Sertoli Cell Function in Diabetic, Insulin-treated Diabetic, and Semi-Starved Rats

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SUMMARY

It is well documented that long-term diabetes mellitus results in numerous deleterious consequences. However, considerable controversy exists concerning male reproductive function in diabetes. The purpose of this investigation was to study several endocrine parameters in diabetic male rats with emphasis on Sertoli cell function. Male Wistar rats were injected with streptozotocin and then either left untreated for 30 days or injected with insulin so as to prevent spillover of glucose into the urine. These two groups were compared with control animals that had only been injected with the vehicle for streptozotocin. Semi-starved control animals were included to determine if any of the potential endocrine alterations were related to body weight changes which occur in streptozotocin-injected rats. It was found that FSH, LH, PRL, and GH serum levels were reduced in diabetic animals. Only FSH was restored to normal by insulin injections. The testis, seminal vesicle, and epididymis weights were all reduced in diabetic animals. Insulin injections raised all organ weights; however, only testis weights were fully restored. Levels of epididymal ABP activity were found to be higher in diabetic animals when expressed per mg protein. Similar patterns of organ weight loss and hormonal alterations were observed in semi-starved rats. However, epididymal levels of ABP activity were unaffected by the semi-starved condition. While weight loss should be taken into consideration when interpreting cause and effect relationships in streptozotocin-treated animals, epididymal ABP levels appear to be well correlated with the altered metabolic state characteristic of diabetes. DIABETES 32:112–116, February 1983.

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Diabetes results in reproductive dysfunction; however, the exact consequences are controversial (for review, see ref. 1). This is surprising, since approximately one-half of the men that have had diabetes mellitus for 7 yr or longer develop infertility.[2,3] Similar results have been reported for experimentally-induced diabetic laboratory animals.[4] Diabetes has been reported to result in low,[5,6] or normal,[7] levels of serum gonadotropins. Subnormal size of the prostate, decreased sperm count, reduced fructose concentration in the ejaculate, changes in the levels of urinary steroids[8–10] and histologic abnormalities of the pituitary[11,12] and testis[13–19] have also been reported. However, other reports indicate normal urinary steroids and normal Leydig cell morphology.[20] It has been shown that genetically-selected diabetic rats undergo testicular degeneration characterized by complete absence of germinal elements.[4] However, even in hypophysectomized patients in which gonadotropins are absent, a few spermatogonia and spermatocytes usually remain.[21] Even emotional factors of nonorganic origins have been implicated.[22] However, these neurologic dysfunctions cannot completely explain the reproductive pathology of diabetic patients.

The purpose of the experiments of this investigation was to study several parameters of reproductive function in experimentally-induced diabetic male rats with special emphasis on Sertoli cell function.

MATERIALS AND METHODS

Experimentally-induced diabetic animals. Adult male Wistar rats (160–210 g), which were housed in a controlled environment (light:dark cycle, 12:12, 22°C; 45% humidity), were used throughout this study. Animals were fed Purina rat chow and tap water ad libitum. Experimental diabetes was induced by cardiac injection of streptozotocin (75 mg/kg body wt) in 100 μl cold 0.05 M citrate buffer (pH 4.5) within 10 min after dissolving. Urine glucose levels were monitored with Clinistix (Miles Laboratories, Elkhart, Indiana) 48 h after injection.

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Animals which had positive urinary glucose levels were considered overtly diabetic. Animals which had no detectable urinary glucose were discarded from the study. A separate group of animals received 100 μl of citrate buffer only. The overtly diabetic animals were divided into 3 groups: group A was untreated; group B was maintained by intraperitoneal injections of insulin (U-40 NPH Iletin-Lilly) at 4 U/day; group C received 8–14 U/day (two equal dose injections at 8:00 a.m. and 4:00 p.m.). Group C was included since 4 U/day was not high enough to prevent spillover of glucose into the urine (measured at 10:00 a.m.), as determined by the Clinistix assay. Body weights were determined at the time of streptozotocin injection and at termination of the experiment (30 days). Animals were randomly selected from the treatment groups and killed between 10:00 a.m. and 11:00 a.m. by decapitation 15 s after removal from the cages. Blood was collected from the body and allowed to clot at room temperature for 1 h. The serum was removed following centrifugation at 1400 × g for 15 min at 4°C. The serum was divided into small aliquots and stored in 1-ml glass serum vials at −95°C. The entire experimental procedure was performed twice.

Semi-starved animals. These animals were included as controls since the streptozotocin-injected animals lost considerable body weight. The animals were divided into two groups: group A received food and water ad libitum; group B was restricted to 8 g food/day and received water ad libitum. The animals were killed and processed as described above after 30 days.

Glucose assays. The serum was thawed at room temperature and assayed for glucose with a glucose-oxidase kit (Boehringer).

Hormone assays. The serum samples were stored at −95°C until they were shipped from Lubbock to Omaha on dry ice. The samples were then stored at −70°C until assayed. Serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), and growth hormone (GH) were assayed using the NIAMDD kits for rat hormones. Each unknown was assayed for 2-dose levels in duplicate.

Weight determinations. The epididymides and seminal vesicles were removed at sacrifice and placed in liquid nitrogen. The epididymides were then weighed and quickly placed in a freezer at −95°C until the day of the ABP assay (see below). The seminal vesicles were weighed when frozen and discarded. The testes were blotted dry and weighed immediately after decapitation.

ABP assays. The epididymides were thawed at room temperature and homogenized in cold buffer which included 50 mM Tris-HCl (pH 7.4), 1 mM EDTA, 1 mM p-chloromercuriphenyl sulfonate, 350 nM testosterone, and 10% glycerol (vol/vol).[23] The homogenates were centrifuged at 100,000 × g for 1 h in a Spinco Ti 65 rotor. The supernatants were extracted with dextran-coated charcoal and assayed for ABP by steady-state polyacrylamide gel electrophoresis as previously described by Ritzen et al.[24] with slight modification.[25] Protein determinations were done on the 100,000 × g pellet by the method of Lowry et al.[26]

Statistical analysis. Analysis of variance was used to compare the means of data obtained from diabetic, insulin-injected, and control animals. Duncan's new multiple range test was used to determine which group was statistically different ($P \leq 0.05$) from controls. Student's t test was used to determine the statistical difference between the means of two groups.

RESULTS

Experimentally-induced diabetic animals. The overtly diabetic animals demonstrated a mean body weight loss of 3.6% over 30 days. Animals that received insulin injections of 4 U/day lost 10.5% of their initial body weight (Table 1). These results led us to include the control group of streptozotocin-injected animals which received 8–14 U of insulin/day thereby more closely controlling body weight and glucose levels. The control animals (citrate-injected) and insulin-injected (8–14 U/day) animals gained weight during the same experimental period; means of 91.6% and 40.9%, respectively.

The testis, epididymis, and seminal vesicle weights were all reduced ($P \leq 0.05$) in the diabetic animals. The testis weights were nearly restored to the control weight by insulin treatment (Table 1). The epididymis and seminal vesicle weights were restored to 89.1% and 78.6% of the control values, respectively. In two cases, the testes had a reddish-purple appearance.

Streptozotocin treatment resulted in elevated urinary glucose levels within 24–48 h, as determined by the Clinistix

TABLE 1
Body and organ weights (diabetic study)

Treatment†	Body weight		Testis*		Epididymis*		Seminal vesicle wet wt. (g)
	Start	End	Wet wt. (g)	Protein (mg)	Wet wt. (g)	Protein (mg)	
Control	190 ± 2‡(30)§	364 ± 5(30)	3.38 ± 0.04(12)	91.10 ± 3.34(12)	1.37 ± 0.07(12)	33.24 ± 1.22(12)	1.03 ± 0.07(5)
Diabetic	223 ± 7 (37)	215 ± 8(37)	2.58 ± 0.22(14)#	74.75 ± 5.56(14)#	0.82 ± 0.11(14)#	23.46 ± 4.16(14)#	0.22 ± 0.07(8)#
Insulin‖	258 ± 4 (24)	231 ± 8(24)	—	—	—	—	—
Insulin¶	186 ± 4 (7)	262 ± 18(7)	3.24 ± 0.06	76.54 ± 1.86(14)#	1.22 ± 0.03(14)#	28.90 ± 1.46(14)#	0.81 ± 0.07(8)#

*Both organs.
†Thirty days after streptozotocin treatment.
‡Mean ± standard error of the mean.
§Number of animals.
‖Four units/day.
¶Eight to fourteen units/day.
#Indicates a statistically significant difference from the control animals at the $P \leq 0.05$ level (organ weights only).

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TABLE 2
Glucose and hormone levels* (diabetic study)

Experiment 1	Glucose (mg/dl)	FSH	LH	PRL	
Control	167 ± 13(25)	906 ± 35(29)†	30.8 ± 2.5(25)	50.2 ± 6.9(25)	
Diabetic	675 ± 19(29)‡	620 ± 28(29)‡	17.3 ± 1.3(29)‡	18.5 ± 2.6(29)‡	
Insulin (4 U/day)	612 ± 25(21)‡	593 ± 24(21)‡	13.2 ± 0.5(21)‡	14.9 ± 2.3(21)‡	
Experiment 2	**Glucose (mg/dl)**	**FSH**	**LH**	**PRL**	**GH**
Control	126 ± 6(5)	728 ± 82(5)	16.0 ± 1.8(4)	63.2 ± 15(5)	1096 ± 116(4)
Diabetic	584 ± 56(8)‡	377 ± 48(8)‡	11.9 ± 6.0(8)	17.7 ± 3.6(8)‡	40.2 ± 7.9(8)‡
Insulin (8–14 U/day)	175 ± 88(7)	780 ± 115(7)	8.6 ± 1.9(7)‡	15.5 ± 1.8(7)‡	4.26 ± 1.31(5)‡

*All hormone levels indicate ng/ml.
†Mean ± standard error of the mean (N).
‡Indicates a statistically significant difference from control values at the P ≤ 0.05 level.

assay. The serum glucose levels, obtained at sacrifice, of animals treated with streptozotocin were elevated 404% (Table 2). Animals injected with 8–14 U/day of insulin had mean serum glucose levels near control levels. However, the serum glucose levels in the insulin-maintained animals varied greatly with a mean of 175 and a standard error of 88, while the mean of serum glucose levels of the control animals was 126 ± 6.

FSH, LH, PRL, and GH levels were all reduced (P ≤ 0.05) in the overtly diabetic animals as compared to the control animals (Table 2). FSH levels were restored to normal levels by insulin injections of 8–14 U/day. GH levels were actually 10-fold lower in the insulin-injected animals as compared with overtly diabetic animals (P ≤ 0.001). PRL and LH levels were unaffected by insulin treatment.

The levels of ABP in the epididymis of the overtly diabetic animals were similar to those levels observed in the control animals when expressed per organ (P ≤ 0.2). However, these levels were much higher than control levels when expressed per mg epididymal protein (Table 3).

TABLE 3
ABP determinations (diabetic study)

	pmol ABP	
Treatment	Per epididymis	Per mg protein × 10
Control	5.83 ± 0.69*(5)†	1.12 ± 0.1(5)
Diabetic	4.65 ± 0.46 (8)	2.72 ± 0.77(8)§
Insulin (8–14 U/day)	6.47 ± 0.52 (7)	1.60 ± 0.10(7)§

*Mean ± standard error of the mean (N).
†Number of animals.
§Indicates a statistically significant difference from control values at the P ≤ 0.05 level.

Semi-starved control animals. The control animals gained 118.3% of their initial body weight while the food-restricted animals lost 15.8% of their initial body weight (Table 4). The testis and epididymis wet weights were also significantly reduced (Table 4). The serum glucose levels also decreased significantly in the semi-starved animals (Table 5). The hormone levels were also significantly reduced (Table 5). The ABP levels, however, were not altered, regardless of the unit by which the data were expressed (Table 6).

DISCUSSION

The results of this investigation demonstrate that streptozotocin-induced diabetes results in a multiple endocrine derangement characterized by lowered FSH, LH, PRL, and GH levels. These animals also lose considerable body and accessory sex gland weight. Epididymal ABP levels also appear to be altered in diabetic animals 30 days after streptozotocin treatment. These data are consistent with those of Murray et al.[27] who found a "window" at 3 wk after streptozotocin injection, at which time the epididymal levels of ABP were significantly higher than controls.

Importantly, we have demonstrated that the loss of body weight is an important consideration when interpreting results on experimentally-induced diabetic animals. This concept is supported by our observation that semi-starved animals have similar endocrine dysfunction as the diabetic

TABLE 4
Body and organ weights (restricted food study)*

	Body weight		Testis†		Epididymis†	
	Start	End	Wet weight (g)	Protein (mg)	Wet weight (g)	Protein (mg)
Control (7)‡	143.0 ± 17.5§	312.1 ± 23.2	3.18 ± 0.14	78.59 ± 0.53	1.04 ± 0.07	22.86 ± 1.24
Restricted food (10)‡	137.6 ± 8.7	115.9 ± 1.8‖	2.20 ± 0.12‖	55.51 ± 1.72‖	0.56 ± 0.05‖	20.46 ± 1.23

*Eight grams/day for 30 days.
†Both organs.
‡Number of animals.
§Mean ± standard error of the mean.
‖Indicates a statistically significant difference from control values at the P ≤ 0.05 level.

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TABLE 5
Glucose and hormone levels* (restricted food study)†

	Glucose‡	FSH	LH	PRL	GH
Control (7)§	114.3 ± 2.1‖	646.2 ± 34.1	42.4 ± 5.0	31.3 ± 0.11	327.8 ± 138.4
Restricted food (10)§	94.0 ± 5.3¶	443.9 ± 61.3¶	23.8 ± 5.3¶	8.0 ± 2.0¶	18.4 ± 5.3¶

*Nanograms/milliliter for all hormones.
†Eight grams/day for 30 days.
‡Milligrams/deciliter.
§Number of animals.
‖Mean ± standard error of the mean.
¶Indicates a statistically significant difference from control values at the $P \leq 0.05$ level.

animals. This is in agreement with previous studies which demonstrated decreased pituitary hormone serum levels in nutritionally-altered animals.[28,29] Administration of a low-protein diet has been reported by Herbert and Tindall to decrease epididymal ABP levels.[30] Glass et al.,[31] using a similar low-protein diet, reported that ABP was not detectable in the testis. In the latter study, a semi-starved group was also included in which it was reported that testicular ABP levels were also abolished. However, the studies of Herbert and Tindall, as well as our studies, were conducted on epididymal tissues, while Glass et al. studied testicular ABP levels. Furthermore, the discrepancy between our results, which demonstrated increased epididymal ABP levels, and those of Herbert and Tindall and Glass et al. may also be due to differences in the nutritional makeup of the experimental diets. The low-protein diets of Glass et al. and Herbert and Tindall were both supplemented with vitamins. Our semi-starved regimen merely consisted of restricting ordinary rat chow to 8 g/day/rat. Additional studies are needed to determine if the endocrine alterations observed in our diabetic animals were a specific result of body weight loss, since the degree of weight loss and quantity and quality of food intake in these diabetic animals and the semi-starved animals were different.

It is possible that the increased ABP activity observed in the diabetic animals is a result of decreased turnover of this protein within the epididymis. This would explain the contradictious observations that FSH levels are decreased while ABP levels are increased. It is also possible that there is a defect in the biochemical sequence of events between hormone binding and protein secretion such as an increase in the affinity or the number of FSH receptors on the Sertoli cell. Alterations in receptor behavior have been reported in diabetic animals. Murray et al.[27] demonstrated reduced LH sensitivity in diabetic Leydig cells. Similarly, Charreau et al.[32] found a reduction in the number of LH binding sites in diabetic Leydig cells. These studies may also explain our finding that insulin restored sex accessory organ weights while LH levels remained unchanged. If true, the insulin administered in our study may have restored Leydig cell LH receptors to normal, thereby allowing the production of testosterone with the subsequent stimulation of organ weights. It is also possible that insulin has direct effects on the epididymides and seminal vesicles as has been shown for Sertoli cells.[33] Murray et al.[27] have also demonstrated decreased seminal vesicle weights after streptozotocin treatment; however, no alterations in testis or epididymis weights were observed. They also reported decreased testosterone levels.

TABLE 6
ABP determinations (restricted food study)*

Treatment	pmol ABP	
	Per epididymis	Per mg protein × 10
Control (7)†	9.37 ± 0.43‡	4.31 ± 0.27
Restricted food (10)	9.40 ± 0.20	4.38 ± 0.52

*Eight grams/day for 30 days.
†Number of animals.
‡Mean ± standard error.

Interestingly, of all the hormones which were influenced by streptozotocin treatment, only FSH was restored to control levels with insulin injections. It is possible that the wide variations in glucose levels known to occur using insulin therapy, as described in this study, functionally alter the LH-, PRL-, and GH-secreting cells more than the FSH-secreting cells. This explanation seems plausible since GH levels were actually lower in insulin-injected animals than in the diabetic animals. The suppressed GH levels observed with streptozotocin treatment are in agreement with previous studies.[34]

The results of this study are acute as compared with the length of time of onset of reproductive dysfunction observed in diabetic patients. Thus, the complete characterization of reproductive dysfunction in diabetes awaits long-term studies. However, these studies are significant in that they demonstrate increased ABP levels in the epididymides of diabetic animals. This abnormality may be involved in the increased incidence of infertility which occurs in long-term diabetic patients.

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