Deconstructing and Reconstructing Obesity-Induced Diabetes (Diabesity) in Mice

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Obesity-driven type 2 diabetes (diabesity) involves complex genetic and environmental interactions to trigger disease. Here, we combine variable numbers of known quantitative trait loci (QTL) for obesity and diabetes contributed by New Zealand Obese (NZO/HlLt) and Nonobese Nondiabetic (NON/Lt) strains in the form of 10 interval-directed recombinant congenic strains (RCS), with NON/Lt as the background strain, to dissect the genetic interactions involved. All 10 RCS gain significantly more weight than the NON parental strain, but none are as obese as the parental, diabetes-prone NZO. Diabetes development in these RCS at F12 ranges between 0 and 100%, depending on genetic constitution. RCS-2, -1, and -10 represent a step-wise increase in numbers of specific diabetogenic QTL, resulting in a step-wise increase in diabetes incidence. RCS-10 recreates the 100% incidence seen in (NZOxNON)F1 males, but with less weight gain. Similarly, RCS-6, -7, -8, and -9 represent diabetes-prone strains with different combinations of diabetogenic QTL. RCS-3, -4, and -5 represent obese strains that do not transit to diabetes. Because these obesity and diabetes syndromes reflect different collections of QTL, rather than null mutations in the leptin or leptin receptor genes, they are extremely relevant as models for the polygenic obesity/diabesity syndromes in humans. *Diabetes* 51:825–832, 2002

Although murine monogenic obesity mutations, such as those in leptin (*Lep*^{ob} [obese]) or its receptor (*Lepr*^{db} [diabetes]), have been extensively used to test antiobesity and antidiabetic drugs, the monogenic basis of their obesity is not reflective of the more common human obesities, which are polygenic in origin (1). Type 2 diabetes is known to be a polygenic syndrome, and more polygenic obesity rodent models are needed in which quantitative trait loci (QTL) interact with each other and with the environment to elicit obesity syndromes that are potentially diabetic. The New Zealand Obese (NZO/HlLt) mouse represents one such model. These mice are hyperphagic and develop juvenile-onset obesity, even when fed a low-fat (4–6%) diet. This obesity syndrome is characterized by postpubertal development of hyperleptinemia, hyperinsulinemia, and impaired glucose tolerance (2). Approximately 50% of NZO/HlLt males transit from impaired glucose tolerance to diabetes by 24 weeks of age (3). The strain is very difficult to breed, presumably because of juvenile-onset obesity, but perhaps also because of defects in leptin transport/receptor signaling (4,5). F1 hybrids between NZO and the relatively lean, though glucose intolerant, Nonobese Nondiabetic (NON/Lt) strain have the same obesity phenotype as NZO, but deleterious contributions from both parental genomes "synergize" to force 90–100% diabetes in the F1 males by 24 weeks of age (6). Genetic analysis of reciprocal F2 and backcross between NZO and NON (3,6) has identified a number of QTL for obesity and diabetes subphenotypes contributed by both NZO and NON parental backgrounds. This genetic information allowed development of a set of 10 interval-directed recombinant congenic strains (RCS) by inbreeding after the second backcross generation (N3) to NON/Lt.

Recombinant congenic strains are particularly useful for analysis of polygenic syndromes. For a single gene contributing a major proportion of the variance in a phenotypic trait, a congenic strain will suffice. However, this approach is inadequate when a complex phenotype represents a variable collection of QTL that separately may make relatively small contributions to the variance in the trait. A single congenic region may not recreate the phenotype or even a subphenotype. RCS increase the chance of bringing phenotype-causing genotypes together and limit the donor strain's contribution to ~12.5% at the same time. RCS have been used to dissect complex genetic susceptibilities to cancer (7,8) and insulin-dependent diabetes resistance (9,10).

Obesity QTL have been reported on most of the mouse chromosomes (11). The NZO strain, selected for polygenic obesity, is known to contribute obesity/diabetes QTL on chromosome (Chr) 1, 2, 4, 5, 6, 7, 11, 12, 13, 15, 17, and 18 (3,12,13). RCS development using the genetic background of the relatively lean NON as a platform limits the number of genomic effects contributed by NZO so that their contributions can be more rigorously assessed. Selection for specific genomic intervals in the RCS allows testing of interactions predicted by previous crosses and also allows unknown effects to be unmasked. Ten lines were constructed by selection for certain genotypes and/or phenotypes by interval-directed inbreeding after two backcrosses (N3) to NON. The resulting RCS, analyzed at F12, have differing NZO-derived genomic regions and consequently differing levels of obesity and diabetes susceptibility.

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ALT, alanine aminotransferase; BUN, blood urea nitrogen; Chr, chromosome; NIH, National Institutes of Health; PAS, periodic acid-Schiff; PG, plasma glucose; PI, plasma insulin; PL, plasma leptin; QTL, quantitative trait loci; RCS, recombinant congenic strains.

TABLE 1
Profile of presumed obesity/diabesity QTL directed into the 10 RCS. The microsatellite* shown marks each QTL region (D1 = Chr 1, etc.), with the donor of the diabetogenic allele indicated beneath the allele

	Locus/diabetogenic allele								
RCS	*D1Mit411* NZO	*D15Mit159* NZO	*D13Mit53* NZO	*D11Mit261* NZO	*D11Mit41* NZO	*D12Mit231* NZO	*D4Mit166* NON	*D18Mit60* NON	*D5DMit7* NZO
1	●	●		●	●		●	●	
2	●			●	●		●	●	
3							●	●	
4				●	●		●	●	●
5			●						
6				●	●	●	●	●	●
7				●		●	●	●	●
8	●			●			●	●	
9							●	●	
10	●	●		●	●	●	●	●	●
NZO	●	●	●	●	●	●			●
NON							●	●	

*Microsatellite genetic map positions may be found at the Mouse Genome Informatics website (http://www.jax.informatics.org).

RESEARCH DESIGN AND METHODS

Interval-directed RCS development. The current genome-wide scan profiles for all 10 RCS can be viewed at our website (http://www.jax.org/research/leiter/documents/genomics2.html). The formal designation of the strains is NONcNZO1 through NONcNZO10. However, for brevity, they will be referred to as RCS-1 through -10. Originally, four RCS were created by two backcrosses to NON/Lt of reciprocal (NON/Lt × NZO/HlLt)F1 and (NZO/HlLt x NON/Lt)F1 matings. The remaining six RCS were produced by interval-specific selection as inbreeding of these lines progressed. The four stem lines were ultimately designated NONcNZO3 and NONcNZO6 [from the (NON/Lt × NZO/HlLt)F1 outcross] and NONcNZO4 and NONcNZO5 [from the (NZO/HlLt × NON/Lt)F1 outcross]. At the second backcross (N3), individual mice were identified that were heterozygous for NZO or NON markers previously demonstrated in either F2 or N2 segregation analysis to be associated with the diabetes subphenotypes of elevated plasma glucose (PG), elevated or lowered plasma insulin (PI), and elevated body weight. Such mice were selectively bred to fix the diabetogenic and nondiabetogenic alleles into different RCS. Table 1 shows which NZO-derived QTL were selected for or against in each RCS. With the exception of RCS-5, the RCS are fixed for diabetogenic nonderived QTL on Chr 4 (*Nidd1*) and Chr 18 (*Nidd3*). The presence or absence of NZO-contributed genome on Chr 11 (*Nidd2*) and other NZO QTL identified in N2 backcross analysis (not given "*Nidd* " designations because of their complex epistatic interactions) varied among the 10 RCS, as shown in Table 1. The table shows two different markers on Chr 11 because of the likelihood that each marker may denote a separate QTL (6). RCS-1, -2, -8, and -10 were fixed for NZO alleles around *D1Mit41*, a marker for a NZO-derived diabesity QTL significantly associated with early increase in body weight, elevated PI, and hyperglycemia (3). Similarly, RCS-1 and -10 are homozygous for NZO alleles at and tightly flanking *D15Mit159*, marking a QTL significantly associated in N2 analysis with elevated PG and suggestively with maturity-onset increase in body weight (3). RCS-6, -7, and -10 carry the NZO-derived QTL found in the N2 analysis marked by *D12Mit231* to be significantly associated with increased adiposity index, increased serum leptin, and increased BMI and suggestively associated with increased body weight (3). RCS-4, -6, -7, and -10 are homozygous for an NZO-derived QTL marked by *D5Mit7* that was significantly associated with increased adiposity index and suggestively with elevated serum leptin in N2 analysis (6). RCS-5 carried an NZO-derived QTL at *D13Mit53* that was suggestively associated with increased BMI in N2 analysis (3).

RCS-2 is actually a subline of RCS-1 created by selection against the NZO diabesity QTL on Chr 15 (marked by *D15Mit159* at 49.6 cM) at N3F8. By N3F12, RCS-1 contained NZO markers covering a 12.6-cM span between *D15Mit238* (46.6 cM) and *D15Mit42* (59.2 cM). At this generation, RCS-2 had acquired NON alleles between *D15Mit238* (46.6 cM) and *D15Mit243* (56.7 cM), leaving only a ~2.5 cM residuum of NZO genome distal to the diabesity QTL. RCS-8 and -9 were derived from a group of four N3F3 males aged from RCS-3. It was noticed that all four were very large and hyperglycemic. At 28 weeks of age, they were mated to young (N3F5) RCS-3 females from the same lineage. Three of the four males successfully mated to produce offspring, the females of which were backcrossed to the F3 male parent. Two of these matings were successful, whereupon the offspring were intercrossed and subsequent lineages selected for the development of obesity and diabetes. RCS-8 is distinguished from RCS-9 by the presence of a large segment of NZO genome on Chr 16 and a smaller segment on Chr 14. At N3F13 (after the initial incidence study), RCS-9 stopped breeding. Two males from the last litter produced were backcrossed to the background NON strain (N4). Offspring were intercrossed and selected for the NZO-derived loci known to be present in the RCS-9 at N3F13. However, in the recovery process, the proximal portion of Chr 14 and all of Chr 16, which previously had been NZO derived, had recombined to NON genotype. RCS-4, -6, and -7 had a similar reproductive slowdown, but breeding was recovered by feeding weanling mice regular diet supplemented with a β-3 adrenergic receptor agonist (0.001% CL 316,243; a kind gift of Dr. K. Steiner, Wyeth-Ayerst, Princeton, NJ) for 4 weeks to retard rapid juvenile weight gain (14), a strategy used for the NZO colony as well as RCS-10. RCS-10 was generated by crossing a RCS-1 female at N3F15 with a RCS-6 male at N3F11, then backcrossing a female offspring to a N3F16 male from RCS-1 before intercrossing and selecting for NZO markers on Chr 1 (*D1Mit411*), Chr 5 (*D5Mit7*), Chr 12 (*D12Mit231*), and Chr 15 (*D15Mit159*). Unless otherwise noted, mice not used as breeders were maintained on unsupplemented National Institutes of Health (NIH)-31 diet containing 4% fat (Purina, Richmond, IN) and acidified water. Mice were housed in double-pen Plexiglas boxes on shaved pine bedding and given free access to food and water. All mice shared the same mouse room with controlled temperature and humidity and a 12/12-h light/dark cycle. Males aged for phenotypic analysis and diabetes development were fed NIH-31 diet containing 6% fat and housed two to five per pen.

Genetic analysis. A total of 103 microsatellite markers (Research Genetics, Huntsville, AL) were used both to direct the development of and to characterize the 10 RCS (see http://www.jax.org/research/leiter/documents/genomics2.html). Various thermal cyclers were used (PTC-100; MJ Research, Waltham, MA; or GeneAmp 2400 or 9600; Applied Biosystems, Foster City, CA), and products were separated on 4% Metaphor agarose gels (Biowhittaker, Walkersville, MD) or on an ABI 373A DNA Sequencer (Applied Biosystems).

Phenotypic analysis. A preliminary aging study was undertaken at the N3F12 generation for each RCS (N2F3 for RCS-10). Body weights were measured every 2 weeks beginning at 4 weeks of age. PG levels were determined every 4 weeks beginning at 8 weeks of age (Glucose 2 Analyzer, Beckman Instruments, Fullerton, CA). Hyperglycemia was defined as a PG level consistency >250 mg/dl. PI levels were measured at 24 weeks of age by a rat insulin radioimmune assay kit (Linco, St. Charles, MO). Plasma leptin (PL) levels were measured at 24 weeks of age by a mouse leptin radioimmune assay kit (Linco). A Synchron 5 chemistry analyzer (Beckman Instruments) was used for clinical chemistry. Serum alanine aminotransferase (ALT) was used as an indicator of liver damage, and aspartate aminotransferase was used as an indicator of liver and pancreas damage; amylase was also used to assess pancreatic damage, and blood urea nitrogen (BUN) level was used to assess kidney function. Total cholesterol and triglycerides in sera were also measured. Males of subsequent generations were measured for total carcass fat and percentage of fat using a bone densitometer (Piximus; LUNAR Instruments, Madison, WI) at 8, 16, and 24 weeks of age. The instrument calculates the percentage of fat from total fat/body weight × 100, with the head excluded

TABLE 2
Diabetes and subphenotypes in the males of the RCS and parental strains

Strain	NIDDM by 24 weeks (n)	BW at 16 weeks (g)	Insulin at 24 weeks (ng/ml)	Leptin at 24 weeks (ng/ml)	Food consumption at 6 weeks (g/day)
1	10/18 (56)	37.8 ± 0.6*†	3.2 ± 0.5†§	13.8 ± 3.5†	3.1
2	0/17 (0)	35.2 ± 0.8†	2.9 ± 0.5†§	11.2 ± 2.2†	3.3
3	1/19 (5)	40.9 ± 0.9*†	6.2 ± 1.2†§	10.8 ± 2.2†	4.0
4	1/14 (7)	39.9 ± 0.8*†	2.9 ± 0.2*†	8.2 ± 1.4†	4.0
5	1/19 (5)	40.0 ± 0.8*†	5.4 ± 1.2†§	8.6 ± 1.1†	4.2
6	9/20 (45)	40.7 ± 1.4*†	4.1 ± 0.7†§	11.3 ± 2.6†	4.2
7	6/16 (38)	43.5 ± 1.2*†	11.7 ± 2.9*†	9.7 ± 1.9†	3.6
8	12/18 (67)	45.5 ± 0.7*†	13.5 ± 2.4*†	20.3 ± 3.1†	4.8
9	4/14 (29)	44.8 ± 1.3*†	4.2 ± 0.9†§	8.4 ± 2.2†	3.4
10	12/12 (100)	42.0 ± 1.1*†	5.7 ± 0.4*†	23.2 ± 3.5†§	3.4
F1	29/30 (97)	56.4 ± 0.5*	9.7 ± 4.3†§	54.6 ± 5.4*	4.8
NZO	6/10 (60)	55.6 ± 0.9*	34.5 ± 4.5*	71.8 ± 3.8*	4.9
NON	0/9 (0)	31.7 ± 0.8†	1.5 ± 0.3†	11.6 ± 2.1†	3.3

Data are n (%), means ± SEM, and %. Comparative body weights are shown at 16 rather than 24 weeks because chronically diabetic males between 16 and 24 weeks may lose weight. Food consumption measured at 6 weeks of age during the period of the most rapid weight gain. *Significantly different from NON/Lt ($P < 0.0023$); †significantly different from NZO/HILt ($P < 0.0023$); §suggestive significant difference from NON/Lt ($P < 0.05$).

from the analysis. Food consumption (grams per day) was measured at 6 weeks of age in males and females by calculating the difference in the weight of the diet in the food hopper before and after 3 or 4 days, minus the wastage (sifted from litter and weighed), divided by number of mice in the pen, and then divided by the number of days.

Histopathologic analysis. At sacrifice, pancreas, liver, and kidney tissues were removed and fixed in Bouin's solution. Three separate nonoverlapping layers of pancreas were sectioned so that separate islets were profiled on each section, stained by aldehyde fuchsin, and counterstained with hematoxylin and eosin. Liver and kidney tissues were stained by periodic acid-Schiff (PAS) reagent.

Statistical analysis. Two-way ANOVA was used to compare the significance of differences in RCS weight gain over time. For phenotypic comparisons at single time points, unpaired *t* tests were conducted using Stat View (Abacus Concepts, Berkeley, CA). The significance threshold was determined by dividing 0.05 by the number of comparisons made (Bonferroni correction). In the case of Table 3, the 10 RCS and F1 are compared with NZO and NON, thus making 22 comparisons. Therefore, the threshold *P* value for significance is 0.05/22 = 0.0023. Any value <0.05 but not ≤0.0023 is denoted as suggestive. For simple comparisons between specific strain pairs, e.g., RCS-1 versus -2, the threshold *P* value was ≤0.05.

RESULTS

Genetic analysis. Randomly selected RCS generated at N3 should contain ~12.5% of the donor genome and 87.5% of the background genome. Given the nonrandom interval-directed nature of the RCS construction in this study, NZO genomic contribution in the 10 RCS was estimated (based on genome-wide scan results for each strain) to vary between 8 and 28%. Graphics depicted on our website define the known NZO-derived genomic regions on the NON background for each RCS. Table 1 shows the variable number of known QTL associated with various diabetogenic and subdiabetogenic phenotypes present in each RCS. The RCS have a variable number of susceptibility loci, ranging from RCS-5 with the least to RCS-10 with the most. Human syntenic chromosomal regions are also given.

Phenotypic analysis. Male diabetes frequencies in all 10 RCS are compared with NZO and F1 male frequencies in Table 2. Figure 1 depicts the comparative changes in body weight and PG in the related RCS-1, -2, and -10 strains to illustrate the power of RCS analysis. Males from all three RCS gain weight more rapidly than NON parental males, but not as rapidly as the NZO parental or the F1 males. Although they do not develop the same level of extreme obesity as NZO males, RCS-1 males develop hyperglycemia at a comparable frequency. However, onset is delayed compared with that of NZO or F1 males, probably because of the blunted obesity development in RCS-1. As shown in Table 2, RCS-1 and -2 do not differ significantly from each other in levels of PI, PL, or carcass fat at 24 weeks of age. Although the difference in mean body weight of RCS-2

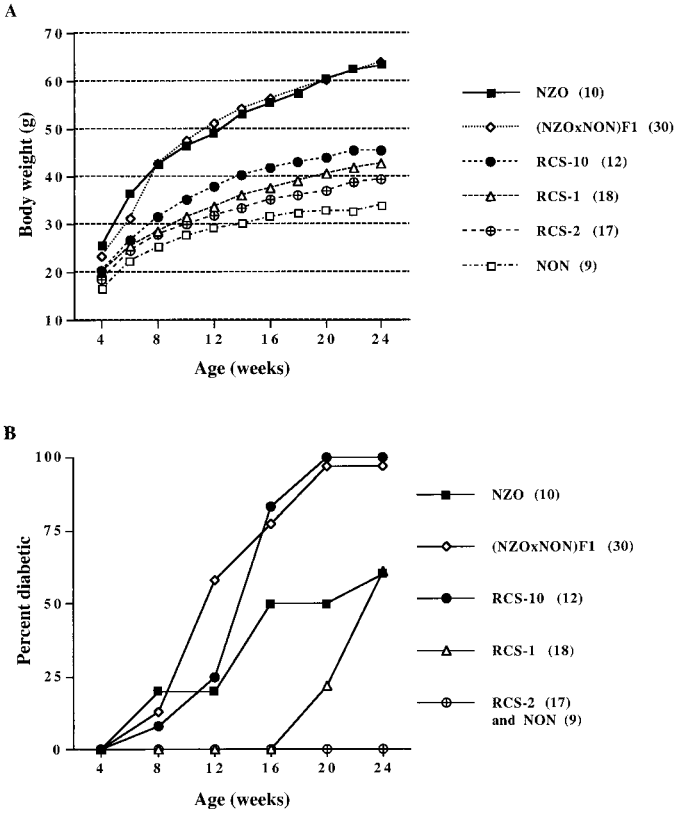

FIG. 1. *A*: Comparative changes in body weight of RCS-1, -2, and -10. Parental (NZO, NON) and F1 weight gains are shown for comparison. *B*: Diabetes development in RCS-1 and -10 compared with that of NZO and NON. Note that RCS-2 exhibits the NON strain resistance to diabetes. Number (*n*) in parentheses.

TABLE 3
Analysis of serum glucose and lipids and dual-emission X-ray absorptiometry analysis of a separate cohort for adiposity: all measurements taken at 24 weeks

RCS	*n*	PG	Total cholesterol	Triglycerides	Carcass fat (g)	Percentage of fat	*n*
1	16	280 ± 26†	113 ± 4*‡	325 ± 15*‡	12.2 ± 1.4§	28.6 ± 2.2§	6
2	16	146 ± 6*‡	98 ± 3*‡	245 ± 11†§	11.8 ± 1.2‡	30.6 ± 2.0§	6
3	19	184 ± 12§	108 ± 6*‡	280 ± 17‡†	13.7 ± 0.6	30.4 ± 0.6§	3
4	11	166 ± 11§	182 ± 11	421 ± 26*‡	16.8 ± 0.6§†	38.6 ± 0.9§	5
5	16	148 ± 9‡†	93 ± 2*‡	265 ± 14†‡	15.9 ± .7†	35.4 ± 0.9	8
6	19	217 ± 21	211 ± 11†	380 ± 17*‡	7.4 ± 1.6‡	23.2 ± 4.0§	6
7	11	257 ± 22	172 ± 12	269 ± 10‡†	17.5 ± 2.0†	37.9 ± 2.2	3
8	17	261 ± 24	155 ± 5‡	282 ± 17‡†	14.4 ± 0.7‡	32.0 ± 0.8§	4
9	14	176 ± 13§	123 ± 6‡†	250 ± 15§	12.2 ± 0.2‡	28.3 ± 0.4§	4
10	5	460 ± 36*§	170 ± 20‡	300 ± 19†§	14.6 ± 1.2§	31.3 ± 1.5§	2
F1	7	375 ± 30*§	169 ± 19	144 ± 33	20.2 ± 0.7*	33.8 ± 0.8§	7
NZO	9	260 ± 40	194 ± 2*	177 ± 20	21.1 ± 1.0*	38.2 ± 1.1§	3
NON	9	189 ± 10	152 ± 14‡	198 ± 19	13.1 ± 1.1‡	32.4 ± 1.4§	5

Data are means ± SEM unless otherwise indicated. *Significantly different from NON/Lt ($P < 0.0023$); †suggestive significant difference from NON/Lt ($P < 0.05$); ‡significantly different from NZO/HlLt ($P < 0.0023$); §suggestive significant difference from NZO/HlLt ($P < 0.05$).

(3.3 ± 0.8 g less than RCS-1 by 24 weeks; $P = 0.019$) is modest, it is significant, as are the higher serum triglycerides in RCS-1 shown in Table 3 ($P = 0.0002$ for triglycerides). As shown in Fig. 1, RCS-2 males, like NON/Lt males, fail to develop overt hyperglycemia on the 6% fat-containing diet. RCS-2 is a subline of RCS-1, constructed to evaluate the epistatic interaction of the NZO-derived QTL on Chr 1 and 15, as previously reported (3). Both RCS share the NZO genomic interval on Chr 1 (*D1Mit411-D1Mit213*, ~14 cM) that marks the major diabesity QTL, but they differ in the length of the NZO-derived genome spanning the diabesity QTL on Chr 15. Whereas RCS-1 has NZO genome proximal and distal to the QTL peak at *D15Mit159*, RCS-2 has lost (through recombination) the majority of the NZO congenic region, including the QTL peak markers. Dual-emission X-ray absorptiometry analysis revealed that these two RCS exhibited differences in their early patterns of fat accumulation. RCS-1 differed significantly from RCS-2 by exhibiting a more NZO-like adiposity development from 8 to 16 weeks (total fat, $P = 0.001$; percentage of fat, $P = 0.03$). But these parameters did not differ significantly between the two RCS in the 16- to 24-week interval, whereas NZO continued to increase total fat and percentage of fat in a nearly linear fashion (data not shown). Thus, RCS-1 has an NZO-like tendency for early weight gain by fat accretion, but not the continual NZO weight gain phenotype in both intra-abdominal and subcutaneous depots. In contrast, RCS-2 exhibited a NON-like pattern for total fat and percentage of fat, wherein levels remain low at 8 and 16 weeks, but then intra-abdominal depots increase sharply by between 16 and 24 weeks. It is interesting to note that both RCS-1 and -2 exhibit a NON level of food consumption at 6 weeks of age (Table 2); this may be a principal reason for their reduced weight gain as compared with the hyperphagic NZO parental strain.

The inference stated above that retarded onset of diabetes in RCS-1 reflects the reduced rate of weight gain/adiposity development is supported by the accelerated rate of diabetes development and the higher diabetes frequency achieved in RCS-10 (Fig. 1). RCS-10 was developed by directing specific NZO-derived genomic intervals containing diabesity QTL in RCS-6 into RCS-1. Specifically, the NZO adiposity/diabesity QTL on Chr 12 and 5 in RCS-6 were combined with those from RCS-1 on Chr 1 and 15 in an effort to initiate an earlier-onset diabesity at the high frequency characteristic of the (NZOxNON)F1. Statistical analysis had previously indicated that this set of NZO-derived QTL interacted epistatically to dramatically elevate PG when heterozygously expressed in the N2 study (3). Homozygosity for these NZO-derived diabetogenic QTL indeed allowed RCS-10 to develop the same 100% male incidence of disease as the F1, but at a lower rate of weight gain (Fig. 1). A group of 12 males (6 from N2F3 and 6 more at N2F5) were all diabetic by 20 weeks. Body weight at 16 weeks in RCS-10 (42.0 g) was significantly lower than that of F1 or NZO males (57.6 and 55.6 g, respectively), yet still significantly higher than that of RCS-1 (37.8 g, $P < 0.0001$ for all comparisons).

Diabetes and obesity in other RCS. RCS-8 develops diabetes at or above the frequency of RCS-1 (Table 2) and, like RCS-1 and -2, contains the diabetogenic QTL from NZO on Chr 1 as well as NZO-derived regions on Chr 11 and 13. However, RCS-8 does not carry the diabesity QTL on Chr 15 carried by RCS-1, nor does it carry the diabesity QTL on Chr 12 carried by RCS-6 and -10. RCS-8 is significantly larger than RCS-1 by body weight ($P < 0.0001$ at all time points). Six-week-old RCS-8 males are as hyperphagic as the parental NZO strain. Hence, RCS-8 must contain additional diabesity QTL from either NZO or NON that are not present in RCS-1. One potential region is around the marker *D11Mit41* (Chr 11), where RCS-8 carries NON markers in a region where RCS-1 carries NZO markers. In a reciprocal F2 cross between NZO and NON (6), homozygosity for NZO markers in this region was associated with low insulin. Hence, the greater hyperinsulinemia of RCS-8 compared with that of RCS-1 (Table 2) may reflect the presence of the NON alleles on Chr 11 in the former. RCS-8 also contains large segments of NZO genome on Chr 14 and 16 that may contain heretofore-unrecognized diabesity QTL.

RCS-6, -7, and -9 are obese strains with a lower diabetes incidence (28–45%) than NZO (Table 2 and Fig. 2*A*). RCS-6 and -7 are poor breeders, similar to NZO, whereas RCS-9 had a near-complete breeding stoppage requiring an additional (N4) backcross to the background NON parental

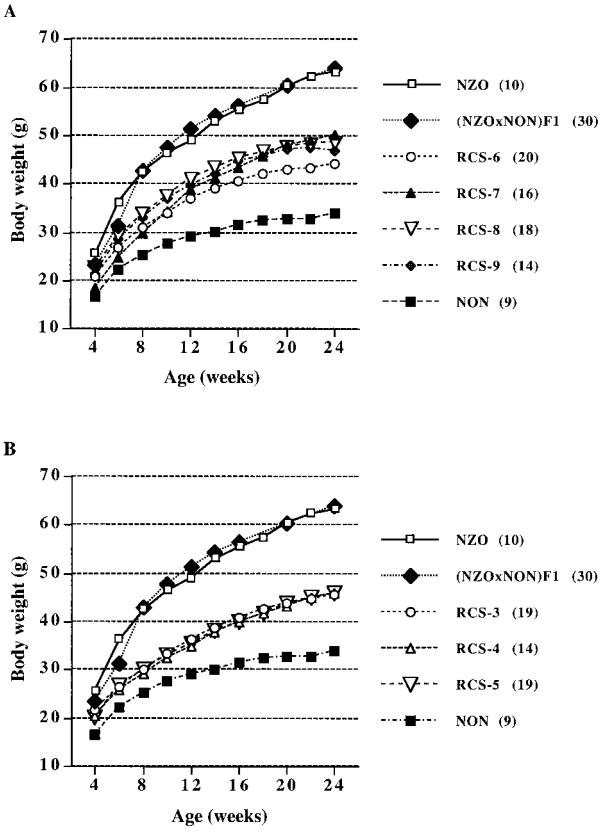

FIG. 2. ***A*****: Comparative changes in body weight of RCS-6, -7, -8, and -9.** ***B*****: Body weight changes in RCS-3, -4, and -5. Parental (NZO, NON) and F1 weight gains are shown for comparison. Number (*n*) in parentheses.**

strain to salvage the line. RCS-9 contains only part of the Chr 1 NZO–derived diabesity QTL carrying a single marker (*D1Mit213* at 25.7 cM) and lacking the more proximal markers *D1Mit123* and *D1Mit411*, probably contributing to the strain's lowered diabetes incidence as compared with RCS-1 or -8.

RCS-3, -4, and -5 are interesting as obese strains that do not transit to diabetes or do so at a very low frequency. All three strains exhibit higher body weight and food consumption than NON but not as high as that of NZO (Fig. 2*B* and Table 2). Table 3 shows that RCS-3 has similar total fat and percentage of fat values as RCS-1 and -8. Although data are only shown for the 24-week time point, this is true at 8 and 16 weeks as well. RCS-4 has the highest total fat and percentage of fat at 24 weeks of all the RCS. RCS-5 has significantly more total fat and greater percentage of fat than RCS-1 ($P = 0.008$ for both), but does not differ significantly for these parameters from RCS-8. That RCS-3, -4, and -5 lack the NZO diabesity region on Chr 1 likely contributes to their diabetes resistance. Hence, these strains harbor many obesity QTL that are not inherently diabetogenic, when the opportunity for epistatic interactions with certain diabesity QTL is precluded.

Histopathologic analysis. Figure 3 presents comparative pancreatic islet and liver morphology for RCS-1, -2, and -10. Pancreatic islet pleiomorphism was evident in both RCS-1 and -2 males necropsied at 24 weeks of age, indicative of earlier islet hypertrophy and hyperplasia. Aldehyde fuchsin staining for granulated β-cells showed partial degranulation of islet β-cells in both RCS; however, the extent of β-degranulation was somewhat less in RCS-2 than -1 (Fig. 3). The exocrine pancreatic tissue of RCS-1 was further distinguished from that of RCS-2 by the presence of numerous foci of fatty infiltration, comparable with that observed in NZO pancreata (not depicted). Livers of RCS-1 males resembled those of NZO parental males in exhibiting moderate fat accumulation in hepatocytes that were also moderately glycogen-depleted, as assessed by PAS staining. RCS-2 livers, like those of the NON parental strain, were not fatty and were PAS-positive, indicating glycogen storage. Serum ALT and amylase values were elevated for RCS-1 compared with those of RCS-2 ($P = 0.014$ and 0.001, respectively), consistent with increased stress on the pancreas and liver. Hence, the histopathology reflected the differential diabetes susceptibilities of these two genetically similar strains. The accelerated onset of diabetes in RCS-10 males was also reflected histologically. Pancreata contained variably sized islets undergoing partial-to-widespread degranulation and exhibited widespread fatty infiltrates. Overall, islet sizes were reduced compared with those of RCS-1 and -2. Some islets were atrophic, whereas others exhibited a loss of structure caused by exocrine cell infiltration (exocrinization). Livers were moderately-to-severely fatty with virtually no glycogen storage, as assessed by PAS staining, and also with varying degrees of focal hepatitis. Serum chemistry showed elevated BUN and triglyceride levels and decreased total cholesterol in RCS-10 compared with those of NZO ($P = <0.0001$ and 0.005, respectively). Total fat and percentage of fat values at 24 weeks are similar to those of NON, which correlates with the decreased body weight of both compared with that of NZO. Histology of the liver and pancreas from the diabesity-prone RCS-8 was similar to that of RCS-1. RCS-8 kidneys, however, exhibited severe glomerular aneurysms and nephritis, a strain characteristic of NON/Lt mice (15).

DISCUSSION

This study demonstrates why generation of a panel of interval-specific RCS, rather than a large number of monocongenic stocks, is required for dissecting the genetic and pathophysiological basis of a complex disease, such as type 2 diabetes. If the susceptibility or resistance effect of a single QTL is not an intrinsic property of the gene, but rather depends on epistatic interaction with other unlinked QTL, then the development of monocongenic stocks is unlikely to reconstitute a susceptibility phenotype on a resistant background or vice versa. For example, in our previous analysis of the epistatic complexity underlying diabesity in our F1 model of accelerated type 2 diabetes, we showed that a complex locus on Chr 1 had a highly significant main effect in contributing ~16% of the variance in body weight, 8% of variance in PG, and 9% in PI (3). Importantly, computational analysis for pairwise interactions of all loci typed in the cross revealed that a gene or genes within this diabesity QTL on Chr 1 greatly enhanced its diabetogenic potency through epistatic interaction with multiple other unlinked QTL, including NZO-contributed loci on Chr 12 and 15 (3). In the present study, the differential susceptibility of RCS-1 and -2 provided formal proof that the epistatic interactions identified by biostatistical methodology were, in fact, genuine. These

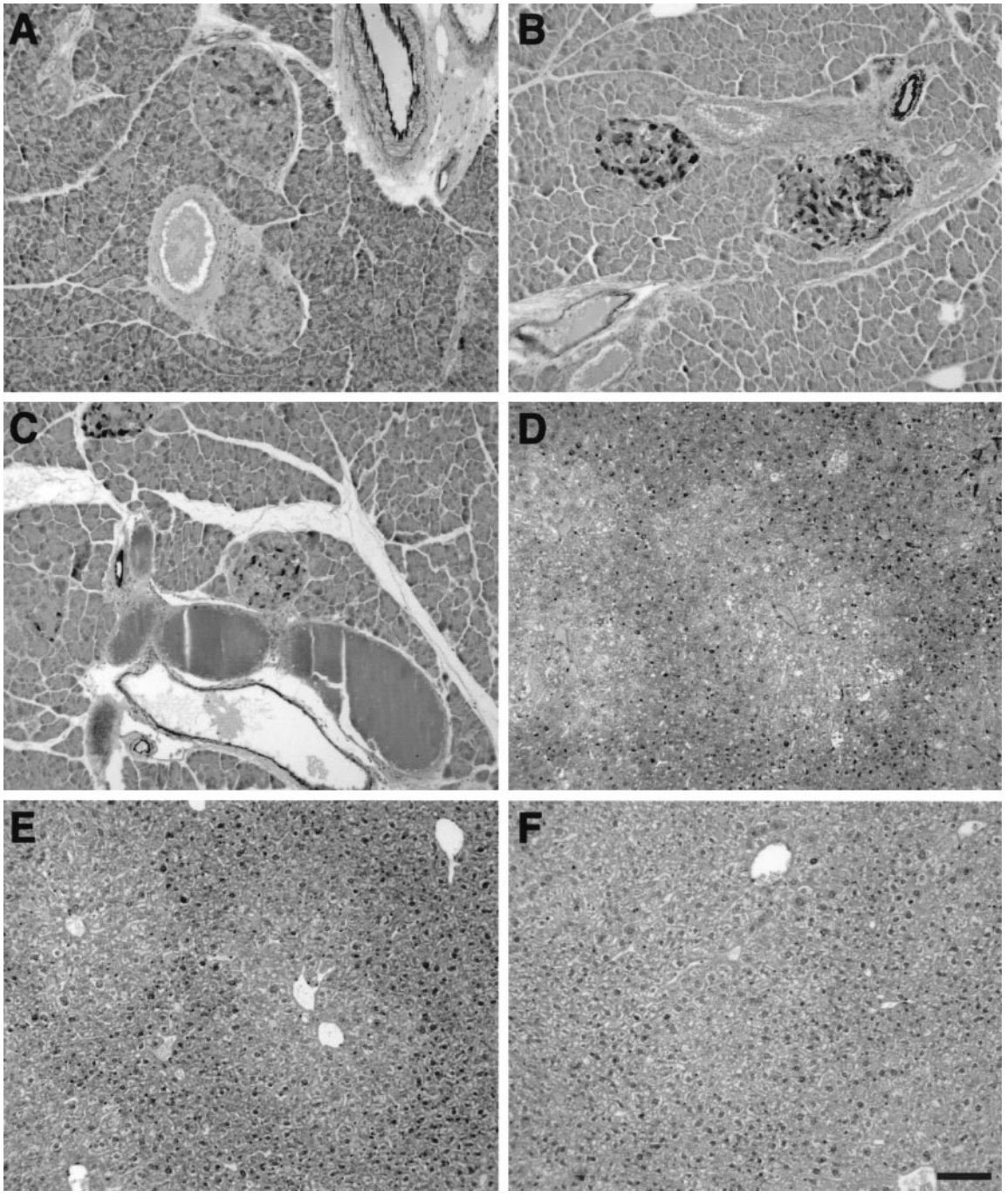

FIG. 3. Comparative differences in pancreatic islet (aldehyde fuchsin stain) and hepatic (PAS stain) histology in RCS-1, -2, and -10 males necropsied at 24 weeks of age. *A*: Islet from a hyperglycemic (PG 470 mg/dl, PI 2 ng/ml) RCS-1 male exhibiting extensive β-degranulation. *B*: Islet from a normoglycemic (PG 113 mg/dl, PI 2.3 ng/ml) RCS-2 male showing an increased percentage of β-cells strongly positive for presence of granules by aldehyde fuchsin staining. *C*: Islet from hyperglycemic RCS-10 male (PG 505 mg/dl, PI = 28 ng/ml) showing atrophic changes and nearly complete β-degranulation. *D*: Liver from RCS-1 showing partial glycogen depletion and fat accumulation. *E*: Liver from RCS-2 showing less extensive glycogen depletion and fat accumulation. *F*: Liver from RCS-10 showing complete glycogen depletion and fat accumulation. Bar = 100 μm.

two RCS were constructed to test the importance of the Chr 1/Chr 15 interaction. RCS-1, selected to contain the diabetogenic QTL on both chromosomes, developed a maturity-onset hyperglycemia, whereas RCS-2, lacking most of the NZO-derived alleles on Chr 15, failed to develop hyperglycemia. The accuracy of the computational predictions of interactive diabetogenic loci was further confirmed through the creation of RCS-10. An NZO-derived QTL on Chr 12 with a significant main effect on adiposity measures and body weight, but not on plasma glucose or insulin, was shown to interact epistatically with the Chr 1 diabesity QTL to exacerbate hyperglycemia. In RCS-10, this NZO contribution on Chr 12, as well as another NZO diabesity QTL on Chr 5, was combined with the diabesity QTL present in RCS-1. Presumably, this Chr 12 "obesity QTL," acting in concert with the diabesity QTL on Chr 1, 5, and 15, accelerated early weight gain and effected a 100% diabetes frequency with earlier onset than

observed in RCS-1. This represents a powerful demonstration of how RCS permit the accumulation of specific complexes of unlinked QTL that individually may make only small contributions to the genetic variance for a phenotype, but large effects in specific combinations.

These recombinant congenic lines, reflecting different levels of obesity and diabesity development, provide a spectrum of new mouse models for the analysis of how different combinations of these QTL can exert diabetogenic stress in the appropriate environment. The penetrance of the diabetogenic combinations of NZO and NON QTL is markedly affected by the early postnatal nutritional environment (3). Further, we have found both NZO and (NZO x NON)F1 diabetic males to be unusually sensitive to the hepatocytotoxic side effects of thiazolidinediones (unpublished data). Although feeding Troglitazone in a 6% fat-containing diet at a dose of 2 mg/kg suppressed hyperglycemia in these two models, the treatment produced unusually severe hepatic steatosis. This effect was limited to NZO and F1 hybrid and was not observed in parental NON/Lt males. Because the 10 new RCS were generated on the NON/Lt background, so that the NZO genetic contribution in each RCS would be relatively limited (and defined), these RCS should provide the necessary resolving power to isolate those obesity/diabesity QTL that facilitate thiazolidinedione-driven lipid accumulation in the liver. The recombination event eliminating the NZO diabesity contribution on Chr 15 in RCS-2 illustrates the power of recombinant congenic analysis to delimit QTL positions and aid the selection of candidate genes. In the vicinity of the NZO-derived *D15Mit159* marker retained in diabesity-developing RCS-1, but not in diabesity-resistant RCS-2, are interesting candidate genes important in lipid metabolism, including peroxisome proliferator–activated receptor-α (*Ppara*), carnitine-palmitoyl transferase 1 (*Cpt1b*), and diacylglycerol acyltransferase (*Dgat*).

Because of the varied genetic contributions from the obese NZO parental and the glucose-intolerant NON parental, we predict that these lines develop obesity through different pathways, modeling diverse obesity syndromes that may, in turn, respond differently to antiobesity agents. RCS-3, -4, and -5 are models for obesity usually uncomplicated by diabetes. These three lines do not share many NZO-derived genomic regions, so it can be inferred that each strain develops obesity by different genes and possibly different physiological pathways. NZO-derived obesity QTL ("*Obq*") have been found on Chr 1, 2, 4, 5, 6, 7, 12, 15, 17, and 18 (3,12,13). RCS-3 may carry *Obq 15* on Chr 7 as well as *Obq 4* on Chr 17 (12). RCS-4 may contain *Nob1*, NZO obesity 1 (13), *Obq 11* and *12* (12), and an adiposity QTL (3), all on Chr 5, as well as an adiposity QTL on Chr 12 and a QTL for body weight on Chr 15 (3). RCS-5 may contain *Obq 9* on Chr 1 and *Obq 4* on Chr 17 (12), as well as a QTL for adiposity on Chr 18 (3). Given their divergent genetics, these three RCS model separate obesity syndromes. RCS-3, -4, and -5 should be useful for research into obesity susceptibility genes and antiobesity agents without the complications that diabetes might present, and thus serve as models for the significant population of humans who are overweight but not diabetic.

These RCS are obviously valuable for more than simply testing gene-specific responses to antidiabetes and antiobesity drugs. It should be noted that, in certain respects, these new models are more reflective of the "typical" type 2 diabetes in humans than the intensively studied monogenic obesity mutant mice. Neither parental strain used to generate the RCS exhibits the hypercorticoidism and failure to cold-regulate that typifies mice homozygous for the Lep^{ob} and $Lepr^{db}$ mutations (16). The RCS provide a solution to several problems associated with the NZO model that have limited its use in the past. After weaning, NZO mice are hyperphagic and develop morbid obesity. Furthermore, in our colony, only half of NZO/HlLt sibmatings produce litters even after dietary supplementation with CL 316,243 for a month before breeding. The RCS, with the exception of RCS-6 and -7, breed at a more prolific rate than NZO, presumably because of reduced obesity and hyperphagia. The reduced breeding performance of RCS-6 and -7, despite the absence of morbid obesity and hyperphagia, indicates that there may be other factors involved, possibly associated with defects in leptin transport or signaling. Whereas all NZO/HlLt males will develop obesity, diabesity is a threshold phenomenon, developing only in the males that exhibit the most rapid rates of weight gain in the peripubertal period (3,6). Despite their reduced obesity, RCS-1 and -8 have maintained a diabetes frequency comparable with that of NZO, whereas RCS-10 has surpassed the diabetes frequency of NZO. Indeed, RCS-10, exhibiting 100% frequency of hyperglycemia by 20 weeks of age, represents a highly reproducible diabetes model of maturity-onset, insulin-resistant diabetes. Finally, because obesity and diabetes in NZO males are determined by multiple genes whose penetrance is environmentally controlled, it is difficult to decide on an appropriate control strain. The obese but nondiabetic female has heretofore been a suitable control for hyperglycemia-induced changes, but not for obesity development. In the case of RCS-1 males, RCS-2 males could be used as control, and for RCS-10 males, RCS-9 males could be considered as a low-incidence control strain.

The key to the development of these new models was the identification of separate susceptibility QTL present in the NON/Lt inbred strain background capable of interacting deleteriously with subsets of the additive or codominant obesity QTL fixed in the NZO inbred background. Although NON/Lt mice of both sexes maintain normal PG levels in the fed state, the strain was selected for high fasting blood glucose values (15). Further, although apparently distinguishing the stock at The Jackson Laboratory from one in Japan wherein impaired glucose tolerance is observed in young NON males but not older ones (17), NON/Lt males exhibit impaired glucose tolerance throughout life. The NON/Lt male also maintains a relatively low-fed PI level compared with other inbred strains, and isolated islets show a blunted glucose-stimulated insulin response in perifusion analysis (18). Hence, those RCS developing hyperglycemia at lower body weights are required for diabesity to develop in NZO males; this may be caused, in part, by NON strain-specific defects at the islet-cell level in the presence of subsets of the NZO "diabesity" QTL.

Given that highly inbred strains of mice accumulate recessive mutations over time, F1 hybrids are normally

considered to be healthier than their highly inbred progenitors. However, there are numerous instances in the literature where combining separate genomes uncovers latent diabesity susceptibility. A classic example of this is the "Wellesley hybrid" produced by outcross of the C3H/f strain with the I/Ln strain (19,20). More recently, outcross of C57BL/6J mice with the BTBR strain produced an insulin resistance syndrome (21). Similarly, outcross of the type 1 diabetes–prone NOD mouse with *Mus spretus* followed by one backcross to NOD produced a male sex biased type 2 diabetes syndrome (22). The NZO strain was selectively bred for polygenic obesity (23), and in outcrosses with lean strains, this segregates numerous QTL associated with obesity development (12). However, this obesity QTL cannot trigger diabesity in all outcross combinations. Outcross of NZO with SJL/J, another Swiss strain related to NON but not heretofore known to harbor glucose intolerance QTL, does not elicit diabesity in F1 males, but rather suppresses it (24). However, like NON, SJL contains a diabesity locus on Chr 4 that is "unmasked" in backcross to NZO (24). Backcross of these resistant F1 mice to NZO also led to the identification of recessive NZO alleles on Chr 5 and 19 contributing to insulin resistance and obesity.

In summary, type 2 diabetes in humans is not commonly associated with extreme hyperphagia, extreme obesity, reproductive failure, hypercorticoidism, or cold insensitivity, factors typically associated with the obesity/diabetes syndromes in the most often–studied mouse and rat models of type 2 diabetes. Our strategy of interval-directed RCS construction has permitted us to develop novel new models of type 2 diabetes that are not complicated by those extreme phenotypes mentioned above. Obesity and diabesity phenotypes are differentially expressed among the RCS, and we have identified genetic markers to define the allelic variants controlling these differences. Hence, these new models are more representative of what is considered "typical" type 2 diabetes in humans. Thus, they should provide much more valid targets for testing the efficacy of antidiabetic drugs than the extreme obesity models currently available, as well as help to define the pathogenesis of disease.

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