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Spontaneous glucose intolerance in the progeny of low dose streptozotocin-induced diabetic mice

T. Linn, E. Loewk, K. Schneider, K. Federlin

Medical Clinic III and Policlinic, Department of Internal Medicine, Justus Liebig University, Giessen, Germany

Summary. Multiple low doses of streptozotocin (LDS) induce low-incidence diabetes mellitus in Balb/cHan and high-incidence diabetes in CD-1 mice. We studied offspring of diabetic parents in both strains. Group 1 consisted of litters from control mice with no streptozotocin treatment. Group 2 litters had an LDS diabetic mother and a control father, group 3 litters had control mother with LDS diabetic father, and group 4 litters had both, LDS diabetic mother and father. Diabetes was induced by 5×40 mg streptozotocin per kg on five consecutive days. Progeny of diabetic mothers showed a state of reduced glucose tolerance associated with reduced glucose disappearance during intravenous glucose tolerance test and increased insulin secretion of isolated islets of Langerhans. These metabolic abnormalities predominated in the male litters of both strains of mice. Amniotic insulin was increased in diabetic mothers during pregnancy. No histologic abnormalities were observed in group 2 progeny. Pancreases in male offspring of LDS diabetic CD-1 fathers (group 3) were studied for insulitis. Insulitis was found in 40% of mice with normal glucose tolerance. A single subdiabetogenic dose of streptozotocin (40 mg/kg) induced insulitis in 90% of pancreases accompanied by reduced insulin release of isolated islets. By contrast, male Balb/cHan progeny of diabetic fathers failed to develop insulitis. In conclusion, we found (1) parental LDS diabetes was transmitted more often to male offspring, (2) maternal LDS diabetes was associated with hyperinsulin secretion and glucose intolerance in the offspring and (3) paternal LDS diabetes was accompanied by insulitis and insulin secretion deficiency in CD-1 progeny.

Key words: Streptozotocin, offspring, insulitis, islet perifusion.

Diabetes mellitus can be induced in rodents by diabetogenic drugs, such as streptozotocin (STZ) or alloxan. STZ interferes with the protective mechanisms of the beta cell that normally scavenge endogenously produced free radicals. Given in multiple low doses, STZ causes insulitis and transient lymphocytic cytotoxicity to pancreatic beta cells in susceptible strains of mice [1, 2]. Diabetes induction is subject to control of the MHC complex and is strain dependent [3, 4].

N-nitroso compounds can induce insulitis and Type 1 (insulin-dependent) diabetes in mice and man [1, 5]. We studied low dose streptozotocin (LDS) diabetes in two mouse strains, because in this murine model Type 1 diabetes is induced by a toxic environmental agent. CD-1 mice are susceptible to LDS diabetes and Balb/cHan mice are not susceptible. In models of spontaneous diabetes the disease is transmitted to offspring, and it is known that this occurs also in models of induced diabetes, but it has not been demonstrated for immune-mediated mechanisms. Consequently, the aims of this study were to determine whether diabetes induced by LDS treatment was transferred to the next generation, and what contribution maternal and paternal diabetes made to the transfer.

Materials and methods

Animals

The CD-1 and Balb/c mice used (Versuchstieranstalt, Hannover, Germany) were comparable in terms of physical activity, litter size (4–8 mice), growth after birth and were free from common mouse virus infections.

The mice were kept under usual animal housing conditions. STZ (Upjohn Co, Brussels, Belgium) was injected i.p. on 5 consecutive days, 40 mg/kg per day and diabetes was defined by fasting blood glucose greater than 10 mmol/l on 3 days. Controls were injected with 0.05 mmol/l citrate buffer. Future parents were made diabetic at 6 weeks of age. About 2 weeks after the last injection, mice that had become diabetic were used for breeding. Five parental groups were formed. In group 1, also termed "controls", neither mothers nor fathers were treated with STZ. In group 2, LDS diabetic mothers were

Table 1. Mouse strain susceptibility to streptozotocin (STZ)

	Control		5 × 40 mg STZ/kg	
	Blood glucose (mmol/l)	Insulitis	Blood glucose (mmol/l)	Insulitis
CD-1 (male)	5.8 ± 1.9 (10)	2 ± 1	13.4 ± 2.7[a] (24)	180 ± 21
CD-1 (female)	5.2 ± 1.3 (9)	0	8.7 ± 1.7 (17)	80 ± 12
Balb/c (male)	4.9 ± 1.0 (11)	0	8.5 ± 1.8 (21)	45 ± 9
Balb/c (female)	4.6 ± 0.9 (10)	0	7.3 ± 1.5 (19)	19 ± 9

After a 12-h fast blood glucose was measured in mmol/l with a Beckman glucose analyser 14 days after the first STZ injection. Insulitis score is explained in the materials and methods. The number of mice studied is in parentheses. Controls were injected with citrate buffer. Values are given as mean ± SEM.

[a] $p < 0.01$ vs Balb/c male and CD-1 female

Table 2. Insulin concentration (pmol/l) of amniotic fluid (gestational day 20)

Parents	Control	Female LDS	Male LDS	Female and Male LDS
Group	1	2	3	4
n	10	9	8	9
CD-1	17.6 ± 4.2	144 ± 17.6[a]	15.6 ± 5.1	123.0 ± 9.7[a]
Balb/c	11.2 ± 3.9	93.2 ± 25.1[a]	14.2 ± 3.1	99.3 ± 16.3[a]

[a] $p < 0.01$ vs control

mated with fathers who had not been treated with STZ. Group 3 progeny had a control mother (no LDS) and an LDS diabetic father, and group 4 litters were born to an LDS diabetic mother and an LDS diabetic father. Group 5 included offspring of group 3 treated with a single subdiabetogenic dose of 40 mg/kg STZ at 4 weeks of age. In all groups offspring were studied for intravenous glucose tolerance, pancreatic histology, and insulin secretion of isolated islets of Langerhans.

Amniotic fluid was collected by direct puncture of the fetal cavity on the 20th day of gestation.

Intravenous glucose tolerance test (IVGTT)

Litters were subjected to an IVGTT at age 12 weeks. After an overnight fast animals were anaesthetized (sodium pentobarbital 75 mg/kg body weight) and the inferior vena cava was catheterized. Glucose (1 g/kg) was injected through the catheter (24 gauge). During anaesthesia blood samples were withdrawn 0, 2, 4, 6, 10, 20, and 30 min after glucose injection by the catheter. Each blood sample of 50 µl was replaced with an equivalent volume of heparin/NaCl. Haemoglobin before and after the IVGTT dropped from 142 ± 2.1 to 98 ± 1.9 g/l. Blood samples were centrifuged and the plasma was analysed for glucose by a Beckman glucose analyser. Glucose concentrations were plotted against time and the area under the curve (AUC) was calculated following the trapezoid rule. Glucose disappearance rate (K-value) was calculated by the least square method and given in % per min (%/min).

Islet histology and islet isolation

After the IVGTT the pancreas was removed. About one-third of the pancreatic volume was used for histology and two-thirds were used for islet isolation. For histological examination, pancreas was paraffin embedded, cut in 10 sections each separated from the previous one by ± 10 slides, and was stained with haematoxylin-eosin. For scoring of insulitis three degrees of insulitis were distinguished: first degree, with lymphocytes present in the periphery of the islet; second degree, with lymphocytes present also inside the islet, without altering its normal architecture; third degree, with massive alterations of its normal architecture. The insulitis score in the 10 sections from each preparation was expressed as the sum of the percentages of islets infiltrated times the degree of infiltration.

Islets were isolated as described previously [6]. Briefly, the pancreas was distended with Hanks' solution and the tissue was minced and digested with 1.5 mg/ml collagenase for 9–11 min at 37 °C. The tissue was stirred by gentle bubbling with 95 % O_2/5 % CO_2. Islets were collected with siliconised constriction pipettes under a stereomicroscope. Only islets with a diameter more than 150 µm were selected. The average yield was 45 ± 21 islets per pancreas.

Islet perifusion

After a culture period of 12 h in RPMI 1640 medium, batches of 100 islets were transferred to chambers consisting of a plastic filter unit fitted with a nylon filter (15 µm pore size).

The basic perifusion medium (Krebs Ringer Bicarbonate) consisted of 119 mmol/l NaCl, 4.7 mmol/l KCl, 2.5 mmol/l $CaCl_2$, 1.2 mmol/l $MgSO_4$, 25 mmol/l $NaHCO_3$, 10 mmol/l HEPES, 3 mmol/l glucose and 0.15 % bovine serum albumin (pH 7.4). Stimulating media consisted of basic medium plus 25 mmol/l glucose or 10 mmol/l arginine. The perifusion system has been described previously [7]. The media were continuously gassed with a mixture of 95 % O_2/5 % CO_2 with pH kept between 7.35–7.45. The dead space of the system was 2.5 ml and the flow rate was 0.25 ml/min. The effluent was collected once per 10 min, except for the interval between the time points 60 min and 70 min of every perifusion experiment. During this time the effluent was collected once per min. Samples were stored at –20 °C.

Insulin in the effluent and in amniotic fluid was determined by a RIA kit (Behring, Frankfurt, Germany) using rat insulin as standard (kindly provided by Novo Industry A/S, Bagsvaerd, Denmark). Non-specific binding was 6.6 ± 0.45 % ($n = 10$). The detection limit of the RIA was 5 pmol/l. The inter-assay coefficient of variation in the perifusion system was 30 % and intra-assay coefficient of variation was 15–20 %.

For calculation of first phase insulin response (FPIR) the area under the insulin concentration curve above steady state between 60 and 70 min, for total insulin response (TIR) between 60 and 120 min was used.

Statistical analysis

Statistical evaluations were made by Student's t-test for unpaired data. Results were presented as mean ± SEM.

Results

Differences between CD-1 and Balb/c mice

Table 1 shows the development of diabetes in two different mouse strains, CD-1 and Balb/c. When given in the form of the multiple injection regimen CD-1 males developed fasting hyperglycaemia (13.4 ± 2.7 mmol/l) on day 14 after the first STZ injection. By comparison, Balb/c

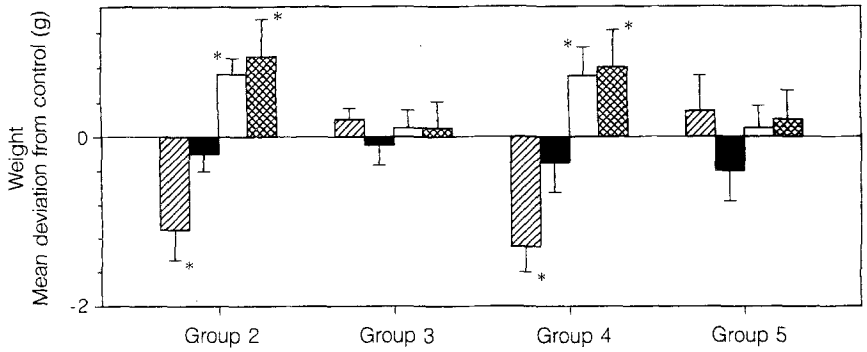

Fig. 1. Body weights (g) in 4-week-old progeny of LDS parents given as deviation from controls (mean ± SEM). $n = 20$ mice per column. Significance vs control mice * $p < 0.05$. ▩ Female Balb/c, ■ Female CD 1, □ Male Balb/c, ▨ Male-CD 1

Table 3. Glucose tolerance of adult male and female offspring of LDS diabetic CD-1 or Balb/c mice

Parents Groups	Control 1	LDS female 2	LDS male 3	LDS female and LDS male 4	LDS male plus STZ 5
CD-1					
Male offspring					
n	22	21	20	22	23
AUC_{glu} (mmol l^{-1}min)	18.2 ± 4.4	26.5 ± 5.0[b]	17.7 ± 5.2	22.9 ± 5.0[a]	33.7 ± 5.0[a]
K-value (% min^{-1})	4.72 ± 2.13	2.71 ± 1.16[a]	4.69 ± 3.1	3.44 ± 1.9	0.91 ± 1.08[b]
Female offspring					
n	17	15	22	20	20
AUC_{glu} (mmol l^{-1}min)	14.3 ± 5.1	21.4 ± 6.2[a]	13.9 ± 3.9	17.2 ± 4.2	17.8 ± 4.7
K-value (% min^{-1})	4.9 ± 3.3	3.1 ± 1.7[a]	4.1 ± 1.5	4.0 ± 3.4	4.0 ± 3.9
Balb/c					
Male offspring					
n	20	20	24	20	20
AUC_{glu} (mmol l^{-1}min)	15.1 ± 2.6	22.5 ± 4.4[a]	12.8 ± 4.4	16.7 ± 3.8	14.2 ± 2.4
K-value (% min^{-1})	2.60 ± 0.11	1.40 ± 0.17[a]	2.57 ± 0.19	1.42 ± 1.19	2.49 ± 1.9
Female offspring					
n	23	21	20	22	19
AUC_{glu} (mmol l^{-1}min)	12.2 ± 1.6	14.3 ± 3.9	13.9 ± 4.1	13.7 ± 4.0	12.8 ± 3.0
K-value (% min^{-1})	2.9 ± 1.0	2.8 ± 1.9	2.5 ± 0.9	2.6 ± 0.9	2.9 ± 1.0

Data show incremental area under the glucose curve (AUC_{glu}) and K-values of intravenous glucose tolerance tests performed at 12 weeks of age. Data are given as mean ± SEM.
[a] $p < 0.05$, [b] $p < 0.01$ vs control

inbred males ($H\text{-}2^k$) developed fasting blood glucose of 8.5 ± 1.8 mmol/l on day 14 after the first LDS injection and had a low insulitis score (Balb/c male 45 ± 9 vs CD-1 male 180 ± 21, $p < 0.01$). Male mice of both strains were more susceptible to STZ than females ($p < 0.05$). LDS treated CD-1 mice developed higher fasting blood glucose compared to LDS treated Balb/c mice ($p < 0.05$). Both males and females made diabetic with the LDS protocol remained fertile in contrast to mice treated with a single high dose (200 mg/kg) STZ (unpublished observation).

Fetal and young age progeny

Mean litter sizes were 6.2 ± 3.0 for non-diabetic mother (groups 1, 3, and 5), 5.6 ± 3.2 for diabetic mother (groups 2 and 4), and 6.4 ± 3.1 for diabetic father.

Table 2 shows that amniotic insulin was increased in diabetic mothers of both strains indicating stimulation of fetal beta cells. Four-week-old male CD-1 offspring of diabetic mothers (groups 2, 4) showed significantly ($p < 0.05$) lower body weights compared to male offspring of diabetic fathers (groups 3, 5) as depicted in Figure 1. In female progeny of CD-1 diabetic mothers, body weight

Table 4. First phase insulin release (FPIR) and total insulin release (TIR) during perifusion of islets from progeny of LDS diabetic CD-1 or Balb/c mice at 12 weeks of age

Parents Groups	Control 1	LDS female 2	LDS male 3	LDS female and male 4	LDS male plus STZ 5
CD-1					
Male offspring					
n	30	15	15	15	20
FPIR	2.9 ± 0.6	1.1 ± 0.8[a]	3.2 ± 0.9	1.4 ± 0.9[a]	1.3 ± 0.4[a]
TIR	13.2 ± 1.1	18.2 ± 2.2[a]	14.2 ± 1.7	17.2 ± 2.0[a]	6.4 ± 1.3[b]
Female offspring					
n	20	15	15	15	14
FPIR	2.4 ± 0.4	1.9 ± 0.5	2.6 ± 0.7	2.2 ± 0.5	1.9 ± 0.7
TIR	12.7 ± 1.6	14.2 ± 1.3	12.9 ± 1.8	14.3 ± 1.3	10.5 ± 1.1
Balb/c					
Male offspring					
n	16	15	14	15	15
FPIR	2.6 ± 1.2	1.1 ± 0.9[a]	2.5 ± 1.1	1.3 ± 0.8[a]	2.2 ± 1.3
TIR	11.1 ± 0.9	14.1 ± 1.1[a]	10.9 ± 1.1	12.5 ± 1.1	10.8 ± 0.7
Female offspring					
n	15	14	15	15	15
FPIR	2.2 ± 0.8	1.7 ± 0.5	2.5 ± 1.5	1.9 ± 0.7	2.2 ± 0.5
TIR	10.7 ± 0.6	13.6 ± 1.0[a]	12.0 ± 1.2	14.2 ± 1.9[a]	10.1 ± 1.1

Basal insulin was measured in the presence of 3 mmol/l glucose. Insulin secretion was stimulated by 25 mmol/l glucose or 10 mmol/l arginine plus 3 mmol/l glucose. FPIR (First phase insulin release, 60–70 min) and TIR (Total insulin release, 60–120 min) are presented in $nmol \cdot l^{-1} \cdot 100\ islets^{-1} \cdot min^{-1}$ as incremental area under the curve.
[a] $p < 0.001$, [b] $p < 0.02$ vs control

Table 5. Insulitis in the pancreas of adult offspring of LDS diabetic CD-1 and Balb/c mice

Parents Groups	Control 1	LDS female 2	LDS male 3	LDS female and male 4	LDS male plus STZ 5
CD-1					
Male offspring	2 ± 1 (19)	11 ± 3 (22)	38 ± 11 (17)[a]	27 ± 13 (20)[b]	115 ± 25 (19)[c]
Female offspring	1 ± 1 (9)	4 ± 2 (12)	6 ± 4 (20)	1 ± 5 (17)	22 ± 9 (18)[a]
Balb/c					
Male offspring	3 ± 2 (10)	3 ± 3 (12)	18 ± 6 (10)[b]	24 ± 19 (10)[a]	29 ± 13 (10)[a]
Female offspring	3 ± 1 (10)	4 ± 3 (11)	6 ± 4 (10)	1 ± 5 (10)	5 ± 6 (10)

For every pancreas a single insulitis score was calculated as described in the materials and methods section. Data are mean score values with number of pancreases in parentheses.
[a] $p < 0.001$, [b] $p < 0.05$ vs control; [c] $p < 0.05$ vs group 3 male offspring

was not significantly different from controls. By contrast, Balb/c diabetic mothers had overweight male and female progeny.

Glucose tolerance, insulin secretion and frequency of insulitis in male offspring of adult age

Diabetic mother

Male offspring of diabetic mothers were glucose intolerant in both strains. CD-1 male offspring of diabetic mothers had 8.5 ± 2.4 mmol/l non-fasting glucose at 12 weeks of age while Balb/c male offspring had 7.6 ± 1.9 mmol/l ($p < 0.05$). Table 3 shows that AUC_{glu} was significantly higher in group 2 ($p < 0.01$) and 4 ($p < 0.05$) compared to controls. K-value of group 2 was significantly ($p < 0.05$) lower than in controls. The insulin secretion pattern of isolated islets of Langerhans in group 2 and 4 of progeny is depicted in Figure 1B and Figure 1D, respectively. Data of FPIR and TIR are given in Table 4. For the CD-1 strain basal insulin before glucose stimulation was 23.4 ± 12.2 $pmol \cdot min^{-1} \cdot 100\ islets^{-1}$ (group 2), 25.6 ± 10.4 $pmol \cdot min^{-1} \cdot 100\ islets^{-1}$ (group 4), and 25.1 ± 11.4 $pmol \cdot min^{-1} \cdot 100\ islets^{-1}$ (control).

FPIR was completely lacking (Figs. 1B and 1D) while TIR was increased in CD-1 groups 2 and 4 ($p < 0.05$) and Balb/c group 2 ($p < 0.05$). Neither basal insulin secretion after glucose stimulation nor arginine induced insulin release in groups 2 and 4 were significantly different from controls.

CD-1 and Balb/c male offspring with a diabetic mother only (group 2) had normal insulitis scores compared to controls.

Diabetic father

Group 3 offspring of both strains and of both sexes had normal non-fasting blood glucose values with no statistical differences compared to controls. AUC_{glu} and K-value were normal in male offspring with a diabetic father only

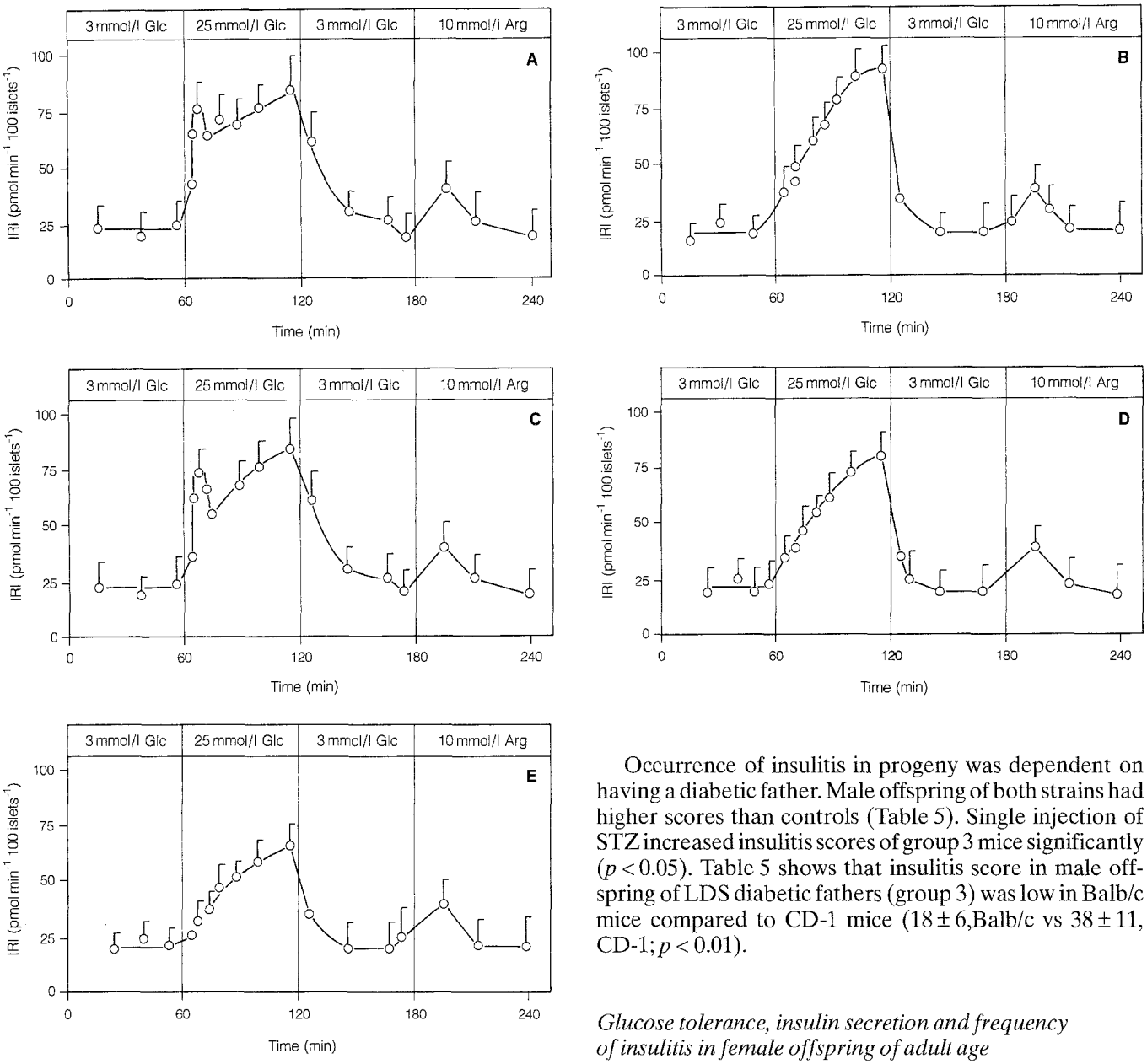

Fig. 2 A–E. Insulin secretory response (IRI) to glucose (Glc) and arginine (Arg) by perifused islets of Langerhans isolated from 12-week-old male offspring (CD-1) of LDS parents. **A–E** correspond to Groups 1–5, respectively. (See Materials and methods section)

(group 3, Table 3) in both strains. Isolated islets showed normal insulin responses (Table 4, Fig. 2 C). However, when a single subdiabetogenic dose of STZ was injected to CD-1 mice (group 5) K-values were markedly reduced ($p < 0.01$) and AUC_{glu} was increased ($p < 0.05$). Islets isolated from group 5 male CD-1 offspring released significantly less insulin in response to a glucose challenge than group 1 (control) islets (Table 4).

Figure 1 E depicts that arginine-stimulated insulin secretion was not decreased. By contrast, glucose tolerance and insulin secretion pattern of islets remained unaltered in group 5 of the Balb/c mice.

Occurrence of insulitis in progeny was dependent on having a diabetic father. Male offspring of both strains had higher scores than controls (Table 5). Single injection of STZ increased insulitis scores of group 3 mice significantly ($p < 0.05$). Table 5 shows that insulitis score in male offspring of LDS diabetic fathers (group 3) was low in Balb/c mice compared to CD-1 mice (18 ± 6,Balb/c vs 38 ± 11, CD-1; $p < 0.01$).

Glucose tolerance, insulin secretion and frequency of insulitis in female offspring of adult age

Diabetic mother

A non-fasting blood glucose level of 8.2 ± 1.7 mmol/l was measured in CD-1 group 2 females (vs control 6.0 ± 1.6 mmol/l, $p < 0.05$, and vs Balb/c group 2 7.33 ± 1.9 mmol/l, $p < 0.05$). Glucose intolerance was found by IVGTT in this group (Table 3) while TIR and FPIR were not impaired (Table 4). Female offspring with diabetic mother and diabetic father (group 4) did not show any abnormalities.

Balb/c female progeny had normal glucose tolerance in all groups. TIR to glucose was slightly, but significantly increased ($p < 0.05$) in Balb/c group 2 and group 4.

Diabetic father

Female offspring of CD-1 and Balb/c LDS diabetic fathers were not glucose intolerant at 12 weeks of age (Table 3). A single subdiabetogenic dose of STZ did not impair glucose tolerance or insulin secretion of islets in group 5.

Insulitis scores in female offspring of diabetic fathers were lower than in male offspring (Table 5). Significant scores were found in CD-1 mice only.

Discussion

Low body weight at young age was associated with glucose intolerance and increased insulin responses in adult offspring of LDS diabetic mothers of the high-incidence strain (CD-1). These abnormalities were predominantly observed in CD-1 males. Balb/c youngsters of diabetic mothers and of both sexes were overweight. At adult age they had lower postprandial blood glucose compared to CD-1 mice of the same age and sex, but nevertheless Balb/c mice had reduced tolerance to an intravenous glucose load, and increased TIR, too.

In male offspring of LDS diabetic fathers we observed spontaneous insulitis and normal glucose tolerance. Although the insulin release profile from progeny of diabetic fathers was indistinguishable from the control group, a single subdiabetogenic dose of STZ induced a general blunting of the secretory response to glucose. Histologically, this phenomenon was accompanied by a dramatically increased incidence of insulitis. Both, insulitis and reduced insulin response to glucose are characteristics of autoimmune prediabetes [8]. Increased Type 1 diabetes susceptibility in children of diabetic fathers has also been reported in man [9]. LDS diabetes in the mother and the father partly protected male CD-1 offspring from insulitis, but not from glucose intolerance. Balb/c offspring failed to develop insulitis and glucose intolerance.

Steele [10] tested the effect of parental exposure to STZ. He described a single spontaneously hyperglycaemic CBA/H mouse among the litters from the cross of LDS male with non-treated female. Spergel et al. [11] reported, that alloxan-induced latent diabetes in the rat could be transmitted to offspring either through the male or female parent with increasing glucose intolerance through the generations. There have been several reports on STZ and other N-nitroso compounds on pancreatic beta cell function after prenatal exposure [3, 12, 13]. Pancreatic insulin stores were decreased and insulin secretion was impaired in response to glucose and arginine in the offspring of STZ treated pregnant rats. In our experimental design, exposure to STZ was not during pregnancy, because mice were mated after the onset of manifest LDS diabetes. We observed reduced insulin response to glucose, but normal insulin secretion to arginine. This implies, that STZ leaves the fetal beta cell non-responsive to different secretagogues, while hyperglycaemia, on one hand, and insulitis, on the other hand, impair insulin secretion selectively to glucose in the progeny. STZ may act on germ cells, both in females and males. No chromosome damage was found in the testes isolated from male mice directly after LDS treatment (unpublished observation). STZ-induced deficits in testosterone production in rats have been reported, but it is not clear whether this is an effect of STZ itself or of hyperglycaemia [14].

During pregnancy of a diabetic mother fetal beta cells are chronically stimulated by hyperglycaemia to produce insulin. As a result, we measured increased concentrations of insulin in the amniotic fluid of pregnant LDS mice. Chronic hyperglycaemia leads to compensatory growth of fetal islet tissue. In our study maternal diabetes during pregnancy resulted in increased probability in the offspring to develop diabetes without significant insulitis. This effect is not immunologically mediated, but is attributable to intrauterine hyperglycaemia as previously described [15, 16]. We observed insulitis predominantly in the progeny of diabetic fathers. Recently, it was reported that prevention of autoimmune diabetes can be achieved by neonatal stimulation of beta cells with glucose [17]. In this study, insulitis in pancreases of offspring of diabetic fathers was partially compensated when both mother and father were diabetic. This supports the hypothesis that perinatal hyperglycaemia sensitizes fetal islets to glucose at an early stage in life and therewith protects them from future immune-mediated impairment of glucose-induced insulin secretion.

In accordance with publications dealing with prenatal STZ exposure we observed defects of insulin release primarily in male litters of LDS diabetic parents. On intraperitoneal challenge with STZ, progeny of group 3 mice responded with insulitis in an enhanced mode. This finding should be considered in the context of the significance of nitrosamine compounds in the pathogenesis of human Type 1 diabetes. Analyses of population-based registries of childhood diabetes have revealed an influence of nutrients, among them nitrosamines [18, 19]. Why do male LDS mice transmit the tendency to develop insulitis to the next generation ? Obviously, this cannot result from hyperglycaemia during fetal development, but must occur genetically. It is known that STZ induces strand breaks in different cell types including beta cells [20] and that it can also activate oncogenes, such as H-ras oncogene [21]. STZ can induce tumour growth in islets, kidney, liver, and mammary gland [22, 23]. Complex effects of STZ including DNA damage on beta cells have been extensively reported. LDS treatment has been shown to result in the expression of retrovirus in beta cells associated with insulitis [24].

Oncogenes or protooncogenes may be activated by STZ directly or by activation of virus oncogenes in susceptible mice. Since males are more susceptible to LDS induced insulitis than females, this could indicate that activation of oncogenes may be modulated by hormones. This mechanism could explain increased susceptibility to STZ in offspring of LDS diabetic fathers [10, 11].

In addition, STZ may also have direct effects on the level of the immune system, because it stimulates T cells and induces the secretion of lymphokines, such as interferon [25]. There is also evidence that subcutaneous STZ injection is followed by lymphoproliferation without diabetes [26]. STZ could use either one or more of these

pathways to transmit diabetes with insulitis in the progeny of LDS diabetic fathers.

In conclusion, this paper represents a systematic study on sex-dependent transmission of diabetes under the conditions of this murine model. Using insulitis as an indicator of immune mechanisms we found that paternal diabetes is associated with increased incidence of insulitis in the offspring as a risk factor for developing insulin-deficient diabetes. Diabetic fathers tend to transmit insulitis to their male progeny while diabetic mothers transmit glucose intolerance, but not insulitis to their male offspring. In maternal diabetes, the influence of uterine environment is more important for the outcome of their progeny than in inflammatory processes which are potentially destructive for the islets of Langerhans.

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Dr. T. Linn
Medical Clinic III and Policlinic
Justus Liebig University
Rodthohl 6
D-35385 Giessen
Germany