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Physiological Changes Associated with Streptozotocin-Induced Experimental Diabetic Rats

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Abstract: The present work is aimed to investigate some of the physiological changes in streptozotocin-induced experimental diabetic rat model with nephropathy. Animals were divided into control and experimental groups. The experimental group was rendered diabetic by intraperitoneal injection of a single dose of 50 mg/kg body weight streptozotocin. Rats with serum glucose levels >200 mg/dl considered as diabetics. Animals of the control group were injected with physiological saline. Streptozotocin-induced diabetic rats showed hypoinsulinemia and hyperglycemia compared to controls. Strong negative correlation (r=-0.8) was found between serum glucose and insulin levels in diabetic rats. Triglycerides and cholesterol levels were significantly increased in diabetic animals. Urea concentrations were also elevated markedly compared to controls. In contrast, uric acid and creatinine concentrations showed significant decrease. The activities of serum aspatate aminotransferase, alanine aminotransferase and alkaline phosphatase were significantly increased in streptozotocin-induced diabetic rats compared to controls. These findings demonstrate the utility of mouse models for identifying and testing novel therapeutic strategies which could translate into better protection against the human disease.

Key Words: Streptozotocin, diabetic, experimental, rats, nephropathy

Introduction: Diabetes mellitus is a group of syndromes characterized by hyperglycemia, altered metabolism of lipids, carbohydrates and protein and an increased risk of complication of vascular diseases (1). The experimental diabetes induced by streptozotocin. Streptozotocin is widely used for inducing experimental diabetes in animals. High doses of β cell toxins like streptozotocin induce insulin deficiency and type 1 diabetes mellitus with ketosis. However, doses calculated to cause a partial destruction of β cell mass can be used to produce a mild insulin deficient state of type 2 diabetes mellitus, without a tendency to cause ketosis (2).

Kidney function is impaired in diabetes mellitus. Diabetic nephropathy is the leading cause of end-stage renal disease (3). The hepatorenal dysfunction and hyperlipdemia are considered the most common pathognomonic factors including the main complications in diabetes mellitus (4).

Liver is an insulin dependant tissue, which plays a provital role in glucose and lipid homeostasis and severely affected during diabetes (5). Decreased glycolysis, impeded glycogenesis and increased gluconeogenesis are some of the changes of glucose metabolism in the diabetic liver (6).

The current work was undertaken to assess the some of the physiological changes in streptozotocin-induced experimental diabetic rat model with nephropathy. The experimental diabetes induced by streptozotocin in the present study seems to be a model of incipient diabetic nephropathy. The findings could open a new avenue of research in identifying and testing novel therapeutic strategies which could translate into better protection against the human disease.

Materials and methods

Experimental animals

Male Sprague-Dawley rats weighting 170±30 gm were used throughout the study. Animals were maintained under the ambient conditions in the animal house in the Department of Biology, The Islamic University of Gaza. They were fed on a commercial balanced diet and water was provided *ad Libitum* with fresh supply daily allover the experimental period.

Animals were divided into two major groups: control and experimental groups. The experimental group of animals was fasted for 24 hours and then intraperitoneally injected with a single dose of 50 mg/kg body weight of freshly prepared streptozotocin dissolved in citrate buffer pH 4.5. Streptozotocin is a mixture of α and β anomers; 2-Deoxy-2-[[(methyl-nitrosoamino)carbomyl] amino]-D-glucopyranose, that produces a selective toxic effect on β cells and induces diabetes mellitus in most laboratory animals (7). Streptozotocin was purchased from Himedia Laboratory Limited, Mumbai, India. Rats with glucose levels >200 mg/dl considered as diabetics.

Physiological studies

At each sampling date, 9 rats were taken from control groups and 11 rats from experimental/week. Animals were decapitated and blood samples were then collected into centrifuge tubes after the termination of 1,2,3 and 4 weeks, respectively. Clear serum were subjected to bioassay including serum insulin using Abbott IMx Insulin assay (8), glucose (9), triglyceride (10), cholesterol (11), urea (12), uric acid (13), creatinine (14), aspartate aminotransferase

and alanine aminotransferase activities (15) and alkaline phosphatase activity (16) using DiaSys reagent kits.

Statistical analysis

Data were statistically analyzed by computer using SPSS 11.0 for windows (Statistical Package for the Social Sciences Inc, Chicago, Illinois). Means were compared by independent-samples t-test. A probability level less than 0.05 were taken as significant. Percentage change was also calculated. Graphs and correlations between different parameters were plotted using Microsoft excel program.

Results

Serum insulin and glucose levels

Streptozotocin-induced diabetic rats showed significant decrease in insulin levels along the whole experimental intervals examined recording a maximum percentage decrease of 33.3% at the end of the third week of the experiment compared to control levels (Table 1). In contrast, serum glucose levels were markedly increased in diabetic rats allover the experimental periods studied recording a maximum percentage increase of 231.1%. The maximum change in glucose level was concurrent with that of insulin level at the end of the third week of the experiment (Table 1). Strong negative correlation (r= -0.8) was found between glucose and insulin levels in streptozotocin-induced diabetic rats allover the experimental periods studied.

Table 1: Serum insulin level (µU/ml) and serum glucose levels (mg/dl) in control, streptozotocin-diabetic albino rats at different time intervals.

Parameter	Experimental period (Week)			
	1	2	3	4
Insulin (µU/ml)				
Control	1.33±0.05	1.28±0.05	1.38±0.06	1.35 ±0.04
Diabetic	1.0±0.09	0.88±0.07	0.92±0.09	0.95±0.07
% change	-24.8	-31.3	-33.3	-29.6
P	<0.01	<0.001	<0.01	<0.01
Glucose (mg/dl)				
Control	101.3±6.4	107.8±7.1	103.5±5.7	104.6 ±5.2
Diabetic	301.2±11.3	331.4±13.1	342.7±12.7	328.2±11.0
% change	197.3	207.4	231.1	213.8
P	<0.001	<0.001	<0.001	<0.001

All values are expressed as means ± Std. Error Mean.

Serum triglyceride and cholesterol levels

Serum triglycerides and cholesterol levels were found to be increased in diabetic rats recording percentage increases of 27.3 and 25.6%, respectively at the end of the experiment (Table 2).

Table 2: Serum triglyceride and cholesterol level (mg/dl) in control, streptozotocin-diabetic albino rats at different time intervals.

Parameter	Experimental period (Week)			
	1	2	3	4
Triglyceride (µU/ml)				
Control	65.4±3.1	59.8±3.2	66.7±2.7	68.2±3.0
Diabetic	73.1±3.7	70.3±2.8	83.4±3.3	86.8±3.6
% change	11.8	17.6	25.0	27.3
P	>0.05	<0.05	<0.01	<0.01
Cholesterol (mg/dl)				
Control	80.3±3.2	82.1±2.8	83.8±2.9	78.6±3.1
Diabetic	88.4±2.9	93.8±3.1	101.5±3.2	98.7±3.0
% change	10.1	14.3	21.1	25.6
P	>0.05	<0.05	<0.01	<0.01

All values are expressed as means ± Std. Error Mean.

Kidney Parameters

Diabetic animal showed significant increase in serum urea concentrations with a percentage increase of 33.0% at the end of the experiment. In contrast, uric acid and creatinine showed significant decreases with percentage of 17.5 and 23.7%, respectively (Table 3).

Table 3: Serum urea, uric acid and creatinine concentrations (mg/dl), and serum level (mg/dl) in control, streptozotocin-diabetic albino rats at different time intervals.

Parameter	Experimental period (Week)			
	1	2	3	4
Urea (mg/dl)				
Control	33.6±1.6	37.4±1.9	34.8±1.8	35.1±1.7
Diabetic	41.5±2.2	51.8±2.7	47.1±2.5	46.7±2.1
% change	23.5	38.5	35.3	33.0
P	<0.05	<0.01	<0.01	<0.01
Uric acid (mg/dl)				
Control	1.72±0.08	1.76±0.07	1.69±0.06	1.77±0.08
Diabetic	1.65±0.10	1.57±0.09	1.44±0.08	1.46±0.10
% change	-4.1	-10.8	-14.8	-17.5
P	>0.05	>0.05	<0.05	<0.05
Creatinine (mg/dl)				
Control	0.62±0.02	0.58±0.03	0.61±0.02	0.59±0.02
Diabetic	0.60±0.03	0.50±0.02	0.48±0.02	0.45±0.03
% change	-3.2	-13.8	-21.3	-23.7
P	>0.05	<0.05	<0.01	<0.01

All values are expressed as means ± Std. Error Mean.

Liver enzymes

Serum aspartate aminotransferase and alanine aminotransferase exhibited significant increases with percentage increases of 20.4 and 26.5%, respectively in diabetic rats at the end of experimental periods studied compared to controls (Tables 4). However, the increment in alanine aminotransferase activity was more pronounced than that observed for aspartate aminotransferase.

Table 4 shows serum alkaline phosphatase activity in control, streptozotocin-induced diabetic rats at different time intervals. The enzyme activity was significantly elevated in diabetic rats compared to controls.

Table 4: Serum AST, ALT and ALP activities (U/L) in control, streptozotocin-diabetic albino rats at different time intervals.

Parameter	Experimental period (Week)			
	1	2	3	4
AST (U/L)				
Control	83.3±3.6	87.9±3.8	86.1±4.2	88.2 ±2.9
Diabetic	92.6±4.3	104.9±4.6	105.7±5.2	106.2±5.5
% change	11.2	19.3	22.8	20.4
P	>0.05	<0.05	<0.05	<0.05
ALT (U/L)				
Control	35.4±1.3	38.5±1.5	36.7±1.4	39.2 ±1.5
Diabetic	41.8±2.1	49.4±2.3	45.8±1.9	49.6±2.5
% change	18.1	28.3	24.8	26.5
P	<0.05	<0.01	<0.01	<0.01
ALP (U/L)				
Control	59.5±2.4	58.0±2.6	59.3±2.1	54.8 ±1.8
Diabetic	66.2±3.2	75.3±3.5	81.3±3.4	69.7±3.0
% change	11.3	29.8	36.8	27.2
P	>0.05	<0.01	<0.001	<0.01

All values are expressed as means ± Std. Error Mean.

Discussion and Conclusion

Most of rats developed hypoinsulinemia and hyperglycemia following streptozotocin injection and it persisted throughout the whole experimental duration. Strong negative correlation (r=-0.8) was found between insulin and glucose levels. Similar results were reported (17, 18).

Serum triglycerides and cholesterol levels were found to be increased in streptozotocin-induced diabetic rats allover the experimental periods studied. This result is in accord with other studies (19, 20). The abnormal high concentrations of serum lipids in the diabetic subject is due, mainly to increase in the mobilization of free fatty acids from

the peripheral fat depots, since insulin inhibits the hormone sensitive lipase (21). These effects may be due to low activity of cholesterol biosynthesis enzymes and or low level of lipolysis which are under the control of insulin. Hypercholesterolemia and hypertriglyceridemia have been reported to occur in streptozotocin diabetic rats (21).

Kidney function is impaired in diabetes mellitus. Diabetic nephropathy is the leading cause of end-stage renal disease. The experimental diabetes induced by streptozotocin in the present study seems to be a model of incipient diabetic nephropathy. This observation is in agreement with several authors (22, 23).

Data revealed marked elevation in serum urea concentrations in streptozotocin-induced diabetic rats. Similar effect was recorded (24). The increase serum urea concentrations may be due to depletion of serum protein, increase in the rate of circulating amino acids and deamination takes place that eventually converted to urea. The breakdown of amino acids during gluconeogenesis in the liver results in increased production of urea, fostering negative nitrogen balance (25).

In contrast, serum uric acid and creatinine concentrations were decreased in diabetic animals. The decrease in serum uric acid concentrations may be due to either a decrease in uric acid production or an increase in its excretion and due to defects in tubular absorption caused by the nephrotoxic effect of streptozotocin. Serum uric acid has been recently associated with insulin resistance (26).

Creatinine is an anhydride of creatine (an end product of protein metabolism). It appears in serum in amounts proportional to the body's muscle mass and easily excreted by the kidneys (27). Creatininuria occurs in any condition associated with extensive muscle breakdown as in starvation and poorly controlled diabetes mellitus (25). The decrease in serum creatinine concentrations may be due to streptozotocin nephrotoxicty.

Serum transaminases and alkaline phosphatase activities were generally increased in streptozotocin-induced diabetic rats during the experimental periods studied. Similar data were recorded (28, 29). Increase in the activities of liver enzymes may be due to streptozotocin toxicity, extensive tissue destructions, disturbances in the transphosphorylation and in the general metabolism of the different cells and tissues of diabetic rats (30). It is known that elevation of transaminases could be a common sign of impairment in liver function.

In conclusion, administration of streptozotocin to albino rats caused damage to pancreatic β cells resulting in hypoinsulinemia and hyperglycemia. In diabetic animals triglycerides and cholesterol levels were elevated; urea concentrations were increased. In contrast, uric acid and creatinine concentration were decreased. Also, transaminases and alkaline phosphatase were elevated In diabetic animals indicating impairment of liver function. The streptozotocin model can be considered as one of the suitable animal models with diabetes mellitus with nephropathy. These findings could open a new avenue of research in the treatment of human disease.

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