Diabetologia 9, 287–293 (1973)

The Influence of Genetic Background on the Expression of the Obese (*Ob*) Gene in the Mouse*

D. L. Coleman and K. P. Hummel

The Jackson Laboratory** Bar Harbor, Maine 04609

Received: January 23, 1973, accepted: April 13, 1973

Summary. A new congenic strain of obese mice, C57BK/KsJ-*ob*, has been developed for comparison with the C57BL/6J-*ob* congenic strain. While obese mice of both strains are characterized by obesity, hyperphagia, and hyperglycemia, the C57BL/Ks obese mice have severe diabetes, marked hyperglycemia, temporarily elevated plasma insulin concentrations, and typical degenerative changes in the islets of Langerhans. In contrast, the C57BL/6J obese mice have mild hyperglycemia and marked hyperinsulinemia coupled with hypertrophy and hyperplasia of the islets of Langerhans. The severe diabetic condition produced by obese (*ob*) on the C57BL/KsJ background is similar, if not identical, to that produced by the diabetes (*db*) gene on the same background. The metabolic disorder produced by these mutations is associated with the capacity of the islets to respond to an increased demand for insulin. The islet response, whether atrophy or hypertrophy, appears to be due to the interaction of the obese and diabetes genes with modifiers in the genetic background rather than the specific consequences of the particular gene. The markedly different diabetic syndromes that result when the obese mutation is on different genetic backgrounds emphasize the importance of strict genetic control in studies with obese-hyperglycemic mutants.

Key words: Diabetes, obesity, modifying genes, genetics, hyperglycemia, islet changes, mice.

Introduction

The obese mutant mouse (*ob/ob*) discovered at the Jackson Laboratory and first described in 1950 [7] has been studied extensively both in the non-inbred heterogeneous stock in which it originated and in the congenic inbred strain, C57BL/6J-*ob*, that was developed by transferring the *ob* gene to the C57BL/6J (BL/6) inbred strain. In the homozygous state this mutation produces marked obesity coupled with overeating, hyperglycemia, hyperinsulinemia, marked insulin resistance and infertility [1, 11]. Because of genetic variability in the non-inbred stock, comparison of results of early studies with later studies in which mice of the BL/6-*ob* strain were used, is difficult and often confusing. Early reports [1, 12] describe the development of hyperglycemia by 9 weeks of age with the maximum blood sugar concentration of over 300 mg/100 ml being reached by 12 weeks of age and remaining at this concentration thereafter. In contrast, obese (*ob/ob*) mice of the BL/6-*ob* strain are characterized by moderate hyperglycemia rarely reaching 300 mg per 100 ml and then only transiently during the period from 8 to 12 weeks, after which the blood sugar concentration returns to near normal levels [3, 5, 6]. The decrease in blood sugar concentration is associated with a dramatic increase in plasma insulin concentration and a marked increase in the number and size of the islets of Langerhans [3, 13]. The elevated plasma insulin concentration [3] (25—50 times normal) persists throughout a nearly normal lifespan although a modest decrease in circulating insulin (from over 4000 to about 3000 μU/ml) is associated with advancing age.

A stock of obese mice, maintained in Sweden and commonly called American-obese (AO), was established in 1959 with breeding stock obtained from the Jackson Laboratory colony prior to the transfer of the gene to the BL/6 inbred strain. This stock is characterized by such massive hypertrophy and hyperplasia of the islet cells that the islets can be readily microdissected from the pancreatic tissue. This feature has made these mice invaluable in fundamental studies involving the metabolism of individual islets and factors regulating insulin secretion [4]. The development of the disease syndrome in these mice and a critical discussion of some of the differences between this stock and those used by others can be found in a comprehensive review by Westman [14]. In most other respects these AO obese mice have clinical characteristics similar to obese mice of the BL/6-*ob* strain. Moderate hyperglycemia is observed only transiently in the early developmental period, after which marked hyperinsulinemia coupled with euglycemia is typical. Again the degree of hyperinsulinemia in the AO obese mice decreases in the period from 6 months until death [14].

Another stock of obese mice, being propagated in France [8] is characterized by marked hyperglycemia (> 400 mg/100 ml) while yet another non-inbred stock is reported to have degenerative changes in the pancreas and duct cell metaplasia [9], features not described in BL/6 obese mice.

* Supported in part by NIH Research Grants AM 14461 from the National Institute of Arthritis, Metabolism, and Digestive Diseases; CA 05873 from the National Cancer Institute and allocations from the South Waite Foundation and the Virginia and D.K. Ludwig Foundation.

** The Jackson Laboratory is fully accredited by the American Association for Accreditation of Laboratory Animal Care.

The variability in clinical expression of the obese gene in the original non-inbred stock led Mayer and Silides [10] to suggest that the time of onset and degree of hyperglycemia was conditioned by the presence or absence of modifying genes that influence the expression of the obese gene. Others [14] have suggested that differences in environmental factors such as diet could account for the diversity of results. Our interest in the influence of genetic background on the expression of obesity genes originated with the observation that the expression of the diabetes-2^J (db^{2J}) allele, which also causes obesity and hyperglycemia, was different depending on the genetic background [6]. Diabetic mice of the congenic inbred strains C57BL/Ks-*db* [2] and C57BL/Ks-db^{2J} [6] have severe diabetes characterized by hyperphagia, obesity, marked hyperglycemia, temporarily elevated plasma insulin concentrations and typical degenerative changes in the islets of Langerhans. In contrast, diabetic mice of the BL/6-db^{2J} inbred strain, although hyperphagic and obese, have a milder disease syndrome, characterized by transitory hyperglycemia, and severe hyperinsulinemia, coupled with hypertrophy of the islets of Langerhans and increased proliferative capacity of the β-cells [6]. Because this mild diabetic syndrome is so remarkably similar to that of obese mice of the BL/6-*ob* strain it seemed possible that the expression of obese on the C57BL/KsJ (BL/Ks) background might be similar to that of diabetes on the BL/Ks background. To assess this possibility we established the obese gene on the BL/Ks background for comparison with obese on the BL/6 background and with diabetes on the BL/Ks background. Preliminary evidence that modifiers in the BL/Ks genotype alter the expression of obese homozygotes has been reported [6].

Materials and Methods

Obese (*ob*/*ob*) mice of two different congenic inbred strains, C57BL/6J-*ob* (BL/6-*ob*) and C57BL/KsJ-*ob* (BL/Ks-*ob*), were born, raised and maintained in our colony to ensure that all environmental conditions were as uniform as possible. The BL/6 obese mice were born to heterozygous parents procured from the Jackson Laboratory Production Department. The BL/6-*ob* congenic strain is maintained by continuous cycles of cross-intercross matings and the parental mice used by us were all offspring of more than 20 such cycles. The BL/Ks obese mice were born to heterozygous parents from our congenic inbred strain, BL/Ks-*ob*, which we had established by 5 cycles of cross-intercross breeding. Controls were male and female mice of inbred strains BL/6 and BL/Ks. All mice were housed in stainless steel pens on pine shavings with pelleted food (19% protein, 6% fat, Emory Morse Company, Guilford, Conn.) and tap water available at all times.

The experimental mice consisted initially of 12 males and 12 females from each strain. Body weights and non-fasted blood sugar concentrations were recorded weekly from weaning age at 3—4 weeks through 6 months of age. At monthly intervals, starting at 4 to 6 weeks, four obese (one male and one female from each group) were bled for insulin assays and killed. Sections of pancreas, liver, and kidney were saved and processed for histological examination. Analytical and histological procedures were as previously described [2].

Results

Control Mice

No differences between normal mice of the BL/Ks and BL/6 inbred strains were observed. Blood sugar concentrations ranged from 140 to 180 mg/100 ml and plasma insulin concentrations from 30 to 100 μU/ml. Typically, female mice had slightly lower blood sugar and plasma insulin concentrations. Body weights increased with age but rarely exceeded 35 g. Islet morphology in normal mice of the two strains was identical and was as previously described [2].

Obese Mice

Table 1 shows the weight, blood sugar and plasma immunoreactive insulin changes with age in obese mice of the two strains. Increases in weight were essentially the same for the first three months. The most rapid post-weaning weight increase occurred between the first and second months when body weight doubled. Thereafter, BL/6 obese mice continued to gain slowly throughout the experimental period and all weighed between 60 and 70 g by 10 months of age, when the experiment was terminated. In contrast, BL/Ks obese mice attained maximum weights of 45 to 55 g at 3 months for males and 4 months for females. After this age, most BL/Ks obese mice lost weight slowly for the rest of their lives.

Blood sugar concentrations were normal at weaning (3 to 4 weeks) in obese mice of both strains (Fig. 1 and Table 1). During the next few weeks, the blood sugar concentrations rose rapidly in both genotypes, reaching a peak in BL/6 obese mice sometime between 2 and 3 months and then gradually decreasing to within the normal range by 4 to 5 months. After this transient period of hyperglycemia, most BL/6 obese mice remained either euglycemic or hypoglycemic for the rest of their lives. Blood sugar concentrations exceeding 300 mg per 100 ml were uncommon in BL/6 obese mice. However two mice, both males, attained values higher than this and sustained these high values until death. The data for these two atypical mice are not included in Table 1 and Fig. 1.

In BL/Ks obese mice the blood sugar concentrations continued to rise and did not return to normal. An average concentration of over 400 mg per 100 ml was attained by 2 months in males and by 4 months in females. The blood sugar concentrations typically remained quite stable after reaching concentrations between 400 and 500 mg per 100 ml. Glycosuria and polyuria were common symptoms. This sustained hyperglycemia was not only in distinct contrast to the transient hyperglycemia typical of BL/6 obese mice but was similar to that observed in BL/Ks diabetic

(*db/db*) mice (Fig. 1). There was one atypical female in which the disease progressed as in BL/6 obese mice, with blood sugar values that gradually returned to within the normal range. The data obtained for this atypical female are excluded from Table 1 and Fig. 1.

In the early stages of the syndrome plasma insulin concentrations were similar in obese mice of both genotypes being elevated from 4 to 10 times normal (Table 1). However, insulin secretion or synthesis, or both, increased rapidly in BL/6 obese mice and plasma insulin concentrations rose to over 50 times normal by 2 to 3 months of age. This dramatic increase in circulating insulin appeared to be sufficient to restore the blood sugar concentrations to near normal values. In contrast, circulating insulin concentrations in BL/Ks obese mice did not increase beyond those observed in the first 2 months after which they fell to near normal levels at the same time that a further elevation in blood sugar concentrations occurred. A similar drop in plasma insulin concurrent with an increase in blood sugar concentration was observed in BL/Ks-*db/db* mice [2, 6].

Table 1. *Average weights and blood sugar concentrations, and immunoreactive insulin concentrations of C57BL/6J-ob/ob and C57BL/KsJ-ob/ob mice*

Strain	Age (mo.)	Mice		Weight g ± SE	Blood sugar mg/100 ml ± SE	Insulin	
		Sex	No.			No.	μU/ml
BL/6-*ob*	1	♀	12	19.6±0.7	164± 9	1	230
		♂	10	20.3±1.2	183±21	1	400
	2	♀	11	42.5±1.0	226±19	1	2630
		♂	9	39.8±1.5	230±20	1	3250
	3	♀	10	52.2±1.5	193±26	1	425
		♂	8	50.8±1.3	247±29	1	2380
	4	♀	9	55.8±1.6	195±25	1	3000
		♂	7	55.8±1.5	184±32	1	2880
	5	♀	8	57.5±2.0	177±32	1	1420
		♂	6	58.5±1.4	184±39	1	3740
	6	♀	7	59.9±2.2	186±37	2	1200
		♂	5	59.8±1.6	187±40	2	2520
BL/Ks-*ob*	1	♀	11	18.7±0.8	187±12	1	400
		♂	12	19.9±1.4	198±13	1	650
	2	♀	10	45.1±1.3	328±27	1	450
		♂	11	45.0±1.1	407±17	1	275
	3	♀	9	52.5±1.9	352±28	1	175
		♂	9	47.5±1.7	429±21	1	150
	4	♀	8	55.4±3.0	384±15	1	330
		♂	7	46.7±2.4	434±20	1	35
	5	♀	7	53.6±3.6	409±16	1	130
		♂	6	45.8±2.8	447±12	1	45
	6	♀	6	49.0±3.7	427±20	2	88
		♂	5	44.0±3.7	441±26	1	175

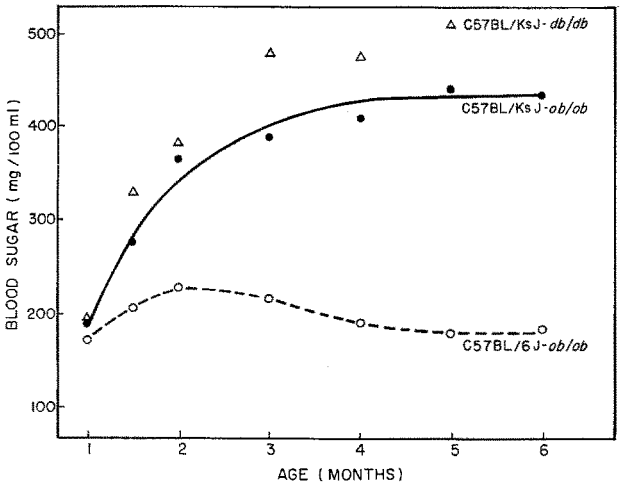


Fig. 1. Average blood sugar concentrations in obese (*ob/ob*) and diabetic (*db/db*) mice as functions of age and background genotype. Each point represents the average value obtained from at least five mice of each sex and genotype

The ability to sustain abnormally high concentrations of circulating insulin is directly related to the length of the period of weight increase and to the ultimate lifespan as well. BL/6 obese mice maintained plasma insulin concentrations at high levels and continued to gain weight throughout life. None had died by 10 months when the remaining mice (5 females and 3 males) were killed for insulin assay and histological examination. At the time of sacrifice (10 months) the BL/6 obese mice were still gaining weight, the plasma insulin concentrations were all well above 1000 μU per ml and no significant decreases in the plasma insulin concentrations had, as yet, become apparent. Persistent hypoglycemia (BS < 120 mg/100 ml) was observed in most of these older mice. In contrast, BL/Ks obese mice attained maximum plasma insulin concentrations during the period of most rapid accumulation of fat (1 to 2 months) and as the plasma insulin concentrations decreased to near normal these obese mice began to lose weight and died prematurely. None

survived to 10 months and by 6 months all survivors were losing weight; 2 males had died by 3 months, 2 more by 6 months, and 1 female died at 7 months.

The morphological changes observed in the islets of Langerhans are consistent with the clinical development of the obese-hyperglycemic syndrome. Increases in size and number of islets and some degranulation of β cells were apparent by 1 month of age in obese mice of both strains. There were, however, more and larger islets with less β cell degranulation in the BL/6 obese compared with the BL/Ks obese mice. Differences in islet morphology and in β cell degranulation were even more apparent at 2 months of age. At this age the BL/6 obese islets were not only more numerous and larger but contained dilated and congested sinusoids indicative of hyperactivity (Fig. 2). BL/Ks obese islets were not as large, β cells were more extensively degranulated, and early signs of degenerative changes such as inclusion of ducts and acinar cells were apparent in many islets (Fig. 3).

With increasing age there was a further increase in number and size of islets of BL/6 obese mice, whereas islets of BL/Ks obese mice became smaller and more atrophic. The characteristic features of the BL/6 obese hypertrophied islets can be seen in Fig. 4 (a portion of an islet of a 6 month old *ob/ob* male). Sinusoids are dilated and β cells somewhat degranulated. Aging and degenerative changes typical of islets of BL/Ks obese mice are depicted in Fig. 5, an islet of a 6 month old *ob/ob* male. The conspicuous features are the small size, marked degranulation of β cells, and the inclusion of ductral structures.

These morphological differences in islets of obese mice on the two backgrounds are remarkably similar to the differences observed in islets of diabetes (db^{2J}/db^{2J}) mice on the BL/6 and BL/Ks backgrounds. Islet hypertrophy with enlargement of sinusoids and β cell hyperplasia was observed in BL/6-db^{2J}/db^{2J} mice (Fig. 6) while islet atrophy with few granulated β cells and the inclusion of acinar and ductal elements were characteristics of BLKs-db^{2J}/db^{2J} mice (Fig. 7).

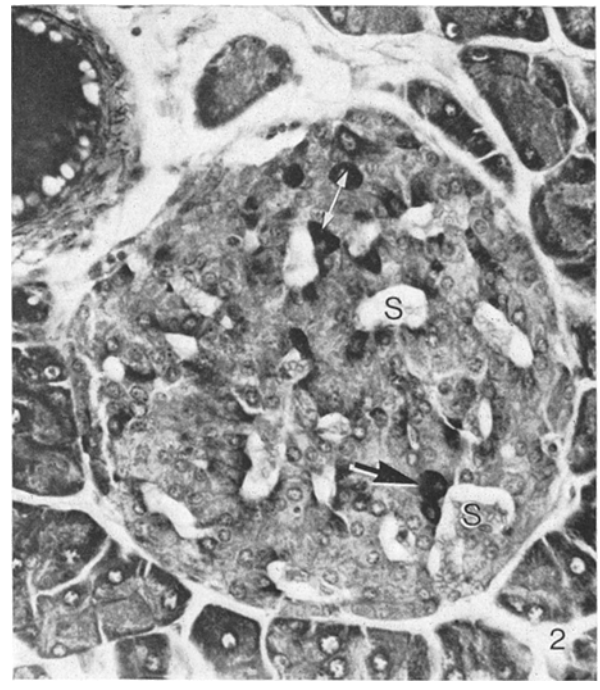


Fig. 2. Islet of BL/6-*ob/ob* ♂ aged 2 months. Blood sugar = 328 mg/100 ml IRI = 3,250 μU/ml. Note dilated sinusoids (S) and arrows to granulated β cells. Aldehyde fuchsin stain × 300

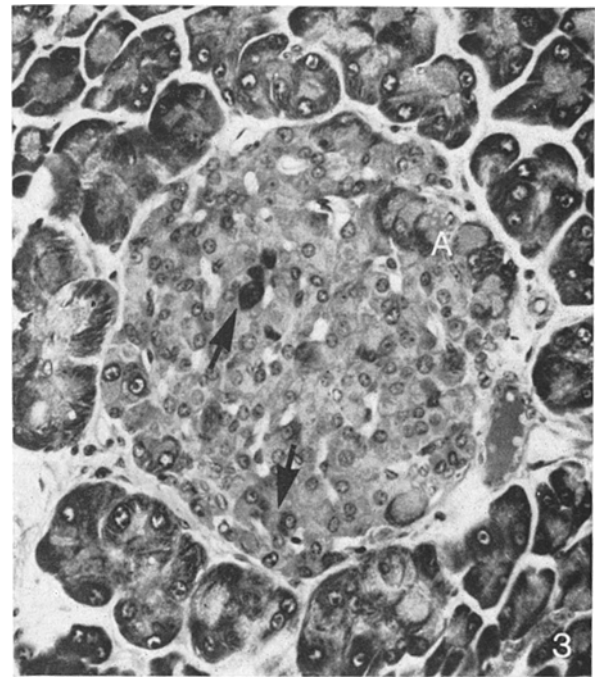
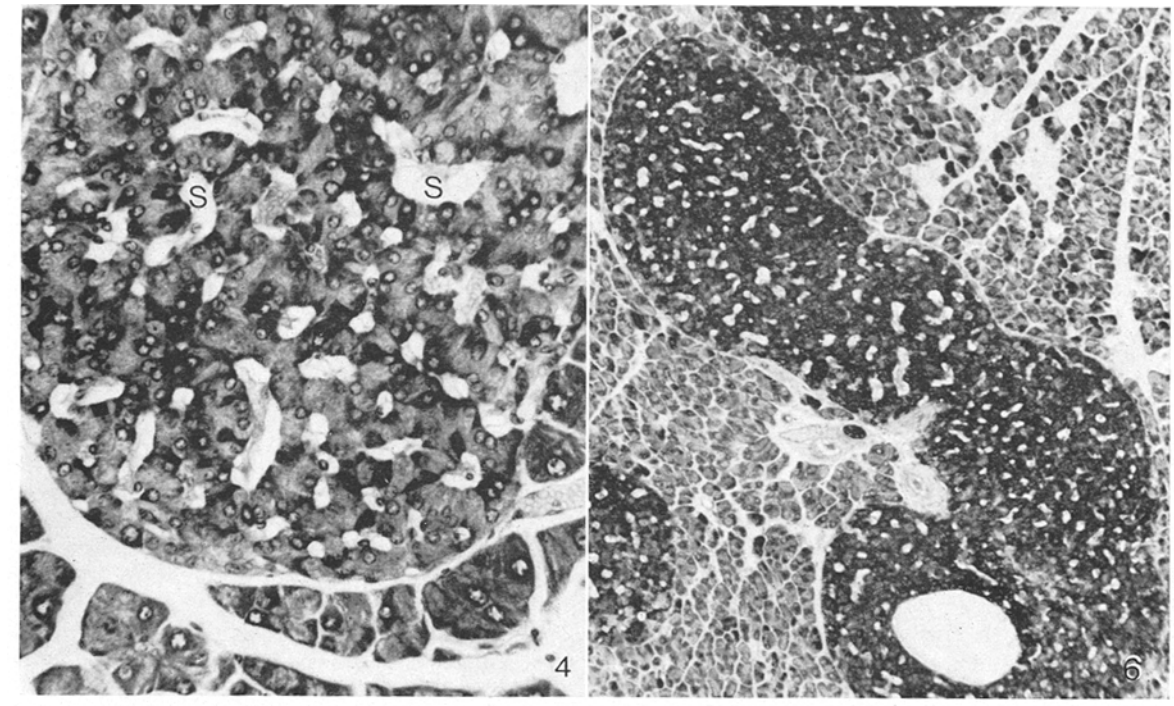


Fig. 3. Islet of BL/Ks-*ob/ob* ♂ aged 2 months. Blood sugar = 407 mg/100 ml IRI = 275 μU/ml. Note inclusion of clump of acinar cells (A) and arrows to granulated β cells. Aldehyde fuchsin stain × 300

Discussion

The metabolic disorder caused by the obese (*ob*) gene on the BL/6 background is distinctly different from that caused by the same gene on the BL/Ks background. The clinical features are similar in the early developing stages at the time when the symptoms include hyperphagia, rapid accumulation of adipose tissue, hyperinsulinemia, and hyperglycemia (Table 1 and Fig. 1). In the later stages, beginning at about 3 months of age, BL/Ks obese mice are characterized by severe hyperglycemia, a return to near normal plasma insulin concentrations and little or no further weight gain. Many die before 6 months of age. In contrast, BL/6 obese mice remain hyperinsulinemic throughout life and continue to gain weight as long as they live. They do not become severely diabetic although many



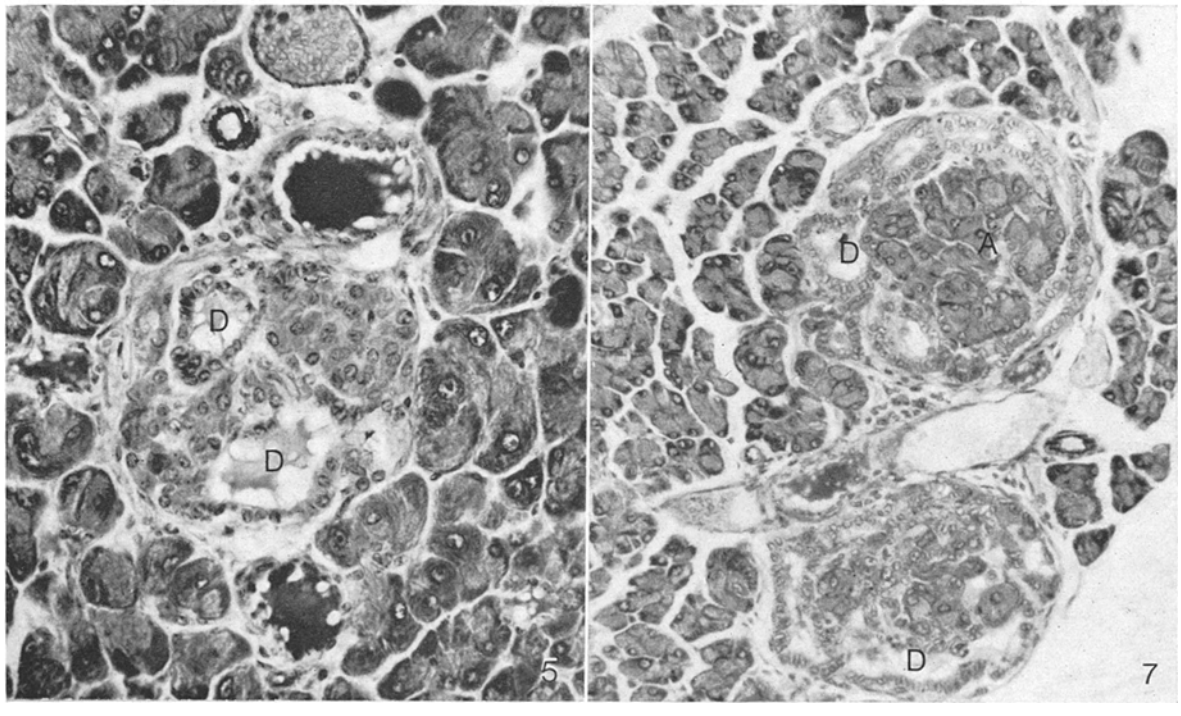


Fig. 4. Portion of an islet of BL/6-*ob*/*ob* ♂ aged 6 months. Blood sugar = 143 mg/100 ml. IRI = 4,700 μU/ml. Note dilated sinusoids (S) and numerous well granulated β cells (black in photograph). Aldehyde fuchsin stain × 300

Fig. 5. Islet of BL/Ks-*ob*/*ob* ♂ aged 6 months. Blood sugar = 518 mg/100 ml. IRI = 175 μU/ml. Note inclusion of ducts (D) and scarcity of granulated β cells. Aldehyde fuchsin stain × 300

Fig. 6. Group of hypertrophied islets of BL/6-db^{2J}/db^{2J} aged 6 months. Blood sugar = 110 mg/100 ml. IRI = 1,250 μU/ml. Note dilated sinusoids and well granulated β cells. Aldehyde fuchsin stain × 100

Fig. 7. Atrophic islets of BL/Ks-db^{2J}/db^{2J} aged 5 months. Blood sugar = 430 mg/100 ml. Note inclusion of acinar cells (A) and ducts (D) and absence of granulated β cells. Aldehyde fuchsin stain × 200

go through a transient period of mild hyperglycemia at about 2 to 3 months of age. Consistent with these clinical differences are striking differences in the islets of Langerhans. In BL/6 obese mice there is a marked increase in both size and number of the islets (Fig. 4), whereas in BL/Ks obese mice there is a typical degenerative atrophy of the islets (Fig. 5). These differences in islet response are direct reflections of the clinical features of the disorder. Normal plasma insulin, high blood sugar concentration, and severe diabetes with shortened lifespan are associated with islet atrophy (BL/Ks obese), whereas extremely high plasma insulin, with mild or transient hyperglycemia are associated with islet hypertrophy (BL/6 obese).

The expression of diabetes (db^{2J}), when maintained on these two different inbred backgrounds [6] is strikingly similar to the expression of obese (*ob*) on the same two genetic backgrounds. Hyperinsulinemia, continued weight increase, transitory hyperglycemia and morphological changes indicative of β cell hyperactivity (Fig. 2, 4, 6) are characteristic of both *ob*/*ob* and *db*/*db* mice on the BL/6 background. Marked hyperglycemia, transitory hyperinsulinemia and morphological changes indicative of loss of capacity of β cells to secrete insulin (Fig. 3, 5, 7) are characteristic of both *ob*/*ob* and *db*/*db* mice on the BL/Ks background. The clinical data and islet morphology suggest that synthesis and secretion of insulin are reduced prematurely in both obese (*ob*/*ob*) and diabetes (*db*/*db*) on the BL/Ks background and enhanced in obese and diabetes on the BL/6 background. In young BL/Ks-*ob*/*ob* and BL/Ks-*db*/*db* mice the β cells are extensively degranulated whereas the numerous enlarged islets of BL/6 mutants contain many well granulated β cells as well as other indications of rapid synthesis and secretion of insulin. The contrast in islet morphology associated with background genotype becomes even more evident in older mice where severe atrophy is typical of islets from both mutants on the BL/Ks background (Fig. 5, 7) and hypertrophy is typical of islets of both on the BL/6 background (Fig. 4, 6).

The striking similarity of the metabolic disorders produced by the two genes (*ob* and *db*) suggests that many, if not all, of the features result not from the presence of these mutant genes themselves but from their interaction with the host genomes. It must be emphasized that no differences in islet morphology have been observed in islets of normal BL/6 and BL/Ks mice and that islet differences are observed only when the mutant genes interact with modifying genes associated with the background genotype. The data presented here indicate that mice homozygous for either the obese or diabetes genes respond in two divergent fashions, the basic difference being that BL/6 obese and BL/6 diabetes mice have the capacity to expand insulin supply indefinitely, whereas the BL/Ks obese and diabetes mice have a limited capacity and cannot meet the demand for an ever-increasing supply of insulin.

Strain BL/6-*ob* has been produced by many cycles of cross-intercross matings, making it unlikely that any residual modifiers from the stock of origin remain in the genome. Thus the occasional atypical response in this strain may be attributable to interaction of the obese gene with unidentified environmental factors. On the other hand, residual BL/6 modifying genes remaining in the BL/Ks-*ob* stain genome after only 5 cycles of cross-intercross breeding, as well as environmental factors, could account for any atypical response in obese of this strain. Uterine environment, as well as availability of food and water, type of food, and other external conditions which are difficult to control completely may be involved. The number of modifying genes is unknown but since the BL/Ks and BL/6 inbred strains are thought to be closely related there is a possibility that only a small number of genes are involved. Since the present studies provide added evidence for the existence of different modifying genes in the two strains we hope to be able to isolate these genes and establish their nature.

These studies emphasize the importance of strict genetic control in attempts to establish the causes of genetic diseases such as obesity and diabetes. We have found that genetic modifiers in two strains, BL/Ks-*ob* and BL/6-*ob*, have different and fairly consistent effects on the expression of both the obese and diabetes genes. We can assume that modifiers in non-inbred stocks will be different from those of inbred strains and also will not be uniformly present. This emphasizes the importance of accuracy of statements regarding the source of any obese mice used in experiments. The fact that the original source of all obese mice was the Jackson Laboratory does not justify the use of terms such as Bar Harbor obese, American obese, and Jackson Laboratory obese in designating the mice used. Such nomenclature implies that the mice were secured directly from the Jackson Laboratory and leaves uncertain their immediate source, as well as whether they were derived from a non-inbred stock or from strain C57BL/6J-*ob*, and how many years or generations they may have been bred in some other laboratory. To avoid confusion we recommend that published reports use the gene name (obese) and symbol (*ob*) and state clearly the immediate source and genetic background on which the mutant is maintained.

Acknowledgements. The skilled technical assistance of Mrs. Dorothy B. Chapman and Mr. R.H. Copp is gratefully acknowledged.

References

1. Bleisch, V. R., Mayer, J., Dickie, M. M.: Familial diabetes mellitus in mice associated with insulin resistance, obesity, and hyperplasia of the islets of Langerhans. Amer. J. Path. **28**, 369–385 (1952).
2. Coleman, D.L., Hummel, K.P.: Studies with the mutation, diabetes, in the mouse. Diabetologia **3**, 238–248 (1967).

3. Genuth, S.M., Przybylski, R.J., Rosenberg, D.M.: Insulin resistance in genetically obese hyperglycemic mice. Endocrinology **88**, 1230—1238 (1971).
4. Hellman, B.: Methodological approaches to studies on the pancreatic islets. Diabetologia **6**, 110—120 (1970).
5. Herberg, L., Major, E., Hennigs, U., Grunecklee, D., Freytag, G., Gries, F.A.: Differences in the development of the obese-hyperglycemic syndrome in *ob/ob* and NZO mice. Diabetologia **6**, 292—299 (1970).
6. Hummel, K.P., Coleman, D.L., Lane, P.W.: The influence of genetic background on expression of mutations at the diabetes locus in the mouse. Biochem. Genet. **7**, 1—13 (1972).
7. Ingalls, M., Dickie, M.M., Snell, G.D.: Obese, a new mutation in the house mouse. J. Hered. **41**, 317—318 (1950).
8. Lemonnier, D., Winand, J., Furnelle, J., Christophe, J.: Effect of a high-fat diet on obese-hyperglycemic and non-obese Bar Harbor mice. Diabetologia **7**, 328—333 (1971).
9. Lever, J.D., Findlay, J.A.: Histological observations on the pancreas of the genetically obese mouse (Birmingham strain) with particular reference to the islets of Langerhans. J. Anat. **108**, 620 Abst. (1971).
10. Mayer, J., Silides, D.N.: A quantitative method of determination of the diabetogenic activity of growth hormone preparations. Endocrinology **52**, 54—56 (1953).
11. Mayer, J., Bates, M.W., Dickie, M.D.: Hereditary diabetes in genetically obese mice. Science **113**, 746—747 (1951).
12. Mayer, J., Russell, R.E., Bates, M.W., Dickie, M.M.: Metabolic, nutritional and endocrine studies of the hereditary obesity-diabetes syndrome of mice and mechanism of its development. Metabolism **2**, 9—21 (1953).
13. Stauffacher, W., Lambert, A.E., Vecchio, D., Renold, A.E.: Measurements of insulin activities in pancreas and serum of mice with spontaneous (obese and New Zealand obese) and induced (gold thioglucose) obesity and hyperglycemia. Diabetologia **3**, 230—237 (1967).
14. Westman, S.: Development of the obese-hyperglycemic syndrome in mice. Diabetologia **4**, 141—149 (1968).

Dr. D.L. Coleman
The Jackson Laboratory
Bar Harbor
Maine 04609
USA