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# EFFECT OF SHORT VERSUS LONG -TERM DIABETES ON ISCHEMIC HEART OF EXPERIMENTAL ANIMALS

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## INTRODUCTION

Coronary artery disease is a major complication of diabetes that correlates with high morbidity and mortality after myocardial infarction **(Abbud et al., 1995)**. Experimental studies of diabetes effects on ischemia/ reperfusion injury have shown contradictory results, including a decrease, **(Feuvray and Lopaschuk, 1997)** increase **(Paulson, 1997)** or no change **(Pijl et al., 1994)** in sensitivity. These contradictory results appear to be due to differences in the duration and severity of the diabetic state. Studies have shown that the diabetic heart is resistant to ischemia/reperfusion injury during early phases of the disease, and becomes more sensitive to ischemia/reperfusion injury as the disease progresses **(Ravingerova et al., 2003; Xu et al., 2004)**.

Several mechanisms have been proposed to explain why diabetic hearts are less sensitive to ischemia/reperfusion injury than control hearts. The diabetic heart may accumulate less sodium and calcium, which could explain protection of contractile function. **(Ramasamy and Schaefer, 1999)** Diabetes may also increase the myocardial content of free-radical-scavenging enzymes, which could also account for this phenomenon **(Wohaieb and Godin, 1987).** Another possibility is that early diabetes may induce angiogenesis, leading to increase capillary artery development, an adaptive response, which could contribute to reducing myocardial ischemia/reperfusion injury. Specific factors are known to stimulate angiogenesis, including vascular endothelial growth factor (VEGF) and nitric oxide (NO) **(Fam et al., 2003)**. Reactive oxygen species (ROS) are also involved in the signaling pathways mediating many stress growth responses including angiogenesis **(Griendling et al., 2000).** In the heart of diabetics, vascular endothelial growth factor expression has been reported to be both decreased **(Chou et al., 2002)** and increased **(Sasso et al., 2003)**. Further study is required to directly relate vascular endothelial growth factor expression to the effect of diabetes on cardiac ischemia/reperfusion injury.

Protein kinase B (Akt) functions as a downstream kinase of vascular endothelial growth factor to promote survival in endothelial cells and medi-

ate nitric oxide production through the direct phosphorylation of endothelial cell nitric oxide synthase **(Six et al., 2002).** Its activation has been linked to protection against ischemia/reperfusion injury and cell death **(Shiraishi et al., 2004).** The relationship between diabetic ischemia/reperfusion injury and P-Akt activation remains to be elucidated.

We hypothesize that short-term diabetes is associated with reduced sensitivity of the heart to ischemia/reperfusion injury. This protection is related to stimulation of angiogenesis and activation of cell survival signals that inhibit cell apoptosis.

## MATERIALS AND METHODS

**Animals.** Male Sprague-Dawley weighing on average 250 g were randomly assigned to different experimental groups: two and six weeks streptozotocin-treated groups (two-week diabetes and six-week diabetes); two and 6 weeks streptozotocin groups subjected to ischemia/reperfusion (2WD+I/R and 6WD+I/R); and time-matched control groups (2WC, 6WC, 2WC+I/R and 6WC+I/R). Diabetes was induced by tail vein injection of streptozotocin (55 mg/kg body weight), which was dissolved in sodium citrate buffer solution (0.01 M, pH 4.5). The time-matched control groups were injected with citrate buffer alone. Blood samples were collected from the tail vein three days after streptozotocin administration and before the ischemia/reperfusion experiment to measure blood glucose. At the same times, urine samples were collected to test urine glucose. Rats were defined as diabetic when blood glucose concentration exceeded 22 mmol/L and urine glucose was positive three days after the streptozotocin injection. Insulin (human insulin, 4 U/day) was administered s.c. to diabetic animals to prevent ketoacidosis.

**Models of Acute Myocardial Ischemia and Reperfusion.** Rats were anaesthetized with ketamine HCl (100 mg/Kg, ip) and xylazine (10 mg/kg, ip). The right jugular vein was cannulated for the delivery of saline. The left carotid artery was cannulated for the measurement of blood pressure and heart rate. Pressure and heart rate measurements were monitored using transducer connected to polygraph. The trachea was then cannulated and connected to a rodent ventilator (model 683, Harvard Apparatus, South Natick, MA). Rats were ventilated with room air at 65 breaths/min supplemented with $O_2$. Atelectasis was prevented by maintaining a positive end-expiratory pressure of 10 mm $H_2O$. Body temperature was maintained at 37°C using a heating pad. Once heart rate and blood pressure stabilized, a left thoracotomy was performed at the fifth intercostal space. A pericardiotomy was then performed and a ligature (6-0 silk suture) was passed below the left descending coronary artery close to its origin immediately below the left atrial appendage to the right portion of the left ventricle. The ends of the suture were threaded through a polyethylene tube to form a snare. Occlu-

sion for a period of 30 minutes was elicited by pulling on the snare and clamping the snare onto the epicardial surface using a hemostat. This resulted in left ventricular ischemia. Coronary artery occlusion was confirmed by epicardial cyanosis and a decrease in blood pressure. Reperfusion for a period of 2-hr was achieved by unclamping the hemostat and loosening the snare.

**Determination of infarct size**. Left ventricle, infarct size (IS) and area at risk (AAR) were determined as described previously **(Barbosa et al., 1996; Maulik, 2002).** After the 2-hr period of reperfusion, the coronary artery was again occluded using the snare. Area at risk was determined by negative staining with Evans blue dye (2%), which was injected into the left ventricle cavity and allowed to perfuse it. The entire heart was excised, rinsed of excess blue dye, followed by trimming of right ventricular and atrial tissue and slicing into transverse sections 2 mm thick. Slices were incubated in a 1% solution of 2, 3, 5-triphenyltetrazolium chloride (TTC) in PBS buffer for 12 minutes to stain viable myocardium to a brick red color. The slices were then fixed in a 10% formalin solution for 24 hours and both sides of each slice were photographed. The ischemic area at risk (unstained by Evans blue dye) and the infarcted area (unstained by TTC) were outlined on each photograph and measured using Metamorph Image Software. The area from each region was averaged from the photographs of each side for each slice. Area at risk was expressed as a percentage of total left ventricle. Infarct size was expressed as a percentage of the area at risk.

**Western blot analysis.** The area at risk of left ventricle from each treatment group was separated from the rest of the heart, and homogenized with cold RIPA buffer containing 1% protease inhibitors cocktail. The tissue lysate then, centrifuged at 10,000 g for 30 minutes at 4°C. Total protein concentration was measured using the Bio-Rad protein assay kits. Protein samples (100 µg) were loaded in a 10% SDS-PAGE gel and separated by electrophoresis. The separated proteins were transferred onto a pure nitrocellulose membrane. The membranes were blocked in 5% milk overnight and then probed with specific antibodies against vascular endothelial growth factor, phospho- Akt, or cleaved caspase 3. The membrane then reacted with 2ry antibody. The reaction was detected using enhanced chemiluminescence and visualized by exposure to X-ray film. Equal loading was detected by re-probing the membrane with the anti-ß-actin antibody as an internal control for protein loading. Densitometric quantification was also performed with the use of Image Quant software.

**Vascular density assay:** Hearts were removed at the end of the experiment, dipped in OCT solution, frozen in liquid nitrogen and stored at -80 °C until used. Vascular density was studied using 20 µm-thick frozen sections from the area at risk in the left ventricle. Cardiac sections were treated

with Isolectin B4; a specific marker of blood vessels that bind endothelial cells. The binding of Isolectin B4 was visualized by reaction with Texas Red. Vascular density then was assessed using Metamorph Image System.

**Statistical measurements.**

All values are expressed as means ± SEM. Statistical analysis was performing using ANOVA factorial test of Stat View Statistical Software. Significant differences were attributed between groups with $P < 0.05$.

## RESULTS

**Animal data:**

Table (1) summarizes the animal data including body and heart weights, and levels of blood glucose in each group. In the two-week diabetes and six-week diabetes groups, blood glucose was elevated, whereas, body weight increased slightly (two-week diabetes) or decreased (six-week diabetes). Heart weights decreased only in six-week diabetes group. The mean blood glucose levels in two-week diabetes and six-week diabetes groups were 29 ± 1.9 and 27.6 ± 1.8 mmol/L, respectively. However, levels in time-matched control groups at 2 and 6 weeks were 6.1 ± 0.3 and 6.3 ± 0.2 mmol/L.

**Hemodynamic data**

The values of mean blood pressure (MBP) and heart rate (HR) in the diabetic and control groups, determined at baseline, and during the course of ischemia and reperfusion are summarized in (Table 2). In two-week diabetes rats, values for heart rate and mean blood pressure at baseline, during ischemia and after reperfusion were significantly decreased compared to time matched controls. In six-week diabetes, values for heart rate and mean blood pressure at baseline, during 30 minutes ischemia and after reperfusion did not show significant differences compared with six-week control rats. Both two-week diabetes and six-week diabetes group mean blood pressure were significantly decreased during ischemia and after reperfusion compared with the values at baseline.

**Infarct size and area at risk decreased in two-week diabetic rats**

Infarct size was determined by TTC staining as a proportion of the area at risk. In the two-week diabetes rats hearts' infarct size was significantly smaller compared with time matched controls (34.5 ± 3% *vs* 50.9 ± 3.3 % of the area at risk in control) (Figs. 1- A, B). The area at risk in these two groups (two-week diabetes & two-week control) were not significantly different (52 ± 2% *vs* 60 ± 4% of left ventricle in control). Both infarct size

and area at risk were not significantly different between six-week diabetes and six-week control rats. The infarct size in both groups (six-week diabetes & six-week control) were significantly larger than that in the two-week diabetes rats (56.1 ± 2.1% and 53.4 ± 2.3% vs 34.5 ± 3%).

**Cardiac Vascular density increased in 2 weeks diabetic rats**

To determine the vascular density in the rat hearts, the cardiac vessels were labeled with Isolectin B4 (Fig. 2-A). Morphometric analysis showed that vascular density in the two-week diabetes heart was significantly greater (28% increase) than in the two-week control group. The cardiac vascular density was the same in six-week control and six-week diabetes rats (Fig. 2-B).

**Effect of diabetes on cardiac vascular endothelial growth factor expression**

Western blot analysis showed that vascular endothelial growth factor expression was elevated in the two-week diabetes group by about 30% compared with two-week control rats (Fig. 3-A). Furthermore, there was 29% less vascular endothelial growth factor expression in six-week diabetes rats compared with six-week control rats.

**Effect of diabetes on cardiac cell survival and apoptosis markers**

a. ***Phosphorylation of Akt (survival marker):*** Phosphorylation of Akt is an indicator of an active prosurvival pathway. Western blot analysis showed that P-Akt in the two-week diabetes group was increased by 35% as compared with the two-week control group. However, in six-week diabetic and time matched control hearts, there was no significant difference in P-Akt expression (Fig. 3 B).

b. ***Caspase-3 (Apoptosis marker):*** Cardiac cell apoptosis was assessed by Western blotting for cleaved caspase-3 (Fig. 4). At baseline, cleaved caspase-3 was not different between two-week diabetes and two-week control groups. However, after ischemia and reperfusion the amount of cleaved caspase-3 in the two-week diabetes group was lower (53%) than that in two-week control group. There were no differences in caspase-3 between six-week control and six-week diabetes rats after ischemia/reperfusion, but baseline levels were markedly elevated (43%) in six-week diabetes rats.

**Table (1):** Body Weight, Left Ventricle Weight and Blood Glucose of Diabetic and Control Rats.

| Time (wk) | Group | N | Initial body weight (g) | Body Weight (g) | Left Ventricle Weight (g) | Blood Glucose (mmol/L) |
|---|---|---|---|---|---|---|
| 2 | Control | 11 | 257 ± 4 | 312 ± 3 # | 0.70 ± 0.02 | 6.1 ± 0.3 |
| | Diabetic | 11 | 259 ± 4 | 276 ± 3 * # | 0.66 ± 0.01 | 29 ± 1.9 * |
| 6 | Control | 8 | 261 ± 5 | 391 ± 6 # | 0.90 ± 0.03 | 6.3 ± 0.2 |
| | Diabetic | 8 | 273 ± 6 | 250 ± 16 * # | 0.60 ± 0.02 * | 27.6 ± 1.8 * |

Values are means±S.E.M.
* $p < 0.05$; Diabetic *vs.* the time matched control group.
# $p < 0.05$ *vs.* initial group

**Table (2):** Heart Rate (Beats/Minute) and Mean Blood Pressure (mmHg) of Diabetic and Control Rats.

| Parameters | Time (wk) | Group | N | Baseline | Ischemia | Reperfusion |
|---|---|---|---|---|---|---|
| Heart Rate (beats/minutes) | 2 | Control | 11 | 246 ± 11 | 247 ± 15 | 240 ± 12 |
| | | Diabetic | 11 | 207 ± 8 * | 211 ± 10* | 191 ± 17* |
| | 6 | Control | 8 | 233 ± 9 | 245 ± 9 | 228 ± 12 |
| | | Diabetic | 8 | 220 ± 14 | 221 ± 15 | 219 ± 9 |
| Mean Blood Pressure (mmHg) | 2 | Control | 11 | 105 ± 4 | 88 ± 3 # | 97± 2 |
| | | Diabetic | 11 | 91 ± 5 * | 76 ± 2 *# | 68 ± 5 *# |
| | 6 | Control | 8 | 111 ± 9 | 85 ± 8 # | 85 ± 7 # |
| | | Diabetic | 8 | 115 ± 6 | 83 ± 6 # | 79 ± 5 # |

Values are means ± S.E.M.
* $p < 0.05$; Diabetic *vs.* control group
# $p < 0.05$ *vs.* Baseline

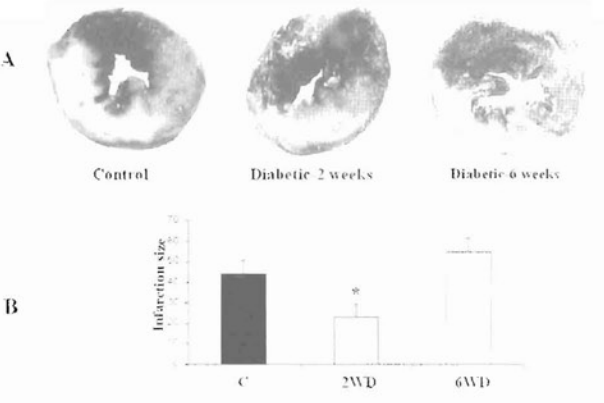


**Fig. (1-A):** Cross sections of hearts from control, two-week and six-week diabetic rats that perfused with Evan blue dye and stained with t-triphenyltetrazolium chloride (TTC). The infarction area (white) and area at risk (pink) was reduced in two-week diabetic rat compared to the normal and six-week diabetic. The area perfused with Evan blue dye was bigger in two-week diabetic. **B:** statistic analysis showed a significant reduction in both the infarction size and area at risk in two-week diabetic rats. (* $P < 0.05$).

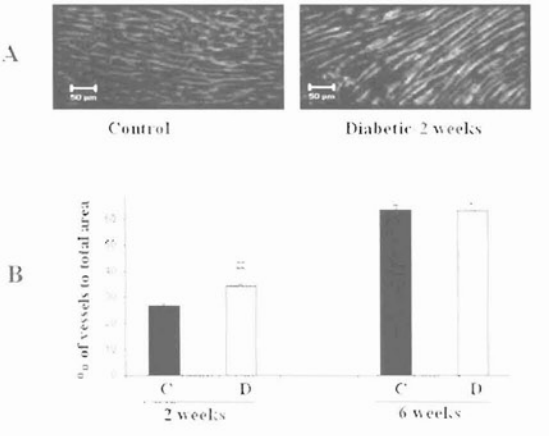


**Fig. (2-A):** Myocardiac vessels are labeled with isolectin B4; a specific endothelial cells marker. Note more labeled vessels in the heart of two-week diabetic rat compared with the control one. **B:** computer-assisted morphometric analysis of vascular density showing a significant increase in vascular density in the heart of two-week diabetic animal versus control while the vascular density is not changed in six-week diabetic group compared to the age-matched control. (* $P < 0.05$).

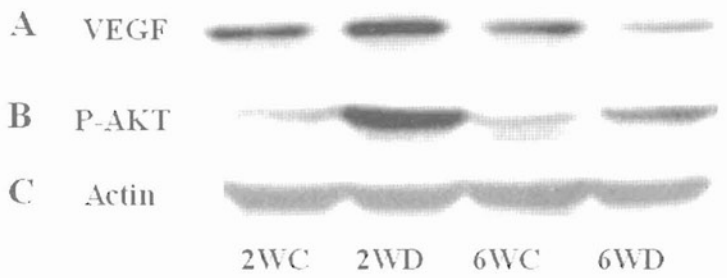


**Fig. (3-A):** Western blotting analysis of vascular endothelial growth factor (VEGF) shows a marked increase in VEGF expression in heart tissues of two-week diabetic rats compared with control and six-week diabetic animals. **B:** western blotting analysis of phospho AKT shows an overexpression in two-week diabetic animal. **C:** Immunoblotting using anti-actin shows equal loading of the protein samples for normalization.


**Fig. (4-*A*):** Western blotting analysis of caspase 3, an apoptotic marker shows a marked decrease in heart tissue of two-week diabetic rats compared to control and six-week diabetic ones. **B:** Immunoblotting with anti-actin shows equal loading of the protein samples for normalization.

## DISCUSSION

Clinical studies have indicated higher rates of mortality of diabetic patients due to cardiac ischemia and reperfusion than non-diabetic patients. Animal studies, on the other hand, have showed varied changes in sensitivity to cardiac ischemia/reperfusion injury in diabetes. One factor that might determine the outcome of the ischemia/reperfusion injury is the duration of the diabetic state. The present study was designed to determine how diabetes affects susceptibility of rat hearts to ischemia/reperfusion injury in early stages of the disease. Our results showed that after two weeks of diabetes, rats had reduced infarct size and cardiac myocyte apoptosis after ischemia/reperfusion as compared to control rats. After six weeks of diabetes, there were no differences in infarct size or cardiomyocyte apoptosis. This result demonstrates that the diabetic heart is more resistant to ischemia/reperfusion injury in the early phase of experimental diabetes (two weeks after onset). However, protection does not extend to hearts of rats that have been diabetic for six weeks. These findings confirmed our hypothesis that early diabetes is associated with reduced sensitivity of the heart to ischemia/reperfusion injury. This result is also consistent with other recent studies **(Ziegelhoffer et al., 2002; Ravingerova et al., 2003; Xu et al., 2004).**

Myocardial ischemia/reperfusion injury is promoted by either an increase in oxygen demand or decrease in oxygen supply. It is notable that our study showed that mean blood pressure and heart rate in the two-week diabetic rats were significantly lower than the control group. It is possible that this might have reduced systolic function and lowered energy consumption and oxygen demand in diabetic hearts.

The presence of adequate blood vessels feeding the myocardial area at risk may limit the infarct size following coronary occlusion and may even provide a survival benefit **(Ramanathan et al., 1995)**. Angiogenesis can contribute to enhanced tissue perfusion **(Ito et al., 1997)**. Generation of new vascular channels by angiogenesis has been shown in both animal models of myocardial ischemia and in patients with coronary disease **(Sasayama and Fujita, 1992; Martin et al., 2003)**. While study of coronary capillary network remodeling in aged obese diabetic rats showed that total capillary density in diabetic was significantly higher than that of controls at early stages, capillary density fell as they progressed to chronic stages **(Sugawara et al., 2003)**. This study showed that cardiac vascular density was significantly increased in the two-week diabetic rats compared with controls, but vascular density in diabetic rats fell to control levels after six weeks. This increase in vascular density was consistent with reduced infarct size in two-week diabetic rats. The increased capillary vessels would preserve perfusion to ischemic myocardium and thereby maintain function. Specific factors are known to stimulate angiogenesis. Thus, we determined vascular endothelial growth factor and endothelial nitric oxide synthase ex-

pression and nitric oxide production in the diabetic and control rat hearts. Vascular endothelial growth factor, an endothelial cell cytokine, is known to attenuate myocardial ischemia/reperfusion injury, in addition to promoting angiogenesis **(Luo et al., 1997)**. Vascular endothelial growth factor also functions as a promoter of endothelial cell migration and an anti-apoptotic, endothelial cell survival factor **(Alon et al., 1995)**. Furthermore, vascular endothelial growth factor has been shown to stimulate the release of nitric oxide and to upregulate the expression of nitirc oxide synthase **(Cooke and Losordo, 2002)**. Vascular endothelial growth factor is known to be elevated in the retina and kidney of diabetic animals **(Cooper et al., 1999; Caldwell et al., 2003)**. Our result showed that vascular endothelial growth factor expression is significantly higher in two-week diabetes rats but it is significantly decreased in the six-week diabetes rats versus control. Vascular endothelial growth factor expression has been reported to be decreased in four weeks diabetes heart **(Chou et al., 2002)**. This means that diabetes causes an early transient increase in vascular endothelial growth factor expression. Furthermore, our results show that the increased vascular endothelial growth factor expression after two weeks of diabetes was also associated with an increase in vascular density.

Protein kinase B (Akt) functions downstream of vascular endothelial growth factor to promote survival in endothelial cells and mediate nitric oxide production through the direct phosphorylation of endothelial nitirc oxide synthase **(Six et al., 2002)**. Activation of Akt has been linked to protection against ischemia/reperfusion injury and cell death **(Shiraishi et al., 2004)**. Activation of Akt significantly elevated levels of phospho-Akt, which could act to inhibit apoptosis. Our result showed that levels of phosphorylated Akt were increased in two-week diabetes rats compared with controls, but no different between six-week diabetes and controls. This finding supports the idea that higher P-Akt levels are related to smaller infarct size and less apoptosis in the heart after ischemia/reperfusion at an early stage of diabetes. **Kajstura et al., (1996)** have argued that apoptosis is the dominant mechanism of cell death in rats undergoing coronary artery occlusion. Recently, hyperglycemia was shown to induce apoptosis in streptozotocin-induced diabetes in rats and mice **(Fiordaliso et al., 2000; Cai et al., 2002)**. In our study, cleaved caspase-3 was not different between the two-week diabetic and control groups but decreased after ischemia/reperfusion in these diabetic rats. However, at six weeks, baseline cleaved caspase-3 levels were higher in diabetic than in control rats.

Our result is consistent with another study **(Backlund et al., 2004)** where the level of myocyte apoptosis was not different between early stage diabetic and control rats, but increased at later stages of the disease. **Ho et al., (2000)**, have also demonstrated that exposure of endothelial cells to high levels of glucose caused significant reactive oxygen species formation in association with caspase-3 activation and apoptosis.

## SUMMARY

The effect of diabetes on ischemia/reperfusion (I/R) injury is controversial. Some studies show resistance to I/R injury during diabetes but others show increased sensitivity to I/R injury. Our aims were to determine the effects of I/R on myocardial infarction in diabetes and to determine if sensitivity to I/R injury is related to diabetes-induced alterations in cardiac vascular density, oxidative stress or cell survival signaling. Diabetes was induced in rats by streptozotocin injection and rats were examined two and six weeks after treatment. I/R injury was induced by occlusion and reperfusion of the left descending coronary artery. Size of I/R-induced infarct was determined using triphenyltetrazolium chloride (TTC) staining. Two weeks after streptozotocin treatment, infarct was decreased in the diabetic hearts (2WD) as compared with time-matched control group (2WC). Whereas after six weeks of diabetes (6WD), the infarct size was increased in the diabetic hearts as compared with the two-week diabetes group, but not significantly different than time-matched control (6WC). Morphometric analysis showed vascular density in the heart was significantly increased in two-week diabetes group as compared with the two-week control, while there was no difference in six-week diabetes compared with six-week control group. Vascular endothelial growth factor expression increased in two-week diabetes group compared with two-week control, but was lower in six-week diabetes rats than in six-week control rats. Phosphorylation of protein kinase B (AKT), a process involved in cellular protection was increased in two-week diabetes group over two-week control values, whereas there was no difference in P-Akt and endothelial nitirc oxide synthase expression in six-week diabetes and six-week control groups. Apoptosis induced by ischemia/reperfusion, determined by cleavage of caspase-3, was decreased in two-week diabetes group compared with two-week control group, while caspase-3 increased in the six-week diabetes group compared with the two-week diabetes group but was unchanged from the six-week control group. These data suggest that short-term diabetes protects the heart from ischemia/reperfusion injury through stimulation of angiogenesis and activation of cell survival signals.

## CONCLUSION

In conclusion our study showed that short-term diabetes induces ischemic preconditioning in the myocardium so, the sensitivity of the heart to ischemia/reperfusion injury decreases. This protection effect of short-term diabetes could be through vascular endothelial growth factor-dependent formation of new collaterals and activation of cell survival signaling and inhibition of apoptosis.

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## الملخص العربى

## مقارنة تأثير مدة مرض السكر على قصور الدموية الموضعية لعضلة القلب فى حيوانات التجارب

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يعتبر قلة الدموية الموضعية / وعودة سريان الدم الناتج عن تأثير مرض السكر محل خلاف بين العلماء. فقد أظهرت بعض الدراسات مقاومة للضرر الناشئ عن قلة الدموية الموضعية / وعودة سريان الدم فى ظل وجود مرض السكر بينما أظهرت دراسات أخرى نتائج مضادة.

**الهدف من الدراسة:** إن الهدف من هذه الدراسة هو معرفة تأثير الضرر الناشئ عن قلة الدموية الموضعية / وعودة سريان الدم على إحتشاء عضلة القلب فى ظل وجود مرض السكر ومعرفة ما إذا كانت الحساسية للضرر الناشئ عن قلة الدموية الموضعية/ وعودة سريان الدم مرتبطة بتأثير مرض السكر على كثافة الأوعية الدموية للقلب والإجهاد التأكسدى هو على إشارات بقاء الخلية.

**خطوات البحث:** تم إحداث مرض السكر فى فئران التجارب عن طريق حقن مادة الستريبتوزوتوسين وتم فحص الجرذان المصابة بالسكر فى الأسبوع الثانى والسادس بعد الحقن مع اختيار مجموعة ضابطة فى الحالتين.

تم إحداث الإصابة الناشئة عن قلة الدموية الموضعية/ وعودة سريان الدم عن طريق غلق ثم فتح الشريان الأمامى النازل وتم قياس المساحة المصابة من إحتشاء عضلة القلب الناتجة عن قلة الدموية الموضعية/ وعودة سريان الدم باستخدام الصبغ بمادة تراى فنيل تترازوليم كلوريد.

**نتائج الدراسة:** أثبتت الدراسة وجود انخفاض ذو دلالة إحصائية فى مساحة الجزء المحتشى من عضلة القلب فى مجموعة الفئران المصابة بالسكر لمدة أسبوعين مقارنة بالمجموعة الضابطة المناظرة. وعلى النقيض من ذلك فان مساحة الجزء المحتشى من عضلة القلب فى مجموعة الفئران المصابة بالسكر لمدة ست أسابيع كانت أكبر بدرجة

ذات دلالة احصائية مقارنة بمجموعة الفئران المصابة بالسكر لمدة أسبوعين بينما لـــم يوجد فرق ذو دلالة إحصائية بين مجموعة الفئران المصابة بالسكر لمدة ست أسابيع و المجموعة الضابطة المناظرة.

أثبت التحليل الشكلى أن كثافة الأوعية الدموية كانت أكبر بدرجة ذات دلالة إحصائية فى مجموعة الفئران المصابة بالسكر لمدة أسبوعين مقارنة بالمجموعة الضابطة المناظرة بينما لم يوجد فرق إحصائي بين مجموعة الفئران المصابة بالسكر لمدة ست أسابيع و المجموعة الضابطة المناظرة.

وجد أيضا من الدراسة أن فسفرة البروتين كاينازB ( ب ) وهو البروتين المسئول عن حماية الخلايا أكثر بدرجة ذات دلالة احصائية فى مجموعة الفئران المصابة بالسكر لمدة أسبوعين مقارنة بالمجموعة الضابطة المناظرة بينما لم يوجد فرق إحصائي بين مجموعة الفئران المصابة بالسكر لمدة ست أسابيع و المجموعة الضابطة المناظرة . بالمقابل فان تركيز مادة كاسباز (٣) وهو البروتين المسئول عن موت الخلية المقدر كانت أقل بدرجة ذات دلالة إحصائية فى مجموعة الفئران المصابة بالسكر لمدة أسبوعين مقارنة بالمجموعة الضابطة المناظرة بينما كان أكبر بدرجة ذات دلالة إحصائية فى مجموعة الفئران المصابة بالسكر لمدة ست أسابيع مقارنة بمجموعة الفئران المصابة بالسكر لمدة أسبوعين بينما لم يختلف بين مجموعة الفئران المصابة بالسكر لمدة ست أسابيع والمجموعة الضابطة المناظرة.

وقد أثبت البحث أن مرض السكر في مراحله الأولى قد يكون له تأثير مفيد فى خفض حجم الاحتشاء الناتج عن القصور وعودة السريان ويعزى هذا إلى زيادة تكوين الأوعية الدموية الناتج من زيادة تركيز العامل المحفز لتكوين الأوعية الدموية فى المراحل المبكرة من السكر لذا يجب تصميم أبحاث إكلينيكية لمعرفة ما إذا كانت هذه الظاهرة موجودة فى الإنسان ومحاولة الاستفادة منها.