Overt Diabetes Induced by Overeating in Neonatally STZ-Treated Impaired Glucose Tolerant Mice: Long-Term Follow Up Study

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Abstract

This study has investigated the effect of a long period of overeating on the glycemic control and pancreatic β -cell function in neonatally streptozocin treated impaired glucose tolerant mice. Neonatally streptozocin (60 mg/kg) treated male ICR mice with 150–200 mg/dl of fed blood glucose levels were divided into two groups at 6 weeks of age. One group was maintained on a cafeteria diet (SZC) and the other on ordinary mouse chow (SZ) until 30 weeks of age. Normal male ICR mice were divided into a cafeteria diet group (CC) and an ordinary chow group (Cont). SZC and CC consumed 134–124% of the caloric intake in SZ and Cont throughout the study. Marked elevation of the fed blood glucose level was observed and the glucose tolerance was progressively impaired in SZC. On pancreas perfusion at 30 weeks of age, insulin secretion to 30 mM glucose in SZC was significantly decreased compared with that in SZ. That in CC was slightly decreased compared with that in Cont. The pancreatic insulin concentration in SZC was significantly less than that in SZ. We conclude that chronic hyperglycemia, induced by the long period of overeating, accelerated the selective loss of β -cell sensitivity to glucose. Even in normal mice that did not have marked hyperglycemia, insulin secretion to glucose was suppressed, probably by chronic stimulation of the β -cell due to the long period of dietary excess.

Hyperglycemia in diabetics could become a secondary pathogenic factor and aggravate

their impaired insulin secretion (Dohan and Lukens, 1947; Gravey *et al.*, 1985; Unger and Grundy, 1985; Kergoat *et al.*, 1987). Recently, Imamura *et al.* (1988) reported that sustained hyperglycemia, which was induced in partially pancreatectomized non-diabetic dogs by the continuous intravenous infusion of glucose, caused an insulin de-

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pendent diabetic state. Whether a similar phenomenon might occur in patients with poorly controlled non-insulin dependent diabetes (NIDDM) is of great interest.

Overeating is one of the risk factors in the development of human NIDDM. We have already reported that overt diabetes is induced by overeating in neonatally streptozocin (STZ) treated impaired glucose tolerant (IGT) mice, using a rather physiological dietary manipulation (Mori *et al.*, 1987). In this study, we evaluated the effect of a longer period of overeating on glycemic control and pancreatic β -cell function, using a pancreas perfusion technique, in these IGT mice.

Materials and Methods

Two-day-old male ICR mice (Charles River Japan, Tokyo, Japan) received 60 mg/kg STZ (Upjohn, Kalamazoo, USA) intraperitoneally, and control mice received the dilutant, citrate buffer, pH 4.5. At 6 weeks of age, STZ treated mice, whose fed blood glucose levels were 150–200 mg/dl, were selected for the study and divided into two groups. One group was maintained on a cafeteria diet (SZC) and the other on ordinary mouse chow (SZ). Control mice were also divided into the cafeteria diet group (CC) and ordinary chow group (Cont). All mice were kept on their diets ad libitum until 30 weeks of age, and had free access to tap water. They were housed in a temperature-controlled room (20–23°C) with a 12-hour light/dark cycle.

Ordinary mouse chow (3.6 kcal/g; Oriental Yeast, Tokyo, Japan) contained 60.6% carbohydrate, 26.7% protein and 12.7% fat by calories. The cafeteria diet was a mixture of chocolate, cookies and ordinary mouse chow (4.0 kcal/g) and contained approximately 57.7% carbohydrate, 19.5% protein and 22.8% fat by calories.

The body weight and fed blood glucose (0900 to 1100 h) were measured every 2 weeks. HbA_{1c}, specific for mouse and rat, was measured at 15, 20 and 30 weeks of age according to the HPLC method (Kondo *et al.*, 1988). The glucose tolerance test was performed at 10, 20 and

30 weeks of age. The mice were fasted overnight and were injected intraperitoneally with a 20% glucose solution in a dose of 2 mg/g·BW. Blood samples were collected at 0, 30, 60 and 120 minutes after the injection. All blood samples were obtained from the orbital vein plexus and glucose was measured by the glucose oxidase method (FUJI DRI-CHEM SYSTEM, Fuji Medical System Co., Tokyo Japan). Urine glucose was examined by the use of TES-TAPE® (ELI LILLY Co., Indianapolis, USA) from fed mice. Measurement of food consumption (expressed as kcal/mouse/day) was done three times each week during the first 4 weeks, and three times every 2 weeks thereafter.

At 30 weeks of age, pancreas perfusion was performed according to the method of Grodsky and Franska (1975) as well as Bonnevie-Nielsen *et al.* (1981). Briefly, the mice were fasted overnight and anesthetized by an intraperitoneal injection of sodium pentobarbital (80 mg/kg·BW). After ligating the arteries around the pancreas (the superior mesenteric artery, the hepatic artery and the splenic artery), the celiac trunk was perfused through a catheter placed in the aorta. The basal perfusate was Krebs-Ringer bicarbonate buffer (pH 7.4) with 1% bovine serum albumin (Miles Laboratories, Elkhart, USA), 3% Dextran T70 (Pharmacia, Uppsala, Sweden) and 5 mM glucose, equilibrated with a continuous supply of 95% O₂/5% CO₂ gas. The pancreas was perfused at 37°C, at a flow rate of 0.6 ml/min. After 20 minutes of equilibration with the basal perfusate, the pancreas was stimulated with 30 mM glucose for 10 minutes. The pancreas was reequilibrated with basal perfusate for 10 minutes, and then stimulated with a combination of 5 mM glucose and 19 mM arginine for 10 minutes. Individual samples, collected from the portal vein at 1- or 2-minute intervals were frozen immediately and stored at –20°C.

After the perfusion, a part of the pancreas tail was fixed in 10% formalin solution for microscopic study. The rest of the pancreas was homogenized in acid-alcohol solution (75% vol/vol of ethanol, 1.5% vol/vol of 11.6 mol/L HCl and distilled water) for the measurement of the insulin concentration. For light microscopy, the pancreas was embedded in paraffin and sequentially sectioned. These sections were stained with hematoxylin and eosin, and by the immunoperoxidase method with primary anti-

bodies to insulin (β -cell) and glucagon (α -cell).

Insulin in the perfusate and the pancreas was assayed by the solid phase radioimmunoassay (insulin RIA kit, Dinabot Radioisotope Lab., Tokyo, Japan). The insulin release from perfused pancreas per minute was expressed as μ U/min, multiplying the insulin concentration of each samples by the flow rate. The total insulin release was calculated by adding the insulin release per minute during the perfusate condition and expressed as μ U/10 min.

The results are shown as the mean $\pm$ SEM. To evaluate the effect of cafeteria diet feeding in STZ treated IGT mice and normal mice, this study allowed the following comparisons: SZC vs SZ and CC vs Cont. Student's *t*-test was used to test for significant differences between these two groups. Comparisons involving four groups were made by analysis of variance (ANOVA).

Results

The mean caloric intake/mouse/day in SZ and Cont was 18.6 ± 0.13 and 18.9 ± 0.19 kcal, respectively, and remained constant throughout the study. SZC and CC

consumed 25.0 ± 0.58 (134% of that in SZ) and 24.9 ± 1.05 kcal (132% of that in Cont), respectively, during the first 12 weeks on the cafeteria diet. Thereafter, they decreased slightly, but maintained 125–128% of the caloric intake in SZ and Cont ($P < 0.001$: SZC vs SZ, $P < 0.01$: CC vs Cont).

The body weight gain in the mice on the cafeteria diet was significantly greater than that in those on ordinary mouse chow ($P < 0.01$: SZC vs SZ, $P < 0.02$: CC vs Cont at 30 weeks of age. Fig. 1).

The fed blood glucose level (Fig. 2) in SZC markedly increased after 4 weeks of cafeteria diet feeding, averaging 270.4 ± 19.5 mg/dl. On the other hand, that in SZ at 10 weeks of age was 205.8 ± 2.7 mg/dl ($P < 0.02$). Thereafter, the fed blood glucose concentration in SZC decreased slightly, but it was maintained at a significantly higher level than that in SZ within the time period studied. Fed blood glucose in CC increased slightly compared with that in Cont after 10 weeks of cafeteria

Fig. 1. Effect of long term cafeteria diet on body weight. Cafeteria diet was fed to SZC and CC from 6 weeks of age. Values represent mean $\pm$ SEM. $\triangle$, Cont (n=5) $\blacktriangle$ —, CC (n=5) $\circ$, SZ (n=6) $\bullet$ —, SZC (n=7) * $P < .05$ ** $P < .01$ vs SZ + $P < .05$ ++ $P < .02$ vs Cont

Fig. 2. Effect of long term cafeteria diet on fed blood glucose and HbA_{1c}. Cafeteria diet was fed to SZC and CC from 6 weeks of age. Values represent mean ± SEM.

△·····, Cont (n=5)

▲——, CC (n=5)

○·····, SZ (n=6)

●——, SZC (n=7)

*P < .02

**P < .01

***P < .001 vs SZ

+P < .05

++P < .02

+++P < .01 vs Cont

diet feeding. Glucosuria was frequently detected only in SZC.

The HbA_{1c} levels (Fig. 2) well reflected the profile of the fed blood glucose concentration in each group. The mice in the cafeteria diet groups had higher HbA_{1c} than in the ordinary chow groups. However, HbA_{1c} did not increase progressively throughout the study.

Glucose tolerance was impaired by cafeteria diet feeding. The longer the period of overeating, the more apparent became the impaired glucose tolerance in the mice on the cafeteria diet (Fig. 3).

On pancreas perfusion at 30 weeks of age (Fig. 4), the insulin secretion to 30 mM glucose was markedly suppressed in the STZ treated groups (SZC and SZ), whereas insulin secretion to 19 mM arginine was relatively well maintained compared to that in controls (ANOVA: not significant). The insulin secretion to 30 mM glucose in SZC was significantly less than that in SZ at all points. Also in CC, the insulin secretion to 30 mM glucose was slightly suppressed compared to that in Cont, and the insulin

secretion to the basal perfusate was greater than that in Cont. Total insulin release to 30 mM glucose in SZC and CC was significantly decreased compared with that in SZ and Cont (Table 1, P < 0.001: SZC vs SZ, P < 0.05: CC vs Cont).

The pancreatic insulin concentration in SZC significantly decreased compared with that in SZ (Table 1, P < 0.01). That in CC slightly decreased compared with that in Cont, although there were no significant differences between the two groups.

In the histological study, the β -cells of SZC and SZ were scattered throughout the islet, whereas the α -cells were usually found at the periphery of the islet as in the normal islets observed in Cont, and there were no differences between SZC and SZ. Also, there were no histological differences between Cont and CC.

Discussion

As shown in the earlier studies on neonatally STZ treated diabetic rats (Weir

Fig. 3. Change in glucose tolerance during cafeteria diet. Cafeteria diet was fed to SZC and CC from 6 weeks of age. Mice, fasted overnight, were administered 2 mg/g·BW glucose intraperitoneally at 0 time. Values represent mean $\pm$ SEM.

$\triangle$ —, Cont (n=5)
 $\blacktriangle$ —, CC (n=5)
 $\circ$ —, SZ (n=6)
 $\bullet$ —, SZC (n=7)
 * $P < .05$
 ** $P < .02$
 *** $P < .01$
 **** $P < .001$ vs SZ
 + $P < .05$
 ++ $P < .02$
 +++ $P < .01$
 ++++ $P < .001$ vs Cont

Table 1. Insulin release from perfused pancreas, and pancreatic insulin concentration.

Group (N)	Total insulin release (μ U/10 min)		Insulin concentration (U/g. pancreas)
	30 mM Glucose	19 mM Arginine#	
SZ (6)	157.0 $\pm$ 11.1	292.2 $\pm$ 20.1	0.43 $\pm$ 0.01
SZC (7)	98.4 $\pm$ 5.9**	279.2 $\pm$ 13.0	0.35 $\pm$ 0.01*
Cont (5)	432.2 $\pm$ 32.3	319.4 $\pm$ 36.2	0.92 $\pm$ 0.05
CC (5)	332.2 $\pm$ 17.0 ⁺	308.9 $\pm$ 52.0	0.79 $\pm$ 0.07

Values are shown as mean $\pm$ SEM.

* $P < .01$, ** $P < .001$ vs SZ

⁺ $P < .05$ vs Cont

ANOVA: not significant ($P > .5$)

Fig. 4. Effect of long term cafeteria diet on insulin response to 30 mM glucose and 19 mM arginine during pancreas perfusion. The pancreas perfusion was performed at 30 weeks of age. Values represent mean $\pm$ SEM. The insulin secretion to 19 mM arginine was not significantly different between the groups at all points (ANOVA, $P > .4$).

$\triangle$ —, Cont (n=5)

$\blacktriangle$ —, CC (n=5)

$\circ$ —, SZ (n=6)

$\bullet$ —, SZC (n=7)

* $P < .02$

** $P < .01$

*** $P < .001$ vs SZ

+ $P < .05$

++ $P < .02$ vs Cont

et al., 1981; Giroix *et al.*, 1983), adult mice treated with streptozocin at birth exhibited a diabetic state similar to that observed in NIDDM with specific failure of insulin release to glucose, as characterized in the perfused pancreas study (Kano *et al.*, 1985).

In this study, overeating caused by the cafeteria diet induced overt diabetes in neonatally STZ treated IGT mice. This finding is consistent with our previous report (Mori *et al.*, 1987). Overt diabetes might be provoked because the reduced β -cell mass in STZ treated IGT mice could not compensate for the increased insulin demand due to dietary excess. The fed blood glucose concentration remained high and glucose tolerance deteriorated in SZC due to the long period of overeating. These

results may be induced by increased insulin resistance due to body weight gain and decreased insulin secretion to glucose. However, the fed blood glucose and HbA_{1C} did not progressively increase, because these mice could secrete insulin to nonglucose stimuli (amino acid), but not to glucose as the following shows. Therefore, body weight loss, one of the symptomatic markers of severe diabetes, was not observed in the overt diabetic mice.

Insulin secretion to glucose from the perfused pancreas at 30 weeks of age was significantly suppressed in these overt diabetic mice, whereas insulin secretion to arginine was well preserved compared to that in the control mice. In our previous report (Mori *et al.*, 1987), overt diabetic mice induced by a 4-week cafeteria diet

demonstrated less insulin secretion to glucose than that in the IGT mice fed ordinary mouse chow, but there were no significant differences between the two groups. Decreased glucose stimulated insulin secretion during chronic glucose exposure has been reported both *in vitro* (Lacy *et al.*, 1976; Bolaffi *et al.*, 1986; Curry, 1986; Hoening *et al.*, 1986) and *in vivo* (Leahy *et al.*, 1986). Therefore, diabetic hyperglycemia, once established, may itself become a secondary pathogenic factor and aggravate β -cell dysfunction. The present study indicated that the chronic hyperglycemia, produced by the long period of overeating in neonatally STZ treated IGT mice, could accelerate the selective loss of β -cell sensitivity to glucose.

It is well recognized that in insulin-dependent diabetes (IDDM) there is virtually no glucose stimulated insulin secretion and there is greatly decreased or nonexistent insulin response to nonglucose stimuli. Also in NOD mouse, the murine equivalent of IDDM, Kano *et al.* (1986) showed that insulin secretion to arginine from the perfused pancreas decreased with the development of insulinitis and a reduction in the number of pancreatic islets. The pattern of insulin response in the present overt diabetic mice was essentially different from that shown in NOD mouse at the onset of diabetes. In the histological study, the loss of pancreatic β -cells in the present overt diabetic mice was not different from that in IGT mice.

Recently, Imamura *et al.* (1988) demonstrated that severe diabetes was induced by means of intravenous glucose infusion in subtotally depancreatized but non-diabetic dogs as a result of rapid β -cell depletion. They exhibited similar ketonuria and severe body weight loss to that observed in human IDDM. A similar mechanism might operate as a consequence of chronic hyperglycemia of poorly controlled human NIDDM. It is well recognized that glucose is the most

potent stimulator of insulin release. Therefore, whether chronic hyperglycemia, found in poorly controlled diabetic patients due to diet failure, has the same effect on β -cells as that induced by enormous glucose overloading intravenously remains questionable. In the present study, we have focused our attention on overeating as an important factor in the development of NIDDM, and succeeded in creating a poorly controlled diabetic state in neonatally STZ treated IGT mice by physiological dietary manipulation. Sustained hyperglycemia induced by overeating in these mice could not produce β -cell depletion. However, it remains possible that a longer period of overeating may cause β -cell depletion at a rather slow rate.

The present study indicated that the long period of overeating induced loss of insulin secretion to glucose on pancreas perfusion even in the normal mice. Leahy *et al.* (1986) have reported that glucose stimulated insulin secretion is partially lost in normal rats made markedly hyperglycemic for 48-hr with continuous glucose infusion. The present results suggest that, without marked hyperglycemia, chronic stimulation of β -cells due to increased insulin demand following dietary excess or body weight gain might induce selective insensitivity to glucose. Normal mice kept on the cafeteria diet had a slightly higher blood glucose level than the mice on ordinary mouse chow (CC: 150 ± 6.9 , Cont: 124 ± 3.9 mg/dl at 30 weeks of age. $P < 0.02$). Therefore, the range of blood glucose levels in which β -cell function is normal might be very narrow (Kergoat *et al.*, 1987).

The pancreatic insulin concentration in overt diabetic mice was significantly reduced compared with that in IGT mice. Regulation of the pancreatic insulin concentration remains poorly understood. In the overt diabetic mice, the rate of insulin secretion might exceed that of insulin biosynthesis due to overeating. Hyperglycemia has been

felt to have the long-term effect of suppressing insulin synthesis (Orland *et al.*, 1985). The pancreatic insulin concentrations in the overt diabetic mice and the control mice differed considerably even though insulin secretion to arginine was not very dissimilar. Thus it is suspected that β -cell secretory capacity may not be determined by the pancreatic insulin concentration.

In conclusion, overt diabetes was induced by overeating in neonatally STZ treated IGT mice. A longer period of overeating caused sustained hyperglycemia and deterioration of glucose tolerance. Furthermore, it accelerated the selective loss of β -cell sensitivity to glucose, but did not result in β -cell depletion. Even in the normal mice, the insulin secretion in response to glucose was suppressed probably by chronic stimulation of the β -cells due to the long period of overeating. By varying the duration of overeating, this NIDDM model mouse, induced by overeating, would provide a useful way to study the abnormal β -cell function found in human NIDDM.

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