradient. The earlier identification of ob/ob individuals during the probosc phase of development hopefully will enable investigators to more precisely identify the sequence of distinctive metabolic patterns in tissues preceding the phenotypic expression of obesity.

Materials and Methods

Heterozygote breeding pairs (C57BL/6J ob/+ ) were obtained from the Jackson Laboratory, Bar Harbor, Maine. The obese and nonobese mice used in these studies were the progeny derived from these breeding pairs at Michigan State University. All animals were housed in plastic cages with heat-treated wood chips as bedding in temperature-controlled rooms at 70-80°F, fed Wayne Lab-Blox (Allied Mills, Inc., Chicago), had free access to water, and were routinely weaned at 21 days of age.

Colonic temperatures were measured in 6-mo-old animals with a rapidly responding thermistor probe and model 43 telethermometer, both manufactured by Yellow Springs Instrument Co., Yellow Springs, Ohio. Oxygen consumptions of individual animals were determined by a manometric procedure previously reported for very young animals (14). To measure the larger volumes of oxygen consumed by animals older than 4 wk of age, a 5-mL gas-tight Hamilton syringe was fastened to the manometer.

Six-month-old animals were used in the experiments measuring both oxygen consumption and colonic tempera-
ture at varying ambient temperature. The usual operational procedure was as follows: the animal was weighed and placed into the special manometric apparatus, lowered into the water bath at the desired ambient temperature, and the apparatus allowed to equilibrate for 5 min; duplicate 3-min determinations of oxygen consumption were made; the animal was then quickly removed from the apparatus, with a lapse of approximately 30 s, and the colonic temperature was immediately determined. In selected instances colonic temperature was measured both before the animal was placed into the apparatus and after oxygen consumption was determined. Measurements were made at only one ambient temperature during a day's work, so that the same animals were exposed to each ambient temperature on different days.

**RESULTS**

Since huge differences between the body weights of adult obese and thin groups are observed (approximately 30 g vs. 60 g), oxygen consumptions were calculated on the basis of body weight to the 0.75 power. This conforms with Kleiber's formulation (15) of the metabolic coefficient for purposes of comparing oxygen consumption in animals with varying body sizes. Oxygen consumptions of adult obese mice were lower than thin mice not only at ambient temperatures of 20 and 25°C, as has been previously reported (6, 7, 14), but also at every ambient temperature studied (Fig. 1). Among the obese animals the changes in oxygen consumption with decreasing ambient temperature were minimal. The only significant differences in oxygen consumption among obese mice were observed between the ambient temperatures of 25 and 30°C ($P < 0.001$). Among the thin mice, a decrease in ambient temperature resulted in a significant increase in oxygen consumption ($P < 0.01$) down to 15°C. A further decrease in ambient temperature to 10°C did not result in a further rise in oxygen consumption.

Colonic temperature was lower in the obese animals at every ambient temperature studied (Fig. 2). A precipitous decrease in colonic temperature among the obese animals was observed with decreasing ambient temperature. Among the thin animals there was a tendency to decrease colonic temperature with decreasing ambient temperature, but the magnitude was considerably less than in the obese group.

Given these observed differences between adult obese and thin mice, particularly the changes in core temperature observed with variation of ambient temperature, we inquired into the possibility that ob/ob individuals may be identified with high reliability at an early age by using one of these indices of energy metabolism. Unfortunately, a thermistor probe suitable for small preweaning mice was not available at a reasonable price. Another alternative was to further explore the role of ambient temperature on oxygen consumption of very young mice, since ambient temperature affected oxygen consumption of adult obese animals.

To this end, oxygen consumption was determined at 25°C ambient temperature over days 17–23 among progeny of ob/+ x ob/+ crosses. The daily oxygen consumption was calculated and the values obtained for days 17–20 were averaged to obtain the preweaning value for each animal. The postweaning values for days 22–23 were obtained in the same manner. All the individual preweaning and postweaning values obtained in this manner were grouped together to obtain the mean oxygen consumption of all animals that eventually became either obese or thin. Distinct differences in body weight and conformation were not
evident at the ages of the animals used in this study. Large
differences in oxygen consumption during the preweaning
phase were evident among those animals eventually
destined to become either obese or thin (Table 1). After
weaning, thin animals showed a 30% decrease in oxygen
consumption while the obese mice exhibited a 40% reduc-
tion in oxygen consumption. The postweaning values were
extremely variable at 25°C and there was considerable
individual overlap in the values of eventual obese and thin
mice.

Since the preweaned animals hardly exhibited any over-
lapping values, animals were labeled as either obese or thin
depending on whether their individual oxygen consump-
tions were below or above 2,000 μl/h per g. At 5-6 wk of
age, when obesity was obviously present, the accuracy of the
predictions was recorded (Table 2). Using the cutoff value of
2,000 μl/h per g, ob/ob mice were identified with ap-
proximately 91% reliability at 18 days of age. After positive
phenotypic identifications of the animals were made their
individual oxygen consumptions were placed on a scatter
diagram (Fig. 3). If the high points for the obese and thin
animals are used as cutoff zones, 22% of the animals exhibit
values below 2,000 μl/h per g, 54% between 2,000 and
3,000 μl/h per g, and 24% above 3,000 μl/h per g. This
corresponds to the familiar 1:2:1 ratio obtained from
heterozygote crosses. A few of the thin mice were mated with
known heterozygotes. Although the number of productive
crosses was not high enough for statistical purposes, all the
heterozygotes from unknown thin mice that were identified
in this manner had oxygen consumptions as preweaning
mice in the 2,000-3,000 μl/h per g range.

DISCUSSION

The display of preweaning oxygen consumptions of obese
and thin animals suggests a gradient of oxygen consumption
with ob/ob animals at the low end, +/+ animals at the
high end, and ob/+ individuals in the middle of the gra-
dient. Although the evidence is very scanty that the middle
zone represents ob/+ individuals, this does suggest a genetic
dosage phenomenon with regard to oxygen consumption. It
would be interesting to know if similar genotypic gradients
exist with regard to other parameters in obese and thin
mice.

Table 1. Oxygen consumption among progeny of ob/+ x ob/+ crosses at 25°C ambient temperature

<table>
<thead>
<tr>
<th></th>
<th>Prewean</th>
<th>Postwean</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>2,789±100 (17)</td>
<td>2,036±124 (17)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Female</td>
<td>2,787±102 (15)</td>
<td>2,188±121 (15)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Obese</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>2,025±98 (7)*</td>
<td>1,313±100 (7)*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Female</td>
<td>2,190±232 (7)†</td>
<td>1,348±246 (7)†</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are means ± SE (μl/h per g of body wt at STP); number of observations in parentheses. Preweaning values were determined at days 17-20; postweaning values were measured at days 22-23. *Significantly different from appropriate group of thin at P < 0.001. †Significantly different from appropriate group of thin at P < 0.02.

The observation of lower colonic temperatures among the
obese animals at every ambient temperature studied (Fig. 2)
would tend to mitigate any notion that the obese animals do
not exhibit marked rises in oxygen consumption with lower
ambient temperatures because they are well insulated by the
abundant subcutaneous adipose tissue. At least three possi-
ble explanations exist: 1) a failure of the mechanisms regu-
lating heat loss; 2) a possible failure of mechanisms regulat-
ing heat production; 3) some combination of failures in heat
conversion and heat production.

Since the heat losses of the animals have not been mea-
sured in an animal calorimeter, remarks regarding heat loss are
mere conjecture. We need this information to intelli-
gently discuss the possibility of failures in the mechanisms
of heat loss. Presumably, vasomotor reactions under neuro-
humoral control would regulate heat loss. A failure of these
mechanisms would indicate some sort of failure in neuro-
humoral mechanisms involved in vasomotor responses.
The failure to observe marked increases in oxygen consump-
tion concomitant with lowered core temperatures sub-
sequent to a decrease in ambient temperature is evidence for
a failure in heat production.

In obese yellow mice, lower oxygen consumptions were
detected among the adult AYa (obese yellow) animals. However, during the preobese phase of development (1) no
differences were observed between AYa mice and their lean
littermates. When expressed on a (body wt)0.75 or (body

Table 2. Predictability of ob/ob individuals from ob/+ x ob/+ crosses

<table>
<thead>
<tr>
<th></th>
<th>Predicted</th>
<th>No. %</th>
<th>Thin</th>
<th>Predicted</th>
<th>No. Obese</th>
<th>% Error</th>
<th>Actual No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thin</td>
<td>36</td>
<td>4</td>
<td>11.1</td>
<td>11</td>
<td>1</td>
<td>9.1</td>
<td>14</td>
</tr>
<tr>
<td>Obese</td>
<td>11</td>
<td>1</td>
<td></td>
<td>5</td>
<td>1</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>47</td>
<td>10.6</td>
<td></td>
<td>16</td>
<td>2</td>
<td></td>
<td>47</td>
</tr>
</tbody>
</table>

Values above 2,000 μl/h per g of body wt at STP were used to identify potentially thin offspring and those below were used to identify potentially obese offspring at 18 days of age. At 5-6 wk of
age, phenotype of each animal was positively identified and errors were tabulated.

Fig. 3. Gradient of preweaning oxygen consumption values in
obese and thin mice. Oxygen consumptions were determined at 25°C.
Phenotypes of individual mice were determined at 5-6 wk of age.
Preweaning values of individual animals were then grouped into
various categories of values.
wt) basis, adult oxygen consumptions of gold thioglucose-induced obese mice (16), obese yellow mice (1), hypothalamic-lesioned obese rats (3), and the fatty rat (2) are lower than in their respective lean controls. Since no other reports indicating the status of oxygen consumption during the preobese phases in these other models are available, the possibility of a lower oxygen consumption during the preobese phase of development cannot be excluded as a generalization among the genetic obesities of rodents. This hypothesis awaits testing. It would be necessary, however, to carry out the determinations at or near the thermoneutral zone, so that problems of thermoregulation can be avoided.

By altering the ambient temperature of the determination of oxygen consumption to 25°C we were able to identify ob/ob individuals at 18 days of age. When the determinations were made at 20°C, ob/ob individuals could not be detected before 22 days of age (14). By day 23 differences between obese and thin mice (13) have been detected only in the insulin sensitivity of muscle, while liver and adipose tissue appeared normal. By identifying ob/ob individuals at an earlier age, it may be possible to determine more precisely the sequence and time of onset of distinctive metabolic patterns in the obese animals and hopefully obtain a clue to the etiology of the disease.

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REFERENCES