Influence of Streptozotocin-Induced Diabetes in Rats on the Lithium Content of Tissue and the Effect of Dietary Lithium Supplements on This Diabetic Condition

Min Hu, Yangsong Wu, and Hanwen Wu

To study the effects of lithium supplementation on the diabetic condition, we measured the lithium concentration in the liver, kidney, and muscle from streptozotocin (STZ)-induced diabetic male Sprague-Dawley (SD) rats that were either treated or untreated with peroral lithium carbonate (0.3 mg/mL). The data showed that the lithium content of the liver and muscle was significantly lower in STZ rats than in normal control rats (0.22 ± 0.05 *v* 1.30 ± 0.15, $P < .01$, and 0.79 ± 0.30 *v* 2.48 ± 2.00 μg/g, respectively). After 4 weeks of lithium carbonate supplementation, we found that (1) the lithium content of the liver and muscle returned to the normal range, (2) the extent of STZ-mediated destruction of β cells in the pancreas decreased, (3) fasting blood glucose (FBG) and 2-hour postprandial blood glucose (PBG) decreased ($P < .05$), (4) among the indicators of oxidative stress and antioxidant defenses, blood lipid peroxidate (LPO) decreased and erythrocyte superoxide dismutase (RBC-SOD) and glutathione (GSH) returned to normal, and (5) hepatic LPO decreased and glutathione peroxidase (GSH-Px) increased. These results suggest that the restoration of lithium to control levels in the liver and muscle of diabetic animals is associated not only with decreased blood glucose but also with reduced oxidative stress, and consequently with the protection of insulin-secreting pancreatic islet cells.

STUDIES HAVE DEMONSTRATED that the diabetic condition can cause a disturbance in the metabolism of certain trace elements such as chromium, zinc, copper, magnesium, etc.[1-10] We previously observed lithium deficiency in some insulin-sensitive tissues of the Chinese hamster with hereditary spontaneous diabetes,[11] and found that lithium supplements eliminate this deficiency and normalize blood glucose and glycosylated serum protein levels. Because lithium appears to exert some insulin-like effects, lithium metabolism and its effect on glucose metabolism have drawn considerable interest.[12-21]

In the present study, we investigate the effect of restoring lithium levels in streptozotocin (STZ)-induced diabetic Sprague-Dawley (SD) male rats. We extend our previous findings[11] on the effect of lithium carbonate treatment and include assessments of the level of oxidative stress and the status of β cells in the pancreas, to further understand the relationship between lithium and diabetes.

MATERIALS AND METHODS

Animals

Twenty-eight male SD rats weighing 200 to 300 g were randomly paired and separated into four groups: controls (C), controls treated with lithium (CT), diabetics (D), and diabetics treated with lithium (DT). Diabetes was induced by intraperitoneal injection of STZ (Sigma, St Louis, MO) in 0.1 mmol/L citrate buffer, pH 4.5, 60 mg/kg body weight. Seventy-two hours after injection, when fasting blood glucose (FBG) levels were twice higher than 13.00 mmol/L, the rats were included in the experiment. Groups C and CT received intraperitoneal injections of the same volume of citrate buffer.

From the Institute of Metabolism and Endocrinology, The Second Affiliated Hospital, Hunan Medical University, Changsha, Hunan, China.

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Address reprint requests to Min Hu, MD, Institute of Metabolism and Endocrinology, The Second Affiliated Hospital, Hunan Medical University, Rd Renmin 156, Changsha, 410011 Hunan, China.

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The Animal Care and Use Committee of Hunan Medical University approved the protocols used for this study.

Handling

The rats were housed in metabolic cages and received 0.1 g regular chow/1 g body weight for 4 weeks. The room alternated between light (60 lux) and dark every 12 hours. The ambient room temperature was maintained at 24°C and the relative humidity at 62%. All animals were allowed to drink water ad libitum, and lithium carbonate (Li_2CO_3 0.3 mg/mL) was added to the drinking water of groups DT and CT.

Blood Samples

Blood samples were taken from a tail cut for measurement of FBG and postprandial blood glucose (PBG) levels at the beginning and end of the experiment. FBG was measured after a 12-hour fast, and PBG was measured 2 hours after feeding. For each sample, the whole-blood glucose level was determined immediately after collection by a glucose oxidase assay. Four weeks after the experiment, the rats were killed and the blood was collected in anticoagulant. Within 1 hour of collection, the blood was separated into plasma and cells for assessment of insulin, lipid peroxide (LPO), erythrocyte superoxide dismutase (RBC-SOD), glutathione peroxidase (GSH-Px) and glutathione (GSH).

Tissue Samples

To measure tissue LPO, SOD, GSH-Px, and GSH levels, 1 g liver was taken from each animal and homogenized in a 10-mL glass tissue grinder. When needed, the volume of homogenates was adjusted with normal saline. Samples (0.5 to 0.6 g) of hepatic, renal, or skeletal muscle tissue were dissected from each animal and dried at 50°C for about 24 hours until a constant weight was achieved. Each sample was placed in a Kjeldahl flask and boiled in 1.5 mL 75% nitric acid (analytical grade). After the sample was completely dissolved, hot 30% hydrogen peroxide solution (analytical grade) in deionized distilled water was added to the sample until the liquid became colorless and clear. The volume was boiled down to 8 mL, and the samples were stored at 4°C until needed for analysis.

Biochemical Assays

Whole-blood glucose concentrations were determined by the glucose oxidase assay method. Insulin concentrations were determined by radioimmunoassay with a reagent kit from the Scientific & Technologic Institute of Diabetes of West China Medical University (Chengdu, China). LPO levels were measured by z-thiobarbituric acid colorimetry, SOD activity was assayed by auto-oxidation of pyrogallol, and the GSH

Table 1. Alterations of Body Weight and Water Consumption Before and After Lithium Treatment (mean ± SEM)

Group	No.	Body Weight (g)		Water Consumption (mL/d)	
		Before	After	Before	After
C	7	233.6 ± 5.0	241.7 ± 5.0	27.4 ± 1.4	27.4 ± 1.3
CT	7	222.9 ± 6.2	242.6 ± 5.2	27.4 ± 1.8	27.4 ± 1.8
D	7	228.7 ± 5.3	157.0 ± 3.1*[c]	92.1 ± 5.5†[c]	87.1 ± 4.8
DT	7	234.4 ± 5.5	169.9 ± 4.5*[b]	91.6 ± 5.7†[d]	67.4 ± 3.7*[b]

*$P < .05$.
†$P < .01$.
[b]DT *v* D.
[c]D *v* C.
[d]DT *v* CT.

level and GSH-Px activity were measured by spectrocolorimetry (Auto-Biochemical Analyzer; Beckman Instruments, Fullerton, CA).[22,23] The lithium concentration was determined by atomic absorption spectrophotometry (AA-680 Spectrophotometer; Shimadzu, Tokyo, Japan).

Histopathology

Part of a pancreas freshly isolated from each animal was fixed in Bouin's solution and then processed by routine histological methods. Sections were stained with hematoxylin and eosin and examined by light microscopy. Pathologist D.Q. Li (Department of Pathology) evaluated the pathologic changes by counting the mean number of islets per field per pancreas, and calculating the mean number of β cells per islet in 10 fields (100× magnification) per section. The β cell was identified by immunohistochemical staining of streptavidin peroxidase–conjugated mouse monoclonal anti-insulin antibody (Zymed Catalog No. CH 18-0066; Zymed, San Francisco, CA).[24] To stain, this antibody was diluted 1:50 in antibody diluent (Zymed No. 00-3118) and incubated 60 minutes at room temperature. Insulin-containing cells were evaluated by light microscopy following incubation with strepavidin peroxidase and staining with a color-forming peroxidase substrate.

For transmission electron microscopy, the other part of each pancreas was fixed in 2.5% glutaraldehyde buffer and routinely postfixed in 1% OsO_4, rinsed in phosphate buffer, dehydrated in acetone, and embedded, sectioned, and stained. Professor L.X. Peng (Department of Electron Microscopy) assessed the structure of pancreatic islets with a Hitachi H-600 high-voltage transmission electron microscope (Tokyo, Japan).

The examiners did not know from which group of rats the islets in each electron micrograph originated.

Statistical Analyses

Data are presented as the mean ± SE and were analyzed with the SPSS (Chicago, IL) PC 5.0 statistical package by ANOVA for comparison among groups, followed by the Newman-Keuls (Q-test) procedure for comparison of two different groups. For comparison of data before and after the experiment within the same group, Student's *t* test was used. A *P* value less than .05 was considered significant.

RESULTS

General Conditions

Water consumption was much higher in diabetic rats (groups D and DT) than in nondiabetic rats (groups C and CT). However, the amount of water intake was significantly less in diabetic rats with lithium supplementation (group DT) versus diabetic rats without lithium supplementation (group D, $P < .05$). Water consumption and body weight were essentially the same in the normal controls (groups C and CT). Table 1 shows that animals in group DT lost weight during the experiment, but they lost less weight than animals in group D.

Changes of Blood Glucose and Insulin Levels

FBG and PBG in group D remained very high throughout the experiment. Both FBG and PBG in group DT were as high as the levels in group D at the beginning, but decreased significantly ($P < .01$ to .05) by the end of the experiment. FBG and PBG in groups CT and C did not change significantly. Insulin levels were not significantly different in groups C and CT. Insulin concentrations in groups D and DT were considerably diminished, but were a little higher in group DT with lithium treatment (Table 2).

Lithium Content of Tissue

In every tissue tested, lithium content was higher in animals treated with lithium. The livers of diabetic rats (groups D and DT) contained considerably less lithium than the livers of their respective controls (groups C and CT). The lithium content of muscle from diabetic rats was also lower, but the difference was not statistically significant. However, the lithium content of the kidney was significantly higher in group DT versus group CT or D. The kidney lithium content increased considerably as a result of lithium supplementation, especially in group DT. The extent of enhancement in the kidney surpassed that in the liver and muscle (Table 3).

Histopathological Examination of Pancreatic Islets

Under light microscopy (100× magnification), pancreatic tissue sections from groups C and CT showed normal structure, size, and distribution of both islets and islet cells. In group D, focal lymphocyte infiltration and hydropic cell degeneration

Table 2. FBG, PBG, and Insulin Before and After Treatment With Lithium (mean ± SEM)

Group	No.	FBG (mmol/L)		PBG (mmol/L)		Insulin (mU/L)	
		Before	After	Before	After	Before	After
C	7	6.3 ± 0.30	6.2 ± 0.30	7.5 ± 0.30	7.5 ± 0.26	16.57 ± 2.49	15.19 ± 1.74
CT	7	6.0 ± 0.53	4.8 ± 0.53	8.3 ± 0.41	8.4 ± 0.41	17.18 ± 2.27	17.08 ± 2.31
D	7	18.3 ± 1.20	18.3 ± 1.06	27.8 ± 0.49	27.4 ± 2.61	5.24 ± 1.40†[c]	5.56 ± 0.95
DT	7	18.2 ± 0.79	7.5 ± 1.85†[a]	28.1 ± 0.91	16.9 ± 1.79*[a]	5.97 ± 1.06†[d]	7.14 ± 0.75

*$P < .05$.
†$P < .01$.
[a]After *v* before.
[c]D *v* C.
[d]DT *v* CT.

Table 3. Lithium Content in Tissues (μg/g dry tissue, mean ± SEM)

Group	No.	Liver	Kidney	Muscle
C	5	1.30 ± 0.15	0.20 ± 0.09	2.48 ± 2.00
CT	5	3.17 ± 1.48	7.41 ± 2.23	4.53 ± 1.08
D	7	0.22 ± 0.05*[c]	0.91 ± 0.42	0.79 ± 0.30
DT	6	2.18 ± 0.61†[b]	9.12 ± 2.50†[b]	2.97 ± 0.61†[b]

*$P < .05$.
†$P < .01$.
[b]DT *v* D.
[c]D *v* C.

resulted in indistinct islets and decreased islet size. Cell numbers decreased significantly. However, in group DT, the extent of degeneration and lymphocyte infiltration was much less severe. The mean number of islets per field in group C, CT, DT, and D tissue sections was 6.10 ± 0.48, 5.20 ± 0.32, 4.70 ± 0.25, and 2.90 ± 0.23, respectively. The mean number of β cells per islet in group C, CT, DT, and D tissue sections was 71 ± 4.6, 52 ± 2.7, 45 ± 2.1, and 24 ± 2.2. The pancreas from diabetic rats had the least number of islets and β cells ($P < .01$), while the pancreas from rats treated with lithium had many more islets and β cells than in untreated diabetic rats ($P < .01$).

Under the electron microscope, α and β cells in the pancreas of group C and CT rats were normal: nuclei were centrally located, nucleoli and chromatin were distributed normally, and large numbers of secretory granules were in the cytoplasm. Secretory granules in α cells were highly electron-dense, surrounded by a gray halo and a limiting membrane. β-Cell secretory granules were of markedly varied electron density. Around the granular core, the haloes appeared bright and transparent and were enclosed by a limiting membrane. Figure 1 shows a β cell from a group C rat pancreas. β Cells from group CT rat pancreas had a similar appearance. The group D β cell in Fig 2 shows degranulation, degeneration of cytoplasmic vacuoles, focal lysis, and indistinct mitochondrial structure. The β cell in Fig 3 is from a diabetic rat that received lithium carbonate treatment (DT). This cell shows more granules and fewer pathological changes.

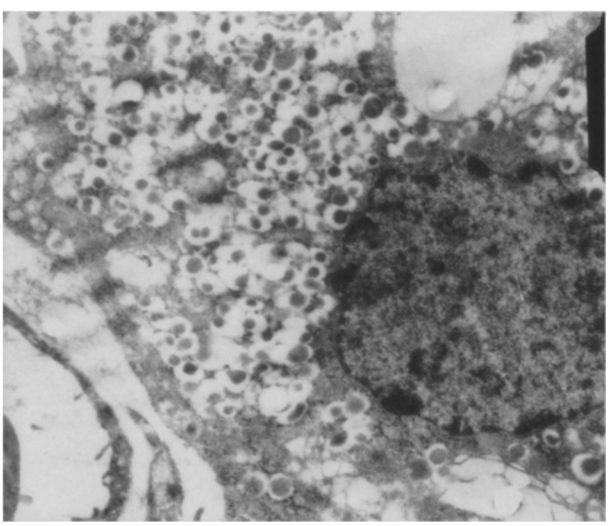

Fig 1. β Cell from pancreas of a normal rat (group C) showing a nucleus and secretory granules (original magnification ×10,000).

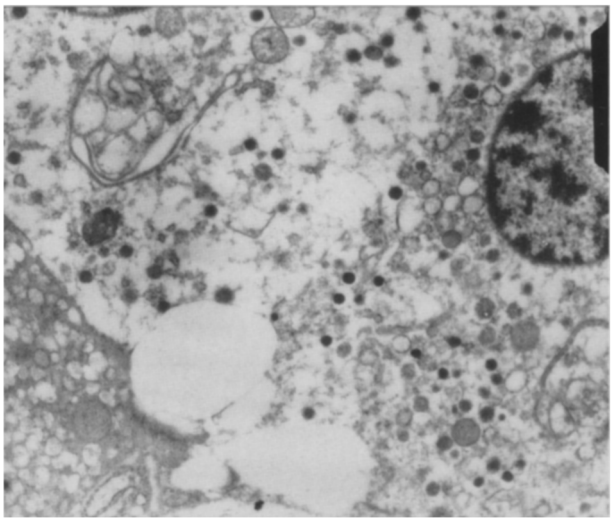

Fig 2. β Cell from pancreas of a diabetic rat (group D) showing the pronounced degranulation and degeneration of cytoplasm, focal lysis, indistinct structure of a mitochondrion, and some dilated rough endoplasmic reticulum (original magnification ×10,000).

Alterations of the State of Oxidative Stress

Table 4 shows the normalizing effect of lithium treatment on whole-blood levels of LPO and RBC-SOD. Diabetic rats had more LPO and less GSH than normal control rats, but GSH-Px levels were the same in both groups. After lithium carbonate treatment, the level of LPO decreased and RBC-SOD returned to near normal. GSH-Px was the same in all groups. Table 5 shows that the normalizing effect also occurred in the liver. LPO levels decreased while SOD and GSH-Px activity increased significantly (group DT *v* D).

DISCUSSION

In an earlier study,[11] we reported lithium deficiency in the liver, kidney, and muscle of Chinese hamsters with hereditary

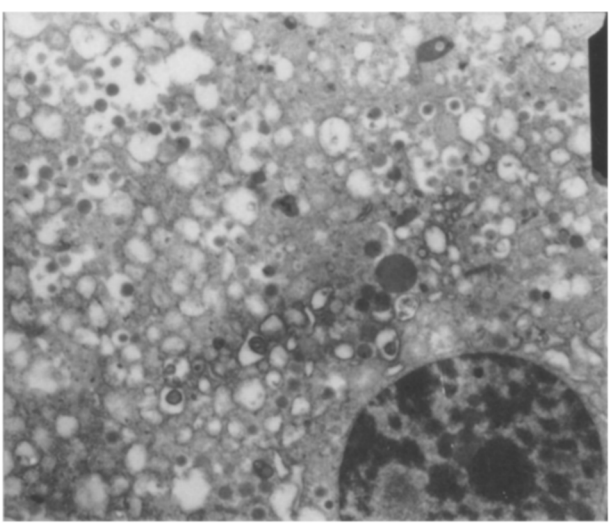

Fig 3. β Cell from pancreas of diabetic rat treated with lithium (group DT) showing more granules and fewer pathologic changes than Fig 2 (original magnification ×10,000).

Table 4. Changes of LPO, RBC-SOD, GSH-Px, and GSH in Blood (mean ± SEM)

Group	No	LPO (μmol/L)	RBC-SOD (U/g Hb)	GSH-Px (U/L)	GSH (μmol/L)
DT	6	7.35 ± 0.49†[b]	1,600 ± 82*[b]	176.9 ± 3.1	19.96 ± 3.74†[b]
D	6	12.60 ± 1.08*[c]	1,257 ± 88	175.7 ± 1.7	9.96 ± 0.75*[c]
CT	5	9.22 ± 0.80	1,651 ± 130	178.4 ± 1.5	18.31 ± 2.40
C	5	7.36 ± 1.19		174.7 ± 3.6	18.04 ± 2.88

*$P < .05$.
†$P < .01$.
[b]DT *v* D.
[c]D *v* C.
NOTE. The activity of RBC-SOD in group C was not determined because of an accident.

spontaneous diabetes. We now report lithium deficiency in the liver and muscle of SD rats with diabetes. However, SD rats and Chinese hamsters are different in that the normal lithium content of rat kidneys is very small and increases in response to lithium administration. Lithium levels in hamster kidneys are already high and increase very little in response to lithium intake. This difference in the lithium content of the kidneys may be explained by a difference in consumption of water. SD rats consume much more water and thus would take in much more lithium than Chinese hamsters. As a result, the kidney of SD rats must excrete much more lithium than the kidney of Chinese hamsters. Another possible explanation for the increase in kidney lithium content in STZ-diabetic rats is the decrease in lithium content in the liver and muscle. The liver and muscle retain less lithium, which results in a great lithium load on the kidney.

It has been reported that the diabetic condition can cause disturbances in the level of certain metal and trace elements.[1-10] We have noted lithium deficiency in tissues of two different types of diabetic animals. Raz and Havivi[7] have studied the effect of diabetes on the status of zinc, copper, and chromium in the liver, kidney, muscle, bone, and serum from sand rats (*Psammomys obesus*). They found that a chronic hyperinsulinemic-hyperglycemic state is associated with a significant reduction of the zinc concentration in the liver, kidney, and muscle but an elevation of the zinc content in bone and chromium in the liver. They considered that in hyperinsulinemic-hyperglycemic sand rats, zinc tends to be depleted in several tissues. This depletion is not solely the result of a hyperinsulinemic state, but may be related to the accompanying hyperglycemia.[9] They also found a zinc deficiency in the plasma and femur of diabetic mice.[2] However, in SD rats with STZ diabetes[4-7] and BB Wistar rats,[8] both characterized by insulin deficiency, it has been shown that despite increased urinary loss of zinc and copper, the content of these metals is increased in various tissues. It is thought that endocrine imbalance, mainly hypoinsulinemia, causes an elevation of metallothionein, which contributes to the alterations of trace metal metabolism and concentrations in STZ-induced or BB-diabetic rats.[8]

Low intracellular magnesium is also found in diabetes, and is thought to result from both increased urinary loss and insulin resistance. A reduced intracellular magnesium content might contribute to the impaired insulin response and action that occurs in type 2 (non–insulin-dependent) diabetes mellitus. Long-term magnesium supplementation can contribute to an improvement in both the islet β-cell response and insulin action in non–insulin-dependent diabetic subjects.[10]

Zhou and Wang[25] reported that serum lithium levels in diabetic patients are decreased. Rossetti et al[13,15] demonstrated that oral administration of a combination of lithium and vanadate to diabetic (90% partial pancreatectomy) rats normalizes insulin sensitivity and muscle glycogen synthesis and the addition of zinc and magnesium further improves glucose metabolism, primarily by stimulating glycolysis. But they did not determine whether changes of lithium metabolism occur in the diabetic state.[13,15]

One mechanism by which STZ induces diabetes is by causing an oxidative stress condition, which destroys β cells.[26] In line with this known mechanism, we observed that in group D the number of pancreatic β cells decreased significantly. And in the residual β cells, degranulation and degeneration of cytoplasmic vacuoles occurred, and the structure of mitochondria was obliterated. The levels of LPO in both the blood and liver increased, which indicates that the ability to combat oxidative stress declined remarkably. This may not be a direct effect of STZ, as it was injected 4 weeks prior to measurement. These changes must be due to the diabetic condition, because hyperglycemia itself can result in oxidative stress.[27] Tatsuki et al[28] recently reported that in the SD rat pancreas, LPO activity was reduced and catalase activity increased at 2 weeks, while these activities were unchanged by 7 weeks.

Lithium is an effective antioxidant.[18] We have shown that dietary supplementation of lithium can diminish pathological changes in pancreatic β cells, decrease LPO levels in both the blood and liver of diabetic rats (group DT), and enhance the antioxidant status of diabetic rats. The reduction of oxidative stress by lithium, especially in the liver, can help to decrease blood glucose levels. As blood glucose levels decrease, the oxidative stress state in diabetes can be further ameliorated.

Although the plasma insulin levels in diabetic rats treated

Table 5. Changes of LPO, SOD, GSH-Px, and GSH in Liver (mean ± SEM)

Group	No.	LPO (μmol/L)	SOD (U/mg protein)	GSH-Px (U/L)	GSH (μmol/L)
DT	6	18.51 ± 0.98†[b]	204.2 ± 45.2	143.0 ± 6.8†[b]	39.48 ± 3.72
D	6	28.91 ± 1.32†[c]	139 9 ± 17.9†[c]	27.9 ± 6.7†[c]	36.69 ± 3.83*[c]
CT	5	14.94 ± 0.85	167.7 ± 23.3	129.7 ± 15.42	78.80 ± 5.48
C	5	15.41 ± 1.02	194.4 ± 19.7	106.7 ± 14.9	66.39 ± 6.70

*$P < .05$.
†$P < .01$.
[b]DT *v* D.
[c]D *v* C.

with lithium (group DT) increased slightly, this increase was not statistically significant. Because hepatic glucose production is extremely sensitive to small increments in the plasma insulin concentration, the slight increase of plasma insulin can contribute to suppress hepatic glucose production, resulting in a modest decrease of elevated FBG.[29] But the lithium supplements greatly decreased both PBG and FBG in diabetic rats, indicating that lithium must have other effects that can decrease blood glucose. Lithium has some insulin-like actions such as inhibition of hepatic gluconeogenesis and phosphoenolpyruvate carboxykinase gene expression, which can reduce blood glucose.[12-21,30] Moreover, the antioxidative stress effect of lithium can also enhance insulin action in target cells. Therefore, the demand for insulin is reduced. A reduction in insulin biosynthesis and secretion can render β cells less susceptible to cytotoxic events.[31,32] Conversely, high glucose potentiated the diabetogenic effects of STZ in vitro[33] and in vivo,[34] and hyperglycemia itself can impair β-cell function and glucose metabolism.[35,36] However, the insulin-like effect of lithium is not strong enough to reduce blood glucose to normal and restore body weight to normal.

In addition to its antioxidant effect, lithium increases the DNA replication, polyamine content, and insulin secretion in rat pancreatic β cells. Lithium mediates these effects through the pertussis toxin–sensitive GPT-binding proteins, stimulating rat pancreatic β-cell replication.[37,38] This effect of lithium may contribute to some of the recovery from STZ toxicity that we observe in β cells. Cam et al[39] demonstrated that partial preservation of pancreatic β cells and apparently minor changes in the islet insulin store in a model of reduced β-cell mass can have profound consequences for glucose homeostasis, and may have important implications for interventions that have "limited" effects on β cells.

When at least 90% of the pancreas is removed, both humans[40] and rats[41] develop overt diabetes, and only when the reduction in islet function is greater than 90% does nonfasting hyperglycemia occur in STZ-treated mice.[42] The pancreatic insulin content in STZ-diabetic mice correlates highly with and is quantitatively equivalent to β-cell function and mass.[43] It is considered that in the unique case of STZ diabetes wherein residual β cells are severely degranulated, improvement in the diabetic state can be much better correlated with the residual insulin store than with β-cell mass.[39,44,45]

In conclusion, our research suggests a lithium deficiency in the hepatic and muscular tissue of SD rats with STZ-induced diabetes, and treatment with lithium can reduce the oxidative stress in tissues, decrease blood glucose levels, and lessen the destructive effect of STZ on pancreatic β cells.

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