Spontaneous mutation in the db gene results in obesity and diabetes in CD-1 outbred mice

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Department of Zoology, North Carolina State University, Raleigh 27695-7617; Department of Genetics, North Carolina State University, Raleigh, North Carolina 27695-7614; and Columbia University, R. Berrie Pavilion, New York, New York 10032

Brown, Julie A., Streamson C. Chua, J R., Shun Mei Liu, Matthew T. Andrews, and John G. Vandenbergh. Spontaneous mutation in the db gene results in obesity and diabetes in CD-1 outbred mice. Am. J. Physiol. Regulatory Integrative Comp. Physiol. 278: R320–R330, 2000.—Five allelic mutants of the diabetes (db) gene have been previously described in mice and rats causing obesity, infertility, and varying degrees of diabetes. We have identified a new, spontaneous mutation resulting in obesity and diabetes in a colony of CD-1 outbred mice, Mus musculus domesticus. Genetic complementation studies indicated that the new mutation was an allele of the diabetes locus. Sequence analysis of cDNA fragments showed a deletion of one G residue located in exon 12 of the leptin receptor gene. The mutation, Lepr\textsuperscript{db-NCSU}, results in a frameshift and reduces Lepr transcript levels 10-fold. Mutant mice drank up to four times more water and were up to two times heavier than wild-type mice. Blood glucose and plasma insulin and leptin concentrations were sexually dimorphic among affected mice, suggesting an effect of sex steroids. Mortality of affected males was 100% by 5 mo, whereas affected females survived up to 10 mo of age.

leptin; phenotype; mouse; insulin

SEVERAL GENETIC MUTATIONS causing obesity and diabetes syndromes in mice have been described (2, 9, 13, 18, 20). The increasing prevalence of these diseases in the human population makes the genetic and phenotypic characterizations of these mutant animals a leading area of interest in the biomedical community. The potential to clarify the genetic and physiological bases of obesity and some forms of diabetes was significantly enhanced by the molecular characterization of the obese (ob) and diabetes (db) genes. Well characterized in the late 1960s and early 1970s, mice with ob and db mutations are obese and often diabetic depending on diet and strain background (13, 14, 18). On the basis of several elegant parabiosis experiments, Coleman (12) hypothesized that ob mutants failed to produce a circulating satiety factor to which db mutants failed to respond.

In recent years, the “satiety factor” in question has been identified. The recently cloned ob gene is expressed in adipose tissue and codes for leptin, a protein that serves as an appetite-regulating signal (15, 36). The effects of leptin, however, are many, and its description as an appetite regulator is an oversimplification. Ahima et al. (1) proposed that leptin’s primary role is, in fact, endocrine regulation during periods of food deprivation; i.e., leptin acts mainly in response to starvation, rather than obesity. Leptin also plays a significant role in fertility. Both ob and db mutants are infertile. However, when treated with exogenous leptin, ob mice regain fertility. Leptin seems to influence reproduction in wild-type (WT) mice as well. Leptin injections accelerated reproductive maturity in WT prepubertal female mice and rats (5, 7). Cheung et al. (7) suggested that leptin acts as a metabolic gate, allowing maturation to proceed only when metabolic resources are adequate.

The presence and severity of overt diabetes in these obese mutants seem to be largely dependent on the genetic background on which the mutation appears. Leptin is directly involved with insulin production via its interactions with neuropeptide Y (NPY), a neurotransmitter produced in the arcuate nucleus of the hypothalamus that stimulates appetite and insulin secretion from pancreatic beta cells (30, 31, 33). Leptin normally holds NPY levels in check, and mice with an effective lack of leptin may lack the inhibitory regulation present in normal animals. Because diabetic symptoms vary greatly among strains, other genes seem to play important interacting roles as well.

The db gene is expressed in the brain and several other tissues and codes for the leptin receptor (Lepr; Ref. 6). Thus leptin and its receptor normally provide an internal signaling system regulating body weight and glucose metabolism in mice. Five extant allelic mutants of the Lepr gene have been identified in inbred mice and rats (2, 10, 13, 18, 32). Here we report the identification and phenotypic characterization of a new spontaneous mutation of the db gene, Lepr\textsuperscript{db-NCSU}, in an outbred CD-1 mouse.

METHODS

Animals. The mice used in this study were derived from Charles River Laboratories outbred CD-1 strain. All animals were individually housed, except when paired, in 28 × 18 cm clear cages at ~23°C and kept on a 12:12-h light-dark cycle.
Breeding experiments. To determine whether the spontaneous obese/diabetic phenotype was the result of a mutation, sibling matings were conducted within a family showing such traits. All animals were paired for 2 wk, and females were examined daily for the appearance of vaginal plugs. All obese animals from the original litter were paired with unaffected siblings. The lean male was mated sequentially to his unaffected sisters.

Mice heterozygous for the db and ob mutations were obtained from the Jackson Laboratory. C57BL/ksj db heterozygotes and C57BL/6j ob heterozygotes were paired with possible lean heterozygotes from our original litter.

Genomic DNA preparation. Genomic DNA was prepared from the brain and liver of mutant and WT mice according to the protocol described in unit 2.2 of Ausubel et al. (3). Total RNA from normal and affected mice was prepared by a modification of the method developed by Chomczynski and Sacchi (8) where brain tissue was homogenized in 4 M guanidinium isothiocyanate followed by addition of sodium acetate to a final concentration of 0.2 M. Samples were extracted by water-saturated phenol and chloroform-isoamyl alcohol (49:1) followed by two extractions with phenol-chloroform-isoamyl alcohol (50:49:1). All extractions were centrifuged through Phase Lock Gel (5′→3′ Prime). The RNA was precipitated with isopropanol and quantified by absorption spectrophotometry. Other molecular biological techniques used (cDNA synthesis, PCR amplification, and sequence analysis) have been previously described (35).

Assay for the detection of the db-NCSU mutation. The db-NCSU mutation can be positively identified in genomic DNA by amplification with a pair of primers: NCSU-Taq (AGA AAA ATG GAT GGG GAC GTT ACT TCG) and Ex12R (GTC AGC TCT GAC AAC CAC AT). Amplification is performed with a hot-start modification of a standard 20 µl PCR reaction [in mM: 50 KCl, 10 Tris (pH 9), 0.1% Tween 20, 2 MgCl2, 0.2 dNTPs, and 0.2 spermidine, and 100 ng genomic DNA and 50 ng of each primer] with 0.5 U Taq DNA polymerase and 35 cycles (94, 55, and 72°C sequentially at 30 s for each plateau). After the amplification, 10 µl of the amplification product is digested with 2 U of Taq I using the manufacturer’s recommendations. The digested amplification product is size fractionated on an agarose gel (2% agarose containing 0.5 µg/ml ethidium bromide) and visualized by ethidium bromide fluorescence on a Bio-Rad Gel Doc 1,000. The fluorescence intensity of the bands was quantified with the QuantOne software (Bio-Rad, Hercules, CA). Lepr cDNA and actin cDNA are presented as fluorescence units. Input RNA (log scale) is plotted against the logarithm of amplified Lepr or actin cDNA to accommodate the disparity of Lepr mRNA copy number.

Primer sequences. Primer sequences were as follows: mLepr-58, ATT TTG GAG AAA AAT GGA TGG G; mLepr-59, AAG GTA AGG TTA AAA TTC ACA AG; actin-3, CTC GTG TAT GAG CTG C; actin-1, CTG GAG AAG AGT TAC ACT CCT; actin-3, CTC GTG TAT GAG CTG C; actin-1, CTG GAG AAG AGT TAC ACT CCT.

Physiological experiments. Because Lepr<sup>db-NCSU</sup> mutants are infertile, a colony of heterozygotes was maintained and bred to produce affected animals. Mice were mated until vaginal plugs were found or pregnancy was palpable. On postnatal day 7, all pups were toe clipped for identification and weighed. Mice were weaned and weighed on postnatal day 21, and pups were tail clipped at this time for DNA analysis. Urinary glucose was measured beginning on day 28 and at 7-day intervals thereafter until age 49 days or glucose was detected. Urinary glucose was measured using reagent strips (Multistix 7, Bayer, Elkhart, IN).

Animals heterozygous for the mutation (+/Lepr<sup>db-NCSU</sup>) were bred throughout the experimental period to ensure the continued production of affected animals. Therefore, most of the phenotypic experiments described below included only WT (+/−) and mutant (Lepr<sup>−/−</sup>) mice. Heterozygous females, aged 6 mo only, were included in measurement of leptin concentrations.

Affected and WT pups were randomly placed into three “treatment” groups. Groups underwent similar tests, but at different ages. Because the db mutation shows autosomal recessive inheritance, approximately one-quarter of the animals per litter were expected to express the diabetic phenotype. Animals were treated as follows.
Group 1 consisted of seven affected males, six affected females, five WT males, and five WT females. Mice were weighed weekly from day 7 through 56 days of age. Response to insulin was measured at 1 and 2 mo of age (~35 and 54 days). Animals were fasted for 10 h, and blood glucose concentrations were determined using tail cuts to obtain blood samples, followed immediately by injection of porcine insulin at a concentration of 1 IU/kg. Blood glucose was measured using a glucometer (One Touch Basic by Lifescan, Johnson and Johnson, Milpitas, CA). Blood glucose was measured at 15 and 30 min after injection (14). Glucose concentrations at time 0 were considered basal fasting concentrations for these animals.

Food and water consumption were measured daily over a 3-day period beginning at ~37 days of age. All mice received 100 g of mouse chow pellets, and the amount of decrease in the weight of the food was measured daily to determine the amount consumed (g). Food spillage was not controlled. Graduated water bottles provided continuous measurement of each animal’s daily water consumption.

At 2 mo (~56 days), mice were killed by cervical dislocation after a 12-h fast. With the use of cardiac puncture, blood was collected for insulin measurement. Insulin concentrations were measured using a rat insulin radioimmunoassay kit (Linco Research, St. Charles, MO).

Group 2 consisted of eight affected males, six affected females, five WT males, and five WT females. Mice were weighed at 84 days (~3 mo) and 112 days (~4 mo). Animals were subjected to tests similar to those described for animals in group 1, using techniques previously described. The ages, however, at which animals underwent each test varied as follows.

Insulin resistance was measured at ~4 mo of age (~110 days). Food and water consumption were measured from days 87 to 90. Mice were killed by cervical dislocation at 4 mo of age (day 112). Blood was collected for insulin and leptin measurement. Plasma leptin concentrations were measured using a mouse leptin radioimmunoassay kit (Linco Research).

Group 3 consisted of five affected females and four WT females. Basal blood glucose and insulin resistance were measured at 6 mo of age (~165 days). Mice were killed by cervical dislocation at 6 mo (168 days). Blood was again collected for insulin and leptin measurement. Due to high morbidity of affected males, all males were killed at 2 or 4 mo of age.

In addition, plasma leptin concentrations of heterozygotes were measured using a mouse leptin radioimmunoassay kit (Linco Research). Four heterozygous females were killed at ~6 mo of age for comparison with homozygous mutant and WT females from group 3. Due to their value in the production of affected mutants, no heterozygotes younger than 6 mo were killed.

For some tests, the sample sizes vary from the numbers indicated above because of experimental error or inadequate amount of blood or plasma obtained for radioimmunoassay. Sample sizes for each test are indicated in the legends of Figs. 4, 6, 7, and 8.

Statistical analysis. All statistical analysis was done with Statistical Application Software programming. Reported means are least square means ± SE.

Body weights were analyzed by age as a two-way ANOVA. Group main effect and interactions were included but removed from the model due to lack of significance. Litters were included as a random effect. Sex and category (affected vs. WT) main effects and interaction were tested against a between-litter error term. Sex and category main effects and interactions were tested against a between-litter error term. Data were analyzed similarly to weight, except that age was included as a within-subject factor. Age main effect and interactions were included in the model.

Insulin response data were analyzed similarly to glucose, except that the means tested were differences in glucose concentration after insulin injection. Differences at both 15 and 30 min after injection were analyzed. Age of onset of hyperglycemia was analyzed for correlation with weight at weaning using linear regression.

RESULTS

New mutation is allelic to Leprdb. An apparent spontaneous mutation in our breeding colony of CD-1 mice resulted in a litter containing two obese females, one obese male, six lean females, and one lean male. At 60 days of age, the affected mice were almost double the weight of their unaffected siblings and age-matched colony members. Obese animals drank approximately four times more water than normal siblings and showed urinary glucose concentrations >2,000 mg/dl.

All obese animals proved to be infertile based on 2-wk matings with unaffected siblings, regardless of sex combinations. Mating the unaffected male sequentially to his unaffected sisters showed that the male was heterozygous for the trait because two of the sisters produced affected offspring at a ratio of three normal to one obese (12 of 49 pups, not different from the expected ratio). This indicated that the male and two females were heterozygous for the trait. A single autosomal recessive gene mutation was the most parsimonious explanation for these results. Complementation mating of our heterozygous mice with mice known to be heterozygous for the db mutation produced 8 of 23 affected pups. When mated with ob heterozygous mice, no affected pups out of 18 born were produced. These results indicated that the spontaneous mutation was allelic to the db mutant because ~34% of the offspring were obese (not significantly different from the expected 25%). In comparison, none of the ob offspring were affected (significantly different from the expected 3:1 ratio; Fisher’s exact test P < 0.005).

Identification of the mutation in the Lepr gene. The coding sequence of the leptin receptor gene was analyzed from genomic DNA and whole brain cDNA of affected animals. Sequence analysis of cDNA fragments obtained by amplification of brain cDNA showed a deletion of one G residue located in exon 12 of the Lepr gene (11). Because the normal sequence is AAGGAG, whereas the mutant sequence is AAGAG, it is impossible to determine whether nucleotide 2022 or 2023 was deleted (Fig. 1A). This deletion results in a shift of the reading frame, causing a substitution of 11 amino acids followed by premature termination of translation before the membrane-spanning domain. No other differences were found in the Lepr coding sequences between affected and lean (+/-) mice.
Two PCR-based assays were developed to detect the missing base pair in genomic DNA without resorting to sequence analysis. One primer, NCSU-Msp (Fig. 1A), was designed to introduce an Msp I site uniquely into the WT sequence, whereas a similar primer, NCSU-Taq (Fig. 1A), was made to introduce a Taq I site solely into the mutant allele. These primers, in conjunction with a primer based on intronic sequence 3' of coding exon 12 (Ex12R), were used to amplify a fragment from the genomes of lean (+/+) and affected mice. Digestion of the amplification products with the appropriate enzymes (Msp I or Taq I) yielded the expected restriction fragments so that use of this assay allowed identification of mice heterozygous for the mutation (Fig. 1B). The mutation, designated Lepr-db-NCSU, terminates translation before the transmembrane region (11). Thus any protein product would not be membrane bound nor would it contain the critical STAT3 box. The mutation should be considered a null allele, similar to the mouse db-Pas and the rat allele fa-f (35). The new allele, expressed on a CD-1 outbred genetic background, produces a novel sexually dimorphic obesity/diabetes syndrome described below.

Effects of the db-NCSU mutation on Lepr expression. We examined the effect of the db-NCSU mutation on Lepr gene expression by RT-PCR analysis of total brain RNA. Initial analysis using NCSU-Taq and mLepr-59 as primers for amplification indicated that the mutation significantly decreased Lepr mRNA levels (data not shown). However, because the NCSU-Taq primer has two mismatches compared with the authentic sequence, it was possible that the mismatches caused preferential amplification of the WT Lepr cDNA. Therefore, we performed a second series of RT-PCR analyses with two primers that flank the db-NCSU mutation, mLepr-58 and mLepr-59. In this case, we obtained amplification products for both the WT and mutant Lepr mRNAs (Fig. 2A). There was significantly less Lepr cDNA product from the mutant brain RNA, whereas amplification of actin cDNA indicated equivalent amounts of total RNA in the WT and mutant samples (Fig. 2B). Densitometric analysis of the Lepr cDNA amplification products showed there was a significant reduction (15-fold) in Lepr mRNA due to the db-NCSU mutation (Fig. 2C and D).

Onset of obesity and hyperglycemia. Mice containing the Lepr-db-NCSU mutation were significantly heavier than WT animals at an early age. Mutant females were heavier than WT females when first weighed at 7 days of age (P < 0.02; data not shown) and remained heavier in body mass through 6 mo of age (Fig. 3). The body mass of mutant males did not differ significantly from WT males until 35 days (P < 0.002). Unlike female mutants, however, male mutant animals showed a steep decrease in body mass from 56 to 70 days of age, which continued to decline throughout the 4-mo data
collection period (Fig. 3). By 84 days of age, body weights of affected and WT males were no longer significantly different. The loss of weight in males was associated with a dramatic increase in glycemia and decrease in circulating insulin (see below). There was no difference between weights of affected males and females through 2 mo of age. However, female mutants were heavier than male mutants at 3 and 4 mo of age ($P$, 0.0002).

The mean age of diabetes onset, as determined by urinary glucose, was 32.3 days for affected mice (Fig. 4). The mean weight at age of onset was 34.7 g, with no sexual dimorphism in either variable. There was also a correlation between age of onset and body weight at weaning ($P < 0.02$; Fig. 5). Mice that were heavier at weaning showed earlier onset of urinary hyperglycemia and higher blood glucose concentrations at 35 and 56 days of age than lighter db weanlings. There were no significant effects of sex on age of onset of glycosuria.

Morbidity and mortality rates of diabetic males were significantly greater than diabetic females at 3–4 mo of age. Mortality of males was 100% by 5 mo of age. Therefore, data obtained at 6 mo were based on female diabetic mice only.

Food and water consumption. Water consumption differed significantly between diabetic mutants and WT mice when tested at both 1 and 3 mo. There was no effect of sex on water consumption. Mean water consumption for affected animals at 1 mo ($n = 12$) was $38.7 \pm 2.4$ ml, whereas WT mice ($n = 10$) consumed a mean of $8.03 \pm 2.7$ ml at this age ($P < 0.0001$). At 3 mo of age, mean water consumptions for affected ($n = 13$) and WT mice ($n = 10$) were $36.8 \pm 2.4$ and $7.15 \pm 2.7$ ml, respectively ($P < 0.0001$). Mean food consumption for affected animals at 1 mo was $\sim 0.290 \pm 0.01$ g/g body wt, whereas mean food consumption was $0.221 \pm 0.01$ g/g body wt for 1-mo-old WT animals ($P < 0.0002$). At 3 mo, mean food consumptions for affected and WT
animals were 0.198 ± 0.01 and 0.129 ± 0.01 g/g body wt, respectively (P < 0.0002). Where standard error bars are absent, errors were too small to appear on graph. Data at 6 mo were based on females only. Sample sizes are as follows: affected females: 1 mo (6), 2 mo (6), 3 mo (6), 4 mo (6), 6 mo (5); affected males: 1 mo (7), 2 mo (7), 3 mo (8), 4 mo (8); WT females: 1 mo (5), 2 mo (5), 3 mo (5), 4 mo (5), 6 mo (4); WT males: 1 mo (5), 2 mo (5), 3 mo (5), 4 mo (5).

Blood glucose and insulin measurement. At 1 mo of age, there were no significant effects of sex on blood glucose concentrations. The mean blood glucose concentrations of mutant males and females were greater than those of sex-matched WT animals at 1 mo (P < 0.02) and remained significantly higher throughout the test period (Fig. 6A).

At 2 mo of age, mean glucose concentrations of affected males were higher than those of affected females (P < 0.04; Fig. 6A). From 1 to 2 mo of age, blood glucose concentrations increased twofold for male mutants, and 1.5-fold for female mutants (P < 0.0001 and P < 0.008, respectively). Again, both affected male and female glucose concentrations were higher than those of WT mice (P < 0.0001 and P < 0.0001, respectively). Insulin concentrations of affected animals were not significantly higher than those of WT animals at 2 mo of age (Fig. 6B).

At 4 mo of age, affected mice again exhibited sex differences in blood glucose, with male mutants displaying higher concentrations than their female counterparts (P < 0.002). Both male and female mutants remained hyperglycemic compared with WT mice (P < 0.0001 and P < 0.0001, respectively; Fig. 6A). However, although insulin concentrations of affected males remained low at 4 mo, the hyperglycemia of mutant females persisted despite high plasma insulin concentrations (Fig. 6B). Plasma insulin concentrations of affected females were higher than those of WT females.
Because of increased morbidity at earlier ages, no males were available for blood glucose measurement at 6 mo of age. Mean blood glucose concentrations of female mutants remained higher than WT females (P < 0.0001; Fig. 6A). Plasma insulin concentrations of affected females also remained higher than WT females at 6 mo (P < 0.0001; Fig. 6B).

Insulin response. There were no effects of sex on insulin response at 1, 2, or 4 mo of age. Affected and WT animals responded similarly to insulin injection at 1 mo of age. Both groups were insulin sensitive, with corresponding decreases in blood glucose concentrations after insulin injection at 15 and 30 min postinjection (Fig. 7).

Insulin sensitivity changed by 2 mo of age. WT mice exhibited mean decreases in blood glucose, whereas mutant animals showed increases in glucose concentration at 15 and 30 min postinjection (Fig. 7). The increase was likely due to both increased insulin resistance and increased production of glucose due to the stress of injection. Thus at 15 and 30 min after insulin injection, mutant animals were significantly more resistant than WT mice (P < 0.002 and P < 0.002, respectively).

At 4 mo of age, diabetic mice again exhibited significantly greater insulin resistance than WT animals (Fig. 7). WT mice showed steady declines in blood glucose concentrations and maintained similar glucose to WT mice. Affected males showed higher glucose concentrations than WT at 4 mo of age (P < 0.0003) and affected males at 4 mo of age (P < 0.002; Fig. 6B).

Fig. 6. A: mean blood glucose concentrations of affected and WT animals. At all ages, glucose concentrations of affected animals were higher than WT mice (P < 0.02). Differences between affected males and females at 2 and 4 mo of age were significant (P < 0.04 and P < 0.002, respectively). Standard errors of some values were too small to appear on graph. Data at 6 mo were based on females only. Sample sizes are as follows: affected females: 1 mo (6), 2 mo (6), 4 mo (6), 6 mo (5); affected males: 1 mo (7), 2 mo (7), 4 mo (8); WT females: 1 mo (5), 2 mo (5), 4 mo (5), 6 mo (4); WT males: 1 mo (5), 2 mo (5), 4 mo (5). B: mean plasma insulin concentrations of affected and WT animals. At 4 mo of age, differences between affected females and affected males were significant (P < 0.0002). At both 4 and 6 mo, differences between affected and WT females were significant (P < 0.0003 and P < 0.0001, respectively). Data at 6 mo were based on females only. Sample sizes are as follows: affected females: 2 mo (4), 4 mo (5), 6 mo (4); affected males: 2 mo (6), 4 mo (5); WT females: 2 mo (4), 4 mo (5), 6 mo (4); WT males: 2 mo (4), 4 mo (4).
glucose at 15 and 30 min postinjection. Conversely, 4-mo-old affected mice again exhibited increases in glucose concentration. Differences in insulin response between affected and WT animals at each time interval were significant (P < 0.05 and P < 0.0005 at 15 and 30 min, respectively). There was no sexual dimorphism in response to insulin injection among affected animals at 1, 2, or 4 mo of age.

At 6 mo of age, mean blood glucose concentrations of WT females again decreased after insulin injection, whereas those of affected females increased steadily, indicating greatly reduced insulin sensitivity. These responses to insulin at 15 and 30 min after injection were significantly different for diabetic versus WT females at 6 mo of age (P < 0.007 and P < 0.0005; Fig. 7).

Plasma leptin concentrations. Plasma leptin concentrations of affected male mice, aged 4 mo, ranged from 5.4 to 31.4 ng/ml, with a mean of 13.5 ng/ml. This value was higher than age-matched WT mice (P < 0.04; Fig. 8). Plasma leptin concentrations of all affected females, aged 4 and 6 mo (n = 11), were higher than the maximum detectable value (20 ng/ml) of the radioimmunoassay kit used. When diluted two times, values of females were still higher than the maximum, indicating that affected females had plasma leptin concentrations >40 ng/ml (Fig. 8). Because of insufficient plasma samples, no further dilutions could be made. The available data suggest that leptin concentration is sexually dimorphic among affected animals at 4 mo of age. The mean leptin concentration of 6-mo-old female heterozygotes (n = 4) was 10.5 ± 2.5 ng/ml. Age-matched WT females (n = 4) exhibited a mean leptin concentration of 5.8 ± 2.9 ng/ml. This difference was not statistically significant.

**DISCUSSION**

History of the Swiss albino mouse. The CD-1 mouse, an outbred stock of albino mice, are based on the Swiss mouse colony maintained at The Rockefeller Institute for Medical Research, starting from two males and seven females in 1926 (24). This stock was derived from a colony of 200 mice obtained by investigators at the Pasteur Institute from a local dealer. Genetic analysis supports the claims of commercial breeders that colonies of outbred Swiss mice have not been supplemented by non-Swiss mice, as Swiss mice are genetically distinct from other mouse stocks (29). The Swiss mouse stocks may be considered an island population, isolated from migration. The C57BL/6 inbred mouse strain was established in 1921 from a breeder pair (female 57 and male 52) from Abbie Lathrop’s mouse colonies. The C57BLKS/J inbred strains are a derivative strain of C57BL/6 that was genetically contaminated, probably by DBA/2J (25). Information about other strains of mice relevant to this discussion can be obtained from a listing by Dr. Michael Festing maintained by the Mouse Genome Informatics Group at the Jackson Laboratories.

Lepr<sup>db-NCSU</sup> phenotype. The purpose of this study was to provide a phenotypic description of outbred mice with a novel, spontaneous mutation in the Lepr gene. The Lepr<sup>db-NCSU</sup> mutation exhibits sexual dimorphism in the CD-1 outbred strain, produces a phenotype in CD-1 females unlike previously described db mutations, and may serve to provide a more complete understanding of leptin's physiological effects.

The earlier appearance of diabetic symptoms in pups that were heavier at weaning indicates that obesity plays a significant role in the disease process of Lepr<sup>db-NCSU</sup> mutant animals. It is also clear that the early onset of urinary hyperglycemia affects disease progress later in life, because mice with the earliest onset of diabetes symptoms had the highest blood glucose at 35 and 56 days of age. However, it is not clear from these data that obesity actually precedes diabetes. Although female mutants displayed greater body mass than control animals by postnatal day 7, male mutants were not significantly heavier than WT males until day 35, at which time affected animals were already hyperglycemic compared with WT animals. Urinary hyperglycemia as an indication of diabetes onset may be problematic as well. Coleman and Hummel (14) found hyperinsulinemia in db mice as early as 10 days of age, long before evidence of hyperglycemia became apparent.

Sex differences became apparent during the study and indicated variability in the progress of diabetes in the mutant mice. Coincident with decreasing weight in diabetic males after 2 mo of age were decreasing plasma insulin and increasing hyperglycemia. Mutant males were insulin resistant at 2 and 4 mo of age, although their plasma insulin concentrations at these ages were low. Insulin resistance increased during the lifetimes of the mutants. This is consistent with the

![Fig. 8. Mean plasma leptin concentrations of affected (Aff) homozygous females (n = 5) and males (n = 5) and WT females and males combined (n = 7) at age 4 mo. All leptin concentrations of affected females were higher than maximum detectable value of radioimmunoassay kit used when diluted 2× (40 ng/ml). Leptin concentrations of affected males were significantly higher than WT mice (P < 0.04).](http://apregu.physiology.org/Downloadedfrom/http://ajpregu.physiology.org/)
observation that mice with severe insulin resistance due to combined haploinsufficiency for the insulin receptor and insulin receptor substrate-1 do not exhibit abnormal insulin tolerance tests until 4–6 mo of age (4). The severe weight loss and early mortality of affected males seem to be due to their decreased insulin production. The mean decrease in plasma insulin concentration of affected males at 4 mo is the probable result of islet degeneration, as affected males were not hyperinsulinemic compared with controls at 2 or 4 mo of age. Data on insulin production of db-NCSU mice aged 1 mo and younger will be necessary to determine if males are hyperinsulinemic before 2 mo of age. In contrast, affected CD-1 females showed oversecretion of insulin without apparent loss of insulin secretory capacity. Although females maintained high glucose concentrations, their continued weight gain and longer life spans seem to be due to maintained insulin secretion.

Sexual dimorphism among Lepr<sup>db-NCSU</sup> mutants may be due to differing sex steroid concentrations. Prochazka et al. (27) reported that homozygous <i>db</i> mutants fed estrone in their diets did not develop hyperinsulinemia, hyperglycemia, or islet atrophy. Leiter (21) also noted a marked sexual dimorphism in the diabetes syndrome of <i>db/db</i> animals of the subline CBA/Lt. Female <i>db/db</i> animals of this strain were obese and hyperinsulinemic, but only slightly hyperglycemic, in contrast to the sustained hyperglycemia in <i>Lepr<sup>db-NCSU</sup></i> mutant females. Males of the CBA/Lt strain exhibited only moderate obesity but severe hyperglycemia and 100% mortality by 6 mo. CBA/Lt <i>db/db</i> mutant males were hyperinsulinemic at 2 mo, but exhibited normal plasma insulin levels by 4 mo, a progression similar to our mutant males. Leiter (21) found that injections of 17β-estradiol and progesterone resulted in remission of diabetes symptoms in males.

The increased plasma leptin concentrations exhibited by male and female <i>Lepr<sup>db-NCSU</sup></i> mutants were not unexpected, because the <i>db</i> mutation blocks leptin signal transduction. Differences in leptin concentrations between affected males and females were most likely due to the decreased body weights, possibly corresponding to fat mass, of mutant males.

The <i>Lepr<sup>db-NCSU</sup></i> mutation is unique in its effects on CD-1 female mice. Obese CD-1 males exhibit the hyperglycemia and early mortality due to loss of insulin secretory capacity similar to <i>db/db</i> males of the CBA/Lt and C57BLKS/J strains. However, obese CD-1 females behave differently than <i>db/db</i> females of the CBA/Lt and C57BLKS/J strains. Obese (i.e., <i>db/db</i>) CBA/Lt females are hyperinsulinemic and normoglycemic, similar to obese (i.e., <i>db/db</i>) C57BL/6J females. Obese (i.e., <i>db/db</i>) females of the C57BLKS/J strain are hyperglycemic and progress to decreased insulin production and body weight. In contrast to these strains, mutant CD-1 females are hyperglycemic but maintain high body weight and insulin secretory capacity. A further analysis of well-characterized <i>Lepr<sup>db</sup></i> strains may be useful for comparison of mutation effects on different genetic backgrounds.

Phenotypic comparisons of 10 congenic <i>Lepr<sup>db</sup></i> strains. The summary data in Table 1 provide an overview of information about the effects of Lepr deficiency in 10 mouse strains at 4–5 mo of age (this paper, 2, 22, 23; insulin values were converted using 25 µU = 1 ng). It is clear that the <i>db</i> mutation causes obesity independent of genetic background, although severe insulinopenia with hyperglycemia is associated with lower body weights. The average body weights for lean mice of all strains is 33 g for males and 30 g for females. Taking into account that the following comparisons are based on historical data rather than data obtained from concurrent experiments, it appears that the diabetes subphenotypes of obese CD-1 mice are similar to the subphenotypes of obese mice of the DBA/2J and CBA/Lt strains. The hyperglycemia and insulinopenia of obese males of these three strains result in high early mortality, whereas the insulin levels in obese females of the three strains minimize mortality. However, the DBA-db congenic strains were discontinued, whereas the CBA-db strain is only available as frozen embryo stocks. This sexual dimorphism is not present in the C57BLKS/J strain, with obese animals of both sexes being hyperglycemic with insulinopenia and having similar mortality rates. Mortality appears to be minimized if insulin levels are maintained near 20 ng/ml (~500 µU/ml), as in the case for obese mice of the C57BL/6J, C3H, MA/Mj, SWR/J, and 129J strains. One should note that high insulin levels (>100 ng/ml) are not strictly associated with euglycemia, as exemplified by a comparison of SWR/J mice and 129J mice. This information regarding the influence of strain background is a useful base for efforts to identify

Table 1. Characteristics of 4- to 5-mo-old obese mice

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<th>Strain</th>
<th>Sex</th>
<th>Weight, g at 4–5 mo</th>
<th>Glucose, mg/dl</th>
<th>Insulin, ng/ml</th>
<th>Mortality, % at 4–5 mo</th>
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<tr>
<td>CD-1 &lt;i&gt;db-NCSU&lt;/i&gt;</td>
<td>M</td>
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<td>100</td>
</tr>
<tr>
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<td>F</td>
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<td>352</td>
<td>6.6</td>
<td>NA</td>
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<tr>
<td>CBA/Lt</td>
<td>M</td>
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<td>517</td>
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<td>219</td>
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<td>M</td>
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<td>452</td>
<td>25</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>F</td>
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<td>211</td>
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<tr>
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<tr>
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<td>F</td>
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<td>22</td>
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<tr>
<td>DBA/2J</td>
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<td>F</td>
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<td>18</td>
<td>25</td>
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<tr>
<td>DW/Pas db-Pas</td>
<td>M</td>
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<td>89</td>
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<tr>
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</table>

Data presented are for obese mice between 4 and 5 mo of age and derived from cited literature (see text). Insulin values were converted to ng/ml using 25 µU = 1 ng. All values are based on 4- to 5-mo-old animals. Data are provided for coisogenic strains (db-NCSU, db-Pas, and db-3J) or congenic db strains. Strains in which mutation is not specified are db congenic strains. NA, not available; M, male; F, female.


