

# Diabetes and Cardioplegia

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**Cardiac surgery with cardiopulmonary bypass and cardioplegic arrest is associated with injury to the vasculature and microcirculation leading to coronary microvascular dysfunction, permeability changes and cardiac dysfunction. In the setting of cardiopulmonary bypass with cardioplegia, poorly-controlled diabetes is associated with significant changes in endothelium-dependent and independent vascular dysfunction, vascular reactivity, vascular permeability, protein expression, cell death, coronary/peripheral microcirculation and reduced vasomotor tone leading to hypotension and impaired endothelial function. The gene expression profiles after cardiopulmonary bypass with cardioplegic arrest is quantitatively and qualitatively different in patients with diabetes. Gene expression profiling capitalizing on the differences between patients with and without diabetes is a good place to identify potential medical targets.**

Diabetes | Cardiac Surgery | Cardioplegia

## Introduction

Type 2 diabetes affects nearly four hundred million people across every country in the world. Between 2012 and 2030 there is projected to be a 69% increase in the number of adults with diabetes in developing countries and a 20% increase in developed countries.<sup>1</sup> Patients with diabetes and its associated comorbidities, otherwise known as metabolic syndrome, have a higher risk of coronary artery disease, overall cardiovascular disease, and total mortality than patients without metabolic syndrome.<sup>2</sup> Patients with diabetes and ischemic heart disease often require coronary artery bypass graft surgery with cardioplegic arrest and cardiopulmonary bypass. These patients have worse outcomes after cardiac surgery than patients without diabetes.<sup>3,4</sup> Furthermore, cardiovascular complications are the leading cause of diabetes-related morbidity and mortality.<sup>5</sup> Understanding the pathophysiology of how diabetes affects patients undergoing cardiac surgery involving cardioplegia is essential in developing a medical therapy for this highly prevalent disease. The purpose of this review is to briefly outline the current research regarding diabetes and its associated endothelial and cardiovascular dysfunction in the setting of cardiopulmonary bypass and cardioplegic arrest.

## Diabetes and Endothelial Dysfunction

Patients with long-standing diabetes are known to have endothelial dysfunction, which results in a diverse range of vascular complications including atherosclerosis of large arteries, coronary artery disease, retinopathy and renal failure.<sup>6,7</sup> Furthermore, patients with type 2 diabetes usually have insulin resistance, which further exacerbates endothelial dysfunction. Interestingly, plasminogen activator inhibitor-1 has been identified as a possible biomarker of diabetes and its associated cardiovascular disease.<sup>8</sup> The retinopathy seen in patients with diabetes is attributed to endothelial progenitor cell dysfunction.<sup>9</sup> Ironically, endothelial progenitor cells have been found to promote vascular repair, but appear to be overactive in this inflamed disease state.<sup>10</sup> The renal dysfunction seen in patients

with diabetes who develop nephropathy is a direct consequence of hypertension and its associated endothelial dysfunction.<sup>11</sup> Nuclear respiratory factor 2 (Nrf2) is considered a master regulator of anti-oxidant genes and suppresses the inflammatory cytokine NFkB. Up-regulation of Nrf2 has been suggested to limit diabetes-associated vascular injury.<sup>12</sup>

In past decade, we and others have extensively investigated the effects of diabetes on microvascular endothelial function in animals and humans.<sup>13–20</sup> Our results consistently demonstrated that diabetes is associated with significant reduction in microvascular endothelial function in coronary and peripheral microvasculature. Patients with diabetes demonstrate decreased contractile response to endothelin-1 in human peripheral microvasculature.<sup>14</sup> The nitric oxide donors and endothelium-derived hyperpolarizing factor (EDHF)-mediated endothelium-dependent relaxation is diminished in the diabetic patients.<sup>14,21</sup> In the setting of cardioplegic arrest and cardiopulmonary bypass, diabetes further impairs the recoveries of microvascular endothelial function.<sup>22</sup>

The exact mechanism of endothelial dysfunction associated with diabetes is multifactorial and complex. To cover all the current material on this topic is beyond the scope of this review.

## Diabetes and Cardiac Function

Metabolic diseases, including diabetes, are associated with cardiac remodeling leading to left ventricular dysfunction. Cardiomyopathy of diabetes is defined as the ventricular dysfunction that occurs in patients with diabetes in absence of coronary artery disease, vascular or congenital heart disease, hypertension or alcoholism.<sup>6,24,25</sup> Patients with diabetes show greater concentric remodeling and high left ventricular filling pressures after acute myocardial ischemia than those without diabetes, suggesting a mechanism by which diabetes causes a higher cardiovascular risk.<sup>26</sup> Furthermore, patients with diabetes demonstrate an increased ratio of end-diastolic left ventricular mass to end diastolic volume compared to patients without diabetes. This difference correlates with length of diabetes.<sup>27</sup> Patients with diabetes and normal coronary function and ejection fraction have subclinical defects in systolic function which can be measured using a two-dimensional speckle tracking echocardiography. Subclinical systolic dysfunction can be determined by measuring left ventricular longitudinal strain (or synchronized myocardial contraction) and time-to-peak systolic strain among left ventricular segments. Patients with diabetes who have normal coronary function and ejection fraction demonstrate decreased segmental and global end-systolic longitudinal strain and increased time-to-peak systolic strain among left ventricular segments, regardless of the presence of diastolic dysfunction.<sup>23</sup>

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The underlying pathogenesis of diabetes-induced cardiomyopathy is only partially understood and likely multifactorial.<sup>6</sup> Autonomic dysfunction, metabolic derangements, abnormalities in ion homeostasis, alteration in structural proteins and interstitial fibrosis likely all contribute to the development of cardiomyopathy of diabetes. Additionally, sustained hyperglycemia may increase glycation of interstitial proteins such as collagen, which results in myocardial stiffness and impaired contractility.<sup>6,28</sup>

Diabetes is also a major risk factor for ischemic heart disease.<sup>29</sup> Myocardial ischemia is known to promote the development of coronary collateral vessels to increase blood flow by bypassing the diseased vessels.<sup>30</sup> Patients with diabetes have been shown to have poorer coronary collateral growth than patients without diabetes.<sup>31</sup> Growth of new blood vessels is dependent upon a variety of cell signaling cascades. Several cytokines have been identified to be involved in the process including fibroblast growth factor, vascular endothelial growth factor, platelet derived growth factor, angiopoietins, reactive oxygen species (ROS), calpain, glycogen synthase kinase-3 $\beta$ , nitric oxide, NADPH, NF $\kappa$ B, TNF- $\alpha$ , MMPs, TIMPs and many others. Not surprisingly, these same molecular markers have been found to be aberrantly expressed in the setting of diabetes.<sup>13</sup> In a porcine model of metabolic syndrome with ischemia and reperfusion, metabolic syndrome was associated with increased myocardial oxidative stress and inflammation, attenuation of cell survival pathways and induction of apoptosis in the ischemic myocardial tissue.<sup>32</sup>

Understanding the mechanism involved in heart disease in diabetes is vital to successful surgical outcomes for these patients.

### Endothelial Dysfunction resulting from Cardiac Surgery with Cardioplegia

Cardioplegia refers to the solution used to induce cardiac asystole. Interestingly, while cardioplegia is accepted as a mandatory vehicle for myocardial protection during on-pump cardiac surgery, there is controversy over the best formula (blood versus crystalloid), temperature (warm versus cold) and mode of delivery (intermittent versus continuous and antegrade versus retrograde). Blood cardioplegia has been found to be superior to crystalloid cardioplegia by improving left ventricular function and endothelium-dependent relaxation of coronary microvessels. This beneficial effect is associated with decreased activation of pro-apoptotic protein caspase 3 and increased anti-apoptotic protein phosphorylated-Bad expression levels.<sup>49-51</sup> A meta-analysis of randomized clinical trials published in *Circulation* in 2006 evaluated the effectiveness of blood cardioplegia in lowering adverse postoperative outcomes compared to crystalloid cardioplegia. It determined that blood cardioplegia provided superior myocardial protection compared with crystalloid cardioplegia by lowering the incidence of low output syndrome and increased creatinine kinase MB release. However, the incidence of post-operative myocardial infarction and death were similar between groups.<sup>51</sup> Another meta-analysis compared cold blood cardioplegia to cold crystalloid cardioplegia and determined that cold blood cardioplegia reduces perioperative myocardial infarction compared to cold crystalloid solution. However, there were no differences between groups in incidence rates of spontaneous sinus rhythm, 30 day mortality, atrial fibrillation or stroke.<sup>52</sup> There is no clear data to suggest that one form of cardioplegia is better than another in patients with diabetes. Current research looking at adding additives to cardioplegia solution to improve myocardial function is an area of active research; however no studies to date have proven to be beneficial in human trials.

Cardiac surgery with cardiopulmonary bypass and cardioplegic arrest is associated with injury to the vasculature and microcirculation. This injury leads to coronary microvascular dysfunction, permeability changes and cardiac dysfunction. Part of the myocardial dysfunction is attributed to the abnormalities in the coronary vasculature which leads to impaired vasorelaxation or vasoconstriction, increased permeability, heart edema and ultimately, overall dysfunction.<sup>33-37</sup>

The abnormalities in microvascular permeability have been attributed to inflammatory changes leading to increased nitric oxide synthase, cyclooxygenase, and vascular endothelial growth factor activity.<sup>33,38,39</sup> Additionally, the degradation of endothelial and cardiomyocyte adherens junctions, has been identified to be increased following cardiac surgery leading to vascular permeability.<sup>36</sup> Vascular permeability is, in part, regulated by thrombin-induced alterations in cellular junctions. Prevention of thrombin protease-activated receptors by aprotinin was found to decrease myocardial infarct size, decrease nitrotyrosine staining (marker of cell damage) and preserve adherens junctions after regional ischemia and cardioplegic arrest resulting in preservation of the vascular endothelial barrier and reduced myocardial tissue edema.<sup>40,41</sup> Cardioplegia and cardiopulmonary bypass also alters the contractile response of human coronary and peripheral arterioles to serotonin, endothelin-1, ERK1/2, COX-2, and thromboxane A-2.<sup>21,42-46</sup> The contractile response to endothelin-1 is in part via the activation of COX-2, endothelin-A receptors and protein kinase C- $\alpha$ .<sup>20,47</sup> The serotonin-induced coronary vascular dysfunction demonstrated after cardiopulmonary bypass with cardioplegia may be mediated by increased expression of serotonin receptors 1B in coronary smooth muscle.<sup>48</sup>

Brief periods of ischemia and reperfusion can increase the ischemic tolerance of the heart to sustained ischemia; this is referred to as ischemic preconditioning.<sup>53</sup> Studies have shown that preconditioning increases tolerance to repetitive balloon inflation during angioplasty and causes better preservation of myocardial adenosine triphosphate after intermittent aortic cross-clamping during cardiac surgery.<sup>54</sup> The exact mechanism of benefit remains unknown; studies suggest that it may be receptor-mediated cardiac protection involving intracellular signal transduction pathways involving protein kinase C, mitochondrial K<sup>+</sup>(ATP) channels.<sup>55</sup> Ischemic preconditioning preserves coronary microvasculature against endothelial dysfunction. The microvascular dysfunction seen in coronary and peripheral arterioles seen following cardioplegic arrest has been partially attributed to impaired function of calcium-activated potassium channels.<sup>56-59</sup> Exogenous bradykinin has been found to mimic the effect of ischemic preconditioning by opening calcium-activated potassium channels.<sup>57</sup> Severe acidosis during cardioplegic arrest increases the percentage of apoptotic cells in myocardial tissue, and independent of ischemia, diminishes recovery of microvascular and left ventricular function.<sup>60</sup> Ischemic preconditioning has been shown to significantly reduce DNA fragmentation and apoptotic myocyte death associated with ischemia-reperfusion.<sup>55</sup>

Mesenteric ischemia is a rare but serious consequence of cardiopulmonary bypass. Mesenteric tissue in a swine model of cardiopulmonary bypass showed decreased microvascular contraction to phenylephrine. ERK1/2 pathway inhibition reduced contractile responses to phenylephrine at baseline.<sup>61</sup> This suggests a potential role for ERK1/2 pathway inhibition to help prevent against cardiopulmonary bypass-induced mesenteric ischemia.

### Diabetes and Cardiac Surgery with Cardioplegia

In the setting of cardiopulmonary bypass with cardioplegia, poorly-controlled diabetes is associated with significant changes

in endothelium-dependent and independent vascular dysfunction, vascular reactivity, vascular permeability, protein expression, cell death, coronary/peripheral microcirculation and reduced vasomotor tone leading to hypotension and impaired endothelial function.<sup>22,62-69</sup> Patients with poorly-controlled diabetes demonstrate impaired arteriolar function both before and after cardiopulmonary bypass surgery.

Patients with diabetes demonstrate both endothelium dependent and independent vascular dysfunction. Cardioplegia exacerbates this dysfunction. Patients with poorly controlled diabetes demonstrate worsened recovery of coronary arteriolar function after cardioplegic arrest and reperfusion, associated with increased expression of protein kinase C, alpha and beta.<sup>66</sup> These changes in vascular dynamics may be partially explained by abnormal expression of hepatocyte growth factor, protein kinase C, vascular endothelial growth factor and its receptor gene FLK-1 which have all been found to be aberrantly expressed in coronary microcirculation after cardioplegia.<sup>69-71</sup> These alterations in signaling lead to increased oxidative and nitrosative stress.<sup>66,69</sup>

Down-regulation of endothelial adherens-junction protein activation, expression, and localization is seen in patients with diabetes after coronary bypass surgery with cardioplegia. The microvessels from myocardial tissue in patients with diabetes demonstrate increased tyrosine phosphorylation and deterioration of VE-cadherin indicating damage to the cell-cell endothelial junctions following cardiopulmonary bypass surgery with cardioplegia. These alterations may lead to an increase in vascular permeability and endothelial dysfunction, and negatively affect outcomes in patients with diabetes after cardiac surgery.<sup>62</sup> Patients with diabetes also demonstrate increased expression of endothelin-1 and greater endothelin-1 mediated vasoconstriction, as well as diminished nitric oxide-mediated vasodilation after cardiopulmonary bypass and reperfusion than do patients without diabetes. Endothelin antagonism attenuates these effects.<sup>72</sup> Interestingly, diabetes is associated with inactivation of endothelial small and intermediate calcium-activated potassium channels isolated from coronary arterioles isolated from human right atrial tissue.<sup>73</sup>

### Gene Expression Profiles and Diabetes in Patients with Cardiac Surgery and Cardioplegia

In patients undergoing cardiac surgery, diabetes is an independent risk factor for morbidity and mortality including early and late cardiac death, stroke, and increased need for hospital readmission and further intervention.<sup>74-77</sup> Therefore, it is important to understand the pathophysiology taking place in patients with diabetes who undergo cardiac surgery and cardioplegia. Transcriptional profiling using high density microarrays provides specific data about disease mechanisms by comparing the level of miRNA transcribed in cells in a pathological state versus a control state. Studying the cardiac gene expression response to cardiopulmonary bypass and cardioplegia in patients with and without diabetes, could lead to the development of tailored cardioprotective strategies for diabetic patients.<sup>78</sup>

In gene expression in human atrial tissue before and after cardiopulmonary bypass and cardioplegic arrest and cardiac surgery, patients with diabetes demonstrated a significantly modified response to surgery than did patients without diabetes. Patients with diabetes demonstrated a larger number of upregulated genes and a smaller number of down regulated genes. Sixty-six percent of the altered genes were upregulated in the group with diabetes, compared with 43% in the group without diabetes. These differences demonstrate the strikingly different gene expression patterns between the two groups.<sup>78</sup>

Looking at these differences more closely showed that patients with diabetes showed upregulation of genes associated with mediators of the inflammatory response, including transcription activators MYC, JUN, IL-8 and IL-1 $\beta$ .<sup>78</sup> Genes that were exclusively upregulated in the group without diabetes involved cell cycle regulators and mediators of apoptosis.<sup>78</sup> Inflammatory cytokines are known to induce cardiac remodeling and fibrosis leading to ventricular dysfunction.<sup>79-82</sup> Inhibition of certain inflammatory cytokines has been shown to improve cardiac function.<sup>80,83</sup> Increased myocardial apoptosis is associated with decreased ventricular function and decreasing pro-apoptotic pathways has been found to be beneficial.<sup>84</sup>

The gene expression profiles after cardiopulmonary bypass with cardioplegic arrest is quantitatively and qualitatively different in patients with diabetes.<sup>78,85</sup> These same patients demonstrate endothelium-dependent and -independent vascular dysfunction of coronary arterioles and worsened recovery of coronary arteriolar function after cardiopulmonary bypass with cardioplegic arrest.<sup>14,66,71</sup> It is well known that patients with diabetes have worse outcomes after cardiopulmonary bypass with cardioplegic arrest. Many of the genes that have been found to be upregulated in patients with diabetes are involved in inflammatory and apoptotic pathways, which have also been shown to be associated with myocardial injury during cardiac surgery.<sup>86</sup> This underscores the importance of the need for tailored myocardial protection and operative strategies for patients with diabetes undergoing coronary bypass with cardioplegic arrest. Currently, there are no therapies tailored to myocardial protection for patients with diabetes who undergo cardiac operations beyond tight perioperative glucose control. However, there is extensive research studying the effects of diabetes on patient outcomes.

### Future Work

There is currently extensive research in progress to improve cardiac function in patients with coronary vascular disease. Much of the research being performed demonstrates success in animal models; however, fail to translate into similar success in humans during phase II or III clinical trials. This is likely due to the fact that patients with diabetes and/or coronary artery disease often have co-morbidities that are not represented in most animal models. Therefore, it is important that research analyzing relevant medical therapies for the coronary artery disease seen in patients is performed using clinically relevant animal models of diabetes that portray both disease processes along with associated comorbidities. Gene expression profiling capitalizing on the differences between patients with and without diabetes is a good place to identify potential medical targets.

### Conclusions

Diabetes affects millions of people around the world and has devastating consequences on vascular and cardiac function over time. Patients with long-standing diabetes suffer from macrovascular and microvascular dysfunction, which leads to multiple organ and tissue dysfunction including ischemic heart disease and cardiomyopathy. Patients with diabetes with ischemic heart disease, therefore, often require coronary bypass grafting with cardioplegic arrest. Unfortunately, patients with diabetes fare worse post-operatively than patients without diabetes. While there are many therapeutic options available to patients with diabetes to help prevent heart disease, there are many studies which must be performed to determine optimal treatments which will be most beneficial in reversing the damaging effects of diabetes on the cardiovascular system. Furthermore, use of current cardioplegia in cardiac surgery needs to be studied further to determine potential

changes or new therapies needed for this uniquely high-risk and prevalent population.

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