Oxygen Consumption in Mice (I Strain) after Feeding[1]

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ABSTRACT To determine if excess heat production can account for the lower fat accumulation in I strain mice, oxygen consumption, a measure of energy expenditure, was measured in I mice and C57BL mice (a control strain) to determine basal metabolic rate (BMR) and resting metabolic rate (RMR) and response to food consumption and acute cold exposure. Oxygen consumption was higher in the I strain than in the C57BL mice only after the dark cycle (feeding period). No difference between I and C57BL mice in spontaneous activity was found during the dark or light cycle. Body temperature was also not different in I and C57BL mice. Oxygen consumption in response to norepinephrine was similar in the two strains. These results indicate oxygen consumption is greater in I than C57BL mice only in response to feeding. Differences in glucose utilization by I and C57BL mice, including a lower glucose tolerance curve, greater deposition of glucose to muscle glycogen and lactate production in I mice also indicate differences in nutrient processing. Higher oxygen consumption after feeding in I mice than in C57BL mice indicates inefficient food utilization and accounts for their lower ability to store energy as fat. J. Nutr. 115: 303–310, 1985.

INDEXING KEY WORDS I strain mice • oxygen consumption • spontaneous activity • glucose utilization

The storage of energy as adipose tissue for an individual or animal is determined by the difference between energy intake and energy expenditure. Thirty years ago studies (1–4) showed that genetic inbred strains of mice vary in their capacity to store energy. Mice of the I strain (1, 2) have a lower body fat content, gain less weight and have a lower food efficiency than other nonobese mouse strains (A, C3H, C57BL). Also, mice of the I strain are resistant to fat accumulation with age (1) or high fat diets (2). In obese animal models (both rats and mice), differences occur in the regulation of energy expenditure (5). Energy expenditure (6) is considered to have four components: *1*) resting metabolic rate (RMR) or energy needed for maintaining the physiological systems of the body at rest; 2) muscular or physical activity; *3*) thermic effect of food or heat produced following a meal; and *4*) adaptive thermogenesis due to cold-induced or diet-induced heat production. In the obese models (6), hyperphagia and inactivity contribute to the obesity, but the component of greatest significance is heat production.

Since I mice are resistant to fat accumulation, the strain provides a unique model to study energy expenditure. The aim of this study was to measure oxygen consumption (an indirect measure of heat production) to determine if excess heat production for basal metabolic rate (BMR), RMR, thermic effect of food or adaptive thermogenesis can account for the unusually low fat accumulation in the I mice.

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METHODS

Animals. The mouse strains used in this study were: I, C57BL/6, and C3H (obtained from L. C. Strong Research Laboratories, La Jolla, CA and Cancer Research Laboratory, University of California, Berkeley, CA). The mice were maintained in a room at 23–26°C with a 12-h alternate light and dark cycle (0500–1700 h) and had access to water and a modified[2] AIN-76™ purified diet (7) or nonpurified diet (Wayne Lab-Blox F6, Allied Mills, Inc., Chicago, IL)[3] ad libitum. The mice were housed individually in plastic cages with wire-mesh bottoms (oxygen consumption experiments) or in plastic cages four or five per cage with pine chip bedding (Long Beach Shavings Co., Long Beach, CA). Mice were weighed weekly, and food intake measured between 6 and 10 wk of age. Oxygen consumption was measured when mice were 8 wk of age, glucose tolerance and glucose utilization experiments were conducted when mice were 8 to 10 wk old, and body composition was determined for mice 10 wk of age.

Oxygen consumption. Oxygen consumption was measured (8) by using a Phipps-Bird (Richmond, VA) apparatus consisting of a Plexiglas chamber fitted with a rubber stopper containing a calibrated inlet tube. The mouse was placed in a wire cylinder, fitted with a dowel to prevent activity and situated within the chamber above a layer of soda lime. After 15 min, to allow for equilibration of the mouse with the chamber environment, the rate of inflow air or oxygen consumed (seconds/milliliter) was determined by measuring the rate of change of a detergent solution in the calibrated inlet tube. The average of five values (seconds/milliliter) was converted to milliliters/minute, corrected to standard temperature (0°C) and pressure (760 mm), and expressed as liters of O_2 consumed per hour per kilogram$^{0.75}$ body weight. Oxygen consumption was measured in mice after a 24-h fast to determine the BMR, before the feeding period (1600 h) to determine the RMR, after the feeding period (0600 h) to determine the response to food consumption, and after acute cold (4 h, 4°C) to determine the thermogenic response to cold exposure. The response of oxygen consumption was also determined to a dose (750 μg/kg body weight, dissolved in a 0.3- to 0.4-ml volume of 0.9% saline, 0.1% sodium metabisulfite, wt/vol) of norepinephrine injected i.p. Oxygen consumption was measured at 10 min (determined experimentally as the average maximum response time for both strains) after norepinephrine administration and at 1-min intervals from 5 to 16 min after administration.

Body temperature. Body temperature was measured by using a thermocouple with a digital readout indicator (Omega Engineering, Inc., Stamford, CT). The probe was inserted 2 cm into the rectum.

Spontaneous activity. Spontaneous activity was determined for I, C57BL and C3H (a control strain) mice in the fed state at ambient temperature (23°C) from 0800 to 0100 h. The mice had unlimited access to food and water during the 17-h period. Spontaneous activity (University of California, San Diego, Department of Psychiatry) was measured as crossovers on the quadrant of the cage floor and recorded by computer for each cage (one mouse/cage).

Glucose tolerance. Glucose tolerance experiments were carried out 6 to 8 h after the dark cycle. Mice were injected i.p. with glucose (3 mg/g body weight) (9), and blood was collected from each mouse from the orbital sinus before glucose administration and 15, 30 or 60 min after the injection. The mice were lightly anesthetized with ether for both the injection of glucose and the collection of the blood. The blood was collected in heparinized capillary tubes, centrifuged, and the plasma was separated. Plasma protein was precipitated with 10% (wt/vol) trichloroacetic acid, and the supernatant fraction was assayed for glucose by the glucose oxidase method of Hugget and Nixon (10) and for lactate according to Lowry and Passoneau (11). In another experiment, in vivo glycogen accumulation was determined before (0) and 1, 2 and 4 h after a dose of glucose (3 mg/g body weight injected i.p.). The animals were decapitated and tissues were removed and immediately

[2]The fat content of the AIN diet was increased to 6 g/100 g of diet and the sucrose component adjusted to compensate.

[3]For the Wayne Lab-Blox, the guaranteed analysis (grams/100 g of diet) was: crude protein, 24.0; crude fat, 6.0; and crude fiber, 4.5.

freeze clamped and stored at −70°C. Tissues were digested with 30% KOH, and glycogen was assayed enzymatically (12) by releasing glucose with amyloglucosidase and measuring glucose with hexokinase (13).

Glucose utilization. To measure glucose utilization, 8-wk-old mice were killed by cervical dislocation, hemidiaphragms were removed and incubated in Krebs bicarbonate buffer, pH 7.4, with [U-^{14}C]glucose, 1 μCi/4 ml radioactive glucose (0.04 μCi/μmol glucose), 6 mM glucose and 2.5 mU/ml insulin (gift of Eli Lilly, Indianapolis, IN). After a 1-h incubation, 0.3 ml of methyl benzethonium hydroxide (Sigma Chemical Co., St. Louis, MO) was added to the center well in the cap of each flask, and 0.5 ml of 6 *N* H_2SO_4 added to the medium. CO_2 was collected in the center wells by shaking the flasks for 60 min. The center wells were then transferred to liquid scintillation (xylene:triton, 2:1) vials, and radioactivity was determined. The amount of lactate released into the medium during the incubation of the diaphragms was determined by the enzymatic method of Lowry and Passoneau (11). Since the glucose utilization studies were done with intact hemidiaphragms (with ribs), the contribution of the ribs attached to the diaphragm to CO_2 or lactate production was evaluated. Ribs from both strains of mice were incubated as above, and the amount of lactate and CO_2 production determined. The potential contribution of the ribs to lactate production was small (<10%). Although the contribution of the ribs to CO_2 production was greater (23%), there was no difference between strains in the incorporation of radioactivity into CO_2 from diaphragms without ribs.

Body composition. At 10 wk of age mice were killed by cervical dislocation between 1000 and 1200 h; fat pads were removed and weighed, and body composition was determined. Aliquots of the whole dried carcass were analyzed for lipid (14) and ash (15), and protein was calculated as the difference between dry matter percentage and sum of the fat plus ash (16).

Statistical analysis. All values are expressed as the mean plus or minus the standard error of the mean. Statistical differences between values for the mouse strains were determined by a two-tailed *t*-test (17).

RESULTS

The I and C57BL mice showed no difference in body weight at birth or weaning (21 d of age), but by d 30 body weight (table 1) was significantly lower in I mice than C57BL and remained lower for the duration of the study (6 wk). Body weight gain from 5 wk to 10 wk of age was also less in the I mice than the C57BL mice. Food intake (grams/day per mouse) at 42 d of age was the same in I (3.0 ± 0.1, n = 15, mean ± SEM) and C57BL mice (3.0 ± 0.1, n = 15), but higher ($P < 0.01$) in the I mice when expressed as grams/day per 100 g body weight (table 1). Thus, body weight gain was less and food intake per body weight was greater in the I mice, indicating a decreased food efficiency. When percent food efficiency (grams body weight gain/grams food eaten × 100) was measured from 49 to 70 d of age, percent food efficiency (3.0 ± 0.5, three cages, five mice/cage) was significantly ($P < 0.001$) less in I strain mice than for C57BL mice (5.0 ± 1, three cages, five mice/cage). In older mice, the difference in food efficiency between I and C57BL mice increased, presumably due to the lower ability of the I mice to accumulate fat with age. Body composition

TABLE 1

Body weight, food intake and body composition[1,2]

Measure	I	C57BL
Body wt, g		
Initial (30 d of age)	15.3 ± 0.3* (16)	18.9 ± 0.4 (16)
Final (71 d of age)	23.0 ± 0.5* (12)	27.8 ± 0.6 (13)
Body wt gain, g	7.6 ± 0.6† (12)	9.0 ± 0.9 (12)
Food intake, *g/(d · 100 g body wt)*	14.6 ± 0.4‡ (15)	12.4 ± 0.5 (15)
Body composition		
% Moisture	65.6 ± 0.5* (12)	61.5 ± 0.5 (12)
% Lipid	8.5 ± 1.0* (13)	16.1 ± 0.7 (12)
% Protein	20.7 ± 0.2‡ (12)	18.7 ± 0.5 (10)
% Ash	4.0 ± 0.1* (13)	3.7 ± 0.1 (10)

[1]Values are means ± SEM for the number of mice in parentheses. [2]Symbols indicate a significant difference between mouse strains: *$P < 0.001$; †$P < 0.05$; ‡$P < 0.01$.

(table 1) analyses showed differences between the two strains in all components. The percent moisture, ash, and protein were higher in the I than C57BL mice, but the lipid content was lower in the I mice. The percent lipid in the I mice was half that in the C57BL. The weights of the fat pads removed (table 2) from the I mice were also approximately half that of those from C57BL mice. When expressed as percent of body weight, only the brown interscapular fat was not lower in the I mice than C57BL mice. In general, fat pad size corresponded to body fat content in the two strains.

Oxygen consumption was measured in I and C57BL mice to determine whether BMR, RMR or heat production in response to food consumption and cold were altered in the I mice to account for their lower energy efficiency. The O_2 consumption (fig. 1) was the same in the two strains when the mice were fasted for 24 h (BMR), but was significantly higher in the I mice following the dark cycle or feeding period. No difference between the two strains in O_2 consumption was found before the start of the dark cycle (RMR) or after 4 h of cold exposure at 4°C. Thus, O_2 consumption in I mice was greater than for C57BL mice only after the feeding period. The difference in oxygen consumption between initial values (1600–1700 h) and values measured 4 h (2100–2200 h) after the start of the dark cycle or feeding period also indicated that O_2 consumption was higher for I [0.54 ± 0.12 L $O_2/(h \cdot kg^{0.75})$, $n = 6$, mean ± SEM] than C57BL mice (0.24 ± 0.07, $n = 6$) in response to food consumption. Body temperature was not different for I (39.6 ± 0.4°C, $n = 7$, mean ± SEM) and C57BL (39.6 ± 0.2°C, $n = 11$) mice indicating no difference in body temperature regulation between the two strains.

Since mice are most active during the dark cycle, spontaneous activity (fig. 2) was measured in three strains of mice, I and C57BL and C3H. Activity during the initial exploratory period and during the feeding period was not significantly different in the I and C57BL mice. Thus, a lack of difference in spontaneous activity in I and C57BL mice suggests that the higher O_2 consumption in I mice than in C57BL mice following the feeding period is related to food utilization rather than activity. With the other control strain, C3H, significant differences in activity were noted relative to the I and C57BL strains, a finding that validates the ability to detect activity differences of mouse strains in this apparatus.

To determine whether the higher O_2 consumption in the I mice is due to higher diet-induced thermogenesis, O_2 was measured at 10 min (fig. 1) or for 5 to 16 min (data not shown) after an injection of norepinephrine (750 μg/kg body weight). No difference in the value after injection of norepinephrine minus the value before norepinephrine was found between I [0.65 ± 0.10 L $O_2/(h \cdot kg^{0.75})$, mean ± SEM] and C57BL (0.53 ± 0.11) at

TABLE 2

Fat pad weights, increase in serum lactate after glucose administration, and glucose utilization by diaphragms incubated in vitro[1,2]

Measure	I	C57BL
Fat pad wt	(11)	(7)
Epididymal, *mg*	206 ± 27*	412 ± 27
% body wt	0.87 ± 0.07*	1.51 ± 0.07
Retroperitoneal, *mg*	77 ± 10*	126 ± 13
% body wt	0.31 ± 0.03†	0.46 ± 0.04
Scapular, *mg*	168 ± 20†	264 ± 35
% body wt	0.68 ± 0.07†	0.97 ± 0.06
Brown interscapular, *mg*	73 ± 4†	111 ± 14
% body wt	0.30 ± 0.01	0.04 ± 0.05
Serum lactate,[3] *mg/dl*	(12) 22.09 ± 3.91	(15) 7.46 ± 2.25
Glucose utilization, μmol glucose converted/ (g tissue · h)	(5 or 6)[4]	(7)
CO_2	13.06 ± 2.26	11.63 ± 1.80
Lactate[5]	81.58 ± 7.37‡	61.71 ± 5.63

[1]Values are means ± SEM for the number of mice in parentheses. [2]Symbols indicate a significant difference between mouse strains: *$P < 0.001$; †$P < 0.01$; ‡$P < 0.05$. [3]The increase in serum lactate is the concentration at 15, 30 or 60 min after glucose administration minus the concentration prior to glucose administration. No significant differences ($P > 0.05$) between the 15, 30 or 60 min lactate values were observed for either mouse strain and, therefore, values at the different time points were combined. [4]By CO_2, five mice; by lactate, six mice. [5]Lactate found in medium after 60-min incubation and converted to micromoles glucose by calculation.

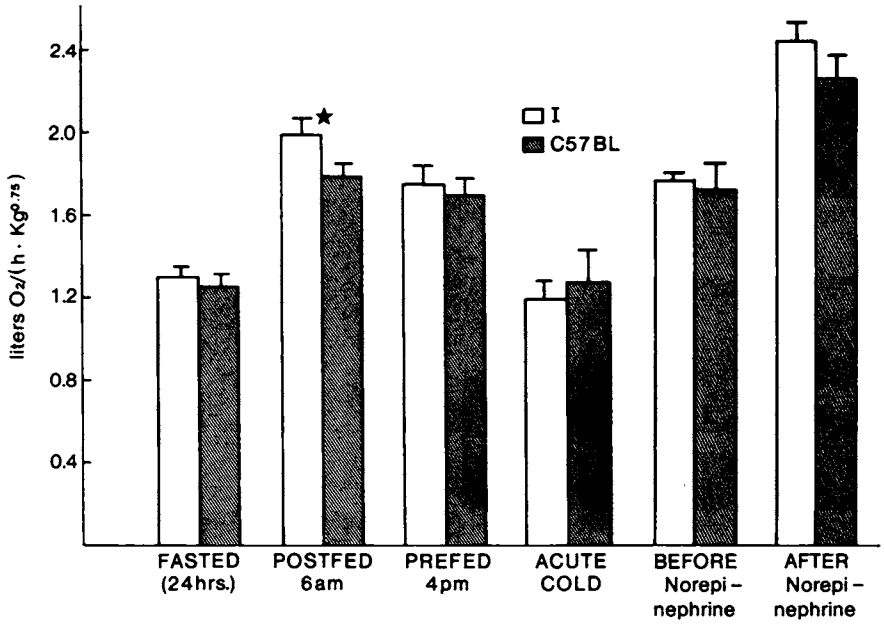

Fig. 1 Oxygen consumption. Bars are the means ± SEM of 10–12 mice. The symbol (★) indicates a significant ($P < 0.05$) difference between the mouse strains.

10 min even when values from two I mice that did not respond were excluded (fig. 1). Since the peak time of response after the norepinephrine injection may have varied in the two strains, O_2 consumption was measured from 5 to 16 min for 12 mice of each strain after the norepinephrine administration. In both strains of mice O_2 consumption began increasing at 5 min, reached a plateau at 8–12 min and then declined. The

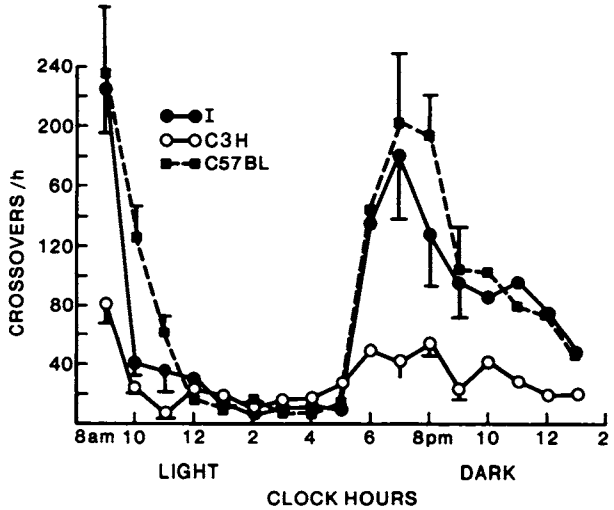

Fig. 2 Spontaneous activity. Values are the means ± SEM of five mice. Crossovers per hour for I and C57BL mice are not significantly different. Crossovers per hour for C3H were significantly ($P < 0.05$) lower during the initial period 0800–1000 h (8–10 a.m.) and dark period 1800–2200 h (6–10 p.m.).

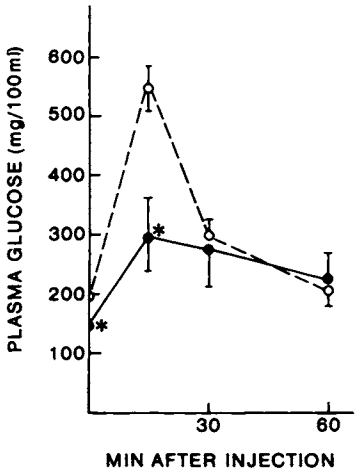

Fig. 3 Serum glucose concentration before and after an injection of glucose (3 mg/g body weight) in I (●) and C57BL (○) mice. Each time point is the mean ± SEM of 3–16 different mice. Significant ($P < 0.01$) differences between the two strains at the same time are indicated by the symbol (*).

lack of difference between the two mouse strains in oxygen consumption after norepinephrine administration suggests that the brown adipose tissue response to norepinephrine is similar in the two strains.

Since differences in nutrient processing in the I mice might account for the higher oxygen consumption associated with feeding, glucose utilization was measured in vivo and in vitro. The plasma glucose curve was lower (fig. 3) in I mice (a similar pattern was found when glucose was injected i.v., Hoover-Plow, J. and Nelson, B., unpublished observations), which indicated that a glucose dose is removed from the blood by tissues at a faster rate. Glucose accumulated in muscle rather than liver (fig. 4), and lactate production (table 2) was higher in the I mice than in the C57BL mice. In muscle incubated in vitro, lactate production was higher in I mice than C57BL mice (table 2). These results indicate glucose storage and utilization are different in the I mice.

DISCUSSION

The results found in this study confirm earlier observations (1–4) of lower food efficiency and fat accumulation in I strain mice than in C57BL. The results also suggest I strain mice utilize more energy to process food (i.e., greater diet-induced thermogenesis) as demonstrated by the higher O_2 consumption following the feeding period. Differences between the two strains in glucose utilization—faster disposal of glucose, storage of glucose in muscle rather than liver glycogen, higher lactate production by the diaphragm, and higher glucose oxidation (18) by the adipose tissue in the I mice—also support the concept that differences

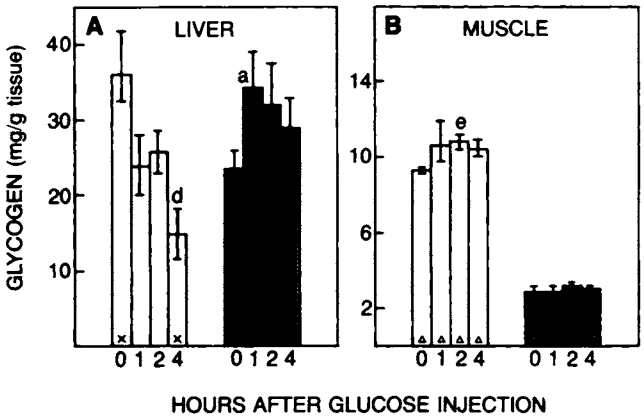

Fig. 4 Glycogen concentration in liver (4*A*) and mixed leg muscle (4*B*) of I (open bars) and C57BL (hatched bars) after a dose of glucose (3 mg/g body weight). Values are the mean ± SEM of four to nine mice. Significant differences between the two strains are indicated by × ($P < 0.025$), or Δ ($P < 0.001$), and significant difference for each strain between initial value (0) and time after glucose injection is indicated by: a ($P < 0.05$), d ($P < 0.005$), e ($P < 0.001$).

between I and C57BL mice in energy balance are related to differences in nutrient utilization.

Differences between animals in fat accumulation may be related to differences in energy intake or energy expenditure (6). The importance of the contribution of energy expenditure to animal models with abnormal energy balance (5) has been realized in recent years. In the two mouse strains, I and C57BL, no difference was observed in the basal metabolic rate (O_2 consumption at fasting) or resting metabolic rate (O_2 consumption prior to the dark cycle or feeding period). Body temperature was also not different in the two strains. These results indicate that the lower fat accumulation in I mice cannot be attributed to a higher metabolic rate or the maintenance of a higher body temperature.

No difference was observed in spontaneous activity between I and C57BL mice. The earlier studies that reported higher activity in I strain, had used measures of exercise capacity, such as length of swimming time (9) or revolutions in activity cages (19). The greater exercise capacity in I mice may be due to higher muscle glycogen (fig. 4). The lack of difference in spontaneous activity between I and C57BL mice measured in this study indicates activity cannot account for their difference in energy balance. Clearly, for C3H mice, lower activity is a contributing factor to their greater body weight and fat accumulation (1).

The greater O_2 consumption in the I mice is quantitatively important in accounting for the smaller weight gain in this strain. An estimation of the caloric cost of the growth (20) during the 41-d experimental period based on the body composition (table 1) of both strains is 13.8 and 16.1 kcal/$(d \cdot kg^{0.75})$ final body weight for the I and C57BL mice, respectively. An estimation of total caloric expenditure based on a value of 4.8 kcal/L of O_2 (21) was made by assuming the prefed and postfed O_2 values occurred for 12 h and then calculating the sum of these two values. The efficiency of energy utilization for growth is 6.0% for the I strain and 7.6% for the C57BL mice. Thus, the I strain mice were only 79% as efficient as the C57BL in utilizing energy for body weight gain.

Diet-induced and cold-induced thermogenesis have been shown to be defective in both obese rat and mice animal models (6). No difference between I and C57BL in oxygen consumption was found when the mice were exposed to 4 h of acute cold. Oxygen consumption, however, was higher in the I mice 4 h after the feeding period began and continued to be elevated at the end of the feeding period. Earlier studies (1–4) examining the effects of high fat diets in various mouse strains indicated I mice did not have higher body weight or body fat content when a diet high in fat (50%) was fed than when a low fat diet (25%) was fed from weaning to 6 mo of age. Oxygen consumption was not measured in the I strain mice in these feeding experiments. The higher O_2 consumption by I mice, observed in this study after the feeding period, and the ability to maintain body weight when fed high fat diet indicate that the I mice may have a higher capacity for diet-induced thermogenesis. A higher O_2 consumption in response to norepinephrine by the I strain mice would have supported the concept of higher diet-induced thermogenesis in the I mice compared to C57BL. However, the lack of difference in O_2 consumption in response to norepinephrine observed in the present study does not exclude the possibility of a higher diet-induced thermogenesis until the brown adipose tissue has been examined directly. The abnormal glucose utilization in I mice indicates that the storage and processing of nutrients are altered, require more energy and are less efficient than in C57BL mice.

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