Diabetes mellitus (DM) affects more than 10% of the United States population and is increasing dramatically in prevalence. Patients with diabetes have significantly higher risk for coronary microvascular or macrovascular diseases than individuals without diabetes. Cardiopulmonary bypass (CPB) and cardioplegic arrest and cardiopulmonary bypass (CP/CPB) are associated with increased vasoconstriction and endothelial dysfunction, which contribute enhanced coronary vasoconstriction, impaired blood flow, and increased cardiovascular morbidity and mortality in DM patients. Vasopressin receptors in the mediastinum region are upregulated after the effects of vasopressin in the coronary circulation of diabetes. 1, 2

The goal of this research is to delineate the role of vasopressin in coronary mediastinum vasoactivity of DM patients after CPB and cardiac surgery.

**Hypothesis**
Diabetes mellitus may upregulate vasopressin receptor expression and alter contractile response of coronary arteries and myocardium as measured by western blots and immunohistochemistry.

**Specific Aims**
To examine whether diabetes alters coronary artery response to Vasopressin.
To examine whether diabetes and CP/CPB affect Vasopressin receptor expression localization in coronary arteries and myocardium as measured by western blots and immunohistochemistry.

**Methods**

**Criteria For Case Selection**
Hemoglobin A1C (HbA1C) was measured in all patients and the patients were then divided into the following two groups: 1) those with a normal HbA1C and no history of treatment for diabetes were considered non-diabetic (ND); 2) Diabetic patients with an HbA1C ≥ 8.5 were considered poorly controlled diabetes (DM). Patients who also underwent valve surgery were excluded from the study. Patients with cross-clamp time greater than 120 minutes and/or CPB time greater than 180 minutes, the patient will be excluded from the study. N=8/group.

**CPB Prime and Cardioplegic Solution**
The standard pump-prime solution (total 1700ml) was combined a 1200ml Plasma-Lyte A (Baxter Healthcare, Deerfield, IL) with 10 ml heparin (1000 iu/ml), 2.5 ml NaHCO3 (8.4%), 250 ml albumin (5%), 60 ml mannitol (300 mg/ml) with target flow rates of 2.4 to 2.8 L/min/m2 for all patients.

**In-Vitro Microvessel Preparation**
Cardioplegic arrest and cardiopulmonary bypass (CP/CPB) are associated with increased vasoconstriction and altered contractile response of coronary vessels to vasopressin in the setting of CPB: Methods. The right arterial tissue samples of diabetic (DM) and non-diabetic (ND) patients were harvested before and after CP/CPB. The included coronary arteries (8-10 mm in diameter) from the harvested right atrial tissue samples were cannulated and perfused. The changes in diameter were measured using video microscopy. The protein expression was determined by using a known vasoconstrictor (vasopressin receptors (V1A) and (V1B)) in the arterial tissues were measured by immunofluorescence staining. The protein expression of (V1A) and (V1B) contractile responses of the coronary arteries to vasopressin were significantly increased compared to the N.D group. The result was more pronounced in the diabetic patients than the non-diabetic patients.

**Results**

**Immunoblotting**
Arterial tissues from six patients were procured and cleaned of connective tissues, then embedded in OCT embedding buffer. Total proteins (40 mg) was functioned on an 8-10% SDS-PAGE gel, then transferred to a nitrocellulose membrane (Millipore Corporation, Bedford, MA). Membranes were incubated for 1 hour at room temperature with 1:1000 dilutions of primary antibodies to anti-V1A and V1B receptor antibodies (ABCAM, Cambridge, MA) at 4°C. The membranes were then washed in PBS and incubated with the appropriate HRP-secondary antibody and reacted using fluorcent mounting medium (Vector Labs, Burlingame, CA). These were visualized using a Zeiss LSM510 confocal microscope system (Carl Zeiss Microimaging, Inc. Thornwood, NY).

**Immunofluorescence Staining**
Arterial tissues from five sections from five patients were depauplified in cyclohexan, embedded in graded ethanol and paraffin-embedded tissues sections (PAB), and antigen-unmasked with sodium citrate (10 mmol/L, pH 6.8), followed by PBS wash and blocking with 0.7% bovine serum albumin in PBS at room temperature for 2 hrs. After PBS wash, sections were incubated overnight with V1A and V1B antibodies (each used at 1:200) (ABCAM, Cambridge, MA) at 4°C. Sections were then washed in PBS and incubated with the appropriate Alexa Fluor secondary antibody and mounted using fluorescent mounting medium (Vector Labs, Burlingame, CA). Three were visualized using a Zeiss LSM510 confocal microscope system (Carl Zeiss Microimaging, Inc. Thornwood, NY).

**Conclusion**
Increased Coronary Arteriolar Reactivity to Vasopressin in Diabetic Patients After Cardiac Surgery

**Abstract**

**Introduction**
Cardiac arrest and cardiopulmonary bypass (CP/CPB) are associated with increased vasoconstriction and altered contractile response of coronary vessels to vasopressin in the setting of CPB. Methods. The right arterial tissue samples of diabetic (DM) and non-diabetic (ND) patients were harvested before and after CP/CPB. The included coronary arteries (8-10 mm in diameter) from the harvested right atrial tissue samples were cannulated and perfused. The changes in diameter were measured using video microscopy. The protein expression was determined by using a known vasoconstrictor (vasopressin receptors (V1A) and (V1B)) in the arterial tissues were measured by immunofluorescence staining. The protein expression of (V1A) and (V1B) contractile responses of the coronary arteries to vasopressin were significantly increased compared to the N.D group. The result was more pronounced in the diabetic patients than the non-diabetic patients.

**Conclusions**
Vasopressin may induce coronary arterial constriction via V1A receptors. This alteration may lead to increased coronary arterial constriction in diabetic patients undergoing CP/CPB and cardiac surgery.

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References

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ENHANCED CORONARY ARTERIOLE REACTIVITY TO VASOPRESSIN IN DIABETIC PATIENTS AFTER CARDIAC SURGERY