

# Type 2 Diabetes–Like Hyperglycemia in a Backcross Model of NZO and SJL Mice

## Characterization of a Susceptibility Locus on Chromosome 4 and Its Relation With Obesity

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A backcross model of New Zealand obese mice (NZO) with the lean, atherosclerosis-resistant SJL strain was established to locate genes responsible for obesity, insulin resistance, and type 2 diabetes–like hyperglycemia. In male NZO × F1 backcross mice, a major susceptibility locus for the development of hyperglycemia and hypoinsulinemia (*Nidd/SJL*) was identified on chromosome 4 between the markers D4Mit278 and D4Mit232, 10–28 cM distal of the previously described *Nidd1* locus. The diabetogenic allele has presumably been contributed by the SJL genome, and it appeared to be responsible for ~60% of the total prevalence of hyperglycemia. The presence of *Nidd/SJL* did not alter body weight or weight gain by week 12. Thereafter, it was associated with reduced weight gain or weight loss, presumably as a consequence of decompensated hyperglycemia. In all male backcross mice, the prevalence of hyperglycemia at week 22 increased with the body weight at week 12, suggesting that the development of hyperglycemia was dependent on the degree of obesity. In the absence of *Nidd/SJL*, mice weighing <50 g at week 12 did not develop hyperglycemia by week 22. In contrast, in animals carrying the diabetogenic allele, the prevalence of hyperglycemia was 20 and 64% when the 12-week weight was <45 and 45–50 g, respectively. These data are consistent with the conclusion that *Nidd/SJL* represents a diabetes gene that lowers the obesity threshold for the development of hyperglycemia and hypoinsulinemia. *Diabetes* 49:1590–1596, 2000

New Zealand obese (NZO) mice exhibit a polygenic syndrome of hyperphagia, obesity, and insulin resistance (1–5). In the course of the syndrome, some NZO mice develop islet cell failure, hypoinsulinemia, and overt hyperglycemia. Thus, the NZO strain is an ideal model for the identification of the genes that are responsible for aberrations of glucose metabolism and insulin action in mice. Recently, a cross between NZO and NON mice was established, and it was shown that susceptibility loci derived from both NON (*Nidd1* and *Nidd2*) and NZO genomes (*Nidd3*) contributed to the development of hyperglycemia and hypoinsulinemia (6). Furthermore, it has been shown that leptin resistance might be the primary cause of obesity in NZO mice (7,8). Interestingly, a leptin receptor variant with several amino acid exchanges (*Lepr*<sup>A720T/T1044I</sup>), including 2 nonconservative substitutions (A720T; T1044I), was found in the NZO strain (7). However, the contribution of *Lepr*<sup>A720T/T1044I</sup> to the metabolic syndrome of the NZO mouse was unclear, because the allele was also present in the related nonobese New Zealand Black (NZB) strain.

To assess the contribution of *Lepr*<sup>A720T/T1044I</sup> and identify other susceptibility loci for obesity and insulin resistance, we established a backcross model of NZO mice with the lean and atherosclerosis-resistant SJL strain (9,10). Surprisingly, we found that the prevalence of hyperglycemia and hypoinsulinemia in the male NZO × F1 backcross mice was higher than expected and that this effect was due to a diabetogenic allele (*Nidd/SJL*) contributed by the SJL genome. This allele appears to lower the obesity threshold for the development of hypoinsulinemia and hyperglycemia.

### RESEARCH DESIGN AND METHODS

**Animals.** SJL (SJL/NBom) and NZO mice (NZO/HIBom) were obtained from Bomholtgard (Ry, Denmark). Female SJL and male NZO mice were used to found an F1 generation, and backcrosses (SJL × F1, female SJL mated with F1 males; NZO × F1, female NZO mated with F1 males) were performed. After weaning (3 weeks of age), mice received rodent food (C1057; Altromin, Lage, Germany) with 16% fat, 46.8% carbohydrates, 17.1% protein, and 15.4 kJ/g digestible energy. Throughout the study, mice had free access to food and water. Three to 6 mice were kept per cage (Macrolon, type III) in a temperature-controlled room (20° C, 55 ± 5% relative humidity) with a 12-h light-dark cycle and lights on at 6 A.M. Animals (fed ad libitum) were killed at the age of 22 weeks at 9 A.M. in isoflurane anesthesia by exsanguination followed by decapitation. The study was approved by the committee for ethics of animal experimentation at the Regierungspräsidium Köln, Germany.

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IRI, immunoreactive insulin; LOD, logarithm of odds; QTL, quantitative trait locus.

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**Body weight, BMI, and total body fat.** Body weight and food consumption were determined once every week during the study period. Body length (not including the tail) was measured once after 22 weeks. Because of differences in the length of the backcross animals ( $\pm 5\%$ ), BMI was calculated as an additional measure of obesity ( $\text{BMI} = \text{body weight [g]} / (\text{body length [cm]})^2$ ). For determination of total body fat, carcasses were dried for 48 h at 105°C and extracted for an additional 48 h with tetrachloroethylene. Aliquots of the filtered extracts were measured with the Fosslet MKII fat analyzer (type 15300/15500; N. Foss Elektronik, Hamburg, Germany).

**Serum parameters.** Blood glucose, serum cholesterol and serum triglycerides were measured by autoanalyzer (Johnson & Johnson, Neckargemünd, Germany). Serum insulin was determined by radioimmunoassay (Amersham-Pharmacia, Freiburg, Germany) with anti-rat insulin antiserum and  $^{125}\text{I}$ -labeled rat insulin as tracer. Free and bound radioactivity were separated with an anti-IgG antibody. Duplicate samples were assayed and, if necessary, reassayed after appropriate dilution.

**Genotyping, genetic maps, and quantitative trait locus analysis.** DNA was prepared from mouse tails with a DNA isolation kit (InViTek, Berlin, Germany) based on a salt precipitation method. Animals were genotyped for microsatellite markers by polymerase chain reaction with oligonucleotide primers obtained from Research Genetics (Huntsville, AL), and microsatellite length was determined by nondenaturing polyacrylamide gel electrophoresis (10% gels). A quantitative trait locus (QTL) analysis was performed as described (11) with the program Mapmaker/QTL 1.1 (12) after construction of the genetic map with Mapmaker/EXP 3.0 (13).

## RESULTS

**Characterization of the parental strains.** Table 1 summarizes the characterization of some metabolic parameters in the parental strains NZO and SJL at 22 weeks of age. As compared with the lean normoglycemic SJL strain, male as well as female NZO mice exhibited markedly higher body weights, serum cholesterol, triglycerides, insulin, and glucose levels (Table 1). It should be noted that male and female NZO mice differed considerably, in particular in the abnormalities of glucose homeostasis. Some male NZO mice developed hyperglycemia between 12 and 22 weeks of age because of failure to compensate for the increasing insulin resistance; in these animals, high blood glucose levels were correlated with low serum insulin levels. In contrast to NZO

males, none of the females developed hyperglycemia and hypoinsulinemia by week 22. However, all female NZO mice exhibited markedly increased serum insulin levels, reflecting the severe insulin resistance of this strain (Table 1).

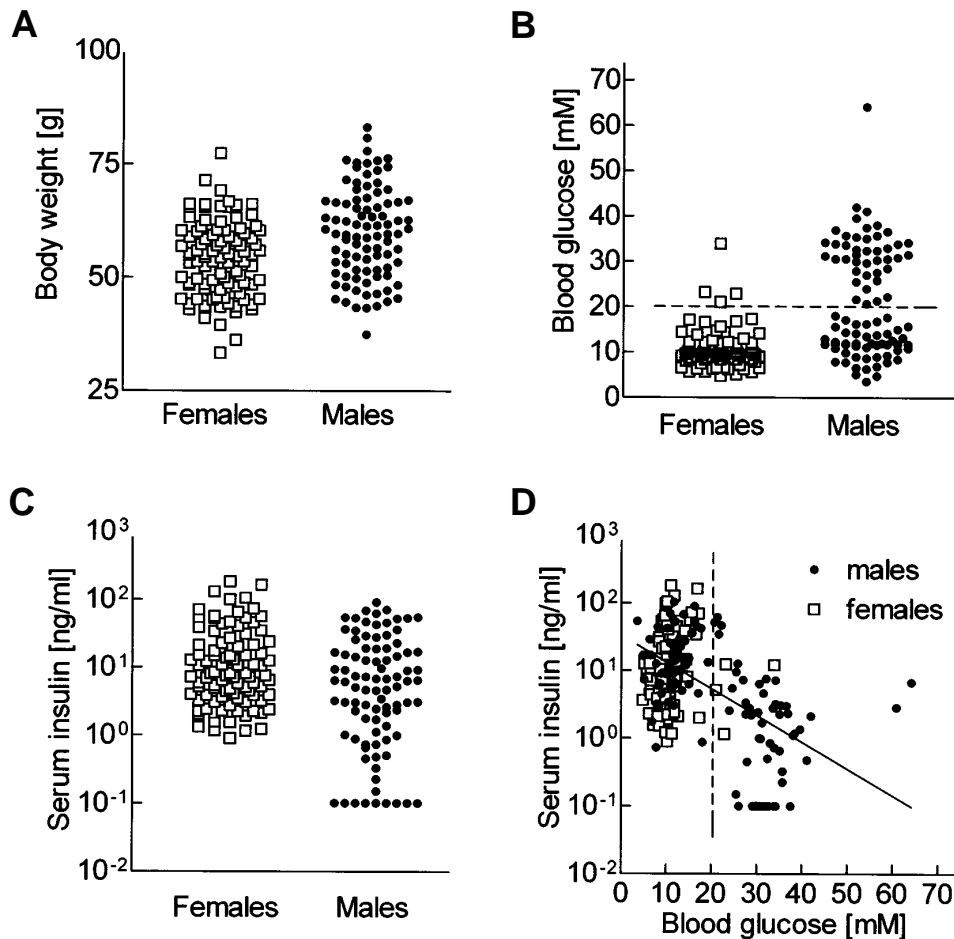
**Characterization of F1 and NZO  $\times$  F1 backcross progeny.** F1 mice were generated by mating SJL females with NZO males, because the reciprocal backcross produced a very low number of litters. As shown in Table 1, all obesity-related traits and most serum parameters were significantly higher in the F1 (SJL  $\times$  NZO) progeny than in parental SJL, but lower than in NZO. Serum insulin was moderately elevated in males but normal in females, and blood glucose at week 22 was normal in male and female F1 mice.

Two backcross populations (SJL  $\times$  F1, SJL females mated with F1 males; NZO  $\times$  F1, NZO females mated with F1 males) were established. According to an initial characterization of the backcross populations, obesity-related traits were unstable in the SJL  $\times$  F1 backcross and exhibited seasonal variations as well as dependence on the caging conditions. In contrast, the NZO  $\times$  F1 backcross appeared more suitable for detection of obesity and diabetes genes. Furthermore, we expected a possible effect of the  $\text{Lepr}^{\text{A720T/T1044I}}$  variant to be recessive and, therefore, to be detectable in the NZO  $\times$  F1 backcross only. Thus, a total of 111 male and 96 female NZO  $\times$  F1 backcross mice were characterized. Figure 1 illustrates the distribution of body weights, blood glucose levels, and insulin levels in the NZO  $\times$  F1 backcross population. Body weights were distributed over a range of 40–75 g in females and 35–85 g in males (Fig. 1A). Blood glucose exhibited a bimodal distribution in males, with  $\sim 50\%$  of the animals exhibiting overt type 2 diabetes-like hyperglycemia (Fig. 1B). From this distribution, a threshold blood glucose level of 20 mmol/l (dashed line) for the onset of the diabetes-like hyperglycemia can be deduced. Consistent with their hyperglycemia, a significant portion of the male backcross animals exhibited low serum insulin levels (Fig. 1C). Furthermore, insulin levels

TABLE 1  
Comparison of body weights and serum parameters in parental (NZO and SJL) and F1 mice

	SJL	NZO	F1 (SJL $\times$ NZO)
<b>Males</b>			
Body weight at week 12 (g)	23.3 $\pm$ 1.5	50.3 $\pm$ 2.9*	32.7 $\pm$ 2.7*
Body weight at week 22 (g)	27.4 $\pm$ 2.2	50.2 $\pm$ 9.4*	45.8 $\pm$ 3.3*
BMI ( $\text{g}/\text{cm}^2$ )	0.290 $\pm$ 0.01	0.391 $\pm$ 0.06*	0.377 $\pm$ 0.02*
Total body fat (g)	2.6 $\pm$ 0.8	13.1 $\pm$ 4.9*	18.9 $\pm$ 7.5*
Blood glucose (mmol/l)	9.5 $\pm$ 1.1	22.7 $\pm$ 7.1*	12.5 $\pm$ 2.0*
Serum insulin (ng/ml)	1.4 $\pm$ 1.2	6.8 $\pm$ 8.4†	11.9 $\pm$ 7.6†
Serum cholesterol (mmol/l)	3.14 $\pm$ 0.3	4.85 $\pm$ 0.9*	4.19 $\pm$ 0.2*
Triglycerides (mmol/l)	1.48 $\pm$ 0.1	2.47 $\pm$ 0.9†	1.58 $\pm$ 0.3
<b>Females</b>			
Body weight at week 12 (g)	19.8 $\pm$ 1.2	43.4 $\pm$ 4.9*	26.8 $\pm$ 1.9*
Body weight at week 22 (g)	21.6 $\pm$ 0.8	58.6 $\pm$ 8.3*	33.6 $\pm$ 2.9*
BMI ( $\text{g}/\text{cm}^2$ )	0.242 $\pm$ 0.01	0.463 $\pm$ 0.06*	0.293 $\pm$ 0.02*
Total body fat	2.1 $\pm$ 0.3	24.5 $\pm$ 4.2*	9.2 $\pm$ 2.3*
Blood glucose (mmol/l)	8.5 $\pm$ 1.7	11.6 $\pm$ 3.4‡	11.6 $\pm$ 1.8‡
Serum insulin (ng/ml)	1.1 $\pm$ 0.3	39.5 $\pm$ 36.5*	1.6 $\pm$ 0.7
Serum cholesterol (mmol/l)	1.71 $\pm$ 0.4	4.37 $\pm$ 0.4*	2.65 $\pm$ 0.2*
Triglycerides (mmol/l)	0.78 $\pm$ 0.2	1.78 $\pm$ 0.4*	1.10 $\pm$ 0.2†

Data are means  $\pm$  SD of 10 animals. Differences from SJL were tested for statistical significance by *t*-test analysis. \* $P < 0.001$ ; † $P < 0.01$ ; ‡ $P < 0.05$ .



**FIG. 1.** Characterization of the NZO  $\times$  F1 backcross population with regard to body weight, blood glucose, and serum insulin levels. **A:** Distribution of body weights at 22 weeks in male and female mice. **B:** Distribution of blood glucose levels at 22 weeks in male and female backcross mice. The dashed line represents the cutoff blood glucose level for decompensated hyperglycemia (20 mmol/l) derived from the bimodal distribution. **C:** Distribution of serum insulin levels at 22 weeks in male and female backcross mice. Values at or below the level of detection of the assay (in 11 animals) were given as 0.1 ng/ml. **D:** Correlation between blood glucose and serum insulin levels in male and female backcross mice. The continuous line represent the result from a linear regression including male animals only; the dashed line depicts the threshold of decompensated hyperglycemia.

were inversely correlated with blood glucose in the male animals (Fig. 1). In contrast, only 4 of the female NZO  $\times$  F1 backcross mice developed hyperglycemia by week 22.

#### **A susceptibility locus for the type 2 diabetes-like hyperglycemia at D4Mit278/D4Mit232 on chromosome 4.**

Backcross animals were genotyped for 92 polymorphic markers distributed over the whole genome. A comparison of the genotypes with the prevalence of hyperglycemia ( $>20$  mmol/l) indicated that linkage disequilibrium was present at markers on chromosome 4 (D4Mit278–D4Mit232). Male heterozygous backcross mice exhibited a significantly higher blood glucose and a 2.5-fold higher prevalence of hyperglycemia ( $>20$  mmol/l) at week 22 (Table 2). This effect was also visible when the more common threshold of 16.5 mmol/l for hyperglycemia in fed mice was used (32.8 vs. 73.6% in N/N versus S/N). Heterozygous mice also had lower serum insulin levels: the prevalence of mice with severe hypoinsulinemia ( $<0.1$  ng/ml) was 17% in the heterozygous group but only 3.5% in the homozygotes. Because the diabetogenic allele appeared to be contributed from the SJL

genome, we designated the locus *Nidd/SJL*. *Nidd/SJL* was apparently responsible for ~60% of the prevalence of diabetes in the backcross population [69.8% – (69.8/2)  $\times$  27.6%, with 69.8 and 27.6% being the diabetes prevalence in the heterozygous or homozygous animals, respectively].

Table 2 also summarizes other characteristics of the NZO  $\times$  F1 backcross animals separated by their genotype at D4Mit278. Consistent with the lower serum insulin levels, serum triglycerides were markedly elevated in the heterozygous group. Furthermore, body weight at week 22, BMI, and total body fat content were significantly lower in the heterozygous animals. In contrast, there was no difference between the genotypes in body weight at 12 weeks ( $P > 0.3$ ). Also, there was no significant difference in any of the investigated parameters in female animals. However, it should be noted that out of the 4 hyperglycemic females, 3 were heterozygous for the D4Mit278 allele.

After genotyping for 5 additional microsatellite markers on chromosome 4, a QTL analysis of the backcross population was performed with the program Mapmaker/QTL (11–13). In

TABLE 2

Effects of the genotype at D4Mit278 on blood glucose, serum insulin, prevalence of hyperglycemia, serum lipids, and parameters of body weight development in NZO x F1 backcross mice

	Males		Females	
	N/N	S/N	N/N	S/N
<i>n</i>	53	58	46	47
Blood glucose (mmol/l)	17.6 ± 11.0	26.0 ± 11.7*	10.3 ± 3.5	11.4 ± 4.7
Prevalence of hyperglycemia (%)	27.6 (16/58)	69.8 (37/53)†	2.2 (1/46)	6.4 (3/47)
Serum insulin	18.1 ± 22.6	9.7 ± 15.6*	22.1 ± 38.5	21.1 ± 27.1
Triglycerides (mmol/l)	1.89 ± 1.6	3.97 ± 3.3*	1.52 ± 0.4	1.66 ± 0.6
Serum cholesterol (mmol/l)	4.78 ± 0.9	4.35 ± 0.8‡	3.87 ± 0.5	3.83 ± 0.7
Weight at week 12 (g)	51.6 ± 7.8	50.3 ± 5.6	41.4 ± 5.5	39.8 ± 5.8
Weight at week 22 (g)	65.7 ± 11.4	57.6 ± 10.3*	54.6 ± 8.2	53.9 ± 8.4
Weight gain				
Weeks 12–22 (g)	14.1 ± 10.2	7.0 ± 10.8*	13.3 ± 4.6	14.0 ± 4.4
BMI (g/cm <sup>2</sup> )	0.47 ± 0.07	0.42 ± 0.06*	0.41 ± 0.05	0.41 ± 0.05
Total body fat (g)	21.4 ± 6.8	16.9 ± 7.2†	20.5 ± 3.7	20.6 ± 4.9

Data are *n* or means ± SD of *n* animals in each column. \**P* < 0.0005; †*P* < 0.005; ‡*P* < 0.05.

this analysis, a QTL predisposing for hyperglycemia was found on chromosome 4 with high logarithm of odds (LOD) scores for blood glucose, serum triglycerides, log (immunoreactive insulin [IRI]/blood glucose) as a surrogate parameter for insulin secretion, and body weight over a range of ~20 cM between the markers D4Mit278 and D4Mit232 (Fig. 2). Maximum LOD scores were 3.6 (blood glucose), 3.8 (log [IRI/blood glucose]), 3.6 (triglycerides), 3.2 (log IRI), and 3.0 (body weight at week 22). Furthermore, the presence of a peak close to the marker D4Mit214 suggested the presence of an additional QTL for body weight (LOD score 3.3).

**Effect of *Nidd/SJL* on body weight development.** Because the presence of *Nidd/SJL* was associated with a lower body weight at week 22, we compared the body weight development between weeks 3 and 22 for the genotypes N/N and S/N at D4Mit278 (Fig. 3A). Weight gain was identical in the 2 groups between weeks 3 and 12. Thereafter, mean body weights increased more slowly in mice presenting *Nidd/SJL* (S/N at D4Mit278). This finding led us to suspect that the reduced weight gain was secondary to the higher prevalence of diabetes in heterozygous mice and not to an obesity gene contributed from NZO. Thus, we analyzed the relationship between blood glucose and weight gain between weeks 12 and 22. As illustrated in Fig. 4, this analysis produced a bell-shaped correlation. Above the 20 mmol/l threshold, blood glucose levels were inversely correlated with weight gain, and severe hyperglycemia was even associated with weight loss. Consistent with the data presented in Table 2, this phenotype was predominantly presented by animals heterozygous at D4Mit278. It should be noted that the percentage of heterozygous animals was highest in the group with the lowest weight gain: 42.5% of all S/N versus 8.6% of all N/N were in the group with weight gain <6 g and blood glucose >20 mmol/l. Furthermore, weight loss and hyperglycemia were associated with very low serum insulin (0.93 ± 0.3 ng/ml in animals losing weight between week 12 and 22 vs. 17.4 ± 2.2 in all others). Thus, the data indicate that the exchange of one NZO allele at D4Mit278 for the SJL allele produces hyperglycemia and hypoinsulinemia and, consequently, reduces weight gain between weeks 12 and 22.

To further support the conclusion that the effect of *Nidd/SJL* on body weight is secondary to the diabetes syndrome, diabetic animals (blood glucose >20 mmol/l) were excluded from an additional analysis of the LOD ratios. This exclusion markedly reduced the effect of *Nidd/SJL* (body weights at 22 weeks: N/N for D4Mit278, 65.0 ± 10.3 g; S/N, 62.3 ± 8.2; *P* = 0.29). However, the LOD ratio distribution (not shown) exhibited a maximum with a suggestive LOD score (2.5) at the marker D4Mit214 that maps 20 cM proximal to *Lepr* and 28 cM proximal to D4Mit278 (*Nidd/SJL*). The differences in body weight at D4Mit214 were 8.7 g (N/N, 67.1 ± 9.4 g; S/N, 58.4 ± 7.8 g; *P* < 0.0005) at week 22 and 3.8 g (N/N, 49.7 ± 6.9 g; S/N, 45.9 ± 6.1; *P* < 0.05) at week 12. Thus, the possibility cannot be excluded that an additional susceptibility locus that affects body weight independent of the development of hyperglycemia is located 28 cM proximal of *Nidd/SJL*.

**Lack of effect of the *Lepr*<sup>A720T/T1044I</sup> allele on parameters of obesity in NZO × F1 backcross mice.** The diabetogenic effect provided by *Nidd/SJL* renders it difficult to detect an independent effect of the neighboring *Lepr* locus on obesity. The LOD scores for body weight at the *Lepr* locus (D4Mit175) were 2.4 (week 22) and 0.2 (week 12). There was no significant difference between the genotypes in the nondiabetic animals. Furthermore, there was no significant difference between the genotypes in the female animals (body weight at 12 weeks: N/N, 41.1 ± 5.9 g; S/N, 40.1 ± 6.0 g; body weight at 22 weeks: N/N, 54.0 ± 8.5 g; S/N, 53.2 g ± 7.6 g; BMI: N/N, 0.402 ± 0.047 g/cm<sup>2</sup>; S/N, 0.409 ± 0.045 g/cm<sup>2</sup>). Thus, the data do not support the conclusion that the *Lepr*<sup>A720T/T1044I</sup> allele contributes to the body weight development of the backcross mice.

**Interaction of obesity and *Nidd/SJL* in the NZO × F1 backcross mice.** Obesity has previously been shown to be of critical importance in the development of the type 2 diabetes-like syndrome in mice (14,15). Consistent with this concept, the body weights of animals developing hyperglycemia by week 22 were significantly higher between weeks 6 and 12 (Fig. 3B, *P* < 0.0001). Therefore, we analyzed the relationship between the body weights at 12 weeks and blood glucose levels at 22 weeks (Fig. 5). According to this analysis, the body weight at week 12 appeared to be a predictor for the

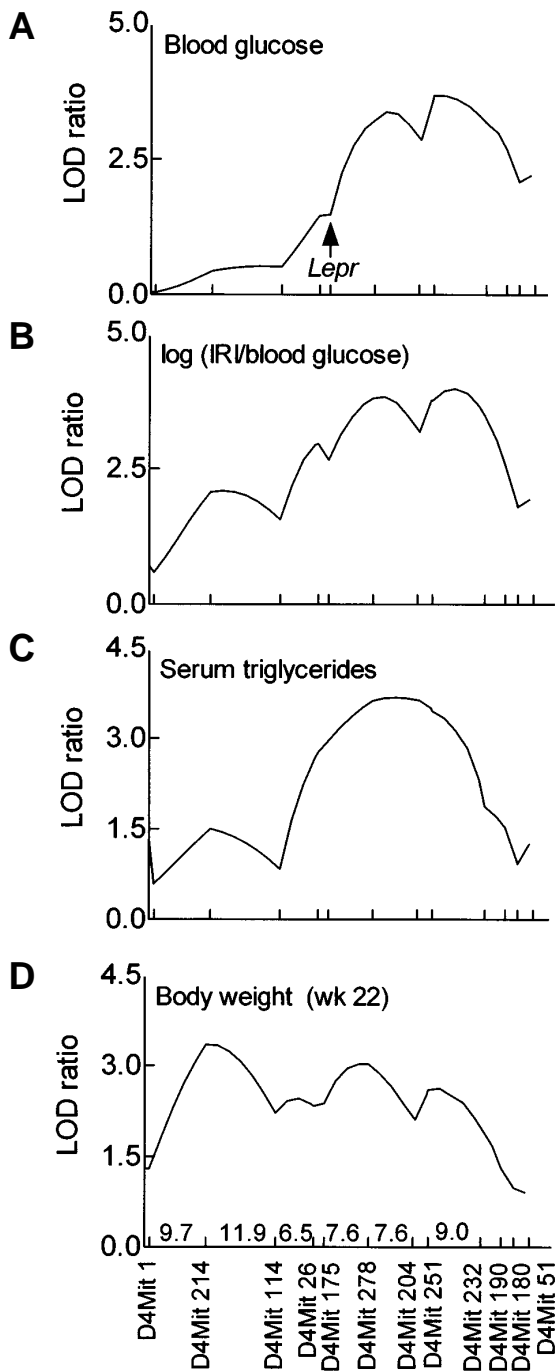


FIG. 2. Distribution of LOD ratios for blood glucose (A), log (IRI/blood glucose) (B), serum triglycerides (C), and body weight (D) in the male NZO x F1 backcross population. A genetic map of the chromosome was generated for the genotyped markers with the Mapmaker program, and LOD scores were subsequently calculated with Mapmaker/QTL. The position of *Lepr* (shown in A) was mapped with 110 male backcross mice.

prevalence of hyperglycemia at week 22. Only 5% of all animals weighing <45 g at week 12 developed hyperglycemia, whereas 66% of all mice with body weights >55 g at week 12 became diabetic (90% in animals heterozygous for D4Mit278). Given that body weight at week 12 reflects the aggregate effect of the obesity genes present in an animal, the data mirror a threshold effect of, or a dose-response relationship between, the obe-

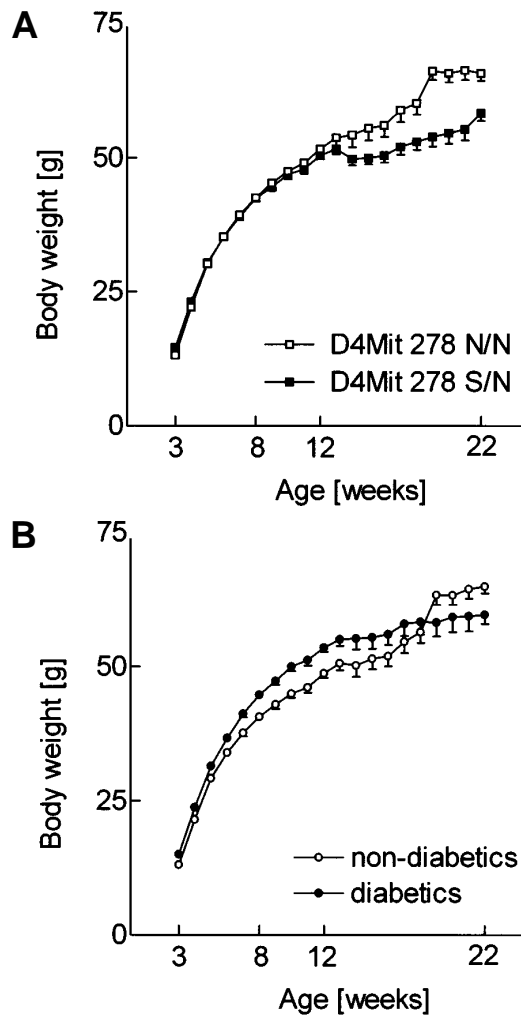


FIG. 3. Body weight development between weeks 3 and 22 in male NZO x F1 backcross mice. A: Effect of *Nidd/SJL*. B: Comparison of diabetic and nondiabetic animals. ○, Blood glucose <20 mmol/l at week 22; ●, >20 mmol/l. Data are means ± SE.

sity genes and the prevalence of diabetes. The presence of *Nidd/SJL* (genotype S/N at D4Mit278) appears to lower the obesity threshold required for the development of diabetes. However, the data also indicate that the presence of *Nidd/SJL* is not sufficient to produce hyperglycemia, but requires a certain degree of obesity for its deleterious effect.

#### DISCUSSION

The present results identify a major diabetes susceptibility locus (*Nidd/SJL*) on chromosome 4 that was responsible for ~60% of the prevalence of diabetes in the male backcross mice. The penetrance of the diabetogenic allele was markedly dependent on the obesity: it produced a 20% diabetes prevalence in backcross animals weighing <45 g at 12 weeks and a 90% prevalence in animals >55 g. Thus, the present data are consistent with the concept that the combination of obesity and diabetes genes (diabesity) provides the critical diabetogenic effect (14–16). The effect of *Nidd/SJL* is substantial and can be separated from that of other diabetogenic genes when the degree of obesity is taken into consid-

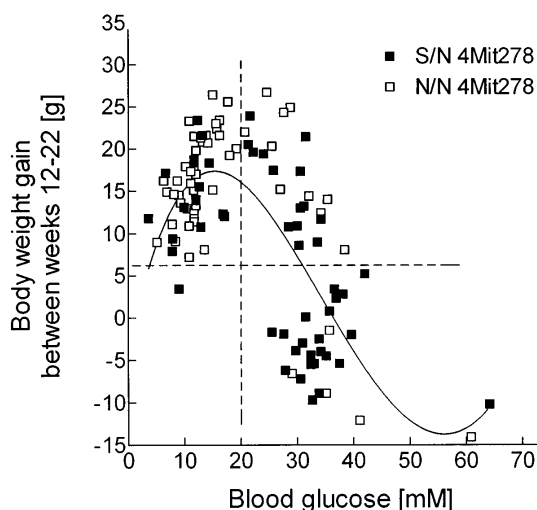


FIG. 4. Correlation between blood glucose levels at week 22 and weight gain between weeks 12 and 22 in male NZO  $\times$  F1 backcross mice. The continuous line represents the result of a nonlinear regression analysis (fourth-order polynomial). The vertical dashed line depicts the threshold of decompensated hyperglycemia; the horizontal dashed line marks the mean  $-2$  SD of weight gain in nondiabetic animals.

eration. Thus, the locus seems to be a good candidate for further efforts to identify the responsible gene(s).

In the absence of further data characterizing the metabolic effects of the diabetogenic allele, we can only speculate on the functions of the responsible gene(s). The decompensation of blood glucose homeostasis in the backcross animals was ostensibly caused by failure of the islet cells to compensate for insulin resistance. Thus, it appears possible that the gene plays a role in islet cell function and produces islet cell failure directly. Alternatively, the possibility cannot be excluded that *Nidd1/SJL* aggravates insulin resistance, thereby indirectly producing islet cell failure. It has been shown previously in mice heterozygous for a null allele of the insulin receptor and IRS1 that severe insulin resistance produces islet failure in the absence of obesity (17).

Recently, a diabetogenic locus on chromosome 4 (*Nidd1*) was identified in an intercross of NZO with the NON strain (6). The diabetogenic gene had apparently been contributed by the NON genome. Maximum LOD scores were obtained for the T1044I polymorphism within the *Lepr* gene. For the diabetogenic locus described here, maximum LOD scores for blood glucose were found 8–28 cM distal of *Lepr*. However, the QTL analysis indicated that the LOD ratios formed a remarkably broad peak, raising the possibility that more than 1 gene contributed to the diabetogenic effect of this locus.

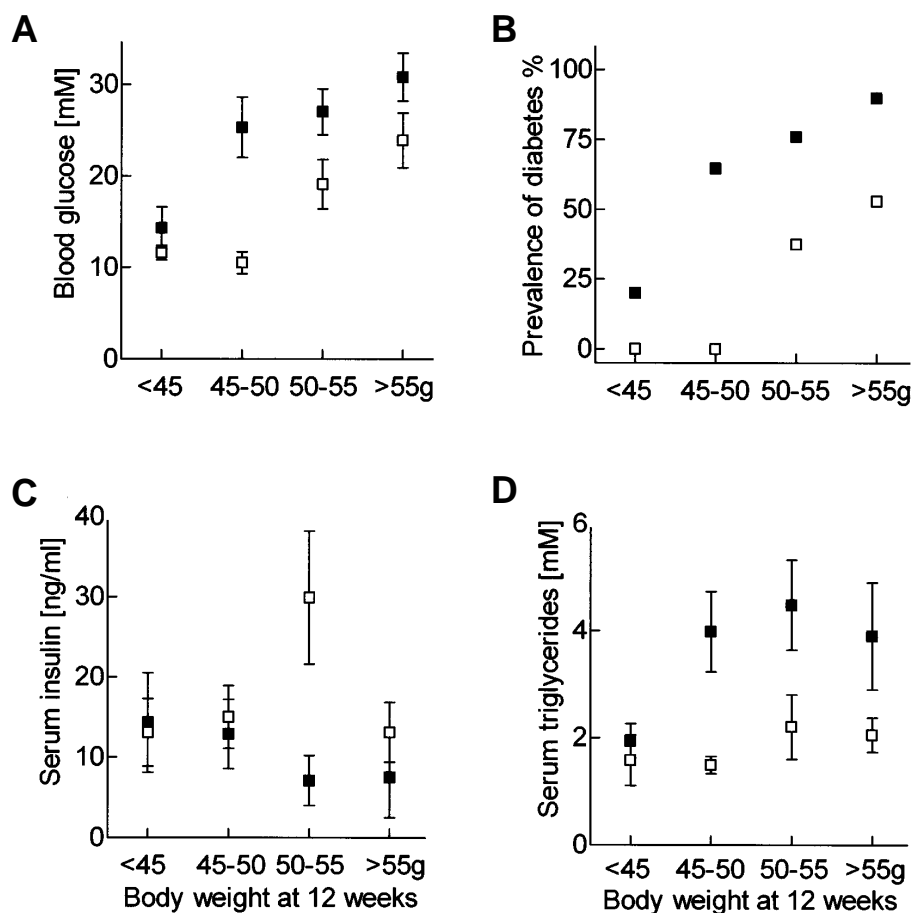


FIG. 5. Correlation between early obesity and blood glucose (A), prevalence of decompensated hyperglycemia (B), serum insulin levels (C), and serum triglycerides (D) in male NZO  $\times$  F1 backcross mice. Mice were divided into 4 groups according to their body weight at week 12, and the indicated parameters were calculated for the different genotypes. ■, Heterozygotes for D4Mit278 (S/N); □, homozygotes (N/N). The numbers of animals included in the analysis were 19 (<45 g), 26 (45–50 g), 37 (50–55 g), and 29 (>55 g).

Thus, the possibility cannot be excluded that *Nidd/SJL* comprises the same variant gene as *Nidd1*, possibly in addition to a second diabetogenic gene.

A second known diabetogenic locus on chromosome 4, *Idd11*, has been mapped close to the Na<sup>+</sup>/H<sup>+</sup> exchanger (18), 7.6 cM distal from D4Mit278, where we mapped *Nidd/SJL*. This diabetogenic locus was identified in NOD mice, and appeared associated with increased Na<sup>+</sup>/H<sup>+</sup> exchange activity in this strain (18). It should be noted that the NOD, NON, and also the SJL strains are derived from Swiss outbred colonies (Mouse Genome Informatics, The Jackson Laboratory). NOD was separated at F6 from the ICR/Swiss-derived CTS strain. NON was separated from NOD at F13. Furthermore, SJL originated from a Swiss Webster outbred stock brought to the Jackson Laboratory between 1938 and 1943. It is therefore conceivable that NOD, NON, and SJL still carry the same diabetogenic genes.

We have previously reported that NZO mice carry a leptin receptor variant with several amino acid exchanges (7). Thus, we intended to define the role of the *Lepr*<sup>A720T/T1044I</sup> variant in this study and anticipated linkage disequilibrium for body weight at *Lepr* which mapped ~7.5 cM proximal to D4Mit278. However, the difference in body weight at 22 weeks between the genotypes at D4Mit278 appeared entirely due to the diabetes-induced weight loss. Furthermore, only small and insignificant differences between the genotypes at *Lepr* were observed for the body weights at 12 weeks and in the female animals. Thus, we failed to detect an independent contribution of the *Lepr*<sup>A720T/T1044I</sup> variant to the obesity of the NZO mice. This conclusion is consistent with the finding that lean NZB mice, a strain related to NZO (19), also presented the *Lepr*<sup>A720T/T1044I</sup> allele (7). The data do not exclude, however, the possibility that *Lepr*<sup>A720T/T1044I</sup> may enhance the effect of other obesity loci.

We have no data showing that an abnormality of glucose homeostasis exists in SJL mice, although the SJL genome ostensibly contributed the diabetogenic allele. Thus, the presence of a diabetogenic locus in a strain without the diabetic phenotype needs some comment. According to the present data, the diabetogenic allele requires a certain degree of obesity; therefore, it cannot produce diabetes in the exceptionally lean SJL strain or in the moderately obese F1 animals. It is tempting to speculate, however, that the allele is responsible for other phenotypic features characteristic for the SJL strain (e.g., atherosclerosis resistance [10], spontaneous amyloidosis [20], and increased muscle regeneration [21]). Interestingly, the *Pax 7* gene, which is considered responsible for the increased muscle regeneration in the SJL strain (22), maps 2 cM proximal of D4Mit232.

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