Heat Production of Lean and Obese (Ob/Ob) Mice in Response to Fasting, Food Restriction or Thyroxine[1] (40432)

JERRY G. VANDER TUIG, NAOMI TROSTLER, DALE R. ROMSOS, AND GILBERT A. LEVEILLE

Department of Food Science and Human Nutrition, Michigan State University, East Lansing, Michigan 48824

Genetically obese-hyperglycemic mice (ob/ob) have a number of metabolic and endocrine disorders which could either be responsible for the development of the syndrome or could occur as a result of the obesity (1, 2). Obese mice have a lower body temperature (3), a greater energy efficiency (4, 5) and a decreased oxygen consumption (6–9), all of which can be detected before the obesity becomes visibly apparent. Obese mice are also sensitive to a cold environmental temperature (10–12) and it has been suggested that obese mice have an alteration in thermogenesis (11) which could partially explain the above observations.

The present study was designed to determine heat production of 3- to 4-month old lean and obese male mice by direct calorimetry under conditions which are known to affect thermogenesis, namely, fasting or food restriction (13) and administration of thyroid hormones (14, 15). Food intake and body weight were also monitored throughout each experiment.

Materials and methods. Obese (ob/ob) C57BL/6J male mice and their lean littermates were obtained from litters of heterozygous (ob/+) breeding pairs[2] or were purchased directly from the supplier. All animals were housed in individual cages in a temperature controlled room (25 ± 2°) with automatically controlled lighting (on from 7 AM to 7 PM). Water was provided *ad libitum* and a stock diet[3] was made available according to the conditions of each study.

Heat production was measured directly with a gradient layer calorimeter[4] (16) which had been calibrated with a small heating apparatus supplied by the manufacturer. For each measurement, one or two animals from the same treatment group were placed in the calorimeter and after a 45- to 60-min acclimation period, heat production was recorded and averaged over a span of 10 min. Because of the time required for each measurement, pairs of mice were frequently placed in the calorimeter to accommodate all animals within each group. Heat production was calculated as total heat produced per animal and per body weight to the 0.75 power (13). Oxygen was provided with a volume meter[5] and carbon dioxide was removed from the chamber with soda lime. Mice from different experimental groups were placed alternately in the calorimeter during the day (8 AM–7 PM).

To study the effect of fasting and refeeding on heat production, 3 month old lean and obese mice were fasted for 72 hr and refed for 48 hr. Heat production and body weights were measured both before and daily throughout the fasting-refeeding period. Before fasting and during refeeding, diet was provided ad libitum and food intake was measured each day.

The second experiment was designed to eliminate any effect of unequal food intake and to evaluate the influence of reduced food intake on heat production of lean and obese mice. Either 2 g or 4 g of diet were provided daily for 6 weeks to 11 week old lean and obese littermates. Heat production and body weights were measured at 2, 4, and 6 weeks after the start of the experiment.

In the third experiment the response of lean and obese mice to L-thyroxine (T_4) was investigated in 3 to 4 month old animals. T_4

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[2] Jackson Laboratories, Bar Harbor, Maine.

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

[4] Thermonetics Corporation, San Diego, California.

[5] Med Science Electronics, Inc., St. Louis, Missouri.

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was prepared daily in alkaline 0.15 *M* NaCl. Mice were divided by weight into groups receiving either T_4 or the alkaline saline vehicle. T_4-treated lean mice and one-half of the T_4-treated obese mice received intraperitoneal injections of 20 μg T_4 per 100 g body weight per day. The remaining T_4-treated obese mice received an amount of T_4 equal to that injected into lean mice (5–6 μg per day). All injections were delivered in a volume of 0.1 ml at 4 PM each day over a 16-day period. Heat production and body weight were recorded for all animals every third day. Ad libitum food intake was monitored for the first 12 days.

Data were analyzed by analysis of variance (ANOV). A split-plot design with repeated measurements over time (17) was used for the analysis of data from the fasting-refeeding experiment. Separate analyses were used for data obtained during fasting and for data obtained during refeeding. Thus, values obtained before and during fasting were included in one ANOV, and values obtained on the third day of fasting and during refeeding were included in a separate ANOV. In the other two experiments, a 2 × 2 factorial design (17) was used for a separate ANOV at each time point. Because they were not statistically different, values from both groups of T_4-injected obese mice were combined for each ANOV.

Results. Body weight, food intake, and heat production of fasted-refed mice are presented in Table I. Both lean and obese mice lost a similar amount of body weight during 3 days of fasting (6.2 and 6.0 g), but this represented a greater percentage of prefasting body weight in lean than in obese mice. During 2 days of refeeding, lean mice regained considerably more body weight than obese mice (4.1 and 2.8 g, $P < 0.001$). The composition of neither the weight loss nor the weight gain was determined. Prior to fasting, obese mice consumed significantly more diet than lean mice ($P < 0.001$), which is in agreement with the characteristic hyperphagia of adult obese mice reported previously (4, 18, 19). Following the 3-day fast, lean mice consumed more diet per day ($P < 0.001$) while obese mice consumed less diet per day ($P < 0.001$) when compared to their respective prefasting food intake.

Total heat production (kcal/hr/animal) of

TABLE I. EFFECTS OF FASTING AND REFEEDING ON BODY WEIGHT, FOOD INTAKE, AND HEAT PRODUCTION OF 3-MONTH-OLD LEAN AND OBESE MICE.[a]

		Days fasted					Days refed			
	Fed	1	2	3	MS_E[b]	ANOV[c]	1	2	MS_E[b]	ANOV[c]
					Body weight (g)					
Lean (21)	27.3	24.2	22.6	21.1	0.24	F, P	24.1	25.2	0.29	R, P, R × P
Obese (21)	45.4	42.3	40.8	39.4			41.6	42.2		
					Food intake (g/day)					
Lean (21)	3.4	—	—	—	—	—	4.5	4.9	0.27	R, P, R × P
Obese (21)	5.8	—	—	—			4.5	4.8		
					Heat production (kcal/hr/animal)					
Lean (11)	0.45	0.38	0.32	0.30	0.001	F, F × P	0.38	0.45	0.001	R
Obese (11)	0.48	0.45	0.41	0.36			0.45	0.48		
					Heat production (kcal/hr/kg$^{0.75}$)					
Lean (11)	6.67	6.20	5.52	5.43	0.218	F, P	6.17	7.05	0.162	R, P, R × P
Obese (11)	4.85	4.82	4.47	4.05			4.87	5.13		

[a] Values represent the means obtained for the number of observations indicated in parentheses. Data were analyzed statistically using an analysis of variance (ANOV) of split-plot design. Separate ANOV's were used to compute fasting effects and refeeding effects. Fasted values were compared with fed values and refed values were compared with the final fasted value (Day 3). Food intake during refeeding was compared with that measured before fasting.

[b] Mean square error.

[c] Analysis of variance. F and R indicate significant ($P < 0.05$) fasting and refeeding effects, respectively. P (phenotype) indicates a significant difference ($P < 0.05$) between lean and obese mice. F × P and R × P indicate a significant difference ($P < 0.05$) between the responses of lean and obese mice to fasting and refeeding, respectively.

obese mice was similar to or slightly greater than that of lean littermates throughout the fasting-refeeding period (Table I). This was true even though obese mice had a much greater body weight ($P < 0.001$) and consumed more diet prior to fasting. When expressed per unit of metabolic body weight (kcal/hr/kg$^{0.75}$) (13), heat production was greater in lean mice than in obese mice. Two days of fasting significantly reduced total heat production in lean mice, but obese mice produced less heat only after 3 days of fasting. Total heat production of lean mice was reduced to 85, 72 and 68% of prefasting values after 1, 2, and 3 days of fasting, respectively; corresponding values for obese mice were 94, 85, and 75%. After one day of refeeding, total heat production of both lean and obese mice had returned to prefasting levels even though both groups had not regained all of the body weight lost during fasting.

Table II contains body weight and heat production data from lean and obese mice fed either 2 g or 4 g of diet per day for 6 weeks. Obese mice weighed significantly more than lean mice throughout the experiment. After 2 weeks of food restriction, both lean and obese mice fed 2 g of diet per day weighed less than their lean and obese counterparts fed 4 g of diet per day. Total heat production per animal was less in mice fed the decreased amount of diet. This reflects the differences in body weight, since heat production per unit of metabolic body weight was similar between animals consuming the two amounts of diet within either the lean or obese group. Obese mice produced slightly more total heat per animal than lean mice, but heat production of obese mice per unit of metabolic body weight was significantly less than that of lean animals.

Body weight, food intake, and heat production of lean and obese mice injected with T_4 or 0.15 M NaCl are presented in Fig. 1. The responses of both groups of T_4-treated obese mice were similar, even though the absolute amount of T_4 administered per day was at least 1.5 times greater in one group than in the other. T_4-injected lean mice gained slightly more body weight during the 16 day period than did control lean mice (2.8 ± 0.3 and 1.4 ± 0.4 g, $P < 0.05$). In contrast, both T_4-injected obese groups of mice tended to lose body weight when compared to control obese mice. Administration of T_4 to lean mice resulted in a marked increase (19%) in food intake over that of control animals during the first 12 days of the study (56.5 ± 1.1 and 47.4 ± 1.3 g, $P < 0.001$). Except for a small increase in food intake by one T_4-treated, obese group of mice (days 10–12), T_4 did not affect total food consumption of obese mice. Throughout the entire 16-day period, both body weight and food consumption of

TABLE II. Body Weight and Heat Production of 11-Week-Old Lean and Obese Mice Fed Either 2 g or 4 g of Diet per Day for 6 Weeks.[a]

	2g/day		4g/day			
	Lean	Obese	Lean	Obese	MS_E[b]	ANOV[c]
Body weight (g)	(7)	(8)	(7)	(8)		
Initial	27.7	50.4	27.5	49.7	5.7	P
2 Weeks	19.7	38.7	23.6	46.4	12.3	P, I
4 Weeks	19.3	36.9	26.3	47.3	7.1	P, I
6 Weeks	19.5	36.4	27.7	48.4	6.3	P, I
Heat production (kcal/hr/animal)	(4)	(4)	(4)	(4)		
2 Weeks	0.29	0.41	0.37	0.48	0.003	P, I
4 Weeks	0.37	0.41	0.44	0.47	0.002	I
6 Weeks	0.42	0.46	0.51	0.53	0.0004	P, I
Heat production (kcal/hr/kg$^{0.75}$)						
2 Weeks	5.59	4.74	6.16	4.79	0.32	P
4 Weeks	7.20	4.87	6.81	4.66	0.21	P
6 Weeks	7.99	5.54	7.68	5.13	0.41	P

[a] Values represent the means obtained for the number of observations in parentheses. Data were analyzed at each separate time point using a 2 × 2 factorial analysis of variance (ANOV).

[b] Mean square error.

[c] Analysis of variance. P (phenotype) indicates significant differences ($P < 0.05$) between lean and obese mice. I (intake) indicates significant differences ($P < 0.05$) between the two levels of food intake.

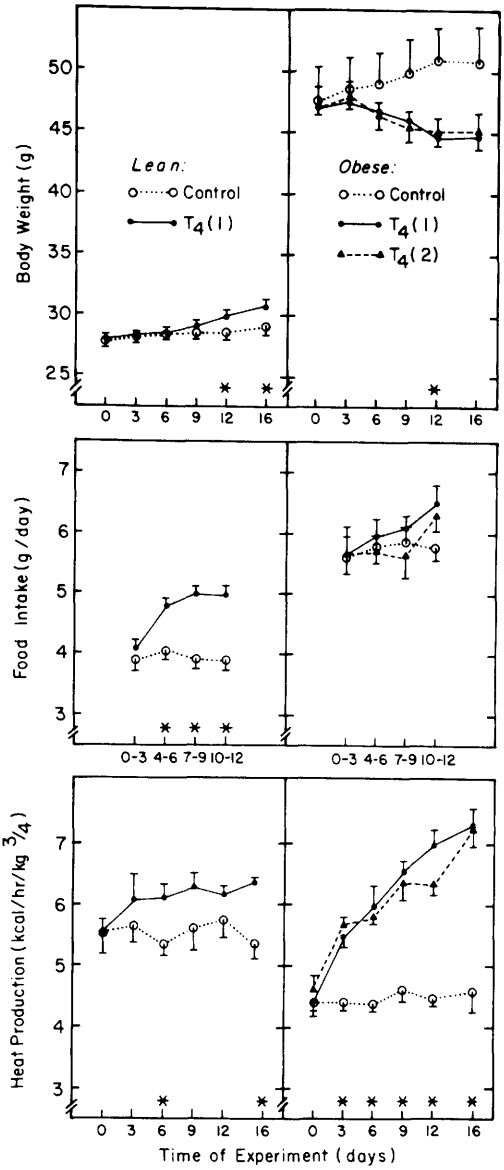

FIG. 1. Body weight, food intake and heat production of 3 to 4 month old male lean and obese mice injected with either thyroxine (T_4) or 0.15 M NaCl for 16 days. Each point in the upper two sets of graphs represents the mean ± SEM for 9–12 mice. Each point in the bottom set of graphs represents the mean ± SEM for 5–6 observations. T_4 (1) indicates mice injected with 20 μg T_4 per 100 g body weight per day. T_4 (2) indicates obese mice injected with same amount of T_4 injected into lean mice (5–6 μg/day). The asterisks indicate significant differences ($P < 0.05$) between control and T_4-injected mice. Total body weight gain, total food intake, and heat production at each time point were analyzed by a 2 × 2 factorial analysis of variance (ANOV). Significant effects ($P < 0.05$) due to phenotype (P), thyroxine (T), and P × T interaction were observed for total body weight gain. For total food intake, significant P and T effects were observed. For heat production (kcal/hr/kg$^{0.75}$), significant P effects were observed on days 0, 3, and 6; significant T effects were observed on days 3, 6, 9, 12, and 16; and significant P × T interactions were observed on days 9, 12, and 16.

all lean mice were significantly less than those of all obese mice.

In agreement with the first two experiments (Tables I and II), obese control mice produced slightly more total heat (kcal/hr/animal) than lean control mice during this experiment (data not presented). However, lean control mice produced significantly more heat per unit of metabolic body weight than did their obese counterparts (Fig. 1). When compared with respective control values, heat production of obese mice was more responsive to T_4 administration than was that of lean mice during the final week of the experiment. T_4-treated lean mice produced more heat than control lean mice, but this difference was significant ($P < 0.05$) only on days 6 and 16 of the study. Obese mice treated with T_4 produced much more heat than control obese mice throughout the study ($P < 0.01$).

Discussion. This study has demonstrated that 3 to 4 month old male obese mice produce at least as much and possibly more total heat per animal than lean littermates. These results are in agreement with earlier observations of oxygen consumption in these mice (6, 20, 21). Obese animals in this study were 60–70% heavier than lean mice; consequently, the amount of heat produced per unit of body weight was considerably less in obese mice. Since a large portion of this increased body weight is fat (4, 22, 23), it has been suggested that either this excess fat is relatively inert (21) or obese mice have a depressed metabolism relative to body weight (7). Reduced oxygen consumption before weaning, when body weights of lean and obese mice are comparable, provides evidence for a depressed metabolic rate in obese mice (6–9). Because obese mice are unable to survive acute exposure to cold (10, 11) and have a lower body temperature than lean mice (3), they apparently have a depressed thermogenesis.

It has been known for some time that

fasting or food restriction reduces heat production in experimental animals (13). As shown in Table I, 3 days of fasting reduced total heat production in both lean and obese animals. Heat production per unit body weight also decreased during fasting which indicates that the reduction in heat production is not simply due to a loss of body weight, but to an actual decrement in thermogenesis. Lean animals were more responsive to fasting with respect to decreased heat production even though loss of body weight was similar to that of obese animals (Table I). This suggests a more sluggish control of thermogenesis in obese mice in response to changes in energy intake. Previous work has demonstrated that restricted food intake leads to a reduction in secretion of thyroid hormones (24, 25) and peripheral thyroxine metabolism (26). It may be that a problem exists within the framework of thyroid hormone secretion and metabolism and that this is in part responsible for the sluggish control of thermogenesis in fasted obese mice.

Both lean and obese mice responded to 2 days of refeeding by producing as much total heat as before fasting; but, as in other studies (27, 28), lean mice were able to regain more lost body weight (Table I). The differences in food intake measured before fasting and during refeeding in lean and obese mice also support the suggestion of greater responsiveness of lean mice to a fasting-refeeding regimen. Previous studies have demonstrated similar results with respect to adaptive enzymes in adipose (27) and liver tissue (28) from lean and obese mice.

By feeding lean and obese mice equal amounts of diet per day (Table II), any effect of unequal food intake on heat production should have been minimal. Based on other data presented (Table I and Fig. 1), 4 g of diet per day approximated ad libitum and 70% of ad libitum intake for these adult lean and obese mice, respectively. Under these conditions obese mice produced at least as much total heat as lean mice at both levels of food consumption (Table II). This is in contrast to an earlier study (29) in which obese rats produced less heat than pair-fed lean rats; but in that study the obese rats did not weigh significantly more than the lean rats. Prolonged restricted food intake did not appear to affect thermogenesis of either lean or obese mice, since both produced the same amount of heat per unit of body weight when fed 4 g of diet as when fed 2 g. The difference in total heat production between levels of food intake can be explained by the difference in body weight.

Obese mice exhibit characteristics which indicate a hypothyroid state (3, 30, 31) but conflicting evidence has been presented (12). Data from the present study demonstrate a definite hypersensitivity of obese mice to T_4 with respect to heat production. These results support similar observations of increased responsiveness to thyroid hormones of body temperature (3) and oxygen consumption (31) of obese mice. Nearly identical responses to thyroxine were observed in both groups of T_4-injected obese mice even though the absolute amount of T_4 administered daily was greater in one group than the other. This suggests that the amount of T_4 injected was above that necessary for a maximum response.

Lean animals adjusted their food intake in response to thyroxine administration so that increased heat production did not result in a loss of body weight (Fig. 1). In contrast obese mice apparently could not adjust energy intake to balance the marked increase in heat production and this was reflected in a loss of body weight. In a similar study (32) weight gain and loss by T_4-treated lean and obese mice, respectively, were correlated with gain and loss of lipid stores. These results suggest that 3 to 4 month old obese mice have less ability than lean mice to control food intake in response to changing energy needs.

Recently the possibility of a decreased selective peripheral response to thyroid hormones has been considered as an explanation for the sensitivity of obese mice to cold environmental temperatures (12). It is apparent from this study that further investigation is needed to relate circulating thyroid hormone levels and peripheral thyroid hormone metabolism to rates of thermogenesis in obese mice.

Summary. Direct measurements of heat production were made in 3 to 4 month old lean and obese mice during 3 days of fasting and 2 days of refeeding, 6 weeks of dietary restriction, and 2 weeks of thyroxine (T_4)

administration. Obese mice produced slightly more total heat per animal at this age but weighed 1.5 to 2 times as much as lean mice and consumed more diet ad libitum. Heat production of obese mice was not as responsive to fasting as that of lean mice. When fed equal amounts of diet, obese mice continued to produce at least as much total heat as lean mice. Heat production of obese mice was more sensitive to T_4 administration when compared to that of lean mice and unlike the lean mice, T_4-treated obese mice were unable to adjust food intake to maintain body weight during a T_4-induced increase in thermogenesis. These data suggest that metabolic responses of obese mice to changes in energy intake or output do not occur as rapidly as those of lean mice.

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