Section I: Genetic Factors in Type 2 Diabetes—In Search of New Links

Differential Levels of Diabetogenic Stress in Two New Mouse Models of Obesity and Type 2 Diabetes

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The genetic basis for the more common forms of human obesity predisposing to insulin resistance and development of type 2 diabetes is multigenic rather than monogenic in origin. New mouse "diabesity" models have been created by combining independent diabetes risk–conferring quantitative trait loci from two unrelated parental strains: New Zealand Obese (NZO/HlLt) and Nonobese Nondiabetic (NON/Lt). F1 hybrid males, heterozygous at all polymorphic autosomal loci distinguishing the two parental strains, are driven to obesity-induced diabetes (diabesity) at high frequencies. This review focuses on two new recombinant congenic strains (RCSs) developed by introgressing multiple NZO/HlLt chromosomal segments into the nominally diabesity-resistant NON/Lt strain background. Both RCSs gain more weight than NON animals. Although exhibiting comparable weight gain and adiposity, only one of the two RCSs develops diabetes. Hence, these two RCSs will be instructive in elucidating genetic and pathophysiological differences underlying uncomplicated obesity syndromes versus diabetogenic obesity (diabesity) syndromes. Unlike mice with null mutations in a single gene producing morbid obesity, the new models develop a more moderate obesity produced by the interaction of numerous genes with relatively small effects. These RCSs are differentially sensitive to adverse side effects of thiazolidinediones and thus should be particularly useful for pharmacogenetic analyses.
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Genetic architecture is a term used to describe the full range of genetic effects on a phenotype. This architecture is not a fixed entity but changes according to age- and sex-mediated shifts in gene expression, as well as changes in the physical environment. Most common forms of type 2 diabetes entail a complex interaction between multiple genes and the nutritional environment. Obesity represents a major phenotypic risk factor for type 2 diabetes susceptibility. Subphenotypic components of human obesity, such as body weight (BW) and percent fat, are continuously varying traits reflecting contributions from numerous quantitative trait loci (QTL) (1). Geneticists are currently grappling with the molecular genetic basis for variation in a complex phenotypic trait such as obesity that confers type 2 diabetes susceptibility (2). This effort requires development of new computational frameworks to enhance power to detect gene-gene interactions in genome-wide screens (3). However, understanding how these genomic interactions translate into dysregulated metabolic pathways (physiogenomics) is still difficult in humans. Currently, mutant stocks with defects in the leptin receptor or leptin genes ($Lepr^{db}$ and Lep^{ob} mice) represent the most intensively studied mouse models of obesity-associated type 2 diabetes ("diabesity"). Although these mutant stocks are very useful, the genetic basis for their morbid obesity, as well as the pathophysiological basis for their insulin resistance, is not reflective of what is sometimes referred to as "garden-variety type 2 diabetes" in humans. For example, the monogenic basis for the obesity, plus the hyperphagia, hypercorticism, loss of cold-adapted thermogenesis, and complete loss of either leptin or the leptin receptor function because of genetic mutation are not characteristics of the human at-risk population. Hence, new strains of mice developed specifically for dissecting deleterious gene-by-gene and gene-by-environment interactions are urgently needed to model for etiopathogenic processes in humans. The ability to control both genotype and environment in inbred populations of mice makes such an undertaking feasible.

There have been two basic approaches to this problem using mouse models. One approach is completely reductionist in concept. It entails genetic targeting of specific candidate genes on separate chromosomes and then recombining the separate mutations in heterozygous and/or homozygous knockout mice in an effort to generate an insulin insensitivity of sufficient magnitude to trigger type 2 diabetes. For example, targeting of genes in the insulin receptor signaling pathway (e.g., mice with targeted mutations in genes encoding both the insulin receptor substrate 1 and the insulin receptor) (4) illustrated how defects in several genes in a common pathway could

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BW, body weight; DEXA, dual-energy X-ray absorptiometry; IGT, impaired glucose tolerance; PAS, periodic acid Schiff; PG, plasma glucose; QTL, quantitative trait loci; RCS, recombinant congenic strain; TZD, thiazolidinedione.

precipitate diabetes in the absence of obesity. Although these models involving targeting of a pair of candidate genes are superficially simple, much greater complexity is revealed when the phenotypic effect of combinations of targeted genes are compared on different inbred strain genetic backgrounds (5). At the other end of the spectrum are the complex polygenic models typified by obese male mice of the New Zealand Obese (6) and Tallyho (7) inbred strains and nonobese (BTBR × B6)F1 (8) and SMXA (9) males. Unlike knockout models producing complete null mutations, the polygenes contributing to insulin resistance and type 2 diabetes in these latter mice represent QTL. Because QTL generally reflect relatively common natural variants, the assumption is that they can be inherited in combinatorial sets that establish a metabolic predisposition (giving rise to the concept of common genes for common diseases). Because these polygenic models develop obesity and type 2 diabetes through natural rather than through investigator-instigated elimination of specific gene functions, they afford special insight into genetic architectures that produce uncomplicated polygenic obesity or obesity syndromes complicated by the development of diabesity.

THE NZO/HlLt MOUSE

NZO is an inbred strain originally selected in New Zealand for polygenic obesity (10). NZO neonates have high birth weights, and mice of both sexes are large and, at weaning, exhibit an elevated carcass fat content (11). The adiposity is more reflective of adipocyte hypertrophy than hyperplasia (12). NZO males develop hypertension when fed a diet with increased fat content (13). NZO mice have not been extensively studied because they are difficult to breed and only recently have been available from commercial suppliers. Because the obesity is polygenic, there is no simple control (although NZW and NZB are closely related nonobese strains and share certain hematological anomalies with NZO) (14). Diabetes frequencies vary among the various NZO substrains. The NZO/Wehi stock develops comparable obesity as observed in NZO/Hl and NZO/HlLt mice (15); however, only males of the latter two strains develop chronic nonfasting hyperglycemia. Approximately, 50% of group-caged virgin NZO/Hl and NZO/HlLt males, but not females, transit from impaired glucose tolerance (IGT) into overt type 2 diabetes between 12 and 20 weeks of age when maintained on a diet containing 4.5% fat (16). The diabetic subset was comprised of those males showing the greatest rate of BW gain between 4 and 8 weeks of age. Genetic backcross analysis between NZO/HlLt and either NON/Lt or SJL/J confirmed that diabesity in this polygenic obesity model represents a complex threshold phenomenon wherein the rate of early adiposity development establishes a diabetogenic level of insulin resistance (17,18).

Obesity in NZO/HlLt mice is characterized by widespread accumulation of subcutaneous as well as visceral fat and is juvenile in onset. Mean BW for 4-week-old males was 25.8 g (compared with 13–15 g for many inbred strains). By 8 weeks, mean BW had increased to 42.7 g. At this age, NZO males remain normoglycemic, and plasma insulin (2–3 ng/ml) and leptin (15–20 ng/ml) concentrations are not yet markedly increased above a normal range. Plasma insulin and leptin levels are not elevated until a later maturational stage (9–12 weeks of age). By 16 weeks of age, when BW is ≥50 g (total carcass fat = 20 g or higher by dual-energy X-ray absorptiometry [DEXA] measurement), a range of plasma insulin values between 4 and 16 ng/ml are observed (normal range for insulin in most strains is 1–3 ng/ml). Those mice with the highest BW and plasma insulin values at 16 weeks generally exhibit plasma glucose (PG) values >250 mg/dl and develop a more pronounced hyperglycemia by 20–24 weeks (PG ranges rising to between 300 and 400 mg/dl). Untreated diabetic males maintain chronic hyperglycemia at these levels for many months without weight loss. Hence, hyperglycemia is late onset and chronic once it is established.

Hepatic insulin resistance and excessive glucose output have been documented as early abnormalities in NZO males (19). Yet the mechanism of this hepatic resistance is quite different from the insulin resistance developing in the more intensively studied Lep^{ob} and $Lepr^{db}$ mice. In the latter, genes encoding glycolytic (glucokinase, pyruvate kinase) and gluconeogenic (PEPCK, glucose-6-phosphatase) enzymes that should be suppressed in chronically hyperinsulinemic mutant mice are not. In contrast, these same genes are responding normally to insulin in NZO mice (20). This difference may relate to the absence in NZO/HlLt mice of the severe hypercorticism characteristic of Lep^{ob} and $Lepr^{db}$ mice (E.H.L., P.C.R., unpublished data). Indeed, metabolic analysis of NZO mice suggests that hepatic insulin resistance is the consequence of increased lipid availability, particularly from glycerol gluconeogenesis due to early postpartum increases in the hepatic activity of fructose-1,6-bisphosphatase (21,22). In author E.H.L.'s colony, hepatic glycogen depletion and lipidosis are consistent histopathologic features and are particularly severe in those males crossing a threshold into overt type 2 diabetes.

Because NZO mice are hyperphagic, difficult to breed, and progressively more leptin resistant as they age, the strain was analyzed for defects in signaling through the leptin receptor. NZO mice express the same leptin receptor variant ($Lepr^{A720T/T1044I}$) as the related NZB strain, which is neither hyperphagic nor markedly obese (23). This variant appears to signal normally after activation by the ligand (24). NZO mice were found to be resistant to peripheral leptin administration but sensitive to centrally administered leptin (25). This finding suggested that NZO mice were defective in leptin transport across the blood-brain barrier, a suggestion recently confirmed (26). Concentrations of leptin receptor splice variant mRNAs were not reduced in NZO brain microvesicles (26). The defect resides in the blood-brain barrier membrane because the same defect was produced in B6 males made obese by being fed a high-fat diet (26). Outcross of NZO/HlLt mice with either NON/Lt or SJL/J mice identified a type 2 diabetes susceptibility QTL on chromosome 4 in the vicinity of the leptin receptor locus. However, the diabetogenic allele in both outcrosses was not from NZO, but rather from the NON/Lt and SJL/J outcross partners (16,18).

UNUSUAL ASPECTS OF TYPE 2 DIABETES PATHOGENESIS IN NZO/HL AND NZO/HLLT MICE

Changes in islet morphology in NZO males are predicated on the duration of chronic hyperglycemia. Islet profiles

exhibiting β-cell hypertrophy and hyperplasia at 3–6 months of age are replaced by profiles of islet atrophy due to β-cell loss at later time points. The diabesity syndrome developing in NZO/Hl and NZO/HlLt males exhibits aspects of what has been termed "latent autoimmune diabetes of adults" or "type 1.5 diabetes" (27). NZO originates from the same outbred stock as the autoimmune-prone NZB and NZW strains and presents an amalgam of interesting immunodeviant phenotypes found in both related strains. For example, NZO shares the same rare $H2^z$ haplotype expressed in NZW mice and shares hematological anomalies found in the NZB strain (14). We confirmed that NZO/HlLt mice, like NZO/Wehi mice, produce anti–insulin receptor autoantibodies (28) and, like NZB mice, develop peri-insular leukocytic infiltrates that are unique in containing an unusually high percentage of B-cells, including plasma cells (29). Type 2 diabetes did not develop in a group of four B-cell–deficient (and insulin receptor autoantibody–null) males homozygous for a targeted mutation in the immunoglobulin heavy chain gene. This suggested that the humoral autoimmunity in NZO mice might not only be a component of the insulin resistance, but potentially also a contributor to the failure and ultimate loss of pancreatic β-cells (29). Because these severely immunodeficient males did not survive beyond 20 weeks (because of opportunistic infections in the urogenital tract), it could not be rigorously established that the absence of B-cell functions, including autoantibody production, explained the absence of diabetes. More recent analysis of an incipient NZO congenic stock, in which B-cell–deficient and B-cell–intact males were aged in pressurized, individually ventilated caging systems and given antibiotic-supplemented drinking water (which prevented infections), showed that the B-cell–deficient males weighed significantly less at weaning and developed diabesity at a more protracted rate. However, by 20 weeks of age, type 2 diabetes frequency did not differ among wild-type and B-cell–deficient males. Hence, although humoral autoimmunity in NZO/HlLt males may contribute to the progressive pancreatic β-cell loss, such immunity is not required.

THE NON/Lt MOUSE: A MODEL FOR IGT AND IMPAIRED β-CELL SECRETORY FUNCTION

NON (Nonobese Nondiabetic) is an inbred mouse strain produced in Japan by selection at each generation for high fasting blood glucose (30). NON mice are not hyperphagic and reproduction is normal. NON/Lt males at The Jackson Laboratory are intolerant to glucose loading throughout life, and islets from these mice exhibit an impaired glucose-stimulated insulin secretory response in vitro (31). As NON/Lt males age, they accumulate intra-abdominal, but not subcutaneous, fat. Interestingly, serum immunoreactive insulin levels in young NON/Lt mice are at the low end of the normal range ($\leq$2 ng/ml). Low leptin levels in adipocytes and in serum of young NON/Lt mice have also been reported (32). However, NON/Lt males fail to transit into overt type 2 diabetes, even when fed a high-fat diet. This strain may therefore represent a mouse counterpart of the GK rat, a model of latent type 2 diabetes associated with low glucose-stimulated insulin secretion by β-cells coupled with IGT (33).

THE FIRST STEP IN NEW MODEL DEVELOPMENT: COMBINING SEPARATE GENETIC TYPE 2 DIABETES SUSCEPTIBILITIES IN AN F1 HYBRID MODEL

When reciprocal outcrosses are performed between NZO/Hl and NON/Lt mice, the separate genetic susceptibilities present in both parental genomes are not suppressed, as might be expected if the diabesity in NZO was controlled by a collection of null alleles comparable to the known recessive obesity-producing mutations in mice. On the contrary, genomic interaction of the two parental genomes induces a predictable type 2 diabetes syndrome in F1 males, converting the latent diabetes of NON/Lt males, and the unpredictable diabetes of NZO/Hl males into overt type 2 diabetes at a highly predictable (90–100%) frequency (16). F1 hybrids are as obese as parental NZO males but lack the reproductive problems of NZO mice. Thus, the NZO (poly)genes contributing to obesity were strongly penetrant in the heterozygous state. Genetic analysis of F2 and first backcross males in author E.H.L.'s laboratory permitted identification of NZO-derived obesity and/or diabesity QTL on chromosomes 1, 5, 11, 12, 13, and 15 and NON contributions on chromosomes 4 and 18 (16,17). One or both of the NON-contributed QTL may control the β-cell insulin secretory defect. Indeed, outcross of NZO with SJL, a Swiss-derived strain related to NON, revealed a SJL-contributed diabetogenic QTL on chromosome 4 in the same region as the diabetogenic NON QTL (34). Outcrosses between NZO/HlLt and other inbred strains identified additional NZO-derived dominant or additive obesity QTL on chromosomes 1, 2, 5, 6, 7, and 17 (35). In the course of the genetic analysis defining the chromosomal locations of the diabesity QTL contributed by both parental strains, complex epistatic interactions were uncovered not only among the QTL (gene-by-gene interactions), but also between many of these QTL and the postparturitional maternal environment (gene-by-gene-by-environment interactions) (17).

THE NEXT STEP: RECOMBINANT CONGENIC STRAINS, THE METHOD OF CHOICE TO DISSECT COMPLEX DIABESITY GENE INTERACTIONS

Recombinant congenic strains are particularly well suited for analysis of polygenic syndromes of the complexity outlined above. 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, but through additive or epistatic interactions with other QTL, nevertheless make important pathogenic contributions. In such a case, a single NZO diabesity-promoting quantitative trait locus transferred onto the diabesity-resistant NON background may not recreate the phenotype or even a subphenotype. Recombinant congenic strains (RCSs) are typically made by backcrossing twice to a recipient strain background (e.g., NON/Lt in this case) and then inbreeding to fix all alleles to homozygosity. At second backcross, the genome of any segregant would average ~87.5% NON alleles and 12.5% NZO-contributed alleles. Consequently, RCSs increase the chance of bringing phenotype-modifying diabesity QTL together in different combinations while

TABLE 1
Diabesity QTL distinguishing RCS-5 and RCS-10

	Chromosome							
Marker	1 *D1Mit411*	4 *D4Mit166*	5 *D5Mit7*	11 *D11Mit261/D11Mit41*	12 *D12Mit231*	13 *D13Mit53*	15 *D15Mit159*	18 *D18Mit60*
QTL donor	NZO	NON	NZO	NZO	NZO	NZO	NZO	NON
RCS-5	No	No	No	No	No	Yes	No	No
RCS-10	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes

NZO or NON indicates donor of the diabetogenic allele; Yes/No denotes presence/absence of diabesity QTL.

at the same time limiting the donor strain's contribution to ~12.5%. This approach has proven successful for dissecting complex genetic susceptibilities to cancer (36) and resistance to type 1 (autoimmune) diabetes (37). We have used the RCS strategy successfully to demonstrate how a quantitative trait locus whose main effect was on adiposity rather than on diabesity proved to be a diabesity quantitative trait locus when combined with other known diabesity QTL (38). Of equal importance, this breeding strategy allowed the development of 10 new obesity/diabesity strains that do not exhibit the massive obesity and hyperphagia characterizing NZO mice (38). Thus, the new diabesity-developing models with moderate rather than morbid obesity are not only considerably easier to maintain, but they also are more reflective of the phenotypes associated with the common forms of type 2 diabetes in humans. Although the formal descriptor for these new RCSs is NONcNZO1 through NONcNZO10, for brevity, we will use the abbreviation RCS-1 through RCS-10 in the discussion to follow below.

RCS-5 VERSUS RCS-10: ILLUSTRATION OF COMPARABLE PHENOTYPIC OBESITY BUT DIFFERENT LEVELS OF DIABETOGENIC STRESS

Table 1 lists the differential content of known diabesity QTL contributed by NZO and NON and present in RCS-5 and RCS-10. It is clear that RCS-5 has been selected for markers of resistance alleles at all but one known diabesity QTL, whereas RCS-10 was selected for the susceptibility markers for most of the known diabesity QTL. As published previously, all RCSs on the NON/Lt inbred strain background gain more weight and gain it more rapidly than do standard NON/Lt males, but none show the rapid development of extreme obesity characteristic of NZO or F1 males (38). As shown in Fig. 1*A*, RCS-5 and RCS-10 males gain weight at a comparable rate. This attests to the large number of as yet unidentified adiposity/BW QTL that segregated in this cross. NZO RCS-5 is fixed for a NZO quantitative trait locus on chromosome 13 marked by *D13Mit53* and contributing to increased BMI, whereas RCS-10 is fixed for the NON allele at this quantitative trait locus (Table 1). This quantitative trait locus, among those not yet identified, would contribute a different genotypic mixture, producing a comparable phenotype. DEXA was used to compare differences in percent total fat between the two RCSs at 8, 16, and 24 weeks. Both strains resembled NZO rather than NON in terms of development of early postmaturational adiposity (Table 2). Yet these percentages were very comparable between the two RCSs, with RCS-5 actually being a few percentage points higher at each time point. Clearly, different combinations of QTL are contributing to a comparable phenotype of increased BWs and early development of adiposity, but the diabesity-inducing potencies of these QTL are markedly different. As shown in Fig. 1*B*, RCS-5 males do not differ from the NON/Lt parental strain males in exhibiting complete resistance to the development of spontaneous type 2 diabetes. On the contrary, RCS-10 males reflect an even higher

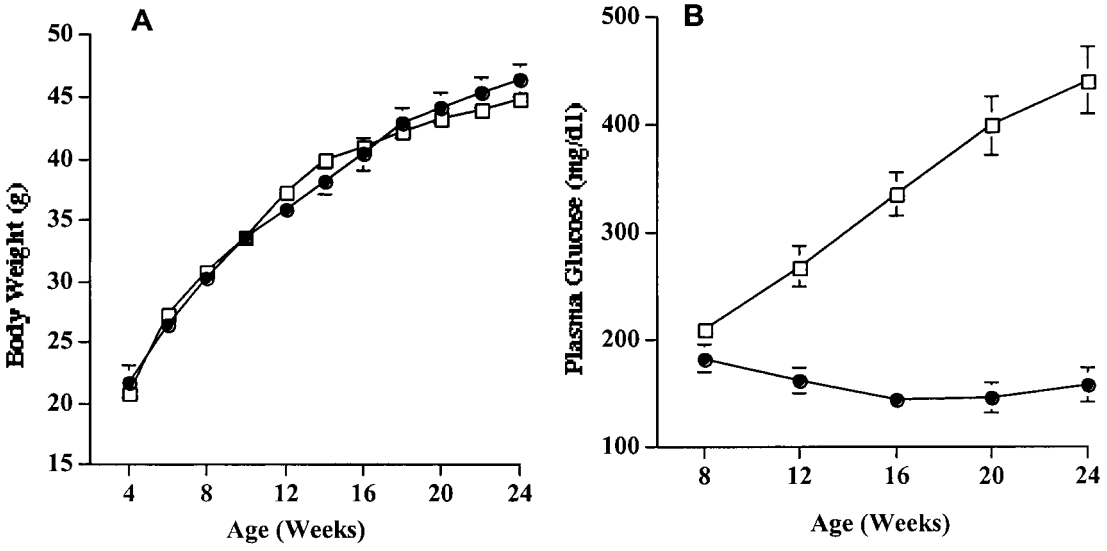

FIG. 1. *A*: Temporal changes in BW in male NONcNZO5 mice (RCS-5; ●) (n = 12) versus NONcNZO10 mice (RCS-10; □) (n = 28). *B*: Temporal changes in PG in the same two cohorts. Mice were maintained on NIH-31 diet with a 6% fat content.

TABLE 2
DEXA analysis of body fat percentage

	Mean body weight (mean fat %)		
	8 weeks	16 weeks	24 weeks
NZO	40.7 (27.9)	54.0 (30.3)	58.4 (38.2)
NON	27.3 (18.2)	30.9 (18.4)	43.0 (32.4)
RCS-5	34.3 (25.3)	44.2 (37.6)	46.9 (35.4)
RCS-10	31.5 (22.9)	42.5 (31.6)	50.1 (31.3)

Group-caged virgin male mice (n = 3/group) were maintained on NIH-31 diet, 4% fat.

frequency of diabesity development than the NZO parental strain, confirming deleterious contributions from both parental genomes. Indeed, the diabesity frequency of RCS-10 males is essentially the same as that for (NZO × NON)F1 males, but with a significantly more moderated rate of weight gain and the absence of hyperphagia. In the study cohort illustrated in Fig. 1*B*, 24 of 28 males (86%) developed PG values >250 mg/dl by 24 weeks of age. Differences in serum metabolites and hormones (insulin and leptin) are shown in Table 3. The serum insulin and leptin levels are clearly more NON-like in both RCS-5 and RCS-10 and are very similar to each other. Serum triglycerides in RCS-10 are significantly higher than in either parental strain, whereas serum cholesterol was significantly lower in both RCSs compared with parental strains. It is noteworthy that the differences in many of these metabolic parameters are not more pronounced between the two RCSs in view of the markedly different glycemic status. The finding that hyperglycemia develops in RCS-10 but not RCS-5 males, and at a frequency comparable to that reported for F1 males, validates genetic predictions from earlier crosses with regard to the chromosomal positions of diabesity QTL.

HISTOPATHOLOGIC COMPARISONS OF PANCREAS, LIVER, AND KIDNEY

Figure 2 shows comparative morphology for pancreas, liver, and kidney of male mice at the study termination point of 24 weeks. NON/Lt rather than NZO/HlLt is used as the basis for comparison of pathologic changes because it is the recipient strain background for the NZO-derived diabesity QTL. The pancreatic histopathology in the latter strain has recently been described in detail (29). Pancreatic islets in 24-week-old NON/Lt males are pleomorphic, showing a spectrum of islet sizes from normal to moderately hyperplastic. Most islet profiles exhibit normal β-cell granularity (aldehyde fuchsin staining, Fig. 2*A*). A small amount of fat infiltration into the exocrine parenchyma is observed. Sporadic focal peri-insulitis and peri-vasculitis, a morphologic characteristic of pancreases of aging NON/Lt mice, was noted in some sections. Livers from these males were unexceptional (Fig. 2*B*); most cases showed moderate to heavy glycogen content (periodic acid Schiff [PAS] staining) and little or no lipidosis. Kidneys of aging NON/Lt mice of both sexes develop a progressively more severe glomerulosclerosis with widespread interstitial nephritis (Fig. 2*C*). This nephropathy is a known NON strain characteristic (39).

Comparative pancreatic, hepatic, and kidney morphology for RCS-5 is shown in Fig. 2*D–F*. A larger spectrum of islet sizes was noted in comparison to NON/Lt pancreases. RCS-5 pancreases also exhibited more fatty replacement of exocrine parenchyma, and, whereas most islet profiles contain β-cells that are strongly aldehyde fuchsin–positive (Fig. 2*D*), some islet profiles show a low to moderate level of β degranulation. Livers from RCS-5 males generally exhibit normal to moderately depleted glycogen storage in hepatocytes and mild to moderate centrolobular lipid accumulation (Fig. 2*E*). Kidneys do not exhibit the severe glomerulosclerotic lesions found in the NON/Lt parental strain. Mild glomerulosclerotic changes, increased PAS staining of glomerular basement membranes, and focal interstitial nephritis, however, are features observed in this strain (Fig. 2*F*).

We have previously reported detailed histopathologic observations of RCS-10 at earlier generations of sib mating (38). The original findings have not changed in current generations; specifically, by 24 weeks of age, islet hypertrophy and hyperplasia observed in pancreases from younger RCS-10 males (and still present in RCS-5 males) had evolved into atrophic changes (Fig. 2*G*) similar to those observed in chronically diabetic NZO/HlLt males (29) and in (NZO × NON)F1 males. These changes were consistent with the pathophysiological phenotype of chronic hyperglycemia and included extensive β-cell degranulation (aldehyde fuchsin staining, Fig. 2*G*). Reduction in islet sizes and extravasation of exocrine cells into decomposing islet profiles were also observed. Focal peri-insulitis and peri-vasculitis were present in RCS-10 pancreases at 24 weeks. A feature distinguishing RCS-10 male pancreases at 24 weeks from both age-matched NON/Lt and RCS-5 male pancreases was the presence of widespread fat infiltration of the exocrine parenchyma (38). The degenerative changes observed in islets in the pancreases of RCS-10 males were male sex–specific; the islets in age-matched normoglycemic (but moderately obese) female pancreases were structurally intact and filled with well-granulated β-cells. The focal peri-insulitis observed in males was also present in females at a comparable level; however, fat infiltration was considerably less extensive. Hence, the normoglycemic females can serve as a control for RCS-10 males in studies of islet

TABLE 3
Serum metabolites and hormones in 24-week-old males

Strain	n	Glucose (mg/dl)	Insulin (ng/ml)	Leptin (ng/ml)	Cholesterol (mg/dl)	Triglycerides (mg/dl)	Amylase (units/ml)
NZO	9	260 ± 40†	34.3 ± 4.5†	59.8 ± 4.8†	194 ± 14	177 ± 20	1,892 ± 34†
NON	5	189 ± 10*	4.1 ± 0.4*	8.8 ± 1.4*	152 ± 14	198 ± 19	1,556 ± 69*
RCS-5	12	140 ± 10*	5.5 ± 0.5*	28.2 ± 3.2*	105 ± 3*†	208 ± 9	1,686 ± 23
RCS-10	21	466 ± 35*†	6.0 ± 0.6*	17.5 ± 3.2*	124 ± 4*†	297 ± 15*†	1,925 ± 65†

Data are means ± SE. *Significantly different from NZO at $P \leq 0.01$. †Significantly different from NON at $P \leq 0.01$.

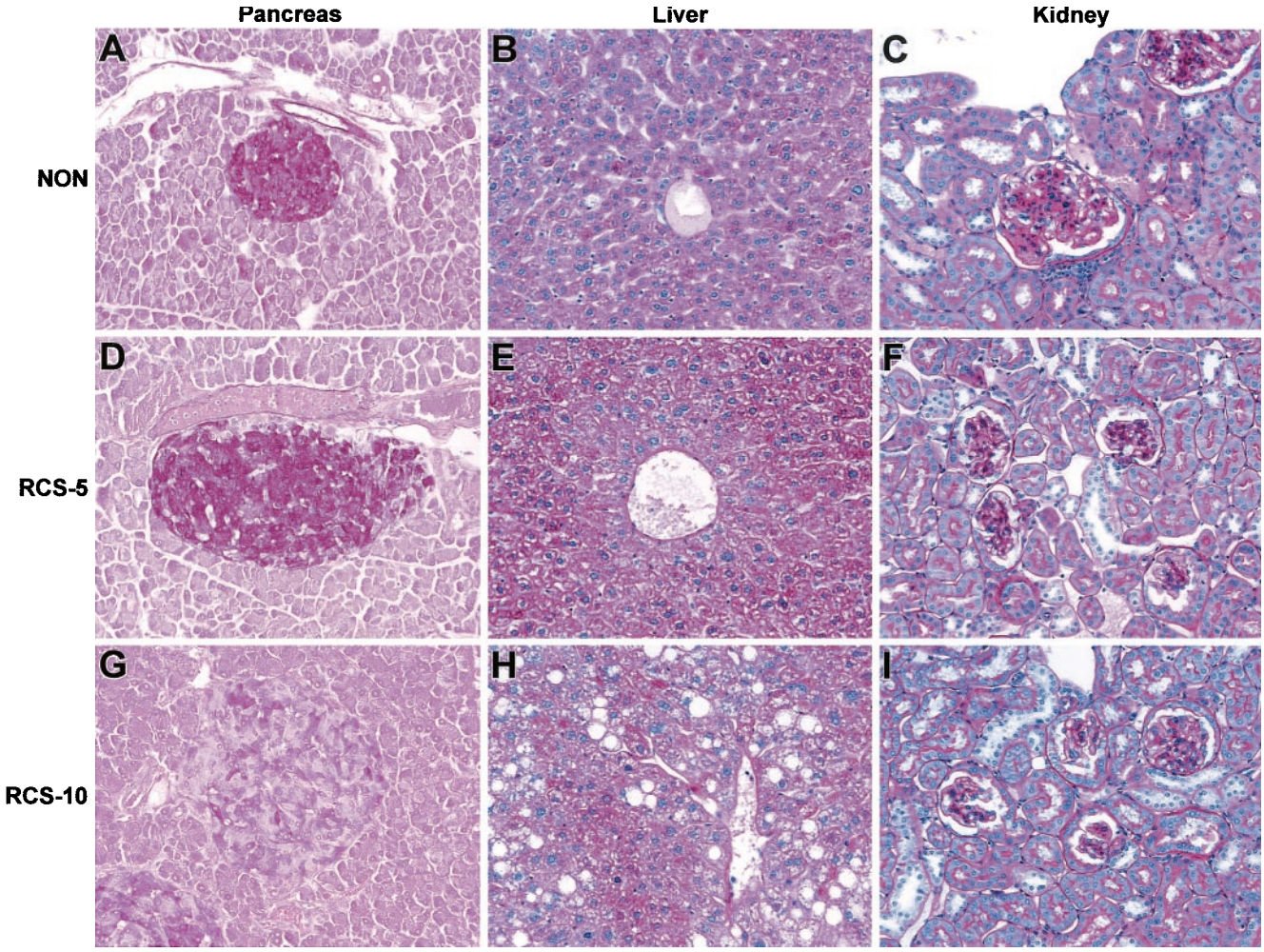

FIG. 2. Comparative histology of pancreas, liver, and kidney from age-matched (24-week-old) males from the parental NON/Lt strain (*A*, *B*, and *C*, respectively), from NONcNZO5 (RCS-5) males (*E*, *F*, and *G*, respectively), and from NONcNZO10 (RCS10) males (*G*, *H*, and *I*, respectively). Pancreases were stained with aldehyde fuchsin to detect granulated β-cells, livers were stained with PAS to detect glycogen, and kidneys were stained with PAS to detect glycoprotein deposition on glomerular basement membranes. Note that the severe glomerulosclerotic lesions present in the NON/Lt kidney were not present in either of the two RCSs. All images were photographed at 200× magnification.

gene expression and/or secretory functions in the presence or absence of glucotoxic or lipotoxic stresses.

Liver morphology of 24-week-old RCS-10 males sharply distinguished these chronically hyperglycemic mice from both the parental NON/Lt and RCS-5 strains. In contrast to the absence of lipid accumulation in most NON/Lt livers, and the presence of relatively mild lipidosis, if any, in RCS-5 livers, all RCS-10 livers exhibited moderately severe to severe hepatic steatosis (Fig. 2*H*) with occasional foci of hepatitis and hepatic necrosis. Kidneys of RCS-10, like those of RCS-5, did not exhibit the more extreme NON strain characteristic of glomerulosclerotic lesions. However, mild glomerulosclerotic changes and increased PAS staining of glomerular basement membranes were noted. Further, interstitial nephritis, sometimes diagnosed as pyelonephritis, was common.

DISCUSSION

Both the NONcNZO5 (a.k.a., RCS-5) and NONcNZO10 (a.k.a., RCS-10) strains have been rederived from author E.H.L.'s nonbarrier specific pathogen–free colony into a full barrier specific pathogen–free resource colony at The Jackson Laboratory and are available as stock numbers 004455 and 004456, respectively. The parental NON/LtJ (stock number 002423) and NZO/HlLtJ (stock number 002105) strains, as well as their F1 hybrids (stock number 100937), are also available for distribution from the same source. As noted in the introduction, these new RCS models offer investigators important new tools for studying the pathophysiology of diabetogenic obesity syndromes. This is because the RCSs do not reflect the more extreme phenotypes exhibited by the commonly studied mice homozygous for monogenic obesity mutations. In that regard, they are more relevant to the more common forms of obesity-associated human type 2 diabetes. In terms of the (poly)genetic basis for the diabesity in these models, we feel that these models are certainly of high relevance to understanding the complexity of human type 2 diabetes etiopathogenesis. NONcNZO10 was developed by introgressing five known NZO genomic intervals containing diabesity QTL onto the NON/Lt genetic background. Whereas parental NZO males exhibited the unwanted phenotypes of hyperphagia, morbid obesity, poor fertility, and a variable frequency of hyperglycemia, NONcNZO10 males are not hyperphagic, develop a more moderate level of obesity, and reproduce normally. They

weigh more than NON males but significantly less than NZO males. However, early accumulation of fat measured by DEXA is NZO-like. Despite the reduced rate of weight gain compared with NZO, all NONcNZO10 males develop chronic hyperglycemia with onset between 12 and 20 weeks on a 6% fat diet. Serum insulin and leptin values, although higher than NON, are less extreme than NZO. Pancreatic islets show the same atrophic changes seen in diabetic NZO/HlLt males. Serum triglycerides are high, and liver shows moderate to severe steatosis. Metabolic analysis shows that NONcNZO10 males differ from NZO males in exhibiting more normal capacity to shift from lipid to carbohydrate metabolism at night (as demonstrated by failure to increase the respiratory exchange ratio in the dark cycle). Females from this RCS are diabetes resistant and can serve as a normoglycemic control. Because diabetic males maintain elevated BWs despite chronic hyperglycemia, they should prove useful for analysis of diabetic complications and testing of antidiabetic pharmaceuticals.

Parental NZO/HlLt and (NZO × NON)F1 males have proven very interesting from a pharmacogenetic standpoint. F1 males have shown earlier development of diabetes in response to modest increases in dietary fat (from 4 to 6%). The NZO parental males develop hypertension on a 16% fat diet (13), and we have observed elevated systolic blood pressures in (NZO × NON)F1 males maintained on a standard 6% fat diet. As noted earlier, the maternal postparturitional environment is also a key factor in phasing the diabesity process. Male pups suckled by obese F1 dams gained weight more rapidly and attained a twofold higher diabetes frequency in first backcross (to NON/Lt) than did males suckled by nonobese NON dams (17). Since the publication of this finding, we have obtained data showing impaired phosphatidylcholine content in F1 milk and reduced hepatic production (E.H.L., P.C.R., unpublished data). Mice with defects in hepatic phosphatidylcholine biosynthesis are highly sensitive to hepatosteatosis (40). This may account for the high basal level of moderate hepatic steatosis in livers of NZO/HlLt and (NZO × NON)F1 males and the acute sensitivity of both NZO/HlLt and (NZO × NON)F1 males to exacerbation of this phenotype by chronic treatment with thiazolidinediones (TZDs) (41). The remarkable sensitivity of the F1 model to the adverse side effects of TZDs apparently distinguishes the model from the $Lepr^{db}$ and Lep^{ob} models and most probably is associated with the unusually high triglyceride accumulation in F1 liver (41). The NON/Lt strain, free of the basal lipidosis observed in F1 male liver, does not show these adverse side effects in response to chronic TZD treatment. Hence, it is reasonable to predict that combinations of QTL from both parental strains leading to dysregulated lipid metabolism in the F1 liver have been isolated in at least one RCS. Preliminary experiments indicate that NONcNZO5 mice of both sexes, with only mild if any basal hepatic lipid accumulation, do not develop TZD-associated hepatosteatosis. In contrast, this pathology is observed in TZD-treated NONcNZO10 male mice. Understanding the pharmacogenetics of differential TZD responsiveness in these RCSs is of clear medical importance.

In conclusion, recently developed RCSs wherein QTL conferring type 2 diabetes susceptibility have been inherited from both sides of the pedigree should prove to be highly relevant for understanding the metabolic perturbations produced by diabetogenic obesity genes contributing to the common forms of type 2 diabetes in humans. Further, these new models, as well as their parental strains and F1 hybrids made from them, provide valuable new models for testing pharmaceuticals.

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