Food intake and body weight regulation in diabetes (*db/db*) and obese (*ob/ob*) mice

ISRAEL RAMIREZ and RICHARD L. SPROTT
The Jackson Laboratory, Bar Harbor, Maine 04609

Diabetes and lean control mice of two strains (C57BL/6J and C57BL/KsJ) were tested for their ability to regulate food intake and body weight when subjected to caloric deficits and surpluses. Diabetes mice regulated food intake and body weight as well as or better than control mice when given milk solutions to drink. Recovery from food deprivation was impaired in older (more than 13 weeks of age) but not in younger (8 weeks of age or less) diabetes and obese (C57BL/6J-*ob/ob*) mice. These results suggest that overweight itself is responsible for some of the behavioral disturbances in genetically obese mice.

Diabetes (*db*), an autosomal recessive gene, is one of several genes producing obesity in the mouse. The behavior of diabetes mice has rarely been investigated, presumably because the name is misleading. The name diabetes was given because it was first discovered in the C57BL/KsJ strain, in which it produces severe degeneration of the islets of Langerhans leading to severe hyperglycemia and insulinopenia. When diabetes was transferred to the C57BL/6J strain, it produced hypertrophy of the islets of Langerhans with mild hyperglycemia and marked hyperinsulinemia. The gene obese (*ob*) is similar to diabetes in both strains (Herberg & Coleman, 1977). The few investigations of the behavior of diabetes mice suggest that it is similar to that of obese mice (Fuller, 1972; Sprott, 1972).

The purpose of the present set of experiments is to repeat some observations that we have previously reported with obese mice (Ramirez & Sprott, in press) on diabetes mice. The first is that obese mice reduce food intake as much as or more than lean mice when given fixed amounts of nutritive solutions to drink. The second is that obese mice eat less than lean mice immediately following food deprivation. These may be regarded as response to caloric surpluses and deficits, respectively. The theoretical rationale for these measures has already been discussed (Ramirez & Sprott, in press).

GENERAL METHOD

Subjects

Two different strains of mice were used. C57BL/KsJ-*db*+/*db*+ mice and their lean controls, C57BL/KsJ-*db*+/*+*+*m*, were obtained from the Animal Resources Department of the Jackson Laboratory. C57BL/6J-*db*+/*db*+ mice were provided through the generosity of Dr. D. L. Coleman. Their controls, C57BL/

6J-*+*+/*+*+, were obtained from the Animal Resources Department of the Jackson Laboratory. The controls for the diabetes mice of the C57BL/KsJ strain (abbreviated KsJ) were heterozygous for diabetes and the closely linked coat color locus misty (*m*), which is used as a marker to identify heterozygotes for diabetes (misty mice are homozygous normal at the *db* locus). The controls for diabetes mice of the C57BL/6J strain (abbreviated 6J) were homozygous for normal alleles at both the diabetes and misty loci. In the last experiment, C57BL/6J-*ob/ob* and C57BL-*?*+/*+* mice were obtained from the Animal Resources Department of the Jackson Laboratory. The controls for the obese mice were either heterozygous or homozygous for the normal allele at the obese locus; there is no way to tell without breeding tests. All the mice were female.

Procedure

The mice were housed individually in clear plastic cages (13 × 28 × 15.2 mm deep) and maintained under 12 h dark/12 h light at 21° ± 2°C. Measurements were always taken in the light part of the light/dark cycle. The mice were given tap water and Old Guilford 911 food (20% protein, 10% fat, and 4.35 kcal/g) ad lib, except where otherwise indicated. Food consumption was measured by weighing to the nearest 0.1 g. An attempt was made to collect and weigh spillage but some diabetes mice ground their food so finely that this was impossible. In every experiment, body weight changes were recorded to validate any conclusions about food intake. Although food intake and weight gain should be correlated, they do not measure precisely the same thing. Weight gain is partly a function of food intake and partly a function of efficiency of utilization of food, water intake, defecation, urination, and other factors.

EXPERIMENT 1

The first experiment was an attempt to demonstrate that two of our most reliable findings with obese mice also applied to diabetes mice; obese mice recover from food deprivation more slowly than do lean mice, and obese mice compensate for surplus calories from a nutritive solution as well as or better than do lean mice.

Method

Subjects. A total of 22 subjects were used: 6 diabetes KsJ (38 to 50 g at the start of the experiment), 6 lean KsJ (18 to

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21 g), 5 diabetes 6J (31 to 36 g), and 5 lean 6J (16 to 18 g) mice. All groups were tested simultaneously, but the KsJ mice were 9 weeks old and the 6J mice were 7 weeks old at the start of the experiment.

Procedure. The mice were weighed on alternate days, beginning 10 days before they were food deprived, to habituate them to handling. They were then food deprived for 2 days and given food again ad lib. Food intake was recorded 1 h and 24 h following food deprivation, and body weights were recorded immediately before and 24 h after food was restored.

The test of the ability to compensate for calories from solutions began 6 days after food was restored. This test is identical to that used by Ramirez and Sprott (in press) except that each mouse was given 8 ml of each solution twice instead of three times. Three solutions were used, all containing 0.35% Na-saccharin water, Carnation brand evaporated milk, and sweetened nonfat milk. The nonfat milk was made by mixing 21.6 g Carnation brand nonfat powdered milk and 17.54 g sucrose, with enough water to make 100 ml. Both milk solutions have about 0.076 g protein/ml and about 1.44 kcal/ml. About 50% of the calories in evaporated milk comes from fat. During each trial, the mice were given 8 ml of solution to drink and its effects on 2-day food consumption and weight gain were recorded. Two-day rest periods were provided between each test, and 4-day rest periods between the two series of tests. The solutions were presented in the following sequence: water, evaporated milk, nonfat milk, nonfat milk, evaporated milk, and water.

The mice were food deprived for 2 days a second time, beginning 4 days after the last time saccharin solution was given. The procedure was identical to that of the first food-deprivation test.

Statistical analysis. Five different kinds of data were obtained and analyzed: food intake in the first hour following food deprivation, food intake in the next 23 h following food deprivation, weight gain in the 24 h immediately following food deprivation, food intake when each of the three solutions was present, and weight gain when each of the three solutions was presented. Each measure was analyzed by an appropriate repeated measures analysis of variance. Separate analyses were computed for each strain.

Results

A summary of the results of the food deprivation tests is presented in Table 1. Diabetes mice of the KsJ strain ate slightly more food during the first hour following deprivation in the first test and slightly less in the second test. This interaction was significant in the KsJ strain ($p < .01$) but not in the 6J strain ($p > .20$). A similar pattern appeared for weight gain in the first 24 h following food deprivation (KsJ, $p < .001$; 6J, $p > .20$). Diabetes mice appeared to consume more food than lean mice in the next 23 h following food deprivation (KsJ, $p < .05$; 6J, $p < .02$), but this may be due to spillage, since very finely

ground food was found in the cage bottoms of some of these mice.

Food intake and weight gain in the caloric compensation experiment are shown in Table 2. Diabetes mice of both strains ate more ($p < .001$) and gained more weight ($p < .05$) than did lean mice, regardless of which solution was given. However, when given milk solutions to drink, diabetes mice showed a greater reduction in food intake in response to the presentation of a nutritive solution than did lean mice (KsJ, $p < .005$; 6J, $p < .01$). This effect was not as apparent in the weight change measure, because there was a great deal of interindividual variability (KsJ, $p > .20$; 6J, $p = .06$). There were no significant differences between the two milk solutions ($p > .20$).

Discussion

When provided with surplus calories, diabetes mice regulate food intake and body weight as accurately as do lean mice. This was true for both fat-free and evaporated milk solutions. These results are identical to those we have previously found with obese mice (Ramirez & Sprott, in press). In our view, this result indicates that diabetes and obese mice have a normal ability to inhibit eating, although they regulate food intake and body weight at an elevated level.

The food deprivation tests produced a more complex picture. Diabetes mice eat as much or more than lean mice following the first deprivation and less following the second deprivation. In order to interpret this finding, it is necessary to determine whether this difference between the first and second deprivation is due to practice or to aging. The next experiment is designed to answer this question by testing response to food deprivation two times in diabetes mice of various ages. Diabetes mice of the KsJ strain were used because they were more readily available than 6J diabetes mice.

EXPERIMENT 2

Method

Subjects. Diabetes and lean control mice (KsJ strain) of three age groups were used: eight lean (17-20 g) and eight diabetes (28-38 g) mice, 5-6 weeks of age; six lean (21-23 g) and six diabetes (60-68 g) mice, 13-15 weeks of age; and four lean (20-22 g) and

Table 1
First Food Deprivation Experiment

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N			1-h Food Intake*				23-h Food Intake				24-h Weight Gain			
			Test 1		Test 2		Test 1		Test 2		Test 1		Test 2	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
6	C57BL/KsJ	Control	1.0	.2	1.2	.4	5.6	2.0	5.2	1.8	3.6	.4	3.5	.5
		Diabetes	1.6	.5	1.0	.4	8.2	1.5	6.9	1.8	3.7	.5	1.7	.7
5	C57BL/6J	Control	1.0	.2	1.4	.3	4.4	.6	4.9	.7	3.4	.3	4.0	.3
		Diabetes	1.3	.4	1.7	.5	6.4	.9	5.3	1.1	3.7	.5	3.2	.7

*Following deprivation, means per mouse in grams.

Table 2
Caloric Compensation

Group		Food Intake/Day*						Weight Gain/Day					
		Solution Given						Solution Given					
		None		Evaporated Milk		Nonfat Milk		None		Evaporated Milk		Nonfat Milk	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
C57BL/KsJ	Control	2.0	.3	.9	.3	.9	.3	−.08	.3	.06	.2	.04	.2
	Diabetes	5.6	1.1	3.8	1.0	4.0	1.3	.05	.5	.27	.4	.18	.1
C57BL6J	Control	2.4	.3	1.1	.3	1.4	.4	.00	.2	−.01	.2	.02	.3
	Diabetes	4.6	.9	2.5	.4	2.4	.4	.42	.2	.22	.1	.14	.1

*All values are group means in grams.

four diabetes (43-65 g) mice, 21 weeks of age. The weights given are ranges immediately before the first deprivation. These numbers do not include mice that died while aging or during the experiment. Diabetes KsJ mice often die prematurely. The number of lean mice was adjusted to equal the number of diabetes mice.

Procedure. The mice were weighed on alternate days for 8 days prior to food deprivation (4 weighings per mouse). They were then food deprived for 2 days and given food ad lib. Food intake was recorded 1 h and 24 h following food deprivation, and body weights were recorded immediately before and 24 h after food was restored. The mice were allowed to recover from food deprivation for 6 days, weighed on alternate days, and then food deprived for another 2 days. Food ad lib was again restored and the same measures were taken.

Results

The results are summarized in Table 3. Some of the mice appeared to spill large amounts of food. Since this was particularly pronounced in the oldest group, analyses of variance for food intakes were performed with and without the oldest mice (21 weeks of age). Main effects are not reported here because the existence of significant interactions makes them meaningless.

The Trials by Genotype interaction was significant for 23-h food intake (with oldest group, $p < .025$; without oldest group, $p = .05$) and postdeprivation weight gain ($p < .001$), but was not significant for 1-h food intakes ($p < .20$). This interaction is opposite to that which was predicted from the practice explanation of Experiment 1; diabetes mice actually improved on the second test relative to lean mice.

On the other hand, postdeprivation weight gain ($p < .001$) and 1-h food intake (with oldest group, $p = .20$; without oldest group, $p = .06$) showed the expected interaction with age; younger diabetes mice did slightly better than lean mice, while older diabetes mice did worse than lean mice of the same age. Diabetes mice consumed more food in the 23-h periods regardless of age (probably spillage), but as predicted, the difference between diabetes and lean mice was greatest in the youngest group (with oldest group, $p < .025$; without oldest group, $p < .05$). Thus, the trials effects in Experiment 1 are attributable to aging rather than to practice.

EXPERIMENT 3

It is apparent that age influences postdeprivation food consumption and body weight gain in diabetes mice. It is not known whether this is so for obese mice. The following experiment is designed to answer this question. A group of diabetes mice was included for comparison.

Method

Subjects. Four obese and four lean control mice were tested beginning at 6 weeks of age (lean, 18 to 20 g; obese, 30 to 32 g) and again at 21 weeks of age (lean, 25 to 30 g; obese, 57 to 62 g). Four diabetes and four lean controls were tested beginning at 8 weeks of age (lean, 17 to 19 g; diabetes, 30 to 32 g) and again at 23 weeks of age (lean, 23 to 25 g; diabetes, 51 to 67 g). All of the mice were of the 6J strain.

Table 3
Age and Practice Effects on Response to Food Deprivation in KsJ Mice

N Age* Group			1-h Food Intake				23-h Food Intake				24-h Weight Gain			
			Test 1		Test 2		Test 1		Test 2		Test 1		Test 2	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
8	5-6	Control	.9	.2	1.1	.2	5.6	1.0	5.9	1.6	3.5	.4	3.0	.3
		Diabetes	1.0	.4	1.3	.3	7.9	2.4	9.0	1.7	3.0	.3	3.3	.6
6	13-15	Control	.9	.2	1.0	1.0	4.4	.4	5.6	1.1	3.2	.4	3.1	.7
		Diabetes	.6	.3	.8	.8	5.0	1.1	7.8	3.2	1.0	.7	2.1	.3
4	21	Control	.8	.1	1.3	.4	4.3	.3	4.9	.4	3.8	.7	3.6	.3
		Diabetes	.5	.3	1.4	1.4	5.2	1.1	7.8	2.2	1.7	.6	2.3	.4

*Age in weeks.

Table 4
Age and Type of Obesity Effects on Response to Food Deprivation in 6J Mice

N	Group		1-h Food Intake*				23-h Food Intake				24-h Weight Gain			
			Younger		Older		Younger		Older		Younger		Older	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
4	Diabetes	Control	1.1	.3	1.1	.1	5.0	.3	4.8	.5	4.4	.4	4.8	.3
		Diabetes	1.1	.1	.8	.2	4.6	1.2	5.3	1.9	2.9	.2	3.6	.5
4	Obese	Control	1.0	.2	1.3	.1	4.6	.7	4.6	.5	3.6	.8	4.4	.6
		Obese	1.1	.1	.6	.1	5.4	.9	4.8	.5	3.4	.5	3.0	.2

*Each number is the mean of two tests.

Procedure. The subjects were food deprived twice at the younger age (6 or 8 weeks of age) using the same procedure as in Experiment 2. They were then group housed for 12 weeks and then tested twice more in the same fashion. Thus, each mouse was food deprived a total of four times.

Statistical analysis. The same measures were analyzed as in Experiment 2, except that trials within an age group (younger or older) were averaged.

Results

The results are summarized in Table 4. One of the diabetes mice spilled large amounts of food after the first hour following food deprivation. Young genetically obese mice consumed as much food as lean mice in the first hour following food deprivation, but old genetically obese mice consumed less food than lean mice in each case (obese, $p < .005$; diabetes, $p = .055$). The same relationship appeared for weight gain ($p < .05$) and 23-h food intake ($p = .06$) in obese mice but not in diabetes mice ($p > .15$). Thus, the deficit in food intake and body weight regulation may be more severe in obese 6J mice and in diabetes KsJ than in diabetes 6J mice.

GENERAL DISCUSSION

On the basis of parabiosis experiments, Coleman (1973) has suggested that obese mice lack and diabetes mice are unresponsive to some substance which regulates food intake. His hypothesis led us to predict that obese and diabetes mice exhibit similar behavior. The present experiments support this prediction for compensation for surplus calories and short-term (1 h) recovery from food deprivation. Experiment 3 suggested that there may be a difference between obese and diabetes mice of the C57BL/6J strain in long-term (23 h) recovery from deprivation. A second difference between these two mutants is more difficult to understand. In nearly all of our experiments, obese mice spill little or no food while diabetes mice spill excessive amounts of food. Other researchers have noticed this as well (D. L. Coleman and B. Moisset, personal communications).

The present experiments provide a valuable clue about the origin of one of the behavioral disturbances in diabetes and obese mice. Some authors (e.g., Schachter & Rodin, 1974) have argued that low

responsiveness to hunger in obese animals is indicative of a motivational disturbance which leads to overeating. We (Ramirez & Sprott, in press) and others (e.g., Sclafani, 1976) have argued that low responsiveness to hunger is a result of obesity. Ramirez and Sprott (in press) have previously reported that obese mice which are allowed to eat for only 30-60 min a day, initially eat less than lean controls but gradually increase their food intake until it does not differ from that of lean control mice. They ruled out learning as a possible explanation and hypothesized that this result was due to the substantial weight loss in the obese mice. If this explanation is correct, young obese and diabetes mice should not show as much of a deficit in food intake and weight gain following food deprivation as older heavier obese and diabetes mice. The present results confirmed this prediction; low responsiveness to hunger appeared only after the mice were extremely obese. It is likely that the results of other experiments with genetically obese mice (e.g., Fuller, 1972; Sclafani, 1976) may be attributable to overweight. This complicates the interpretation of experiments with overweight animals but may eventually lead to a deeper understanding of adaptation to altered physiological conditions such as overweight.

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