Oxidative Stress and Nerve Function After Cardiopulmonary Bypass in Patients With Diabetes

Robina Matyal, MD, Sruthi Sakamuri, BS, Thomas Huang, BA, Khurram Owais, MD, Samir Parikh, MD, Kamal Khabbaz, MD, Angela Wang, BA, Frank Sellke, MD, and Feroze Mahmood, MD

Departments of Anesthesia, Medicine, and Surgery, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts; and Division of Cardiothoracic Surgery, Alpert Medical School of Brown University, Rhode Island Hospital, Providence, Rhode Island

Background. Chronic hyperglycemia has been associated with increased oxidative stress in skeletal muscle and sympathetic nerve dysfunction. We investigated the effect of chronic hyperglycemia on the myocardium of patients with uncontrolled diabetes (UD) compared with patients with well-controlled diabetes (CD) and patients without diabetes (ND) after cardioplegic cardiopulmonary bypass (CP/CPB) with acute intraoperative glycemic control.

Methods. Atrial tissue and serum were collected from 47 patients (ND=18 with glycated hemoglobin [HbA1c] of 5.8 ± 0.2; CD = 8 with HbA1c of 6.1 ± 0.1; with UD = 21 with HbA1c = 9.6 ± 0.5) before and after CP/CPB for immunoblotting, protein oxidation assays, immunohistochemical evaluation, and microarray analysis.

Results. The uncontrolled group had increased total protein oxidation (p < 0.05) and decreased levels of anti-oxidative enzyme manganese superoxide dismutase (MnSOD) (p < 0.05) after CP/CPB compared with the controlled group. Collagen staining revealed increased fibrosis in patients with UD (p < 0.05) compared with patients with CD and patients without diabetes. The uncontrolled group also showed a decrease in the neurogenic and angiogenic markers nerve growth factor (NGF) (p < 0.05), neurotrophin (NT)-3 (p < 0.05), and platelet-derived growth factor (PDGF)-β (p < 0.05) compared with the other groups after CP/CPB. Atrial and serum microarray analysis showed increased oxidative stress and sympathetic nerve damage, increased fibrosis, and a decrease in angiogenesis in patients with UD (p < 0.03) compared with patients without diabetes.

Conclusions. CP/CPB led to higher oxidative stress in patients with UD before surgical intervention, even after normal glucose levels were maintained intraoperatively. Thus, controlled HbA1C in addition to acute intraoperative glucose control may be a more suitable end point for patients with diabetes undergoing cardiac operations.

© 2014 by The Society of Thoracic Surgeons

Accepted for publication June 9, 2014.


Address correspondence to Dr Khabbaz, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02215; e-mail: kkhhabbaz@bidmc.harvard.edu.
systematically investigated. A correlation would also offer an opportunity to modify myocardial oxidative stress with tighter long-term blood glucose control in patients with diabetes. Therefore, we hypothesized that compared to uncontrolled diabetics (UD) and controlled diabetics (CD), patients without diabetes are better protected against oxidative stress, thus reducing nerve and angiostatic damage. We sought to demonstrate the relationship between chronic hyperglycemia and enhanced oxidative stress in the myocardia of patients with UD undergoing cardiac operations with CP/CPB.

**Patients and Methods**

**Human Participants and Tissue Harvesting**

After institutional review board approval and written informed consent, patients undergoing elective coronary artery bypass grafting with CP/CPB were enrolled in the study. Patients were categorized as non-diabetics, with a glycated hemoglobin (HbA1c) value of less than 6.0 and no clinical diagnosis of diabetes; as CD with an HbA1c value ranging from 6.1 to 6.5 and a clinical diagnosis of diabetes; or as UD with an HbA1c value greater than or equal to 6.6. Exclusion criteria consisted of pulmonary disease, valvular disease, cancer, and refusal to participate.

Initial right atrial tissue was harvested after the induction of general anesthesia and median sternotomy, before 600 to 800 mL of cold blood (8°C–4°C) hyperkalemic (15 mmol/L K+) cardioplegic solution was delivered antegradely into the aortic root. After exposure to cold blood cardioplegia, CPB, reperfusion at the completion of the procedure, and removal of the aortic cross-clamp, a second right atrial tissue sample was collected. The venous cannula was kept in place by a loose suture to prevent ischemia from physical pressure at the site of tissue harvest. Tissue samples (50–100 mg each) were snap frozen in liquid nitrogen for microarray and immunoblot analysis. Tissues for immunohistochemical analysis were fixed in 10% buffered formalin for 24 hours, embedded in paraffin, and then sectioned into 5-mm slices.

Blood was collected through a radial arterial line in the holding area. Ten milliliters of blood was collected and

### Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients Without Diabetes</th>
<th>Patients With Controlled Diabetes</th>
<th>Patients With Uncontrolled Diabetes</th>
<th>p Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/Female, n</td>
<td>16/2</td>
<td>4/4</td>
<td>13/8</td>
<td>0.067</td>
</tr>
<tr>
<td>Age (y)</td>
<td>69.7 ± 2.3</td>
<td>66 ± 3.2</td>
<td>63.6 ± 1.4</td>
<td>0.031</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>5.8 ± 0.2</td>
<td>6.1 ± 0.1</td>
<td>9.6 ± 0.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cross-clamp time, min</td>
<td>59.8 ± 5.4</td>
<td>57.1 ± 8.5</td>
<td>62.4 ± 6.8</td>
<td>0.876</td>
</tr>
<tr>
<td>Duration of CPB, min</td>
<td>83.3 ± 6.2</td>
<td>74.1 ± 10.1</td>
<td>86.8 ± 6.9</td>
<td>0.876</td>
</tr>
<tr>
<td>Patient blood glucose level, mg/dL, before CPB</td>
<td>143.0 ± 7.3</td>
<td>153.4 ± 19.8</td>
<td>174.4 ± 9.1</td>
<td>0.005</td>
</tr>
<tr>
<td>Patient blood glucose level, mg/dL, after CPB</td>
<td>125.3 ± 3.7</td>
<td>125.6 ± 10.6</td>
<td>136.1 ± 8.0</td>
<td>0.279</td>
</tr>
<tr>
<td>Diabetes control, n</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diet</td>
<td>18</td>
<td>3</td>
<td>1</td>
<td>...</td>
</tr>
<tr>
<td>Oral therapy</td>
<td>0</td>
<td>4</td>
<td>9</td>
<td>...</td>
</tr>
<tr>
<td>Insulin</td>
<td>0</td>
<td>2</td>
<td>11</td>
<td>...</td>
</tr>
<tr>
<td>Preoperative aspirin</td>
<td>18</td>
<td>8</td>
<td>21</td>
<td>1.000</td>
</tr>
<tr>
<td>Statin drugs</td>
<td>18</td>
<td>8</td>
<td>20</td>
<td>1.000</td>
</tr>
<tr>
<td>β-blockers</td>
<td>16</td>
<td>8</td>
<td>21</td>
<td>0.640</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>8</td>
<td>1</td>
<td>4</td>
<td>0.181</td>
</tr>
</tbody>
</table>

* Data expressed as mean ± standard error of the mean.

CPB = cardiopulmonary bypass;   HbA1c = glycated hemoglobin.
centrifuged at 10,000 g for 12 minutes. Serum was extracted and frozen in liquid nitrogen.

**Microarray Data Protocol**

The cDNA was prepared according to the protocol provided with the Affymetrix U95 GeneChip system (Affymetrix, Santa Clara, CA). Purified cDNA was incubated at 37°C for 5 hours in an in vitro transcription reaction to produce cRNA using the BioArray High-Yield RNA transcript labeling kit (Enzo Life Sciences, Inc, Farmingdale, NY) [18]. The expression arrays were preformed on Affymetrix U95 chips and developed on the manufacturer’s platform.

The U95 Affymetrix gene chip arrays contain 63,000 probe sets interrogating approximately 54,000 UniGene clusters. The presented p values are nominal and therefore unadjusted. To limit false-positive results, the following criteria were used to filter the results: p value
less than 0.01, false discovery rate less than 0.05, and fold change greater than or equal to 2.

**Sirius Red Staining**

Sections were deparaffinized and hydrated. Nuclei were stained with Weigert’s hematoxylin for 10 minutes and washed in water. Slides were stained with Picro Sirius red (0.5 g Sirius red powder [F3B], 500 mL picric acid [saturated] solution; Abcam, Cambridge, MA) for 1 hour and washed twice with acidified water (5 mL glacial acetic acid, 1 L distilled water). Slides were dehydrated 3 times in 100% ethanol, cleared in xylene, and mounted with Permount (Thermo Fisher Scientific, Inc, Waltham, MA). The deparaffinized slides from patients without diabetes and patients with UD were costained with 4,6-diamidino-2-phenylindole (DAPI) and general nerve marker protein gene product 9.5 (PGP 9.5) from Cedarlane (Burlington, NC). Images were analyzed using ImageJ.

**Protein Carbonyl Content Assay**

The experiment was conducted according to the instructions on the OxyBlot Protein Oxidation kit (Millipore, Billerica, MA).

**Immunoblotting Protocol**

Whole-cell lysates were made from the frozen atrial tissue for all groups from samples obtained before and after CP/CPB. Samples were run simultaneously to ensure identical conditions. Sixty micrograms of total protein was fractioned by a 4% to 12% gradient sodium-dodecyl-sulfate polyacrylamide gel electrophoresis (Life Technologies, Grand Island, NY) and transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA). Images were analyzed using ImageJ.
Billerica, MA) with a semidry transfer cell (Bio-Rad Trans-Blot; Bio-Rad Laboratories, Hercules, CA). Ponceau staining was used to ensure equal protein loading. Each membrane was incubated with specific primary antibodies overnight at 4°C and secondary antibodies for 1 hour at room temperature. Carnitine palmitoyl transferase (CPT1)-M, platelet derived growth factor (PDGF)-β, angiostatin, nerve growth factor (NGF) (Santa Cruz Biotechnology, Santa Cruz, CA), manganese superoxide dismutase (MnSOD) (R&D Systems, Minneapolis, MN), ADIPOR1 (Biorbyt, Cambridge, UK), and neuropeptide (NT)-3 (Abbiotec, San Diego, CA) were examined as markers for mitochondrial function, angiogenesis, and neurogenesis. Immune complexes were visualized with an enhanced chemiluminescence detection system (Amersham, Thermo Fisher Scientific, Inc, Waltham, MA).

Statistical Analysis

One-way analysis of variance and multiple comparison tests were used to compare Western blot, protein oxidation, and immunohistochemistry data. \( p \) less than 0.05 was considered significant. For microarray data analysis, Affymetrix CEL files were uploaded into GenePattern, web-based software offered through the Broad Institute (Cambridge, MA). After the recommended quality control and normalization steps, pairwise differential gene expression was sought with the following measurements: \( p \) less than 0.01, false discovery rate less than 10\%, and greater than 2-fold difference between cases and controls. The differences in gene expression in atrial tissue before and after CP/CPB were compared between patients without diabetes and patients with UD, whereas serum values were compared between the 3 groups only at baseline before CP/CPB.

Results

Patient Characteristics

A total of 47 patients were enrolled; 18 did not have diabetes, 8 had CD, and 21 had UD. Detailed characteristics are listed in Table 1.

Mitochondrial Function and Metabolism

Complete results of analyses before and after CP/CPB are shown in Fig 1. Levels of CPT1-M, an indicator of carnitine shuttle function, were significantly higher in patients without diabetes compared with patients with CD and patients with UD (\( p = 0.043 \) and \( p = 0.006 \), respectively) in atrial tissue at baseline before CP/CPB (Fig 1A). Similarly, the levels of ADIPOR1, a receptor for fatty acid oxidation, were significantly higher in the nondiabetic group compared with the UD group before CP/CPB (\( p = 0.025 \)) (Fig 1B).

Levels of MnSOD after CP/CPB were significantly decreased in patients with UD compared with their levels before CP/CPB (\( p = 0.04 \)) and compared with patients with CD (\( p = 0.014 \)) (Fig 1C). Nuclear factor kappa beta (NF-kβ) was greatly increased in patients with CD and in patients with UD in the tissue obtained after CPB compared with samples obtained before CPB (\( p = 0.005 \))

---

**Fig 3.** Representative immunoblots of human atrial tissue for angiogenic proteins. (A) Platelet-derived growth factor (PDGF)-β was significantly increased in patients without diabetes (no diabetes [ND]) compared with patients with undiagnosed diabetes (UD) in atrial tissue after cardiopulmonary bypass (Post-CP/CPB) and reperfusion. (B) Thrombospondin-2, an antiangiogenic protein, was not significantly decreased in patients with UD, but (C) angiostatin was significantly elevated in patients with UD in atrial tissue after CP/CPB and reperfusion. (D) Immunoblots of corresponding proteins are displayed. Data are mean ± standard error of the mean (*\( p < 0.05 \) versus Pre-CP/CPB; †\( p < 0.05 \) versus Pre-CP/CPB ND; \( \ddot{\alpha}p < 0.05 \) versus Post-CP/CPB ND; \( \dddot{\alpha}p < 0.05 \) versus post-CP/CPB ND).
and \( p = 0.002 \), respectively) (Fig 1D). Total protein oxidation was significantly decreased in patients with CD (\( p = 0.018 \)) (Fig 1E) and slightly increased in patients with UD (\( p = 0.20 \)) after CP/CPB.

Neurogenesis

Analyses of nerve markers are reported in Fig 2. Before CP/CPB, levels of NT-3 were significantly lower in patients with UD compared with patients without diabetes (\( p = 0.016 \)) (Fig 2A). After CP/CPB, levels of NGF significantly decreased in patients with UD compared with patients with CD (\( p = 0.002 \)) and patients without diabetes (\( p < 0.001 \)) (Fig 2B). Endothelin-1 (ET-1) levels in patients with UD were significantly different from those in patients without diabetes (\( p = 0.004 \)) (Fig 2C). After staining atrial tissue for sympathetic nerves with PGP9.5, there were more nerves in the atrial tissue from patients without diabetes than in patients with UD (Fig 2E).

Angiogenesis

Angiogenic marker levels are shown in Fig 3. After CPB, levels of PDGF-\( \beta \) significantly increased in patients without diabetes compared with patients with UD (\( p = 0.049 \)) (Fig 3B). Antiangiogenic factor thrombospondin-2 significantly decreased in the patients without diabetes after CP/CPB, whereas it remained unchanged in patients with UD after CPB (Fig 3B). Levels of angiostatin were significantly lower in patients with CD compared with patients with UD before CPB (\( p = 0.025 \)) (Fig 3C).

Fibrosis

Immunohistochemical analysis results showed significantly increased fibrosis in patients with UD compared with patients with CD (\( p = 0.050 \)) and patients without diabetes (\( p = 0.021 \)) (Fig 4).

Microarray Analysis

Atrial Tissue. Results of analysis of commonly known genes are listed in Table 2. Compared with patients without diabetes, PARK7 (protective against oxidative stress) and SMAD5 (involved in fibrosis and antiapoptosis) were expressed significantly less in patients with UD (\( p = 0.006 \) and \( p = 0.002 \), respectively) after CP/CPB. Expression of proangiogenic genes MEF2C, PDGF\( \alpha \), and FGF12 was also significantly lower (\( p = 0.002, p = 0.002, \) and \( p = 0.032, \) respectively). Angiotensin II receptor type 1 (\( p = 0.004 \)) and angiotensinogen gene expressions (\( p = 0.002 \)) were increased. The monoamine oxidase A and SEMA3C associated with neurotransmitter release, normal sympathetic nervous system function, and axonal growth and differentiation were significantly downregulated (\( p = 0.002 \) and \( p = 0.020, \) respectively). Nerve damage marker neural cell adhesion molecule 1 was significantly elevated (\( p = 0.002 \)).

Serum Levels. Table 3 shows a selection of genes analyzed. Oxidative stress gene NOX4 was upregulated in patients with UD compared with patients without diabetes (\( p = 0.036 \)). The NOS1 (\( p = 0.940 \)) and OX11L (\( p = 0.047 \)) nitric oxide synthase and cytochrome oxidase activating genes were downregulated in patients with UD when compared with patients with CD. Serum levels of neurotrophins NGF and endothelin receptor type B were downregulated and NPY receptor Y1 was upregulated in patients with CD compared with patients with UD (\( p = 0.022, p = 0.049, \) and \( p = 0.041, \) respectively).

Comment

In this study, we examined the biochemical, cellular, and genetic effects of poorly controlled DM on oxidative...
with evidence of increased oxidative stress in human atrial tissue after CP/CPB. Compared with patients without diabetes and patients with CD, those with poorly controlled diabetes exhibited decreased nerve growth and angiogenesis-associated proteins in the atrial tissue. At the biochemical level, there was impaired carnitine shuttle activity and decreased levels of ADIPOR1 causing impaired fatty acid metabolism. Above all, there was significantly increased protein oxidation further aggravating nitrosative/oxidative stress. At the cellular level, there was increased fibrosis and mitochondrial dysfunction while at the genetic level there was decreased expression of genes that regulate oxidative stress, fibrosis, angiogenesis, and sympathetic nerve function. Concurrently, there was an increased expression of genes that enhance fibrosis and nerve dysfunction, e.g., angiotensin II receptors and angiotensinogen (Table 2). The downregulation of genes involved in nerve growth (monoamine oxidase and sema domain 3C) may have further decreased NGF and NT-3 and neurotrophins.

Our findings imply that chronicity of hyperglycemia is an important factor in diabetes-related pathophysiologic processes in patients undergoing cardiac operations. Prolonged uncontrolled hyperglycemia is directly related to mitochondrial damage in these patients [19–21]. Our study is clinically significant in that it highlights the relationship between preoperative glucose control and oxidative stress during cardiac operations as a potential area of further investigation. Although our investigation focused on cellular and biochemical derangements, the implications of our findings are clinical.

Blood glucose levels sufficient to increase mitochondrial reactive oxygen species–induced epigenetic alterations in NF-κB genes have been reported in the past [20]. Oxidative stress related to uncontrolled HbA1c has been shown to affect DNA breakage and possibly result in alterations leading to decreased nerve growth factors

### Table 2. Atrial Tissue Microarray

<table>
<thead>
<tr>
<th>Genes of Interest</th>
<th>Fold Change</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxidative stress protective marker</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PARK7</td>
<td>−1.52</td>
<td>0.006</td>
</tr>
<tr>
<td>Profibrotic markers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AGTR1</td>
<td>1.61</td>
<td>0.004</td>
</tr>
<tr>
<td>AGT</td>
<td>2.02</td>
<td>0.002</td>
</tr>
<tr>
<td>Antifibrotic markers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SMAD5</td>
<td>−1.52</td>
<td>0.002</td>
</tr>
<tr>
<td>Growth and angiogenic factors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEF2c</td>
<td>−2.83</td>
<td>0.002</td>
</tr>
<tr>
<td>PDGFA polypeptide</td>
<td>−1.93</td>
<td>0.002</td>
</tr>
<tr>
<td>FGF12</td>
<td>−1.76</td>
<td>0.002</td>
</tr>
<tr>
<td>Sympathetic nerve function and dysfunction markers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAOA</td>
<td>−1.88</td>
<td>0.002</td>
</tr>
<tr>
<td>SEMA3C</td>
<td>−1.51</td>
<td>0.020</td>
</tr>
<tr>
<td>NPPB precursor</td>
<td>2.72</td>
<td>0.012</td>
</tr>
<tr>
<td>Nerve damage–related marker</td>
<td>1.53</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Values expressed as means with p values reflecting the comparison between the groups.

AGTR1 = angiotensin II receptor, type 1; FGF12 = fibroblast growth factor 12; MAOA = monoamine oxidase A; MEF2c = myocyte enhancer factor 2C; NCAM1 = neural cell adhesion molecule 1; NPPB = natriuretic peptide B; PARK7 = Parkinson disease (autosomal recessive, early onset) 7; PDGFA = platelet-derived growth factor alpha polypeptide; SEMA3C = sema domain (sema domain) 3C.

### Table 3. Serum Microarray

<table>
<thead>
<tr>
<th>Genes of Interest</th>
<th>ND Versus UD</th>
<th>CD Versus UD</th>
<th>ND Versus CD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fold Change</td>
<td>p Value</td>
<td>Fold Change</td>
</tr>
<tr>
<td>Oxidative stress markers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NOX4</td>
<td>1.11</td>
<td>0.036</td>
<td>1.17</td>
</tr>
<tr>
<td>NOS1</td>
<td>−1.00</td>
<td>0.981</td>
<td>1.37</td>
</tr>
<tr>
<td>OXAIL</td>
<td>1.02</td>
<td>0.744</td>
<td>−1.22</td>
</tr>
<tr>
<td>Profibrotic markers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMP19</td>
<td>1.05</td>
<td>0.325</td>
<td>1.19</td>
</tr>
<tr>
<td>Sympathetic nerve function and dysfunction markers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NGF</td>
<td>1.19</td>
<td>0.102</td>
<td>1.39</td>
</tr>
<tr>
<td>EDNRB</td>
<td>−1.01</td>
<td>0.903</td>
<td>1.12</td>
</tr>
<tr>
<td>NPY1R</td>
<td>1.04</td>
<td>0.567</td>
<td>−1.25</td>
</tr>
</tbody>
</table>

Values expressed as means with p values reflecting the corresponding comparison between indicated groups.

CD = controlled diabetes; EDNRB = endothelin receptor B; MMP19 = matrix metalloproteinase 19; ND = no diabetes; NFG = nerve growth factor (β-polypeptide); NOS1 = nitric oxide synthase 1 (neuronal); NOX4 = nicotinamide adenine dinucleotide phosphate (NADPH) oxidase 4; NPY1R = neuropeptide Y receptor Y1; OXAIL = oxidase (cytochrome c) assembly 1-like; UD = uncontrolled diabetes.
and decreased sympathetic nerves [22, 23]. However, specific mitochondrial oxidative stress–related epigenetic changes in genes regulating fibrosis and NGFs in response to hyperglycemia are unique findings of our study. The upregulation of NOX4 and downregulation of OXATL and NOX1 genes further cause mitochondrial damage, oxidative stress, and a decrease in nitric oxide, which is an important neurotransmitter. Furthermore, we have demonstrated the altered gene effects on profibrotic proteins and proteins involved in angiogenesis.

Our findings suggest that the resultant oxidative stress possibly leads to upregulation of endothelial peptide ET-1 and the renin-angiotensin hormonal system after CPB. In addition to increasing the hemodynamic stresses, renin and angiotensin exert a direct adverse effect on cardiomyocytes, causing apoptosis and stimulation of fibrosis [24]. The downregulation of genes involved in nerve growth (monoamine oxidase and sema domain 3C) could also further decrease NGFs and neurotransmitters. This observation is corroborated by previous studies that have shown the reduction in neurotransmitters and sympathetic nerves as a possible mechanism for neuropathy in diabetes [6]. Decreased nerve growth results in decreased NPY, which is considered the “master switch” for angiogenesis and cardiac remodeling under stress and ischemia [9, 25].

Our observations imply that the deleterious effects of chronic elevation in glucose levels in the months and weeks before surgical procedures remain at play even if glucose levels are brought within normal limits intraoperatively. This brings into question the policy of intraoperative glycemic control in the presence of preceding UD. The end point of glucose control should be controlled HbA1C rather than controlled glucose levels in the acute intraoperative setting. Our study raises the question of whether there exists a critical threshold of

---

**Fig 5.** Summary of the proposed mechanism for observed altered mitochondrial function, nerve expression, fibrosis, and angiogenesis in right atrial tissue from patients with uncontrolled chronic diabetes. Increased advanced glycosylation end products and hexosamine and polyol pathways cause impaired fatty acid metabolism. The use of glucose for sorbitol formation exhausts nicotinamide adenine dinucleotide phosphate (NADPH), a substrate for glutathione reductase, an enzyme that prevents oxidative stress. Similarly, there are decreased levels of antioxidant manganese superoxide dismutase (MnSOD). The increased reactive oxygen species lead to upregulation of inflammation and increased fibrosis in the cardiomyocytes. Importantly, there are decreased levels of nerve growth factor (NGF) and neurotrophin (NT)-3 (neurotrophins) from cardiomyocytes effecting nerve growth and differentiation angiogenesis. The inflammatory markers, decreased sympathetic nerves, and decreased levels of neurotrophins cause increased antiangiogenic markers (angiostatin) and decreases angiogenic protein platelet-derived growth factor (PDGF).
chronic hyperglycemia for cellular oxidative stress. Future studies are needed to address whether tight glycemic control during cardiac operations can modify the observed myocardial oxidative stress in this particular patient population and possibly improve outcomes.

The number of participants in the study is relatively small and patients have not been followed for a long time to allow for outcomes analysis. Also, there is a possibility of local ischemia at the site of tissue harvest where the venous cannula was sutured, and we used right atrial tissue only for our study. However, the atrial tissue differs from ventricular tissue only in the relative distribution of neuronal tissue, myocytes, and endothelium. In addition to being of only partial thickness, ventricular biopsies are associated with possible myocardial injury and associated morbidity, compromising their feasibility during the surgical procedure.

In conclusion, chronic hyperglycemia leads to increased oxidative stress and decreased vessel and nerve growth in the diabetic myocardium after CP/CPB. These effects may be countered by strict long-term glucose control before surgical intervention.

We thank Debbie Bennett and Bejan Abaspour for obtaining samples, Drs Lay-Hong Ang and Yi Zheng for help with immunohistochemical staining, and the Ronald M. Weintraub family research fund for their support.

References


DISCUSSION

DR PAVAN ATLURI (Philadelphia, PA): I’ve actually got a quick question for you. Looking at your demographics table, it looks like the preoperative hemoglobin A1c clearly was significantly different between the controlled and the uncontrolled diabetics, but looking at the preop glucose, it looked like your p values were essentially 0.2.
DR MATYAL: Yes, the preop glucose was not different, so hemoglobin was seen, actually, that’s what the whole point is, it’s showing the chronicity of those patients. Most of these patients where we could follow their hemoglobin, all of our patients get hemoglobin A1c when they come for cardiac surgery, and some of the patients we proceed that hemoglobin A1c prior 3 months, so basically that’s showing you the chronicity of their uncontrolled diabetes. That’s why it’s interesting that their levels of blood sugar at that time was not that much different. It’s over for the last actually 3 or 4 months they were above that level.

DR ATLURI: Do you think this is more of a chronic influence not the acute preoperative state influencing it?

DR MATYAL: Yes. Chronic and uncontrolled most probably. That one particular time point they were not different; maybe for the last 3, 4 months they might be uncontrolled.

DR ATLURI: And I guess the next question, which is sort of the million-dollar question is: Do you think that tight glycemic control can actually reverse your oxidative stress? Because it looks like your levels of NF-kβ or ADIPOR1 really weren’t statistically different between your uncontrolled and controlled diabetics, especially in correlation with the nondiabetics, so is tight diabetic glycemic control something that could actually reverse the oxidative process?

DR MATYAL: Most probably, that’s what we think. At least it reverses to this extent where we don’t see the rest of the damage, especially those growth factors, most probably, and then angiogenesis. It might not, but there is a subtle, looks like a subtle change that will cause the more adverse effects in uncontrolled diabetics causing more effect on those growth factors, especially in angiogenic growth factors and neurogenic factors might not be that much different than like impaired metabolism, but it might—that subtle point might be enough to cause no damage.

DR MUATH BISHAWI (Stony Brook, NY): Thank you, I enjoyed your talk. Just 2 quick questions.

One, you probably didn’t have enough patients, but did you try to stratify by the pulmonary bypass time to see if there is a dose-response relationship with longer times correlating to the expression? And then the second one is, your microarray slide, are those numbers indexed for the baseline microarray findings? Because I’m sure you took baseline tissue and then tissue after bypass.

DR MATYAL: Yes, these are the difference between the baseline and the after. And to answer to your question, these are all the patients we selected who came for cardiopulmonary bypass. So basically I didn’t look at exactly the p value, but the cardiopulmonary bypass time was not different in those. It was because they all came for 1 single procedure and basically done by the same 2 surgeons, so not that much difference, but in our text we should incorporate it.

DR MICHAEL JESSEN (Dallas, TX): I enjoyed your study. Are you able to control for some of the other variables? For example, the diabetic patients may be receiving drugs like metformin or oral hyperglycemic agents that the control group is not. Could that be a factor affecting your results? And another thing is that our diabetic patients receive a lot more insulin. While nondiabetic patients often get insulin, even with the same glucose control protocol, a lot higher insulin dose is given to those who start with the higher glucose levels. Could that be a factor in your study?

DR MATYAL: I don’t know, maybe, but with these patients, at least our controlled diabetics and uncontrolled diabetics, the drugs, they were on insulin and metformin, almost there was not that much difference. We have in our text we wrote down, but they both got insulin. That’s why I’m showing that in our post-bypass period, the sugar level would decrease actually in our uncontrolled diabetics because, of course, as you said, they got more insulin. I’m not sure whether that will affect the oxidative stress. I can ask Dr Sellke.

DR JESSEN: It’s something to look into. Were there any differences in outcomes including things like cardiac enzyme release amongst the groups? Since 1 group clearly had higher oxidative stress, did that show up in any of the other conventional markers that we look at?

DR MATYAL: Well, it was a small group. I didn’t look at the outcome. There was definitely a difference in the postop A-fib, but I didn’t have many patients where I could see the difference in their postop enzyme release.

DR JESSEN: And which group had more atrial fibrillation, was that the group with the most oxidative stress?

DR MATYAL: No, the higher A-fib was in the uncontrolled group, yes, but it was not significant, it was like 1 or 2 higher, so I think we need to do a bigger study to look at all those postop outcome differences.

DR JESSEN: Thanks very much.

DR ATLURI: Along those lines, did you actually, I mean, clearly you show a very nice difference in your fibrosis markers, did you see that that correlated with postoperative ejection fraction or myocardial function? Was there more myocardial dysfunction in the uncontrolled diabetics compared with the other 2 groups?

DR MATYAL: No, I did not. We should have done the echo, like I can go back and look at their echo findings and see if there is any more diastolic dysfunction in those patients. I didn’t look at it.