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Relationship between the Severity of Experimental Diabetes and Altered Lung Phospholipid Metabolism (41869)

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Abstract. Glucose intolerance was induced in rats by iv infusion of streptozotocin (STZ) in doses of 30, 40, 50, and 100 mg/kg. Serum glucose concentrations were elevated versus controls and weight gains were reduced in a dose-dependent fashion up to 50 mg/kg. Urine outputs and blood urea nitrogen (BUN) values were higher than control values in the animals treated with 40 and 50 mg/kg and serum albumin concentrations were decreased after infusion with 50 mg STZ/kg. Lung phosphatidylcholine (PC) concentrations and dry-to-wet weight ratios were unchanged by STZ treatment, while lung protein and disaturated phosphatidylcholine (DSPC) concentrations were depressed in the 50-mg/kg group. Animals surviving treatment with 100 mg/kg demonstrated increased fasting blood glucose levels, BUN values, and 48-hr urine outputs, and decreased lung protein levels. However, these alterations were less than those found in the 50-mg/kg animals. Pulmonary concentrations of PC, DSPC, and lung dry-to-wet weight ratios were unchanged. It was found advantageous to express the results relative to fasting blood glucose levels. This demonstrated that urine output and BUN values increased and weight gain decreased with rising glucose concentrations, but serum albumin decreased only in moderate and severe hyperglycemia. Fasting glucose concentrations greater than 400 mg/dl were associated with reduced lung DSPC and protein levels, while pulmonary PC and dry-to-wet weight ratios demonstrated no change with increasing hyperglycemia.

In order to study the metabolic effects of diabetes mellitus, several methods have been developed to produce glucose intolerance, but streptozotocin (STZ) is now generally used as the preferred agent (1–4). STZ causes β-cell destruction and disruption of islet architecture (1, 5, 6), leading to insulin insufficiency (7–9), increased urine output (7–9), glycosuria (1, 7–9), and abnormal kidney and liver function (10, 11). Chemically induced diabetic animals also demonstrate impaired pulmonary carbohydrate metabolism (12), altered lung ultrastructure, as evidenced by changes in Type II pneumocyte and Clara cell morphology (13, 14), and disturbed pulmonary lipid and surfactant synthesis (11, 15–17). Thus, STZ-induced diabetes is a useful model for studying the effects of carbohydrate intolerance in a variety of organ systems. The studies performed to date, however, have employed a wide range of drug dosages; also, the duration of the experimental diabetes has varied greatly. These and other differences in methodology make it difficult to compare results between studies. Experimental protocols also differ with respect to species, age, and sex of animals analyzed. Furthermore, there have been very few reports which attempt to relate the effects of STZ to specific blood glucose levels. These experiments were therefore undertaken in order to define a dose–response relationship between STZ and the severity of the resultant diabetes, as judged by its effects on several organ systems in the rat. These studies were also designed to correlate these effects with blood glucose levels in fasting animals. The responses of lung metabolism to these varying levels of chemically induced diabetes were then examined to further investigate the effects of diabetes on pulmonary carbohydrate and phospholipid metabolism.

Materials and Methods. Glucose intolerance was induced in 150- to 200-g male Sprague–Dawley rats (Harlan Sprague–Dawley, Inc., Indianapolis, Ind.) after an overnight fast by the intravenous infusion of STZ via the tail vein in doses of 30, 40, 50, or 100 mg/kg. Streptozotocin was a gift of Dr. W. A. Dulin, Upjohn Company, Kalamazoo, Michigan. The volume of the STZ infusions was kept constant at 0.5 ml. On the same day that STZ infusions were performed, control rats

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received an equal volume of diluent (0.4 *M* citrated saline, pH 4.5). A second control group consisted of rats that received no infusion. Because the effects of STZ are generally manifest by 48 hr, the urine output of the experimental animals was measured on Days 2 and 4 following STZ treatment. The urine volumes were used as a crude estimate of the effectiveness of the infused agent and a monitor of the severity of the diabetes-like state. Urine volumes were used because they were easily obtained, readily measured, and noninvasive. Urine outputs were obtained by placing the animals in individual metabolic cages for overnight urine collection. Urine volume was subsequently measured and the presence of glucose and ketones in urine was determined semiquantitatively with Labstix Reagent Strips (Ames Co., Elkhart, Ind.).

One week after infusion, overnight fasted rats were anesthetized with sodium pentobarbital (50 mg/kg) and weighed. Blood samples were drawn via cardiac puncture, and the lungs quickly removed and frozen in liquid nitrogen. Lung samples were stored at −70°C until used for biochemical assays. Serum samples were analyzed for blood urea nitrogen (urea nitrogen diagnostic kit No. 535, Sigma Chemical Co., St. Louis, Mo.) and albumin (albumin diagnostic kit No. 630, Sigma). Serum glucose concentrations were measured by the glucose oxidase method on a Beckman Glucose II analyzer (Beckman Instrument Company, Fullerton, Calif.).

Lung protein was measured according to the method of Hartree (18), with bovine serum albumin as standard. Lung dry-to-wet weight ratios were determined in the following manner. Approximately 50–75 mg of lung tissue was placed in a weighing vial and dried in a 110°C oven. After 72 and 96 hr, dry weight values were obtained. Dry weights were utilized when values on two consecutive days were within 0.5 mg. The lipids from whole lung were extracted with chloroform:methanol (2:1 v/v) and washed to remove nonlipid contaminants (19). Further separation of the lipid compounds into separate phospholipid classes was achieved by thin-layer chromatographic methods (20). Saturated phosphatidylcholine was measured after osmic acid treatment as described by Mason *et al.* (21). Recovery of phosphatidylcholine and disaturated phosphatidylcholine from whole lung was routinely assessed by adding [^{14}C]dipalmitoyl phosphatidylcholine (Applied Science Co., State College, Pa.) at the time of extraction of lung tissue samples. This permitted the correction for any losses during processing and allowed ongoing assessment of quality control. The appropriate lipid spots were scraped from the plates and the phospholipids extracted from the gel by the method of Bligh and Dyer (22). Lipid phosphorus determinations (23) were performed on each fraction and the amount of radioactivity in each phospholipid was measured by liquid scintillation counting techniques in a Beckman LS 7000 liquid scintillation counter.

The responses of the controls, treated animals, and fasting blood glucose groupings were compared in a one-way analysis of variance (24), using routines in the BMDP computer package (25). The mean response at each dose or grouping was compared with the mean of the appropriate controls and tested for significance.

Results. All of the animals in the control, 30-mg/kg, and 40-mg/kg treatment groups and 90% of the 50-mg/kg group survived the 7-day experimental period. In contrast, only 55% of the 100-mg/kg STZ-treated rats survived. The deaths in this latter group occurred within 48–96 hr of STZ infusion.

Table I presents the data on the effects of STZ on blood chemistry, growth, and urine output as a function of STZ dose. Since the two control groups did not differ significantly in any of the indices examined, they were combined and represent the normal population in this study. The serum glucose concentrations in rats injected with 30, 40, and 50 mg/kg STZ were all significantly different from those of the control group. The administration of 100 mg/kg STZ also produced significant hyperglycemia, although the average of the fasting blood glucoses of the survivors in this group was less than that found in the group injected with 50 mg/kg.

Table I also demonstrates that infusion of STZ produced a reduction in weight gain that generally paralleled increasing STZ dose. The reduction in weight gain is significant in all treatment categories with the exception of the 100-mg/kg group. As was found with fasting blood glucose, the reduction in weight gain in

TABLE I. STREPTOZOTOCIN DOSE RESPONSE[a]

Effect on	Controls	30 mg/kg	40 mg/kg	50 mg/kg	100 mg/kg
Fasting blood glucose (mg/dl)	88.0 ± 4.4 (20)	129.6 ± 9.4 (20)	159.9 ± 16.3 (20)	388.7 ± 25.2 (18)	213.2 ± 49.3 (11)
P value[b]	—	<0.05	<0.001	<0.001	<0.001
Blood urea nitrogen (mg/dl)	12.48 ± 0.45 (20)	16.44 ± 1.01 (20)	19.44 ± 1.74 (20)	43.35 ± 3.15 (18)	30.91 ± 7.85 (11)
P value[b]	—	N.S.	<0.05	<0.001	<0.001
Albumin (g/dl)	3.27 ± 0.05 (20)	3.63 ± 0.05 (20)	3.44 ± 0.07 (20)	2.53 ± 0.06 (18)	2.70 ± 0.13 (11)
P value[b]	—	N.S.	N.S.	<0.01	N.S.
Weight gain (g)[c]	55.9 ± 2.7 (20)	38.1 ± 2.8 (20)	31.3 ± 4.0 (20)	8.2 ± 4.6 (18)	36.9 ± 8.6 (11)
P value[b]	—	<0.05	<0.001	<0.001	N.S.
Urine output (ml/100 g/24 hr) 48 hr post-STZ	4.4 ± 0.3 (19)	7.7 ± 1.1 (20)	15.8 ± 2.4 (20)	34.7 ± 2.2 (20)	15.5 ± 4.0 (15)
P value[b]	—	N.S.	<0.001	<0.001	<0.01
96 hr post-STZ	4.3 ± 0.2 (19)	9.7 ± 1.6 (20)	19.9 ± 3.1 (20)	50.8 ± 2.7 (18)	21.6 ± 8.9 (11)
P value[b]	—	N.S.	<0.001	<0.001	N.S.

[a] Values represent the means ± SE for (*n*) determinations.
[b] *P* vs controls; N.S., not significant.
[c] The weight gain represents the amount of weight gained during the 1-week experimental period.

this group was less than would be expected. Furthermore, due to the wide range of weight gains in the animals receiving 100 mg/kg STZ, they did not differ significantly from the controls. Urine volumes 48 and 96 hr after STZ administration were significantly higher than control values in the 40- and 50-mg/kg groups. The urine volume of the 30-mg/kg group is somewhat higher than that found in the control group, although the difference is not statistically significant. The 100-mg/kg group produced urine volumes that were generally higher than those found in controls, but due to the large range of volumes encountered these values were statistically significant only at the 48-hr collection.

Serum albumin and blood urea nitrogen (BUN) concentrations were determined as laboratory indices of liver and kidney function. Table I demonstrates that BUN values in the 30-mg/kg group were similar to those found in the controls, while the 40-, 50-, and 100-mg/kg groups had values significantly higher than those of the control group. Although the BUN concentrations generally increased with drug dosage, the 100-mg/kg animals had an average value intermediate between that found for the 40- and 50-mg/kg groups. Serum albumin concentrations, in contrast, were significantly different from controls only in the 50-mg/kg group. The 100-mg/kg group showed serum albumin concentrations which were higher than those in the 50-mg/kg group, but lower than those found in the 40-mg/kg group.

The effects of STZ-induced diabetes on lung tissue are shown in Table II. Total lung phosphatidylcholine concentrations and dry-to-wet weight ratios were unchanged in any of the treatment groups. In contrast, the disaturated phosphatidylcholine levels were significantly depressed in the 50-mg/kg group. This lowered level of disaturated phosphatidylcholine resulted in a 30% decrease in the disaturated phosphatidylcholine-to-total lung phosphatidylcholine ratio. The animals that survived treatment with 100 mg STZ/kg generally had lower levels of disaturated phosphatidylcholine than controls. However, these two groups were not statistically different, due to the wide variability of disaturated phosphatidylcholine concentrations found in the 100-mg/kg group. Lung protein concentrations were also affected by the administration of STZ and demonstrated significant reductions after treatment with 50 and 100 mg/kg.

Although the fasting blood glucose levels generally increased with increasing STZ dos-

TABLE II. EFFECTS OF STREPTOZOTOCIN DOSE ON LUNG BIOCHEMISTRY[a]

Effect on	Controls	30 mg/kg	40 mg/kg	50 mg/kg	100 mg/kg
Phosphatidylcholine	10.66 ± 0.33	11.57 ± 0.27	11.11 ± 0.32	11.37 ± 0.47	11.02 ± 0.57
(μmole/g wet wt.)	(14)	(20)	(19)	(16)	(11)
P value[b]	—	N.S.	N.S.	N.S.	N.S.
Disaturated	5.32 ± 0.21	5.76 ± 0.09	5.46 ± 0.14	3.94 ± 0.33	4.58 ± 0.43
phosphatidylcholine	(14)	(19)	(19)	(18)	(8)
(μmol/g wet wt)					
P value[b]	—	N.S.	N.S.	<0.01	N.S.
Protein	133.79 ± 3.67	134.78 ± 2.80	136.64 ± 2.60	115.50 ± 1.88	117.52 ± 3.46
(mg/g wet wt)	(10)	(20)	(20)	(18)	(11)
P value[b]	—	N.S.	N.S.	<0.005	<0.05
Dry/wet wt ratio	0.2078 ± 0.0037	0.2008 ± 0.0021	0.2006 ± 0.0029	0.2018 ± 0.0023	0.1952 ± 0.0031
P value[b]	N.S.	N.S.	N.S.	N.S.	N.S.

[a] Values represent the means ± SE for (*n*) determinations.
[b] *P* vs controls; N.S., not significant.

age, there was considerable overlap between the groups. Therefore, in order to correlate the effects of STZ-induced diabetes with hyperglycemia, the data are presented in Tables III and IV as a function of the final fasting blood glucose concentration. For these analyses, animals with fasting blood glucose concentrations of 100 mg/dl or less were used as the normal group for statistical comparisons. Table III shows that weight gain and 96-hr urine output were significantly different from controls in all animals with fasting glucose

TABLE III. CORRELATION OF EFFECTS OF STREPTOZOTOCIN-INDUCED HYPERGLYCEMIA WITH FASTING BLOOD GLUCOSE (mg/dl)[a]

	Fasting blood glucose (mg/dl)					
Effect on	≤100	101–125	126–150	151–200	201–400	>400
Mean fasting	83.8 ± 3.0	112.0 ± 1.5	138.7 ± 2.3	171.3 ± 3.4	284.3 ± 20.3	452.9 ± 10.6
blood glucose	(23)	(22)	(12)	(7)	(10)	(15)
(mg/dl)						
P value[b]	—	<0.001	<0.001	<0.001	<0.001	<0.001
Blood urea	11.89 ± 0.39	14.98 ± 0.76	17.44 ± 0.81	24.36 ± 0.95	30.79 ± 2.90	53.12 ± 3.65
nitrogen	(23)	(22)	(12)	(7)	(10)	(15)
(mg/dl)						
P value[b]	—	<0.05	<0.001	<0.001	<0.001	<0.001
Albumin (g/dl)	3.42 ± 0.08	3.42 ± 0.08	3.53 ± 0.08	3.40 ± 0.13	2.93 ± 0.13	2.49 ± 0.08
	(23)	(22)	(12)	(7)	(10)	(15)
P value[b]	—	N.S.	N.S.	N.S.	<0.01	<0.001
Weight gain (g)	54.3 ± 2.0	49.6 ± 2.6	35.0 ± 2.6	16.3 ± 3.8	18.5 ± 4.6	0.1 ± 4.1
	(23)	(22)	(12)	(7)	(10)	(15)
P value[b]	—	N.S.	<0.001	<0.001	<0.001	<0.001
Urine output	4.5 ± 0.3	6.9 ± 1.1	9.7 ± 2.2	31.5 ± 3.6	41.9 ± 3.0	54.4 ± 3.9
(ml/100 g/24 hrs)	(22)	(22)	(12)	(7)	(10)	(15)
96 hr post-STZ						
P value[b]	—	N.S.	<0.005	<0.001	<0.001	<0.001
Glucose in urine,	0/22	6/22	7/12	7/7	10/10	15/15
96 hr,						
post-STZ						
Ketones in urine,	0/22	0/22	0/12	0/7	0/10	3/15
96 hr, post-STZ						

[a] Values represent the means ± SE for (*n*) determinations.
[b] *P* vs ≤100 mg/dl glucose group; N.S., not significant.

TABLE IV. CORRELATION OF EFFECTS OF STREPTOZOTOCIN-INDUCED GLUCOSE INTOLERANCE ON PULMONARY BIOCHEMISTRY WITH FASTING BLOOD GLUCOSE[a]

	Fasting blood glucose (mg/dl)					
Effect on	≤100	101–125	126–150	151–200	201–400	>400
Phosphatidylcholine	11.10 ± 0.29	11.10 ± 0.33	11.14 ± 0.45	11.54 ± 0.42	11.05 ± 0.77	11.36 ± 0.43
(μmol/g)[b]	(18)	(20)	(12)	(7)	(9)	(14)
P value[c]	—	N.S.	N.S.	N.S.	N.S.	N.S.
Disaturated	5.41 ± 0.19	5.58 ± 0.14	5.32 ± 0.25	4.70 ± 0.54	4.87 ± 0.39	4.12 ± 0.40
phosphatidylcholine	(18)	(17)	(12)	(7)	(10)	(14)
(μmole/g)						
P value[c]	—	N.S.	N.S.	N.S.	N.S.	~0.01
Protein (mg/g)	132.8 ± 2.9	127.1 ± 3.3	138.5 ± 3.4	134.3 ± 4.9	125.4 ± 5.8	116.1 ± 1.7
	(16)	(19)	(12)	(7)	(10)	(15)
P value[c]	—	N.S.	N.S.	N.S.	N.S.	<0.01
Dry/wet wt ratio	0.2031 ± 0.0022	0.2013 ± 0.0030	0.1992 ± 0.0027	0.1986 ± 0.0037	0.2006 ± 0.0054	0.2015 ± 0.0026
	(16)	(19)	(12)	(7)	(9)	(15)
P value[c]	—	N.S.	N.S.	N.S.	N.S.	N.S.

[a] Values represent the means ± SE for (*n*) determinations.
[b] All data are expressed per wet weight of tissue.
[c] *P* vs ≤100 mg/dl group; N.S., not significant.

levels higher than 126 mg/dl. Blood urea nitrogen values rose with increasing glucose concentration, while albumin concentrations remained relatively steady at glucose concentrations less than 200 ml/dl. Above this value the albumin concentrations declined to appreciably lower levels. Glucose could be detected in the urine of a limited number of animals with blood glucose values in the range 101–125 mg/dl, but was found in all urine samples from animals with levels greater than 151 mg/dl. Ketonuria, on the other hand, rarely occurred in animals with STZ-induced experimental diabetes.

Table IV documents the relationship between increasing hyperglycemia and lung disaturated phosphatidylcholine concentrations and illustrates that the concentrations of this phospholipid decreased with increasing blood glucose values, but only reached statistically lower concentrations at glucose levels greater than 400 mg/dl. Lung protein concentrations remained relatively constant up to fasting glucose levels of 400 mg/dl; above this concentration lung proteins were decreased approximately 13%. Total lung phosphatidylcholine and pulmonary dry-to-wet weight ratios, on the other hand, demonstrated no change with increasing hyperglycemia.

Discussion. In this study intravenous injection of STZ in a dose of 30 mg/kg or greater produced carbohydrate intolerance in the rat, and fasting blood glucose levels increased coincident with increased drug dose. Higher STZ dosages also resulted in increased urine outputs, decreased weight gains, and perturbed kidney function, as assessed by blood urea nitrogen concentrations. Liver function, as represented by serum albumin levels, was affected only in the 50-mg/kg STZ group. The administration of 100 mg STZ/kg resulted in a diabetes-like state that was less severe than anticipated; this may result from the death of this group's markedly abnormal animals within 96 hr after STZ infusion. In general, the effects on urine output, weight gain, and kidney and liver function reported here are similar to previous findings (4, 8–10, 26–28). The fasting blood glucose levels, however, differ from those reported previously, a variance most likely due to different experimental techniques and/or variability in STZ potency (29).

This study also indicates that alterations in adult lung metabolism are dependent on the extent of the diabetic condition. This is exemplified by the decreased concentrations of pulmonary protein and disaturated phosphatidylcholine found in animals treated with 50–100 mg/kg STZ. Since insulin is recognized as an anabolic hormone, the decreased protein concentrations found in severe diabetes were

not unexpected. The decreased levels of disaturated phosphatidylcholine, however, may not be attributable solely to the general catabolic effects of diabetes, since total lung phosphatidylcholine concentrations were not affected in any of the treatment groups. This preferential decrease in disaturated phosphatidylcholine may indicate that diabetes selectively affects the pathways leading to the formation of the disaturated phospholipids that are particularly characteristic of lung surfactant. The results from the dose–response relationships support and extend those of previous findings, where only large doses of STZ were employed (11–17).

Although the fasting blood glucose levels generally increased with increasing STZ dosages, there was considerable overlap between the groups. Therefore, the data were also analyzed as a function of the fasting blood glucose concentration at the time of sacrifice in order to directly compare the severity of the effects of chemically induced diabetes to the severity of glucose intolerance. Also, the effects of STZ-induced hyperglycemia based on fasting blood glucose concentrations may be a more sensitive indicator of the deleterious effects of diabetes on different organ systems that STZ dose alone. Thus, increasing hyperglycemia is accompanied by decreased weight gains and serum albumin levels and elevated blood urea nitrogen concentrations and urine outputs. Pulmonary protein concentrations remain unchanged until hyperglycemia becomes pronounced, then drop to a significantly lower level. Disaturated phosphatidylcholine content appears to decline with increasing hyperglycemia, but can be differentiated statistically from controls only when fasting blood glucose concentrations are greater than 400 mg/dl. This relationship is further support for the notion that the severity of diabetes is an important variable in studies of adult lung metabolism in diabetic animal models.

Using fasting blood sugar levels as a reference base for data analysis is useful because it reveals that some organ systems are sensitive to even mild or moderate glucose intolerance and deteriorate as the carbohydrate intolerance worsens. Other tissue characteristics, however, are apparently more resistant to the disabling effects of diabetes and are affected only in the severest cases, if at all. The relative sensitivity of different tissues and metabolites should be taken into account when using animal models for the study of diabetes mellitus. These results also indicate the importance of reporting blood glucose levels (preferably in fasted animals) in any study involving experimental diabetes. The necessity of establishing the appropriate level of glucose intolerance has been underscored in several publications concerning the effects of maternal diabetes on fetal development (30–32). These investigators have reported that fetal size, fetal insulin, and fetal glucose concentrations are dependent on the degree of maternal carbohydrate imbalance.

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