Quantitative trait loci for obesity and insulin resistance (*Nob1*, *Nob2*) and their interaction with the leptin receptor allele (*Lepr*^{A720T/T1044I}) in New Zealand obese mice

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Abstract

Aims/hypothesis. To locate genes responsible for obesity and insulin resistance, a backcross model of New Zealand obese (NZO) mice with the lean Swiss/Jackson Laboratory (SJL) strain was established.

Results. In female NZO x F1 backcross mice, two major quantitative trait loci for variables of obesity (body weight, body mass index, total body fat) and insulin resistance (hyperinsulinaemia) were identified on chromosomes 5 (*Nob1*) and 19 (*Nob2*) close to the markers D5Mit392 and D19Mit91. The aberrant alleles have presumably contributed by the NZO genome. Whereas *Nob1* contributed mainly to higher body weight, *Nob2* seemed to mainly aggravate insulin resistance independent of obesity. The leptin receptor variant of NZO (*Lepr*^{A720T/T1044I}) failed to alter

any of the variables of obesity. It seemed, however, to enhance the effect of *Nob1* on body weight and that of *Nob2* on serum insulin concentration. When expressed in COS-7 cells, *Lepr*^{A720T/T1044I} produced a normal basal and maximum activation with a minor increase in the EC₅₀ of leptin.

Conclusions/interpretation. The data identify two new quantitative trait loci that are responsible for a major part of obesity and hyperinsulinaemia as produced by recessive genes in NZO mice. *Lepr*^{A720T/T1044I} alone cannot produce obesity, but may enhance the effects of other obesity/insulin resistance genes in this mouse model. [Diabetologia (2000) 43: 1565–1572]

Keywords Obesity, insulin resistance, New Zealand obese mouse, quantitative trait locus, leptin receptor.

New Zealand obese (NZO) mice show a polygenic syndrome of hyperphagia, obesity and insulin resistance [1–3] that resembles the human metabolic syndrome [4]. Thus, the NZO strain is an ideal model for the identification of the genes that are responsible for aberrations of body weight regulation and insulin

action in mice. Moreover, the NZO strain has been used to characterize the interaction of obesity and diabetes genes ('diabesity') and to identify diabetes genes from other mouse strains that accelerate the development of hyperglycaemia in NZO mice [5].

It has previously been suggested that leptin resistance is a primary cause of the obesity in NZO mice [3, 6]. Notably, a leptin receptor variant with four amino-acid exchanges including two non-conservative substitutions (A720T, T1044I; *Lepr*^{A720T/T1044I}) was found in the NZO strain [3]. The contribution of *Lepr*^{A720T/T1044I} to the metabolic syndrome of the NZO mouse was, however, not clear because the allele was also present in the related non-obese New Zealand Black (NZB) strain.

To further assess the contribution of *Lepr*^{A720T/T1044I} and identify other susceptibility loci for obesity and insulin resistance, we established a backcross model

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Abbreviations: NZO, New Zealand obese mice; SJL, Swiss/Jackson Laboratory mice; QTL, quantitative trait locus; STAT3, signal transducer and activator of transcription 3; LOD, likelihood of the odds; EC₅₀, effective concentration 50.

Table 1. Comparison of body weights and serum variables at week 22 in parental (NZO and SJL) and F1 mice

	SJL	NZO	F1(SJL x NZO)	NZO x F1
Males				
Body weight week 12 (g)	23.3 ± 1.5	50.3 ± 2.9 ^c	32.7 ± 2.7 ^c	51.1 ± 6.7
Body weight week 22 (g)	27.4 ± 2.2	50.2 ± 9.4 ^c	45.8 ± 3.3 ^c	61.8 ± 11.5
BMI (g/cm ²)	0.290 ± 0.01	0.391 ± 0.06 ^c	0.377 ± 0.02 ^c	0.449 ± 0.007
Total body fat (g)	2.6 ± 0.8	13.1 ± 4.9 ^c	18.9 ± 7.5 ^c	19.4 ± 7.6
Blood glucose (mmol/l)	9.5 ± 1.1	22.7 ± 7.1 ^c	12.5 ± 2.0 ^c	21.6 ± 12.0
Serum insulin (ng/ml)	1.4 ± 1.2	6.8 ± 8.4 ^b	11.9 ± 7.6 ^c	14.1 ± 19.9
Females				
Body weight week 12 (g)	19.8 ± 1.2	43.4 ± 4.9 ^c	26.8 ± 1.9 ^c	40.6 ± 5.7
Body weight week 22 (g)	21.6 ± 0.8	58.6 ± 8.3 ^c	33.6 ± 2.9 ^c	54.1 ± 5.8
BMI (g/cm ²)	0.242 ± 0.01	0.463 ± 0.06 ^c	0.293 ± 0.02 ^c	0.411 ± 0.05
Total body fat (g)	2.1 ± 0.3	24.5 ± 4.2 ^c	9.2 ± 2.3 ^c	20.7 ± 4.4
Blood glucose (mmol/l)	8.5 ± 1.7	11.6 ± 3.4 ^a	11.6 ± 1.8 ^a	10.8 ± 4.1
Serum insulin (ng/ml)	1.1 ± 0.3	39.5 ± 36.5 ^c	1.6 ± 0.7	21.3 ± 32.8

Means ± SD of at least ten animals are given. Differences to SJL were tested for statistical significance by *t* test analysis (^a, *p* < 0.05; ^b, *p* < 0.01; ^c, *p* < 0.001). For comparison, means of the backcross population NZO x F1 are given

of NZO mice with the lean and normoglycaemic SJL strain [7, 8]. In the present paper, we report the identification of two new quantitative trait loci (QTLs) for obesity and insulin resistance on chromosomes 5 and 19. Furthermore, the effects of these QTLs seem to be enhanced by the leptin receptor allele (*Lepr*) of NZO.

Materials and methods

Animals. We obtained Swiss/Jackson Laboratory (SJL) (SJL/NBom) and NZO mice (NZO/HIBom) from Bomholtgard (Ry, Denmark). Female SJL and male NZO mice were used to found an F1 generation and backcrosses (SJL x F1 and NZO x F1) were bred. After weaning (3 weeks of age), mice received a rodent chow (C1057; Altromin, Lage, Germany) with 16% fat, 46.8% carbohydrates, 17.1% protein and 15.4 kJ/g. Throughout the study mice had free access to food and water. We kept three to six mice in each 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 6:00 a.m. Animals were killed at the age of 22 weeks in isoflurane anaesthesia by exsanguination followed by decapitation. The study was approved by the committee for ethics of animal experimentation at the district administration office, Köln, Germany.

Body weight, body mass index and total body fat. Body weight and food consumption were measured once every week during the study. Body length (without tail) was measured once after 22 weeks with the millimetre scale of plotting paper on which the carcasses were fixed. Because of differences in the length of the backcross animals (± 5%), a body mass index (BMI) was calculated as an additional measure of obesity (BMI = body weight [g]/(body length [cm])²). To measure 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 analyser (type 15300/15500; N. Foss Elektronik, Hamburg, Germany).

Serum variables. Blood glucose, serum cholesterol and serum triglyceride concentrations were measured by an auto-analyser (Johnson & Johnson, Neckargemünd, Germany). Serum insu-

lin concentrations were measured by radioimmunoassay (Amersham-Pharmacia, Freiburg, Germany) with anti-rat insulin antiserum and ¹²⁵I-labelled rat insulin as tracer. Free and bound radioactivity were separated with an anti-IgG antibody. Duplicate samples were assayed and, if necessary, re-assayed after appropriate dilution.

Genotyping, genetic maps and QTL analysis. We prepared DNA from mouse tails with a DNA isolation kit (InViTek, Berlin, Germany) based on a salt precipitation method. In the initial genome scan, animals were genotyped for 109 polymorphic microsatellite markers by PCR with oligonucleotide primers obtained from Research Genetics (Huntsville, Ala., USA) and microsatellite length was measured by non-denaturing polyacrylamide gel electrophoresis (10% gels). A QTL analysis was carried out as described [9] with the program Mapmaker/QTL 1.1 [10] after construction of the genetic map with Mapmaker/EXP 3.0 [11].

Transfection of COS-7 cells with leptin receptor cDNA and assay of STAT3 activation. Wild-type and NZO leptin receptor cDNA [3] were subcloned into the pSVL expression vector and COS-7 cells were transfected with these plasmids with the aid of diethylaminoethanol (DEAE) dextran as described [12]. Cells were stimulated as indicated with leptin (Prepro-Tech, London, UK) for 15 min 48 h after transfection and harvested by lysis with NP-40 (ICN Biochemicals, Aurora, Ohio, USA). Nuclear extracts were prepared as described [13] and tested for tyrosine phosphorylation and activation of signal transducer and activator of transcription 3 (STAT3) by immunoblotting (Phospho Plus STAT3 Antibody kit, New England Biolabs, Beverly, Mass., USA) and by gel retardation assay with the *sis*-inducible element of the *c-fos* promoter as the probe [14, 15]. Immunoblots were quantitated by laser densitometry, and optical density readings (arbitrary units) were corrected for background values.

Results

Characterization of the parental, F1 and backcross mice. Table 1 summarizes the variables; body weight, body mass index, total body fat, blood glucose and se-

Fig. 1 A–D. Distribution of lod ratios on chromosome 5 for the traits body mass index (BMI) (A), body weight at 12 (B) or 22 (C) weeks and total body fat (D) in the NZO x F1 backcross population. LOD scores were calculated (MAPMAKER/QTL) with data from 95 female (BMI, body weight week 22, total body fat) or from 95 female and 111 male (body weight week 12) NZO x F1 backcross mice

rum insulin in the parental strains, F1 and backcross progeny at 22 weeks of age. Compared with the lean, normoglycaemic SJL strain, male as well as female NZO mice had much higher body weights, serum cholesterol, triglycerides, insulin and glucose concentrations (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 hyperglycaemia between 12 and 22 weeks of age; in these animals low serum insulin concentrations were measured. In contrast, none of the females developed hyperglycaemia by week 22. All female NZO mice had greatly increased serum insulin concentrations, reflecting the severe insulin resistance of this strain (Table 1).

F1 mice were generated by mating SJL females with NZO males and by mating NZO females with SJL males. Because the litter frequency of the latter intercross was very low, only SJL x NZO mice were bred in larger numbers and used for further study. In the F1 females (Fig. 1), body weights and BMI were higher than in SJL, but considerably lower than in NZO mice. Severely hyperglycaemic (> 16.7 mmol/l) male NZO mice failed to gain weight between week 12 and 22 and had a lower total body fat than the F1 progeny. None of the F1 progeny developed severe hyperglycaemia. In male F1 mice, serum insulin concentrations were statistically significantly increased. In contrast to parental NZO, female F1 mice had normal serum insulin concentrations.

We established two backcross populations (SJL x F1 and NZO x F1). The traits body weight, BMI and total body fat were unstable in the SJL x F1 backcross in an initial characterization and we failed to obtain evidence for linkage disequilibrium in this backcross population (150 mice). In the NZO x F1 backcross, 48% of the male mice developed severe hyperglycaemia between weeks 12 and 22 because of a major diabetogenic allele that was contributed by SJL [16]. The severe hyperglycaemia seemed to greatly affect the weight gain of the male backcross progeny. Thus, only female NZO x F1 mice were used in the initial screens for obesity and insulin resistance loci.

Identification of an obesity QTL (Nob1) at D5Mit392 on chromosome 5. In an initial genome wide scan, 54 non-selected female backcross progeny were genotyped for 109 polymorphic markers distributed over the whole genome. A comparison of the genotypes with the variables of obesity suggested that markers on several chromosomes might be in linkage disequilibrium. Therefore, a total of 95 mice were genotyped for microsatellite markers on chromosomes 1, 3–8, 13, 15, 18, and 19. In this analysis, a QTL predisposing for higher body weight was obtained on chromosome 5 with maximum likelihood of the odds (LOD scores) at 32 cM close to the marker D5Mit392 (Fig. 1). High LOD scores were obtained for BMI (3.8; with 16.8% of the variance explained), body weight at week 22 (3.4) and total body fat (3.0). For the 12-week body weights, LOD scores were 1.8 for female and 3.1 for male mice; a combined LOD score of 4.4 was obtained after correction of the female body weights (by addition of the difference between means of males and females to each female). A considerably lower LOD score (1.8) was obtained for serum insulin (log immunoreactive insulin). This LOD score was greatly reduced (0.5) when serum insulin was corrected for body fat with the aid of the correlation between serum insulin concentrations and total body fat [4].

Table 2 summarizes the characteristics of the female NZO x F1 backcross animals separated by their

Table 2. Effects of the genotype at D5Mit392 on the variables of body weight development and on blood glucose, serum insulin and serum lipids in female NZO x F1 backcross mice

	N/N (40)	S/N (55)
Weight at week 12 (g)	42.6 ± 5.8	39.2 ± 5.2 ^b
Weight at week 22 (g)	57.8 ± 8.3	51.4 ± 7.3 ^c
Weight gain week 12–22 (g)	15.3 ± 4.4	12.2 ± 4.4 ^b
BMI (g/cm ²)	0.433 ± 0.05	0.394 ± 0.04 ^c
Total body fat (g)	22.6 ± 4.5	19.3 ± 3.8 ^c
Blood glucose (mmol/l)	11.9 ± 5.1	9.9 ± 3.1 ^a
Serum insulin (ng/ml)	30.8 ± 40.4	14.4 ± 24.2 ^b
Triglycerides (mmol/l)	1.64 ± 0.60	1.54 ± 0.38
Serum cholesterol (mmol/l)	3.87 ± 0.65	3.82 ± 0.50

Means ± SD of a number of animals given in parenthesis are presented. Statistical significance: ^a, $p < 0.05$; ^b, $p < 0.01$; ^c, $p < 0.001$. NN, homozygotes for the NZO allele; SN, heterozygotes for the NZO allele

Table 3. Effects of the genotype at D19Mit91 on the variables of body weight development and on blood glucose, serum insulin and serum lipids in female NZO x F1 backcross mice

	N/N (48)	S/N (47)
Weight at week 12 (g)	41.6 ± 5.0	39.5 ± 6.2
Weight at week 22 (g)	56.4 ± 7.5	51.7 ± 8.6 ^a
Weight gain week 12–22 (g)	14.8 ± 4.3	12.3 ± 4.5 ^a
BMI (g/cm ²)	0.42 ± 0.04	0.40 ± 0.05 ^a
Total body fat (g)	21.7 ± 4.0	19.7 ± 4.7
Blood glucose (mmol/l)	11.0 ± 4.6	10.5 ± 3.6
Serum insulin (ng/ml)	31.5 ± 41.7	10.8 ± 14.1 ^b
Triglycerides (mmol/l)	1.65 ± 0.43	1.52 ± 0.53
Serum cholesterol (mmol/l)	3.91 ± 0.52	3.79 ± 0.61

Means ± SD of a number of animals given in parenthesis are presented. Statistical significance: ^a, $p < 0.05$; ^b, $p < 0.005$. NN, homozygotes for the NZO allele; SN, heterozygotes for the NZO allele

Fig. 2A–C. Distribution of lod ratios on chromosome 19 for the traits serum insulin (A), insulin sensitivity index (B) and body mass index (C) in the female NZO x F1 backcross population. LOD scores were calculated (MAPMAKER/QTL) with data from 95 female NZO x F1 backcross mice. The broken line (A) represents LOD scores for serum insulin concentrations that were corrected for total body fat

genotype at D5Mit392. Homozygotes had significantly higher body weights at week 12 and 22, higher weight gain, body mass index and total body fat. Furthermore, serum insulin was twofold higher in the homozygotes. In contrast, serum triglycerides and cho-

lesterol concentrations did not differ significantly. These data indicate that a major obesity QTL, presumably contributed by the NZO genome, is located near the marker D5Mit392; we designated this QTL *Nob1* (New Zealand obese 1).

Identification of a QTL on chromosome 19 predisposing predominantly for hyperinsulinaemia. The data from the initial screen suggested that markers on chromosome 19 might be in linkage disequilibrium for the trait serum insulin. A QTL analysis with 95 animals genotyped for additional markers indicated a LOD score peak for serum insulin (3.2; 14.2% of the variance explained) at 44 cM in the vicinity of the marker D19Mit91 (Fig. 2); we designated this QTL *Nob2*. The LOD scores for the variables of obesity were considerably lower (body weight 12, < 1; body weight 22, 2.0; BMI, 2.1; total body fat, 1.5). After correction for body fat, the LOD score of *Nob2* for serum insulin was only moderately reduced (2.6), in contrast to that of *Nob1* (0.5).

Table 3 summarizes the characteristics of the female NZO x F1 backcross animals separated by their genotype at *Nob2* (D19Mit91). Homozygous mice had threefold higher serum insulin concentrations than heterozygotes. Furthermore, body weights at weeks 12 and 22, weight gain, body mass index and total body fat were higher in the homozygous animals. In contrast, serum triglycerides and cholesterol concentrations did not differ significantly. Thus, D19Mit91 marks a QTL predisposing predominantly for hyperinsulinaemia.

Relation between body weight and hyperinsulinaemia in NZO x F1 backcross mice. We had anticipated that the effect of each QTL on serum insulin concentrations would be a direct function of its effect on body weight. The finding that *Nob1* and *Nob2* seemed to have contributed differently to hyperinsulinaemia and obesity was unexpected and led us to

Fig. 3 A–C. The relation between obesity and hyperinsulinaemia in the female NZO x F1 backcross population. **A** Correlation between body mass index and serum insulin in female NZO x F1 backcross mice. The straight line represents the result of a linear regression analysis. $r^2 = 0.5490$. **B** Effects of body weight and homozygosity for *Nob1* (D5Mit392) on serum insulin concentrations. The backcross population was divided into four groups according to their 22 week body weight, and mean serum insulin concentrations $\pm$ SEM were calculated. **C** Effects of body weight and homozygosity for *Nob2* (D19Mit91). $\blacksquare$ — homozygotes for the NZO allele, $\square$ — heterozygotes for the NZO allele

analyse the correlation between these two traits in the backcross population. We found a close correlation between BMI and serum insulin ($r^2 = 0.5490$) (Fig. 3). *Nob1* (homozygosity for the NZO allele at D5Mit392) seemed to increase serum insulin concentrations only in mice weighing 55–60 g whereas *Nob2* (homozygosity for the NZO allele at D19Mit105) aggravated hyperinsulinaemia in all weight groups (Fig. 3). Thus, the contribution of *Nob2* to hyperinsulinaemia seemed to be in part independent of obesity.

Fig. 4 A, B. The *Lepr* locus enhances the effect of *Nob1* on body weight (**A**) and the effect of *Nob2* on serum insulin concentrations (**B**). Mice were divided into four groups according to their genotype at *Lepr* and *Nob1* (**A**) or at *Lepr* and *Nob2* (**B**) and the variables body weight (**A**) and serum insulin concentrations (**B**) were calculated (means $\pm$ SEM). +, homozygosity for the indicated locus; -, heterozygosity

Interaction between Nob1, Nob2 and Lepr^{A720T/T1044I} in female NZO x F1 backcross mice. Genotyping for the *Lepr* locus failed to show a statistically significant adipogenic effect of the variant allele in the female backcross animals. We found, however, that *Lepr^{A720T/T1044I}* enhanced the effect of *Nob1* on body weight (Fig. 4). In addition, *Lepr^{A720T/T1044I}* also seemed to enhance the effect of *Nob2* on serum insulin concentration (Fig. 4).

Combined effects of Nob1, Nob2 and Lepr^{A720T/T1044I} on body weight and hyperinsulinaemia. To assess the combined contribution of *Nob1*, *Nob2* and *Lepr* to the obesity syndrome, means of homozygotes and heterozygotes for all three loci were calculated. The 22 week body weight was 48.4 ± 2.1 g in heterozygotes and 63.5 ± 2.6 g in homozygotes (means $\pm$ SEM). Given that a comparison of parental (58.6 ± 8.3 g) with F1 (33.6 ± 2.9) mice estimates the contribution of recessive genes to the obesity syndrome (in that case 25 g of body weight at week 22), the three loci seemed to be responsible for approximately 60% of this contribution. Serum insulin concentration in heterozygotes (all three loci) was 7.5 ± 2.4 ng/ml and 66.1 ± 22.8 ng/ml in homozygotes. Thus, the three loci seem to also contribute a major part of the trait hyperinsulinaemia.

Fig. 5A–C. Signalling potential of leptin receptor variant $Lepr^{A720T/T1044I}$ expressed in COS-7 cells. COS-7 cells were transfected with wild-type (WT) or variant (NZO) leptin receptor cDNA as described and were stimulated with the indicated leptin concentrations. Leptin receptor expression, abundance of STAT3 and STAT3 phosphorylation (P-STAT) was assessed by immunoblotting with specific antisera (**A**). STAT3 activation was assessed in a gel retardation assay with the *sis*-inducible element of the *c-fos* promoter as probe (**B**). Electrophoretic mobility shift assay (EMSA) tyrosine phosphorylation of STAT3 was quantitated by laser densitometry of the immunoblot shown in panel B (**C**). —■— NZO, ...△... WT

Analysis of the signalling potential of $Lepr^{A720/T1044I}$ expressed in COS-7 cells. The data we have described suggested that the leptin receptor variant $Lepr^{A720/T1044I}$ plays a part in the pathogenesis of obesity in NZO mice. Thus, we assessed the signalling potential of the receptor variant in a system of COS-7 cells transfected with leptin receptor cDNA. Incuba-

tion of the transfected cells with leptin produced a concentration-dependent increase in tyrosine phosphorylation of STAT3 (Fig. 5). This increase in tyrosine phosphorylation was accompanied by an increase in its activity to shift the mobility of a STAT3-binding oligonucleotide in the gel retardation assay (Fig. 5). The densitometric analysis indicated that the maximum response produced by wild-type and NZO leptin receptor was identical. At submaximum leptin concentrations the signalling potential of $Lepr^{A720/T1044I}$ seemed, however, somewhat lower (Fig. 5). Consequently, the EC₅₀ of $Lepr^{A720/T1044I}$ was higher than that of the wild-type leptin receptor (23.7 ± 4.7 vs 18.0 ± 5.9 nmol/l in controls, means $\pm$ SEM of three independent transfections).

Discussion

Our study identifies two major susceptibility loci (*Nob1* and *Nob2*) for obesity and hyperinsulinaemia,

presumably contributed by the NZO genome, on chromosomes 5 and 19. Together, these QTLs are responsible for a major portion of the total contribution of the NZO genome to body weight and hyperinsulinaemia in the female backcross on NZO. Furthermore, the *Lepr* locus seemed to enhance the effects of both loci, suggesting that the responsible genes are involved in a common control mechanism regulating body weight and insulin sensitivity. Thus, these loci are candidates for further efforts to identify the responsible genes.

In the absence of further data characterizing the metabolic effects of the adipogenic alleles, we can only speculate on the functions of the responsible gene(s). A survey of the genomic regions close to *Nob1* and *Nob2* indicated that none of the known obesity genes, or components of the insulin signalling mechanism, are located in the vicinity of the loci. Furthermore, none of the previously described obesity QTLs map close to the loci identified here [17]. A few candidate genes that might, however, be involved in the regulation of body weight are located near *Nob1* or *Nob2* (mouse genome database, The Jackson Laboratory). The dopamine receptor 5 maps close to *Nob1* (at 23 cM on chromosome 5). A vesicular monoamine transporter (at 53 cM), the adrenergic beta-1 receptor (at 51 cM) and the adrenergic alpha-2 receptor (at 50 cM) map close to *Nob2* on chromosome 19.

Our data are consistent with the conclusion that *Nob1* and *Nob2* represent genes that are responsible for obesity and hyperinsulinaemia of NZO mice. Alternatively, the possibility cannot be ruled out that these loci represent variant genes contributed by the SJL genome and that these variants suppress obesity and insulin resistance in a dominant fashion. SJL mice are characterized by high activity (running, fighting, climbing) [18] and by resistance to a high-fat diet [8]. Higher activity was apparent in our parental mice and also in the backcross on SJL (not described here because of instability of the obesity traits). The activity of the female NZO x F1 backcross mice analysed here was, however, not higher than that of the parental NZO. Thus, if *Nob1* or *Nob2* represented suppressor genes contributed by SJL, their effect on obesity and insulin resistance would probably not be mediated by an increased activity of the backcross mice.

In most of the mouse models described so far, obesity is usually associated with hyperinsulinaemia [2]. Accordingly, the phenotypic characterization of the backcross population indicated a statistically significant correlation between serum insulin concentrations and BMI. Therefore, we initially assumed that insulin resistance is an invariable consequence of obesity and anticipated that we would locate obesity QTLs with equal contribution to hyperinsulinaemia. In contrast, the data indicated a striking difference

between the contributions of *Nob1* and *Nob2* to body weight and hyperinsulinaemia. Whereas *Nob1* does not seem to exert a primary effect on serum insulin concentrations, *Nob2* increased them independent of body fat. Thus, the data are consistent with the conclusion that hyperinsulinaemia in the NZO mouse is not solely produced by obesity genes but also by genes that affect insulin action independent of obesity.

We have previously reported that NZO mice carry a leptin receptor variant (*Lepr*^{A720T/T1044I}) with several amino-acid exchanges [3]. The data in this study indicate that the exchange of one *Lepr*^{A720T/T1044I} allele alone for the wild-type allele does not statistically significantly reduce the body weight of NZO mice. Consistent with this finding, the signalling potential of the leptin receptor variant seemed little, if at all, reduced compared with that of the wild-type receptor. The data also indicated, however, that the *Lepr* locus of NZO mice enhanced the effects of *Nob1* and *Nob2*. Furthermore, the data do not exclude the possibility that variables other than the STAT3 activation are reduced in *Lepr*^{A720T/T1044I}, e.g. its cellular targetting. It should be noted that leptin resistance in the NZO mouse seems to be due to an impaired (receptor-mediated ?) transport of leptin into the brain [6]. Thus, it cannot be excluded that a functional aberration of the leptin receptor variant *Lepr*^{A720T/T1044I} which is not detectable in the COS-7 cell system contributes to the obesity syndrome of NZO mice.

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