The Newly Inbred Cohen Diabetic Rat

A Nonobese Normolipidemic Genetic Model of Diet-Induced Type 2 Diabetes Expressing Sex Differences

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The newly inbred Cohen diabetic rat is an exceptional experimental model of diet-induced type 2 diabetes mellitus that is the result of secondary inbreeding nearly 30 years after it originally had been established. Animals from the original colony were selectively inbred by stringent criteria for 10 additional generations, bringing overall inbreeding to >50 generations. The metabolic phenotypes of the resulting contrasting strains, designated as the Cohen diabetic-sensitive (CDs) and -resistant (CDr) rats, were characterized. The phenotype of the CDs strain that was fed a regular diet consisted of fasting normoglycemia, normal glucose tolerance to intraperitoneal glucose loading, normal fasting insulin levels, and a normal insulin response to glucose loading. In contrast, CDs rats that were fed a custom-prepared high-sucrose low-copper diabetogenic diet became overtly diabetic: fasting glucose levels were normal or elevated, and the blood glucose insulin response to glucose loading was markedly abnormal. CDr rats that were fed a regular or diabetogenic diet did not develop diabetes and maintained normal glucose tolerance and insulin secretion. A striking sex difference was observed in CDs rats that were fed a diabetogenic diet: males had a lower growth rate and a more severe glucose intolerance pattern than females. Gonadectomy shortly after weaning did not prevent the development of the diabetic phenotype in its early phase in either sex but markedly attenuated its expression in males at a later phase, abolishing the sex differences. Alternate-day feeding, as opposed to daily feeding, also attenuated the metabolic phenotype in males. The development of the diabetic phenotype in CDs rats that were fed a diabetogenic diet was not accompanied by obesity or hyperlipidemia. The genetic profile of the strains was established using 550 microsatellite markers evenly distributed throughout the rat genome. The rate of homozygosity within strain was ≥96%. The rate of polymorphism between the contrasting strains was 43%. We conclude that the metabolic phenotypes of the rebred colony of CDs and CDr rats and their genetic makeup render the Cohen diabetic rat a useful experimental model that is highly suitable for studying the interaction between nutritional-metabolic environmental factors and genetic susceptibility (sensitivity and resistance) for the development of type 2 diabetes. The model is also distinctively useful for investigating the effect of sex on the expression of the diabetic phenotype. *Diabetes* 50:2521–2529, 2001

The Cohen diabetic rat is an exceptional genetically derived experimental model of diet-induced type 2 diabetes that reproduces many features of the disease in humans (1–5). This rodent model stands out among other experimental models of type 2 diabetes in several important ways. Its most outstanding and distinctive feature is that it expresses genetic susceptibility (sensitivity and resistance) to a carbohydrate-rich diet, a central feature of type 2 diabetes in humans (1,2,4,5) that is not present in other major genetically inbred rat strains that simulate type 2 diabetes in humans. The other major rat models of type 2 diabetes, the Goto-Kakizaki (GK) (6,7), the Otsuka Long-Evens Tokushima Fatty (OLETF) (8–10), and the Zucker diabetic fatty (ZDF) rats (11,12) develop diabetes spontaneously, without any important relationship to the composition of diet. Another central feature of the Cohen model is that it consists of two genetically derived contrasting strains, originating from the same parent strain, which is useful for genetic and physiological studies. In contrast, the "control" strains of the GK, OLETF, and ZDF models are unrelated strains that do not share the same ancestry. A third feature that makes the Cohen rat stand out is that it is a nonobese model of diabetes, which allows dissociation of the confounding obesity factor from other diabetogenic genes (13–15).

Although established nearly 30 years ago (16), the Cohen diabetic rat model has been studied until recently only to a limited extent. A major drawback of this model has been that it had never been systematically characterized in terms of phenotype or genotype, resulting in only limited recognition of its value and potential contribution

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AUC, area under the curve; BGL, blood glucose level; CDr, Cohen diabetic-resistant; CDs, Cohen diabetic-sensitive; DF, daily feeding; GK, Goto-Kakizaki; HSD, diabetogenic diet; IF, intermittent feeding; IPGTT, intraperitoneal glucose tolerance test; OLETF, Otsuka Long-Evens Tokushima Fatty; RD, rat diet.

to diabetes research. We undertook the task of reviving the model, aiming to render it suitable for metabolic, pathophysiological, and genetic studies and make it available to researchers worldwide. We initiated a program of secondary selective inbreeding of the original colony to ensure maximum phenotypic and genotypic homogeneity of the respective strains. We subsequently studied the phenotype of the resulting strains and established thereby for the first time an updated reference database for this model. We also determined the genotype of the strains to verify genetic homogeneity, to ensure the lack of cross-contamination between the strains, to determine the degree of DNA polymorphism between the strains, and to identify informative markers for future cross-breeding experiments. We provide details of the phenotypes and genotype of the products of the renewed inbreeding.

RESEARCH DESIGN AND METHODS

Animal maintenance. Animals were housed six per cage and separated by sex, except for breeding intervals. During pregnancy and after litter delivery, females were housed in individual cages. Twelve-hour diurnal light-dark cycles were maintained. Room temperature was kept between 22 and 25°C. These conditions are in accordance with "Principles of Laboratory Animal Care" (NIH publication no. 85–23, revised 1985) and the guidelines of the American Society of Physiology for the care of laboratory animals.

Breeding of the Cohen diabetic rat colony. The original colony of the Cohen diabetic rat had been held since its establishment at the animal facility of the Hebrew University and Hadassah School of Medicine in Jerusalem, Israel. Secondary selective inbreeding was initiated on-site 3 years ago. Part of the resulting colony was subsequently transferred to the animal facility of the Barzilai Medical Center Campus of the Ben-Gurion University in Ashkelon, Israel, where inbreeding was continued. Experiments were performed at both sites. Animal breeding, handling, and experimentation received approval of the local Animal Care committees of both institutions.

Selective inbreeding. Cohen's original selection criteria had been based on an oral glucose tolerance test with blood glucose levels (BGLs) at 2 h of >180 mg/dl for Cohen diabetic-sensitive (CDs) and <180 mg/dl for Cohen diabetic-resistant (CDr) rats (16). We set more stringent criteria for the secondary inbreeding: BGL >230 mg/dl for CDs and <140 mg/dl for CDr rats. Brother-sister mating was within the high-glucose for CDs and the low-glucose groups for CDr rats. Selection by the high/low glucose response was continued for 10 additional generations.

Feeding protocols. Animals were weaned at 5–6 weeks, after which they were fed either "regular" rat diet (RD) or a "diabetogenic" diet (HSD), according to the study protocol. RD consisted of a mixture of ground whole wheat, ground alfalfa, bran, skimmed milk powder, and salts, resulting in 21% protein, 60% carbohydrates, 5% fat, and 0.45% NaCl content (Koffolk). Food and tap water were provided ad libitum. HSD consisted of 18% casein, 72% sucrose, 4.5% butter, 0.5% corn oil, 5% salt No II USP, water, and fat-soluble vitamins. The HSD was copper-poor (1.2 ppm), a requirement for CDs rats to develop the full diabetic phenotype (13,16–18). Food and distilled water for drinking were provided ad libitum.

Phenotypes. Male and female CDs and CDr rats were studied while fed either RD or HSD. The following phenotypes were recorded.

Body weight. Animal growth curve was determined by periodic measurement of body weight, which also served as a reflection of the general state of health of the animal.

Blood pressure. Systolic blood pressure was obtained at 6 months to determine hemodynamic consequences of diabetes, specifically whether hypertension developed in this model. Blood pressure was measured by the tail-cuff method using an IITC photoelectric oscillatory detection device (IITC Life Science, Woodland Hills, CA), as previously described (19).

Glucose tolerance. The intraperitoneal glucose tolerance test (IPGTT) was performed in unanesthetized animals shortly after weaning, at 4 months, and at 6 months. Blood was obtained from the tip of the tail. BGLs were determined using a glucose reagent strip and a standard automated glucometer (Elite, Bayer). After baseline BGL measurements, animals received an intraperitoneal injection of 1 g glucose/kg body wt. BGL was measured again 5, 10, 15, 30, 60, and 120 min later.

Plasma insulin levels. Insulin levels were measured at 4 and 6 months after overnight fasting (fasting insulin) and 15 min after intraperitoneal injection of 1 g/kg glucose (stimulated insulin levels). Blood was obtained by direct puncture from the heart under ether anesthesia. Insulin was measured by radioimmunoassay using I^{125}-labeled anti–human insulin antibodies (Medgenix, Brussels, Belgium) and unlabeled insulin at varying concentrations. Cross-reactivity with purified human insulin (used as standard) was 90–95%.

Biochemistry. Plasma electrolytes, creatinine, and lipid levels were determined in 6-month-old anesthetized (xylazine/ketamine) animals in blood obtained from the bifurcation of the aorta. Twenty-four-hour urinary protein excretion was determined in urine collected in individual metabolic cages by the protein microprecipitation assay.

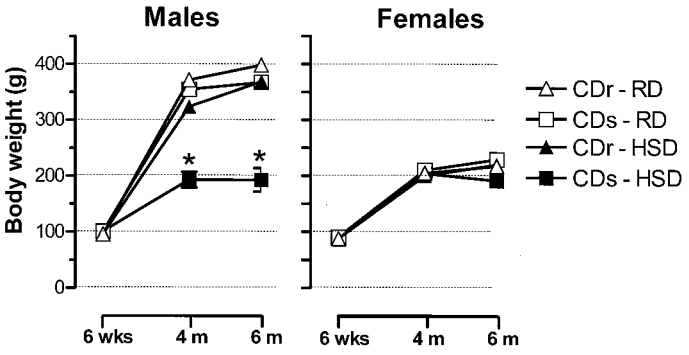

FIG. 1. Body weight of CDs and CDr rats that were fed RD or HSD at ages 6 weeks and 4 and 6 months. Data are presented as mean ± SE; *P < 0.01 compared with all other groups.

Additional experiments. The severity of the diabetic phenotype in CDs males and the sex differences between male and female CDs rats that were fed HSD led us to perform two additional experiments: one designed to produce an intermediate working phenotype and the other to attempt to resolve the issue of a possible hormonal effect on the development of diabetes in this strain.

Alternate-day feeding. We hypothesized that the low body weight and apparent failure to thrive of CDs males that were provided HSD, reflecting a full-blown diabetic phenotype, might be induced by the daily feeding of the high-sucrose HSD and the lack of treatment with insulin, a condition that simulates untreated severe diabetes in humans. We studied this hypothesis with an additional group of CDs rats that were provided the HSD on alternate days only: intermittent feeding (IF) instead of daily feeding (DF).

Gonadectomy. Our second hypothesis was that sex hormones might account for the sex differences observed in the phenotype of male and female CDs rats. Surgical gonadectomy or sham operation was performed in male and female CDs rats shortly after weaning, and animals were fed thereafter with either RD or HSD. Intact nongonadectomized animals served as controls. Growth characteristics were monitored in both sets of experiments, and IPGTT was performed in each group of animals at 4 and 6 months.

Genotype. Genomic DNA was screened for simple sequence length polymorphisms among representative animals from the CDs and CDr strains, using 550 microsatellite markers (Research Genetics, Huntsville, AL) evenly distributed throughout the rat genome. The methods were previously described in detail (19,20). In brief, genomic DNA was extracted from the rat tail by the phenol/chloroform method. Integrity, purity, and quantity of the extracted DNA were assessed by spectrophotometer absorbance (GeneQuant II; Pharmacia Biotech, Cambridge, UK). Polymerase chain reaction was performed on 50 ng of genomic DNA in a final reaction volume of 10 μl containing ^{32}P-labeled primer pairs obtained from Research Genetics. The product of each reaction (3 μl) was loaded onto polyacrylamide gel. Gels were run on a Base Ace apparatus (Stratagene, La Jolla, CA) and exposed to Kodak XAR-5 film for autoradiography. Amplification was determined by the appearance of discrete bands. Homozygosity was established by the presence of a single discrete band of interest for a given strain, polymorphism by the appearance of differential migration of amplification products for the two strains, and heterozygosity by the simultaneous appearance of two bands pertaining to both strains.

Statistical analyses and data management. The results of the IPGTT were analyzed by baseline (fasting), peak (maximal), and 120-min BGL, as well as by the area under the IPGTT curve (AUC). Data are provided as mean ± SE. Statistical analyses for normally distributed data were by analysis of variance. Statistical significance was set at the $P < 0.05$ level.

RESULTS

Phenotype

Growth characteristic. The growth characteristics are

shown in Fig. 1.

Body weight. At weaning, there was no difference in body weight between CDs and CDr rats of either sex. A sex difference developed thereafter. Male CDs and CDr rats that were fed RD continued to grow after weaning at comparable rates, increasing their weight by more than threefold at 4 and 6 months. Male CDr rats that were provided HSD grew at a rate that was comparable to those that were fed RD. In contrast, male CDs rats that were fed HSD failed to thrive and at 4 months only doubled their weight, with no additional growth during the subsequent 2 months. Female CDr and CDs rats that were fed RD or HSD showed similar growth curves, irrespective of strain or diet. It is interesting that the body weight of females, although similar to that of males after weaning and despite similar food consumption, was markedly lower than that of males at 4 and 6 months. Thus, female CDs rats expressed a different pattern of response to the HSD in terms of growth curve than males. In addition, upon termination of the study at 6 months, male CDs rats looked "unhealthy" and emaciated. In striking contrast, all female CDs rats seemed to be in a good state of health at 6 months.

IF experiment. IF of CDs rats with the HSD did not change the growth pattern in terms of body weight gain, when compared to DF. However, animals looked healthier.

Gonadectomy experiments. Orchiectomy or oophorectomy in CDs rats did not significantly affect the growth pattern in terms of body weight in either male or female animals that were fed HSD.

Blood pressure. Systolic blood pressure in male and female CDr rats that were fed RD was 135 ± 1 (n = 11) and 130 ± 1 mmHg (n = 7), respectively (NS) and in animals that were fed HSD was 135 ± 2 (n = 11) and 137 ± 2 mmHg (n = 10), respectively (NS). The values on HSD were not significantly different from those in animals that were fed RD. Blood pressure in male and female CDs rats that were provided RD was 134 ± 1 (n = 10) and 131 ± 1 mmHg (n = 8), respectively (NS), and in those that were fed HSD was 122 ± 1 (n = 3) and 130 ± 1 mmHg (n = 9), respectively. Blood pressure in the 6-month-old CDs males that were fed HSD was lower than in all other groups, probably a reflection of the poor state of health, as also reflected in their body weight. It is noteworthy that in contrast to males, blood pressure in female CDs rats that were fed HSD was not different from that in animals that were fed RD.

IPGTT. The pattern of response of CDr rats to the IPGTT was normal throughout the study period, irrespective of diet or age. The response of CDs rats to the IPGTT in animals that were fed RD showed a similarly normal pattern at 4 and 6 months. In contrast, the response of CDs rats that were provided the HSD was markedly abnormal in all animals studied, with a distinctly more pronounced pattern at 6 months than at 4 months, and more so in males than in females. To quantify and compare the IPGTT data between the groups and by sex, the results were analyzed further by fasting, peak, and 2-h blood glucose levels, as well as by the AUC (Fig. 2).

Fasting blood glucose. At 5–6 weeks of age, fasting BGL of male and female CDs and CDr rats were within the normal range (Table 1). At 4 and 6 months, BGLs remained within the normal range in CDr rats of both sexes and in female CDs rats, irrespective of diet. In male CDs rats that were fed HSD, however, fasting BGLs gradually increased to levels >126 mg/dl at 6 months, and the rats thus became overtly diabetic.

Peak BGL. At 5–6 weeks of age, maximum BGLs of male and female CDs and CDr rats were <200 mg/dl. At 4 and 6 months, BGLs of CDr rats (irrespective of diet) and of CDs rats that were fed RD gradually increased but remained <210 mg/dl, with no differences between the sexes. In contrast, peak BGLs of male and female CDs rats that were fed HSD rose to levels of >210 mg/dl, with significantly higher levels in males than in females (Table 2).

BGL at 2 h. At 5–6 weeks of age, BGLs 2 h after intraperitoneal injection returned to levels below 100 mg/dl in male and female CDs and CDr rats. At 4 and 6 months of age, BGLs in CDr rats (irrespective of diet) and in CDs rats that were fed RD similarly returned to levels below 100 mg/dl. A 4 months, there was no difference in these groups between the sexes, but at 6 months, BGLs were lower in females than in males. In CDs rats that were fed HSD, BGLs at 4 and 6 months remained markedly elevated, with lower levels in females than in males (Table 3).

AUC. At 5–6 weeks of age, the AUC was similar in male and female CDs and CDr rats. At 4 and 6 months, AUC remained unchanged in CDr rats of both sexes, irrespective of diet, and in female CDs rats that were fed RD. In CDs of both sexes that were fed HSD, AUC was double or more than in CDr rats that were fed a similar diet, with significantly lower values in females than in males (Table 4).

IF experiments. IF resulted in an intermediate diabetic phenotype in the 4- and 6-month-old CDs males, with an IPGTT pattern that was significantly attenuated compared with DF: BGLs were lower at fasting, at maximum, and at 2 h; the AUC was lower than in CDs rats that were fed daily but significantly higher than in CDr rats that were fed daily or on alternate days (Fig. 3).

Gonadectomy experiments. The IPGTT pattern of gonadectomized rats showed two phases (Fig. 4). In the early phase at 4 months, the IPGTT curve was not different from that of sham-operated animals in both males and females, with markedly apparent sex differences. At 6 months, however, the IPGTT curve was strikingly attenuated in males compared with sham, with significantly lower fasting, peak, and 2-h BGL as well as the AUC. In females, the IPGTT curve at 6 months was only mildly attenuated by gonadectomy, the only significant difference from sham-operated animals being lower peak BGL. Consequently, there was no longer a difference in the curve between male and female CDs rats that were fed HSD. The sex differences observed in intact animals thus remained unchanged during the early phase of development of diabetes at 4 months but were abolished by gonadectomy in the later phase at 6 months.

Plasma insulin levels. As no sex differences were found in insulin levels in any of the experiments, the data provided are combined for males and females.

Fasting insulin. In 4-month-old animals that were fed RD or HSD, basal fasting insulin levels were significantly higher in CDs than in CDr rats (Table 5). Because fasting

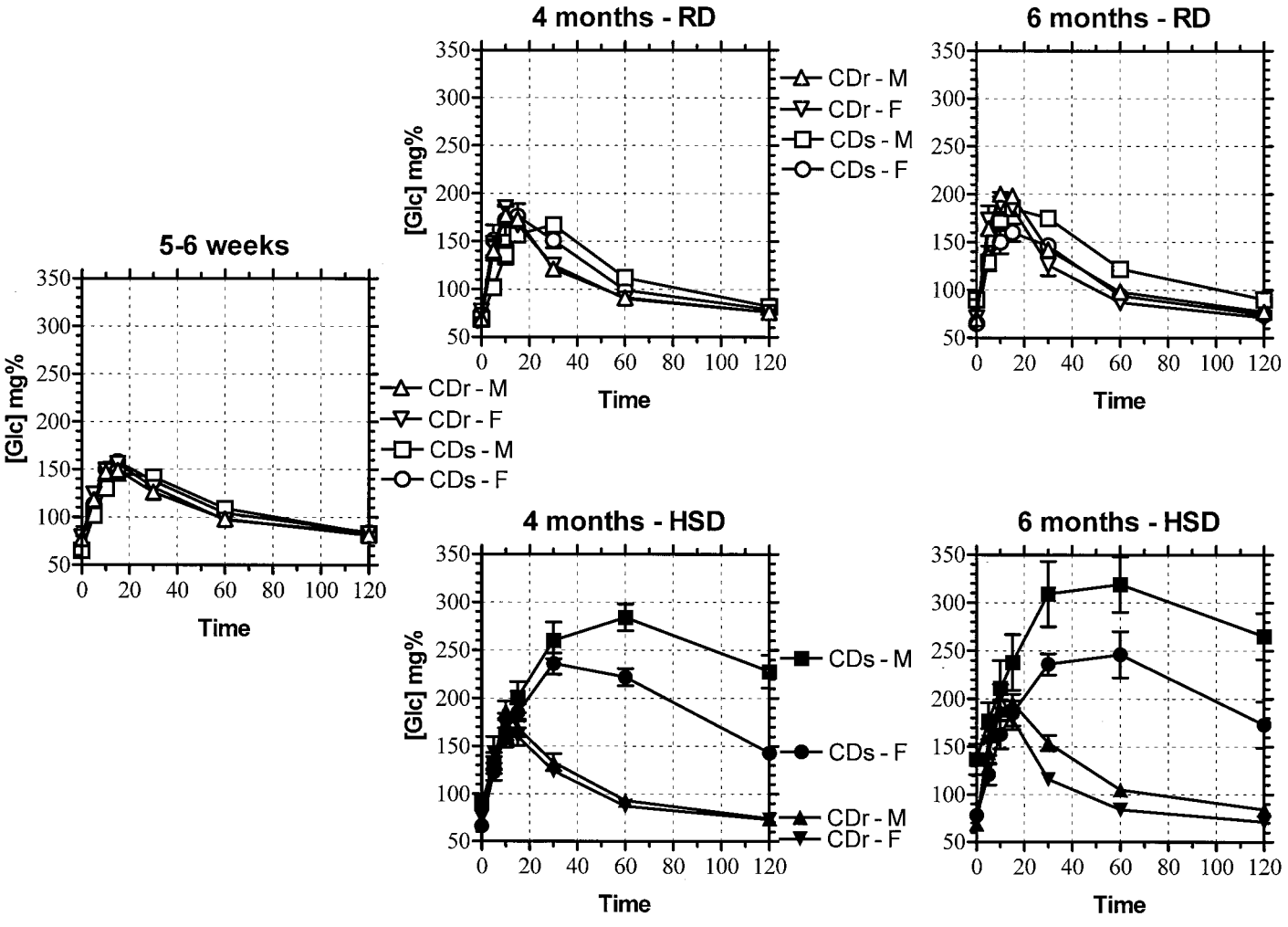

FIG. 2. Results of the IPGTT in male and female CDr and CDs rats aged 5–6 weeks and 4 and 6 months. Animals were fed either RD or HSD. Blood glucose concentration was measured after overnight fasting and immediately before intraperitoneal glucose injection (time 0) and at 5, 10, 15, 30, 60, and 120 min thereafter. Data are presented as in Fig. 1.

BGLs were similar, these findings suggest a hyperinsulinemic state in CDs rats. Insulin levels in both CDs and CDr rats were higher in animals that were fed HSD than when provided RD. In 6-month-old animals, there was no longer a difference in fasting insulin levels between CDs and CDr rats that were fed RD or HSD, even though glucose and insulin levels tended to be higher in CDs rats that were fed HSD, although not to a statistically significant level.

Insulin response to intraperitoneal glucose load. Insulin levels 15 min after injection of 1 g/kg glucose i.p. in 4-month-old CDs rats that were fed RD or HSD were significantly lower than in CDr rats, despite significantly higher glucose levels. At 6 months, a similar pattern of insulin response was observed (Table 5).

Biochemical data. Biochemical data are shown in Table 6.

Plasma electrolytes. There were no differences in plasma sodium, potassium, or chloride levels between CDs and

TABLE 1
Fasting BGL (mg/dl) in CDr and CDs animals aged 5–6 weeks and 4 and 6 months and fed RD or HSD

			5–6 weeks			4 months			6 months		
Diet	Sex	Strain	*n*	Average	SE	*n*	Average	SE	*n*	Average	SE
RD	Male	CDr	15	77	2	15	69	1	15	70	7
		CDs	15	62	2	11	69	2	10	90*	3
	Female	CDr	8	81	1	8	77	3	7	71	1
		CDs	8	71	2	8	75	2	8	65†	2
HSD	Male	CDr	11	76	2	11	73	2	11	69	2
		CDs	8	66	2	8	91*	7	9	137*	16
	Female	CDr	10	78	3	10	75	1	10	71	3
		CDs	9	65	3	9	66†	3	8	78†	6

*$P < 0.01$ for CDs versus CDr within the same sex and feeding group; †$P < 0.01$ for females versus males within the same strain and feeding group.

TABLE 2
Peak BGL (mg/dl) during IPGTT in CDr and CDs animals aged 5–6 weeks and 4 and 6 months and fed RD or HSD

Diet	Sex	Strain	5–6 weeks			4 months			6 months		
			n	Average	SE	*n*	Average	SE	*n*	Average	SE
RD	Male	CDr	15	148	5	15	183	8	15	204	3
		CDs	15	147	4	11	172	7	10	192	6
	Female	CDr	8	152	9	8	188	7	7	202	10
		CDs	8	179	5	8	184	14	8	169	9
HSD	Male	CDr	11	154	6	11	187	10	11	202	9
		CDs	8	158	11	8	289*	15	9	338*	27
	Female	CDr	10	148	11	10	173	13	10	204	12
		CDs	9	148	6	9	244*	9	8	272*	14

*$P < 0.01$ for CDs versus CDr within the same sex and feeding group.

CDr rats that were fed either diet or between sexes.

Lipid profile. Total cholesterol and HDL levels were within the normal range in all groups studied, with no clear pattern differentiating between strains, diet, or sex. Triglyceride levels, although within a normal range, were higher and LDL levels were lower in CDs than in CDr rats in all but males that were fed HSD.

Kidney function. Plasma creatinine levels ranged between 0.4 and 0.6 mg/dl. There were no differences between CDs and CDr rats, irrespective of diet or sex. Twenty-four-hour protein excretion was <15 mg/day in all groups studied.

Genotype

Within-strain homogeneity. The rate of homozygosity, a measure of genetic homogeneity within strains, was 98% in CDs and 95% in CDr rats. The residual heterozygosity was due in most cases to nonsharing allelic mutations rather than to cross-contamination between the strains.

Between-strain polymorphism. The rate of genetic polymorphism between CDs and CDr rats, based on 472 microsatellite markers that amplified in both strains, was 43%. This initial genetic screening thus identified 203 microsatellite markers (Table 7) that are informative for linkage analysis in future cross-breeding experiments.

DISCUSSION

Since Minkowski's creation of an experimental model of diabetes by removing the pancreas (21), a significant number of other models of the disease have been developed, spanning several species (22–26). Despite an apparent abundance of such models, the pathophysiological and genetic basis of diabetes remains in many respects unknown. The Cohen diabetic rat was bred precisely to elucidate the genetic susceptibility to nutritionally induced type 2 diabetes. We selectively reinbred the respective strains from the original colony and established a new homogeneous colony of the Cohen diabetic rat. We revised the nomenclature previously used by renaming the "sensitive" strain, or "upward line," as the Cohen diabetic-sensitive (CDs) rat and the "resistant" strain, or "downward line," as the Cohen diabetic-resistant (CDr) rat. We characterized the two strains of the new colony in terms of the diabetes-related phenotypes. We established thereby for the first time a database that demonstrates that the "new" Cohen rat is a classical model of type 2 diabetes that expresses a major sex effect in terms of the metabolic phenotypes.

The major diabetic phenotype in the current study was the abnormal IPGTT in CDs rats that were fed HSD with a sex effect. Even though fasting BGL became elevated only in males, peak and 120-min BGL and the AUC were distinctly above normal in both male and female CDs rats on HSD, but with significantly higher levels in males. CDs rats that were fed RD handled glucose normally. Thus, the CDs strain is truly sensitive to the HSD, and expression of the sensitivity gene complex allows the development of diabetes in animals that are fed HSD, with differences in the level of expression between males and females. In contrast, the CDr strain is truly resistant to the HSD, and expression of the resistance gene complex prevents the development of diabetes, despite the HSD, without sex differences.

We investigated the insulin profile in this model after overnight fasting and in response to glucose stimulation. Fasting insulin levels in CDs rats that were 4 months of age

TABLE 3
BGL (mg/dl) at 120 min during IPGTT in CDr and CDs animals aged 5–6 weeks and 4 and 6 months and fed RD or HSD

Diet	Sex	Strain	5–6 weeks			4 months			6 months		
			n	Average	SE	*n*	Average	SE	*n*	Average	SE
RD	Male	CDr	15	82	2	15	76	2	15	77	1
		CDs	15	81	2	11	81	2	10	90†	1
	Female	CDr	8	84	2	8	76	2	7	71	1
		CDs	8	80	2	8	79	2	8	74†	2
HSD	Male	CDr	11	82	2	11	74	2	11	84	4
		CDs	8	83	8	8	228*	17	9	265*	24
	Female	CDr	10	82	3	10	73	1	10	71†	2
		CDs	9	89	5	9	143*	4	8	173*†	24

*$P < 0.01$ for CDs versus CDr within the same sex and feeding group; †$P < 0.01$ for females versus males within the same strain and feeding group.

TABLE 4
AUC in CDr and CDs animals aged 5–6 weeks and 4 and 6 months and fed either RD or HSD

Diet	Sex	Strain	5–6 weeks			4 months			6 months		
			n	Average	SE	n	Average	SE	n	Average	SE
RD	Male	CDr	15	12,722	235	15	12,563	285	15	13,897	231
		CDs	15	13,124	318	11	14,182	365	10	15,688	425
	Female	CDr	8	12,966	379	8	12,608	263	7	12,719	276
		CDs	8	13,373	218	8	13,761	348	8	12,908	310
HSD	Male	CDr	11	12,784	285	11	12,895	454	11	14,500	552
		CDs	8	13,369	1,025	8	29,170*	1,370	9	33,936*	3092
	Female	CDr	10	12,928	507	10	12,223	264	10	12,258	375
		CDs	9	13,328	396	9	23,059*†	817	8	25,040*†	1767

*$P < 0.01$ for CDs versus CDr within the same sex and feeding group; †$P < 0.01$ for females versus males within the same strain and feeding group.

and fed either diet were elevated compared with CDr rats, whereas BGLs were not different, suggesting a relative hyperinsulinemic state in young CDs rats in comparison with CDr rats. At 6 months, absolute insulin levels tended to be higher in both strains. The differences in insulin levels between the two strains were, however, no longer apparent at that time point. Stimulated insulin levels were significantly lower in CDs than in CDr rats, despite higher BGLs, suggesting that while insulin was present in the islets, its secretion in response to glucose loading was impaired in CDs rats. Thus, the CDs rats express at least two features of interest, in terms of insulin secretion: a basal fasting hyperinsulinemic state early in the course of diabetes and an inability to secrete sufficient insulin in response to a glucose load at a later stage of the disease. These phenotypic data are in agreement with those published by Cohen et al. (27) in earlier studies, in which they showed in CDs rats that were fed HSD for >2 months a moderate fasting hyperinsulinemia and a subsequent age-dependent reduction in their ability to secrete insulin in response to glucose stimulation. A similar impaired insulin response also has been described in GK rats (7,28). A possible explanation for these findings is prolonged exposure to hyperglycemia per se, which exhausts the insulin-secreting ability of the pancreas (13,17,18,28–31). Any suggestion, however, of absolute insulin deficiency as a result of complete β-cell exhaustion was ruled out by our findings. Also with regards to plasma insulin levels, it is of interest that no sex differences were detected; thus, the observed differences in glucose handling between male and female CDs rats that were fed HSD could not be attributed to differences in insulin secretion per se.

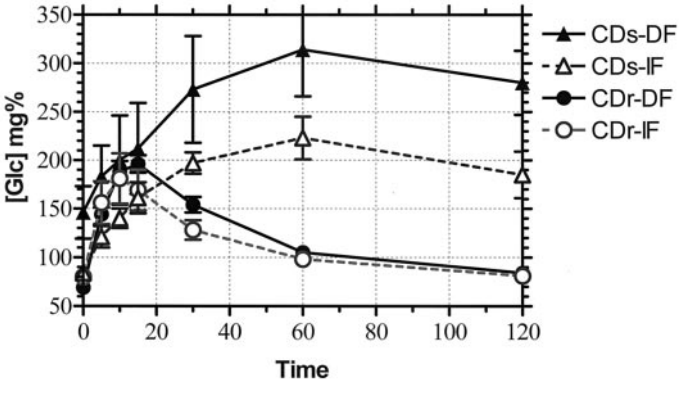

FIG. 3. Results of the IPGTT in male CDr and CDs rats aged 5–6 weeks and 4 and 6 months that were fed daily (DF; $n = 5$) or on alternate days (IF; $n = 5$) with either RD ($n = 5$) or HSD ($n = 5$). Data are presented as in Fig. 1.

In studying animal growth patterns, we confirmed previous observations that neither CDs nor CDr strains of either sex become obese, despite the high-calorie, fat-rich HSD (16). We established thereby the Cohen rat as a nonobese model of type 2 diabetes. A more striking finding, however, was the failure to thrive of male CDs rats that were fed HSD, in contrast to the continuing well-being of female CDs rats that were fed an identical diet. Autopsy of these male CDs rats revealed severe emaciation, with reduced muscle mass and fat tissue. This was not observed in male CDs rats that were fed RD, female CDs rats that were fed HSD, or in male or female CDr rats that were fed either diet. Such findings in the Cohen model have been alluded to previously only vaguely (32). We attributed the failure to thrive of male CDs rats to the more severe expression of the metabolic diabetic phenotype as evidenced by the IPGTT and to the fact that in our experiments, the animals remained untreated with exogenous insulin, allowing the natural course of the disease to develop. In the search for an intermediate diabetic phenotype that could be used in future long-term experiments, we were able to attenuate the phenotype in male CDs rats by IF. We thereby also confirmed our hypothesis that daily provision of HSD to male CDs rats resulted in the severe expression of the diabetic phenotype and that IF would lead to an "intermediate" phenotype, thus establishing a dose-response effect.

The sex differences that we observed in animal growth and glucose handling are novel and of interest. Their relevance to diabetes in humans, however, is unclear, although the issue is intriguing. The importance of sex in diabetes in humans has been raised repeatedly, but mostly in the epidemiological context emphasizing transmission, incidence, and prevalence (33). A sex effect in the "natural" untreated course of the disease, which is what we studied in the Cohen rat model, is almost unexplored in humans. Most patients with diagnosed overt type 2 diabetes are nowadays treated with oral hypoglycemic agents or insulin, which presumably alter the "natural" course of the disease. No patient population is amenable to prospective "untreated" follow-up studies. An exception may be a report on the outcome of type 2 diabetes in Nigeria in which the indigenous population, which may have been undertreated, showed a more malignant course of the

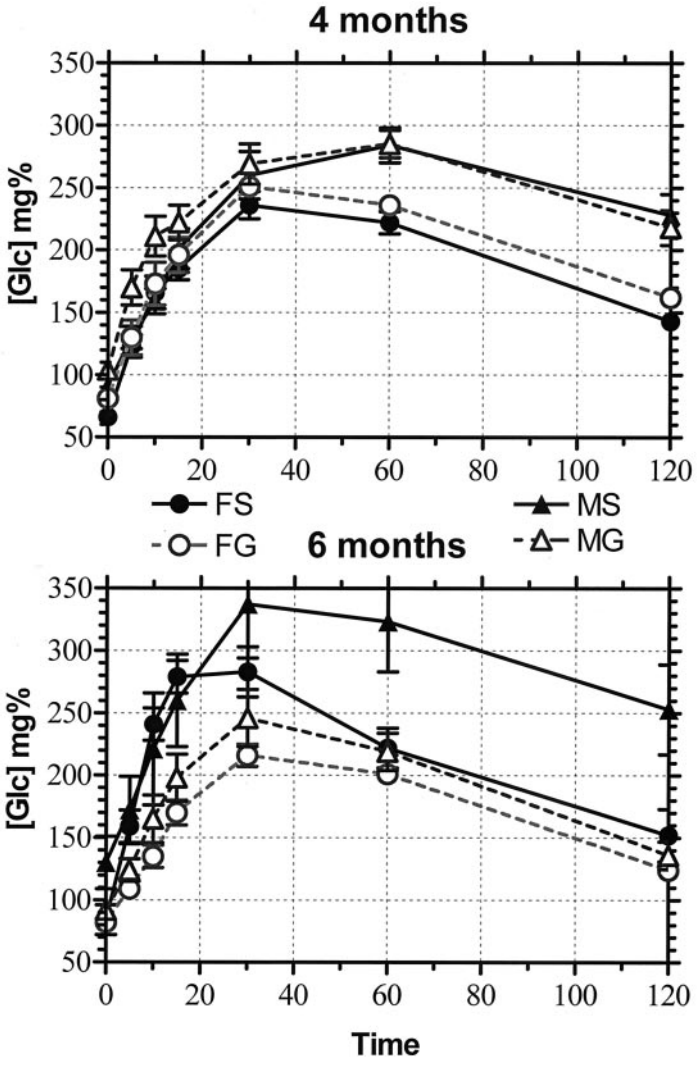

FIG. 4. Results of the IPGTT in CDs male rats that were subjected to orchiectomy (MG; $n = 8$) or sham operation (MS; $n = 5$) and in females that were subjected to orchiectomy (FG; $n = 6$) or sham operation (FS; $n = 5$). Animals were fed HSD and studied at 4 and 6 months. Data are presented as in Fig. 1.

disease in males with higher mortality than in females (34). Thus, the sex effect on the diabetic phenotype may be nonetheless of major importance. We consequently initiated a set of studies in which we began to explore the hypothesis that sex differences might be due in part to differences in sex hormone levels or activity, attenuating the diabetic phenotype in females or allowing more severe expression of the diabetic phenotype in males. Gonadectomy, which removed the sex hormone factor shortly after weaning, did not alter the results of IPGTT in either sex in the early phase of development of diabetes in this model, two and a half months after initiation of the HSD at the age of 4 months. Two months later, however, at age 6 months, while animals continued to be fed HSD, gonadectomy markedly attenuated the severity of diabetes in males and only mildly attenuated in females. These findings suggest that sex hormones do not play a major role in the early development phase of diabetes but are important in determining the severity of the phenotype later on. Further elucidation of these findings, which was beyond the scope of the current studies, remains an important task as it may provide clues to potentially protective measures that may prevent the development of the full-blown diabetic phenotype, such as was observed in males.

We studied additional phenotypes of interest in relation to diabetes. Blood pressure measurements indicated that the Cohen rat model of type 2 diabetes is normotensive, irrespective of diet or sex. These results are consistent with previous reports in this model (4,5,16) and in fact formed the basis for cross-breeding of a hypertensive strain, the SHR, with the normotensive diabetic CDs strain, culminating in the Cohen-Rosenthal model of diabetes with hypertension (3). The fasting lipid profile of male and female CDs and CDr rats indicates that this is a nonhyperlipidemic model of type 2 diabetes. Measurement of postprandial lipid levels and of nonesterified fatty acids, which were beyond the scope of the current study, may nonetheless reveal hitherto undetected abnormalities in lipid handling. Studies of kidney function, as reflected by serum creatinine and urinary protein excretion, did not reveal target organ involvement in either male or female CDs rats. Because the current study was of relatively short duration, considering the length of time required for the development of end-organ damage in diabetes, long-term studies are needed to determine whether diabetic nephropathy indeed develops in this model, as was reported previously by Rosenmann and Cohen (35) in the original colony.

To complete characterization of the new colony of the

TABLE 5
Plasma insulin levels (μU/ml) after overnight fasting (F-Ins) and stimulated plasma insulin levels (S-Ins) 15 min after intraperitoneal glucose injection in CDr and CDs rats at ages 4 and 6 months

			4 months						6 months					
			Glucose			Insulin			Glucose			Insulin		
	Diet	Strain	*n*	Average	SE	*n*	Average	SE	*n*	Average	SE	*n*	Average	SE
F-Ins	RD	CDr	5	76	3	6	16	2	23	70	2	22	28	2
		CDs	11	85	5	11	26	1	18	79	3	18	32	3
	HSD	CDr	5	74	6	6	23	2	21	78	2	21	30	2
		CDs	11	77	5	11	33	3	12	101	13	12	40	8
S-Ins	RD	CDr	9	171	8	9	52	6	18	185	6	18	48	6
		CDs	9	217*	9	9	30*	3	7	214	10	9	25*	2
	HSD	CDr	10	196	9	10	51	10	5	163	12	5	40	6
		CDs	13	273*	15	13	15*	2	12	223	16	12	14*	2

Fasting and stimulated insulin were measured in different animals and in different sets of experiments. Animals were fed before the experiments with RD or HSD. Data are combined for males and females. *$P < 0.01$ for CDs versus CDr within the same feeding group.

TABLE 6
Plasma electrolytes (mEq/l) and lipid profile (mg/dl) in 6-month-old CDs and CDr rats fed since weaning with RD or HSD

Diet	Sex	Strain	n	Electrolytes			Lipid profile			
				Na	K	Cl	Chol	TG	HDL	LDL
RD	Male	CDr	15	142 ± 1	4.7 ± 0.6	105 ± 1	96 ± 5	63 ± 6	53 ± 2	31 ± 2
		CDs	10	145 ± 1	5.1 ± 0.3	106 ± 1	75 ± 3	98 ± 11*	42 ± 1	13 ± 2*
	Female	CDr	8	142 ± 1	4.3 ± 0.6	105 ± 1	79 ± 4	52 ± 3	47 ± 2	22 ± 2
		CDs	8	141 ± 1	4.7 ± 0.3	106 ± 1	73 ± 3	71 ± 5*	44 ± 2	15 ± 1*
HSD										
	Male	CDr	11	143 ± 1	5.1 ± 0.3	105 ± 1	112 ± 4	106 ± 14	61 ± 2	27 ± 3
		CDs	9	145 ± 1	5.2 ± 0.2	104 ± 1	89 ± 7	72 ± 11	53 ± 4	22 ± 2
	Female	CDr	10	142 ± 1	4.6 ± 0.1	105 ± 1	109 ± 9	88 ± 11	58 ± 3	33 ± 6
		CDs	8	140 ± 1	4.9 ± 0.3	100 ± 1	97 ± 6	156 ± 22*	54 ± 2	12 ± 2*

Data are means ± SD. *$P < 0.01$ for CDs versus CDr within the same sex and feeding group.

Cohen diabetic rat, we studied the genetic profile of the strains that resulted from secondary selective inbreeding with microsatellite markers. We screened the entire rat genome with 550 randomly selected microsatellite primer sets, evenly spread over the 20 rat autosome and the X chromosome. We found a 95–98% rate of homozygosity in CDr and CDs rats, suggesting that the two strains are highly inbred. Normally, however, a 100% homozygosity rate would have been expected. Because this residual heterozygosity was due to allelic mutations and not to contamination between the strains, it can safely be assumed that homozygosity among the available animals in our colony is currently maximal. A second finding was that a large number of the microsatellite markers that were tested were found to exhibit simple sequence length polymorphism between the CDs and CDr strains. The polymorphic markers are informative because they enable differentiation of the genotype of CDs strain from that of the CDr strain and allow the correlation between a distinct genotype and phenotype in future genetic linkage studies of type 2 diabetes and its associated metabolic phenotypes. It is noteworthy that, although widely distributed over the entire genome, a few chromosomes still show insufficient coverage. As by now >10,000 rat microsatellite markers have been developed and given the >40% polymorphism rate between CDs and CDr strains, we anticipate that adequate coverage of all of the rat chromosomes will be obtained with the new microsatellite markers. In addition, the high rate of polymorphism between the two strains enables cross-breeding experiments between the CDs and CDr strains, a requirement for future positional cloning studies, without having to resort to the use of more distant strains. Finally, differential expression studies using the CDr genomic background should allow uncovering of important regions in the CDs genome that render them susceptible to develop type 2 diabetes, and, conversely, using the CDs genomic background, the CDr genome should provide useful information as to the reason that diabetes does not develop, despite identical dietary-environmental conditions.

TABLE 7
Genome screen of the CDs and CDr strains that compose the Cohen diabetic rat model

Chromosome number	Number of markers tested	Number of polymorphic markers	Percent polymorphism
1	117	52	44
2	23	13	57
3	25	11	44
4	24	15	63
5	21	13	62
6	21	9	43
7	18	8	44
8	22	16	73
9	16	4	25
10	28	22	79
11	14	3	21
12	13	10	77
13	6	5	83
14	17	7	41
15	26	7	27
16	8	5	63
17	31	7	23
18	14	5	36
19	11	2	18
20	10	5	50
X	21	1	5
Total	486	220	45

In conclusion, the products of the secondary selective inbreeding of the CDs and CDr strains express more distinct phenotypes then the original diabetic phenotypes described by Cohen. Susceptibility (sensitivity or resistance) to the HSD stands out as the hallmark of this model. The sex differences and the role of male sex hormones need to be investigated further. Genome screening demonstrated that this model is highly suitable for genetic studies. Finally, this study provides for the first time a detailed and unified account of the metabolic diabetes-related phenotypes and the genotype of the newly established Cohen diabetic rat colony—the result of a systematic controlled study protocol, to be used as a reference database for future studies in this model.

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