Research Article | **Open Access**

Effect of Diabetes on Myocardial Infarct and No Reflow Size in an Experimental Rat Model and Clinical Trial

Wangde Dai[1,2*], Robert A Kloner[1,2], Jianru Shi[1,2], Juan Carreno[1], Serge Korjian[3], Yazan Daaboul[3], Michael C Gibson[3], Muriel Bouly[4] and Marc Isabelle[5]

[1]HMRI Cardiovascular Research Institute, Huntington Medical Research Institutes, 10 Pico Street, Pasadena, CA 91105, USA

[2]Division of Cardiovascular Medicine of the Keck School of Medicine, University of Southern California, Los Angeles, California 90017-2395, USA

[3]Department of Medicine, PERFUSE Study Group, Cardiovascular Division, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts, USA

[4]Institut de Recherches Internationale Servier, 50 rue Carnot, 92284, Suresnes Cedex, France

[5]Institut de Recherches Servier (IdRS), 3, rue de la République, 92150, Suresnes Cedex, France

*Corresponding author: Wangde Dai, HMRI Cardiovascular Research Institute, Huntington Medical Research Institutes, 10 Pico Street, Pasadena, California 91105, USA, Tel: 626-397-5812; E-mail: Wangdedai@yahoo.com

Abstract

Background: We determined the effect of diabetes on no reflow and myocardial infarct sizes in both an experimental rat model of diabetes and a contemporary trial of subjects with STEMI.

Methods: Adult Zucker Diabetic Fatty (ZDF) and Sprague Dawley (SD) rats (n=15 each group) were subjected to left coronary artery occlusion for 30 min followed by 3 h of reperfusion. In the clinical trial, the myocardial infarct (MI) size and the zone of microvascular obstruction were assessed in 258 non-diabetic MI patients and 34 diabetic MI patients.

Results: There was no difference in infarct size (median) in ZDF rats (49.9%) versus SD rats (59.6%; p=0.32); there was no difference in no-reflow size (mean ± SEM) in ZDF rats (32.5 ± 3.5%) versus SD rats (32.7 ± 4.3%; p=0.97). In the clinical study, CK-MB and Troponin I area under the curve at 72 h were comparable between the 2 groups. Infarct size by MRI on day 4 was 37.9 ± 1.8 ml in 216 non-diabetic patients and 34.8 ± 4.7 ml in 27 diabetic patients (p=0.559). The ratio of micro vascular obstruction on day 4 on the MRI was 0.179 ± 0.018 of the left ventricle in 200 non-diabetic patients and 0.220 ± 0.060 of the left ventricle in 23 diabetic patients.

Conclusions: Both animal and clinical studies demonstrated no evidence for a larger infarct size, or larger area of no reflow in the diabetic compared to non-diabetic conditions.

Keywords: Diabetes; Myocardial infarction; No-reflow

Introduction

Several studies suggest that clinical outcomes after acute myocardial infarction are worse in diabetics than non-diabetics [1,2]. Patients with diabetes have higher rates of heart and renal failure, cardiogenic shock and in hospital mortality compared to non-diabetic patients with acute coronary syndromes. In one pooled analysis from the TIMI group, there was nearly a doubling of 30-day mortality among diabetics compared to non-diabetics who suffered either ST elevation or unstable angina/non-ST elevation myocardial infarction [3]. The exact cause of this worse clinical outcome in diabetics remains controversial. Some studies suggested that diabetes is associated with worse signs of no reflow [4,5]. Other studies did not show that diabetes was associated with bigger infarcts or micro vascular damage [6]; but some studies showed that hyperglycemia on admission was associated with greater myocardial injury, and that this relationship was actually strongest in the non-diabetic patients [7]. Ota et al. [8] also showed that hyperglycemia on admission for ST elevation myocardial infarction was associated with micro vascular obstruction on cardiac magnetic resonance imaging. Thus there is remaining controversy

regarding the effect that diabetes has on myocardial infarct size and micro vascular obstruction (no reflow phenomenon). We determined the effect of diabetes on these two parameters in both an experimental rat model of diabetes as well as in a recent clinical trial in patients with STEMI. Our hypothesis was that since diabetes is associated with micro vascular disease that the size of the myocardial infarction and no-reflow areas in both animal model and in patients would be larger in the diabetic cohorts.

Methods

Experimental study

All rat studies were done in the Dr. Kloner Laboratory at the Huntington Medical Research Institutes, Pasadena, and were approved by the Institutional Animal Care and Use Committees at Huntington Medical Research Institutes. This investigation was performed in accordance with the guidelines for the care and use of laboratory animals (NIH publication No. 85-23, National Academy Press, Washington DC, revised 2011).

Adult male Zucker diabetic fatty (ZDF) rats (n=15) were obtained from Charles River Company. ZDF rats were fed a standard Purina

5008 diet. On the day of surgery, the rats (22.3 ± 1 weeks old) were anesthetized with intraperitoneal ketamine (75 mg/kg) and xylazine (5 mg/kg) and mechanically ventilated. Their necks were shaved and cleaned and cut-downs were performed over the jugular vein and carotid artery. Catheters were inserted into the jugular vein for sampling of blood and drug delivery and carotid artery for arterial wave form monitoring. A sample of blood was obtained for blood glucose levels. Under clean conditions, the chest cavity was opened through an incision in the 4th left intercostal space to expose the heart. The pericardium was gently removed exposing the anterior surface of the left ventricle. A suture was placed under the proximal portion of the left coronary artery as it ran through the interventricular groove just under the tip of the left atrial appendage. The ends of the suture were threaded through a small plastic tube and the tube was clamped to induce 30 min of coronary artery occlusion. Reperfusion of the epicardial coronary artery was induced by releasing the clamp and watching the surface of the heart for reactive hyperemia. The hearts were reperfused for 3 h.

In order to assess the distribution and size of the anatomic no-reflow zone (the zone of micro vascular obstruction) 4% thioflavin S (0.3 ml) was injected into the jugular vein during the last one minute of reperfusion. Thioflavin S is a dye that appears yellow green in areas receiving blood perfusion, when the heart slices are visualize under ultraviolet light; non-fluorescent perfusion defects represent the anatomic zones of no reflow that appear black. At the end of reperfusion, the proximal coronary artery was briefly re-occluded and the blue dye (Super Imperse Blue) was injected into the jugular vein with the coronary artery re-occluded. Blue dye circulates only to the perfused areas and does not reach the ischemic zone (which appears pink when viewing the heart slices under standard white light). At the end of this step, IV KCL is injected intravenously while the rats are under deep anesthesia, in order to stop the heart in a relatively diastolic state. The heart was excised, excess fat was cleared from the surface of the heart, the heart was gently washed in clear saline, and then transected into 4 transverse slices from apex to base. The heart slices were photographed under white light in order to determine the ischemic risk zone (pink) in contrast to the nonischemic regions receiving blue dye. The heart slices were then photographed under ultraviolet light in order to delineate the areas of perfusion by thioflavin S (fluorescent areas) versus the no-reflow zones (non-fluorescent perfusion defects). Finally, the heart slices were incubated in 1% triphenyltetrazolium chloride (TTC) at 37°C for 15 min; TTC is a chemical that stains viable cells brick red, while dead or necrotic cells appear white or pale. The heart slices were again photographed under white light. The photographs were used for planimetry in order to determine the percentage of each heart slice that was at risk, infarcted or contained no-reflow. Planimetered photographs were then corrected for weight of each heart slice and then the percentage of each left ventricle that was at risk (ischemic), demonstrated no-reflow, and was necrotic was calculated. Myocardial infarct size was expressed as the percentage of the left ventricle that went on to develop necrosis; the no-reflow zone was expressed as a percentage of the left ventricular risk zone and percentage of the necrotic zone. Heart rate and blood pressure were monitored throughout the protocol.

We compared the endpoints, including ischemic risk zone, infarct and no-reflow size, body temperature, heart rate, and blood pressure in the diabetic male ZDF rats to the same parameters from a recent study of non-diabetic adult female Sprague Dawley (SD) rats (n=15, 10.7 ± 0.2 weeks old). The SD rats were also subjected to 30 min of left coronary artery occlusion followed by 3 h of reperfusion. All of the surgical procedures and measurements in ZDF and SD rats were performed by the same investigators. All of the data were collected in a blinded fashion. Ischemic risk area less than 15% was excluded from the study.

Clinical study

We then compared infarct size and the extent of reperfusion injury in a subset of patients from a contemporary study of anterior myocardial infarction who underwent successful percutaneous coronary intervention (PCI) (ClinicalTrials.govNCT01572909). The rationale and methods for the EMBRACE STEMI trial have been previously reported [9]. This study was approved by the local institutional review committees, and all subjects provided informed consent.

The clinical study determined myocardial infarct size by both biomarkers (creatine kinase-myocardial band, CK-MB area under the curve over 72 h (258 non-diabetic patients and 34 diabetic patients) and serum troponin I under the curve over 72 h), and using cardiac magnetic resonance imaging (MRI) at 4 days. MRI contrast injection at 4 days was utilized to assess size and the zone of micro vascular obstruction (MVO), which previously was shown to be similar to the no reflow zones assessed in experimental studies using thioflavin S [10]. Here we present the data in MI patients with versus without diabetes (Type 1 or Type 2), on myocardial infarct size assessed by both enzymatic and cardiac MRI as well as the zone of micro vascular obstruction.

Statistical analysis

Statistical analyses for the rat study were performed with Sigma Plot 12 software. Data are reported as mean ± SEM, if the parameter is normally distributed; median, if the parameter is not normally distributed. The Shapiro-Wilk test was performed on each measured parameter in order to evaluate distribution. When the data had a normal distribution then Student's T test was used in order to compare the treated group with the control group. When there was not a normal distribution then Mann-Whitney Rank Sum Test was used in order to compare the treated group with the control group. Statistically significant differences were established at $p < 0.05$. Statistics for the clinical study were previously described [9].

Results

The blood glucose level was measured before anesthesia and was 452 ± 15 mg/dl in the ZDF at the day of surgery. By Shapiro-Wilk test, data of ischemic risk area and no-reflow over risk area were normally distributed and expressed as mean ± SEM; while data of infarct area and no-reflow were not normally distributed and expressed as median. As shown in figure 1, the ischemic risk zone, expressed as % of left ventricle, was comparable in ZDF rats (39.5 ± 2.0%) to SD rats (41.9 ± 2.3%; p=0.43). There was no difference in infarct size, expressed as % of ischemic risk zone, in ZDF rats (49.9%) versus SD rats (59.6%; p=0.32); there was no difference in no-reflow size, expressed as % of ischemic risk zone, in ZDF rats (32.5 ± 3.5%) versus SD rats (32.7 ± 4.3%; p=0.97). Body temperature was maintained at ~37°C during the procedure in both ZDF and SD rats (Table 1). However, the heart rate was significantly lower, and the systolic blood pressure was significantly higher in the ZDF rats compared to in the SD rats during the procedure, but there was no significant difference in mean arterial

pressure between the 2 groups during baseline prior to coronary occlusion, coronary occlusion and reperfusion (Table 1).

In the clinical study, CK-MB area under the curve at 72 h in 258 non-diabetic patients was 5129 ± 221 versus 4503 ± 662 ng h/L in 34 diabetic patients (p=0.34). Troponin I under the curve at 72 h in 254 non-diabetics was 3628 ± 207 versus 3216 ± 504 μg h/L in 34 diabetics (p=0.49). Infarct size by MRI on day 4 was 37.9 ± 1.8 ml in 216 non-diabetic patients and 34.8 ± 4.7 ml in 27 diabetic patients (p=0.559). MRI determined infarct size corrected for mass of the left ventricle did not differ between non-diabetic patients (256.2 ± 8.5) and diabetic patients (223.6 ± 23.3). The ratio of micro vascular obstruction determined by contrast (gadolinium) injection on day 4 on the MRI was 0.179 ± 0.018 of the left ventricle in 200 non-diabetic patients and 0.220 ± 0.060 of the left ventricle in 23 diabetic patients. Similar to the experimental data there was no evidence that diabetic patients had larger myocardial infarctions or more no reflow than non-diabetic patients. In those patients with closed arteries on admission and then successful reperfusion, there also was no difference in infarct size between the diabetic and non-diabetic patients by either enzymatic analysis or MRI.
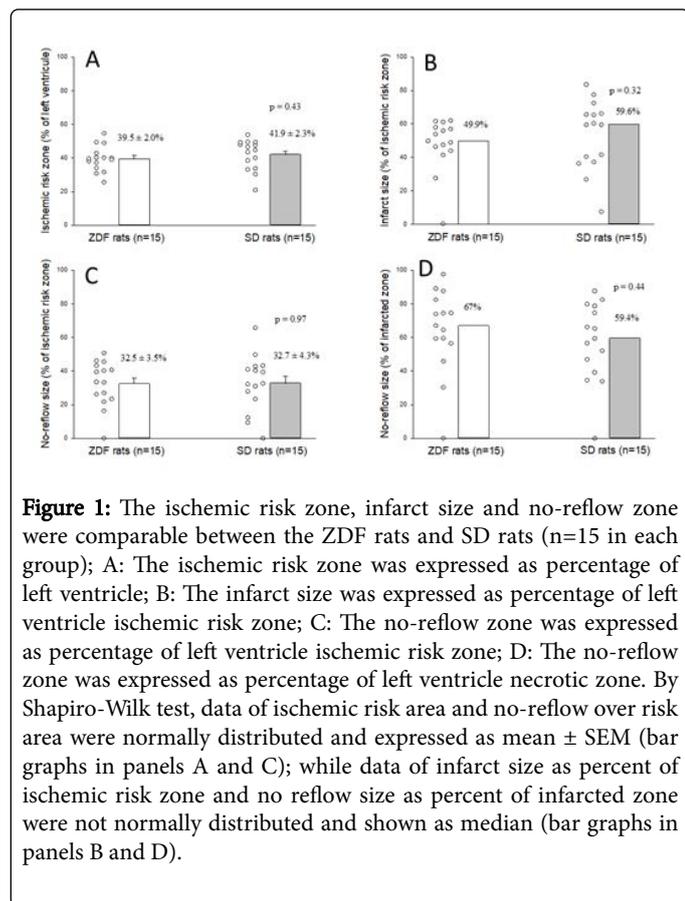
Figure 1: The ischemic risk zone, infarct size and no-reflow zone were comparable between the ZDF rats and SD rats (n=15 in each group); A: The ischemic risk zone was expressed as percentage of left ventricle; B: The infarct size was expressed as percentage of left ventricle ischemic risk zone; C: The no-reflow zone was expressed as percentage of left ventricle ischemic risk zone; D: The no-reflow zone was expressed as percentage of left ventricle necrotic zone. By Shapiro-Wilk test, data of ischemic risk area and no-reflow over risk area were normally distributed and expressed as mean ± SEM (bar graphs in panels A and C); while data of infarct size as percent of ischemic risk zone and no reflow size as percent of infarcted zone were not normally distributed and shown as median (bar graphs in panels B and D).

	ZDF rats (n=15)	SD rats (n=15)	P value
Prior to coronary artery occlusion			
Temperature (°C)	37.1 ± 0.06	37 ± 0.04	0.39
Heart rate (beats/min)	218 ± 6	271 ± 4	5.2×10^{-7}
Systolic blood pressure (mmHg)	98 ± 4	83 ± 1	0.002
Diastolic blood pressure (mm Hg)	59 ± 3	63 ± 1	0.3
Mean blood pressure (mm Hg)	76 ± 4	70 ± 1	0.13
Prior to coronary artery reperfusion			
Temperature (°C)	37.2 ± 0.1	37.1 ± 0.07	0.1
Heart rate (beats/min)	220 ± 5	245 ± 6	0.0047
Systolic blood pressure (mmHg)	90 ± 2	79 ± 2	0.0035
Diastolic blood pressure (mmHg)	52 ± 2	58 ± 2	0.09
Mean blood pressure (mmHg)	68 ± 2	65 ± 2	0.37
End of 3 h reperfusion			
Temperature (°C)	37.1 ± 0	37.3 ± 0.07	0.18
Heart rate (beats/min)	210 ± 5	250 ± 5	2.1×10^{-5}
Systolic blood pressure (mmHg)	77 ± 3	61 ± 1	1.9×10^{-4}
Diastolic blood pressure (mmHg)	37 ± 2	38 ± 2	0.66
Mean blood pressure (mmHg)	52 ± 3	46 ± 1	0.09

Table 1: Body temperature, heart rate, and blood pressure (n=15 in each group).

Discussion

There is no question that diabetes is a risk factor for atherosclerotic disease and myocardial infarction. Diabetes has also been associated with micro vascular disease. As described above, diabetics who have ST elevation myocardial infarction have worse clinical outcomes compared to non-diabetics including worse mortality and more heart failure. The exact mechanism for this worsened outcome is unclear. The results of the present study suggest that diabetic hearts with myocardial infarctions do not have larger myocardial infarctions or larger zones of no reflow compared to non-diabetic hearts. Therefore it is unlikely that the worse clinical outcomes observed in diabetic patients with acute ST elevation myocardial infarctions are due to bigger infarcts or more no reflow (and more micro vascular obstruction). Other factors may be important. It does appear from previous studies that having hyperglycemia at the start of the ST elevation myocardial infarction may be associated with poor outcomes [7,8].

In ZDF rats fed with Purina 5008, non-insulin-dependent diabetes mellitus begins to develop with hyperglycemia and insulin resistance at ~7 weeks of age; and glucose levels typically reach 450-550 mg/100 ml by 9-11 weeks of age [11,12]. Experimental studies of myocardial ischemia/reperfusion tolerance in ZDF rats have been performed in different laboratories, and the effect of diabetes on myocardial injury after ischemia-reperfusion remains controversial. La Bonte [13] subjected male ZDF rats (12-16 weeks old) and aged-matched non-diabetic Zucker lean control rats to 30 min of left coronary artery occlusion followed by 120 min of reperfusion *in vivo*. The infarct size was significantly greater in the ZDF rat hearts compared with their

lean controls. The results demonstrated that there was enhanced myocardial susceptibility to ischemic insult in ZDF rats. In contrast, Kristiansen et al. [14] compared the tolerance to ischemia in hearts from ZDF rats (16 weeks old), and non-obese Zucker. The isolated hearts were mounted in a Langendorff apparatus and perfused retrogradely with Krebs-Henseleit solution, were subjected to regional ischemia by left coronary artery occlusion for 50 min followed by 120 min of reperfusion. The infarct size after a coronary artery occlusion in ZDF rats was significantly smaller than in non-diabetic control animals. Their results suggested that the susceptibility of the type 2 diabetic myocardium to ischemic damage is lower than in non-diabetic hearts. However, Hoshida et al. [15] occluded the left coronary artery for 30 min followed by 24 h reperfusion in male ZDF rats and non-diabetic Zucker lean control rats (27 weeks old). There was no difference in ischemic risk size and infarct size between the ZDF rats and lean control rats. Our present results support the findings of Hoshida in that diabetes had no effect on myocardial infarct size after ischemia-reperfusion in both experimental ZDF rats and in the clinical trial. In addition, our findings extend these studies by showing that in the experimental model and clinical trials that diabetes did not worsen the size of the no reflow zone.

There has been controversy in the literature regarding the effect of diabetes on no reflow. No reflow in our experimental models is due to micro vascular obstruction and the primary ultrastructural abnormality observed with no reflow is endothelial swelling and blebbing that obstructs the lumen of small blood vessels, especially at the capillary level. It does not appear that even well-established diabetes as in our experimental model or in humans exacerbates this phenomenon. The micro vascular damage of diabetes may involve more proximal disease and not the capillaries within the myocardium that are more likely to be affected by ischemia/reperfusion injury. No reflow is now considered an important prognostic marker in patients. Those ST elevation myocardial infarction patients who demonstrate no reflow are more likely to die of their infarction. No reflow is also associated with poor healing of the left ventricle as necrotic debris cannot easily be removed from the infarct and cytokines and cellular elements important to the healing phase of myocardial infarction cannot easily enter the region undergoing healing. As a result, no reflow in both experimental models [16] and in clinical trials [17] is associated with adverse left ventricular remodeling including thin, stretched scars, dilated left ventricles and reduced cardiac function that can lead to heart failure and death. Our hypothesis, that diabetes would be associated with worse no reflow, was, however, disproved in both an animal model and clinical model. The worsened clinical outcomes of diabetic patients suffering an ST elevation MI are likely not related to larger infarcts or more no reflow.

There are certain limitations of our studies. The ZDF rats were studied at one time point during their adulthood, that is about 22 weeks. We cannot rule out the concept that in older rats, diabetes would have caused more no-reflow. The ZDF rats were not aged matched to the non-diabetic rats. The SD rats were females from historical control studies while the ZDF rats were male; however, we previously showed no difference in infarct size by gender in rodent studies [18]. The clinical study did not have large numbers of diabetic patients; however within those patients myocardial infarction size was measured by several techniques: 2 separate biomarkers and MRI imaging.

In conclusion, our results in both animal and clinical studies demonstrated that there was no evidence for larger risk zones, larger infarct sizes, or larger areas of no reflow in the diabetic compared to non-diabetic conditions.

Compliance with Ethical Standards

Conflict of interest: Author Robert A. Kloner has received research grants from Company Stealth BioTherapeutics and Servier. Author Michael C. Gibson has received research grants from Company Stealth BioTherapeutics. Author Muriel Bouly is an employee of company Servier. Author Marc Isabelle is an employee of company Servier. Author Wangde Dai declares that he has no conflict of interest. Author Jianru Shi declares that she has no conflict of interest. Author Juan Carreno declares that he has no conflict of interest.

Ethical approval: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: Informed consent was obtained from all individual participants included in the study.

References

1. Newby LK, Vora AN, Granger CB (2017) Risk stratification in acute myocardial infarction, in Myocardial Infarction. A companion to Braunwald's Heart Disease. Ed DA Morrow. Elsevier, St Louis pages 114-127.

2. Hur SH, Won KB, Kim IC, Bae JH, Choi DJ, et al. (2016) Comparison of 2-year clinical outcomes between diabetic versus nondiabetic patients with acute myocardial infarction after 1-month stabilization: Analysis of the prospective registry of DIAMOND (Diabetic acute myocardial infarctiON Disease) in Korea: an observational registry study. Medicine (Baltimore) 95: e3882.

3. Donahoe SM, Stewart GC, McCabe CH, Mohanavelu S, Murphy SA, et al. (2007) Diabetes and mortality following acute coronary syndromes. JAMA 298: 765-775.

4. Romano M, Buffoli F, Tomasi L, Aroldi M, Lettieri C, et al. (2008) The no-reflow phenomenon in acute myocardial infarction after primary angioplasty: incidence, predictive factors, and long-term outcomes. J Cardiovasc Med (Hagerstown) 9: 59-63.

5. Husser O, Bodi V, Sanchis J, Nunez J, Lopez-Lereu MP, et al. (2013) Predictors of cardiovascular magnetic resonance-derived microvascular obstruction on patient admission in STEMI. Int J Cardiol 166: 77-84.

6. Reinstadler SJ, Stiermaier T, Eitel C, Metzler B, de Waha S, et al. (2017) Relationship Between Diabetes and Ischemic Injury Among Patients with Revascularized ST-Elevation Myocardial Infarction. Diabetes Obes Metab 9: 1706-1713.

7. Eitel I, Hintze S, de Waha S, Fuernau G, Lurz P, et al. (2012) Prognostic impact of hyperglycemia in nondiabetic and diabetic patients with ST-elevation myocardial infarction: insights from contrast-enhanced magnetic resonance imaging. Circ Cardiovasc Imaging 5: 708-718.

8. Ota S, Tanimoto T, Orii M, Hirata K, Shiono Y, et al. (2015) Association between hyperglycemia at admission and microvascular obstruction in patients with ST-segment elevation myocardial infarction. J Cardiol 65: 272-277.

9. Gibson CM, Giugliano RP, Kloner RA, Bode C, Tendera M, et al. (2016) EMBRACE STEMI study: a Phase 2a trial to evaluate the safety, tolerability, and efficacy of intravenous MTP-131 on reperfusion injury in

patients undergoing primary percutaneous coronary intervention. Eur Heart J 37: 1296-1303.

10. Wu KC, Kim RJ, Bluemke DA, Rochitte CE, Zerhouni EA, et al. (1998) Quantification and time course of microvascular obstruction by contrast-enhanced echocardiography and magnetic resonance imaging following acute myocardial infarction and reperfusion. J Am Coll Cardiol 32: 1756-1764.

11. Clark JB, Palmer CJ, Shaw WN (1983) The diabetic Zucker fatty rat. Proc Soc Exp Biol Med 173: 68-75.

12. Bohlen HG, Lash JM (1995) Endothelial-dependent vasodilation is preserved in non-insulin-dependent Zucker fatty diabetic rats. Am J Physiol 268: H2366-2374.

13. La Bonte LR, Davis-Gorman G, Stahl GL, McDonagh PF (2008) Complement inhibition reduces injury in the type 2 diabetic heart following ischemia and reperfusion. Am J Physiol Heart Circ Physiol 294: H1282-1290.

14. Kristiansen SB, Løfgren B, Støttrup NB, Khatir D, Nielsen-Kudsk JE, et al. (2004) Ischaemic preconditioning does not protect the heart in obese and lean animal models of type 2 diabetes. Diabetologia 47: 1716-1721.

15. Hoshida S, Yamashita N, Otsu K, Kuzuya T, Hori M (2000) Cholesterol feeding exacerbates myocardial injury in Zucker diabetic fatty rats. Am J Physiol Heart Circ Physiol 278: H256-262.

16. Reffelmann T, Hale SL, Dow JS, Kloner RA (2003) No-reflow phenomenon persists long-term after ischemia/reperfusion in the rat and predicts infarct expansion. Circulation 108: 2911-2917.

17. Ezekowitz JA, Armstrong PW, Granger CB, Theroux P, Stebbins A, et al. (2010) Predicting chronic left ventricular dysfunction 90 days after ST-segment elevation myocardial infarction: An Assessment of Pexelizumab in Acute Myocardial Infarction (APEX-AMI) Substudy. Am Heart J 160: 272-278.

18. Li Y, Kloner RA (1995) Is There a Gender Difference in Infarct Size and Arrhythmias Following Experimental Coronary Occlusion and Reperfusion? J Thromb Thrombolysis 2: 221-225.