Effects of diabetes and cardiopulmonary bypass on expression of adherens junction proteins in human peripheral tissue

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Background. *We investigated the changes in adherens junction proteins, such as vascular endothelial-cadherin and β-catenin, of skeletal muscle and vessels in patients with or without diabetes in the setting of cardiopulmonary bypass and cardiac operation.*

Methods. *Skeletal muscle tissue samples were harvested pre- and post-cardiopulmonary bypass from nondiabetic (hemoglobin A1c: 5.4 ± 0.1), controlled diabetic (hemoglobin A1c: 6.3 ± 0.1), and uncontrolled diabetic patients (hemoglobin A1c: 9.6 ± 0.3) undergoing coronary artery bypass grafting operation (n = 8 per group). The expression/phosphorylation of adherens junction proteins vascular endothelial-cadherin and β-catenin were assessed by immunoblotting and immuno-histochemistry. Endothelial function of skeletal muscle arterioles was determined by videomicroscopy in response to the vasodilator substance P.*

Results. *The protein expression of total vascular endothelial-cadherin was not changed at baseline or between pre–and post–cardiopulmonary bypass among groups. The pre-cardiopulmonary bypass level of phospho-vascular endothelial-cadherin was found to be significantly increased in the uncontrolled diabetic patients group compared with the nondiabetic or controlled diabetic groups ($P < .05$). The post–cardiopulmonary bypass levels of phospho-vascular endothelial-cadherin were significantly increased compared with pre–cardiopulmonary bypass in all groups ($P < .05$ each), and this increase was greater in the uncontrolled diabetic patients group than that of the nondiabetic or controlled diabetic groups ($P < .05$). Expression of basal β-catenin protein in the uncontrolled diabetic group was decreased compared with nondiabetic or controlled diabetic groups ($P < .05$). There were significant decreases in the β-catenin protein expression between pre– and post–cardiopulmonary bypass in all 3 groups ($P < .05$ each), and this decrease was greater in the uncontrolled diabetic patients group than the nondiabetic group ($P < .05$). There were decreases in the relaxation response of skeletal muscle arterioles to substance P after cardiopulmonary bypass in all 3 groups ($P < .05$), and this alteration was more pronounced in the uncontrolled diabetic patients ($P < .05$).*

Conclusion. *Uncontrolled diabetes causes inactivation and reduction in the expression of endothelial adherens junction proteins in the arterioles of skeletal muscle early after cardiopulmonary bypass. The enhanced phosphorylation of vascular endothelial-cadherin and degradation of β-catenin indicate deterioration of these proteins and damage of the cell-cell endothelial junctions, specifically in the diabetic peripheral vessels. These alterations may contribute to the increases in peripheral vascular permeability and endothelial dysfunction. (Surgery 2016;■:■-■.)*

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Supported by the National Institute of Health (NIH) R01-HL46716 and RO1HL128831 to F.W.S. This work was supported in part by the Institutional Development Award from the National Institute of General Medical Science of the NIH (5P20-GM103652 [Pilot Project]), NIH-1R01HL127072-01A1, and AHA-Grant-in-Aid (#15GRNT25710105) to J.F.; Summer Assistantship Award through Basic and Translational Research Program (NIH T35HL094308) to N.S.; and Summer Research Assistantship Award of Brown University's Program in Liberal Medical Education to J.L.

Presented at the 11th Annual Academic Surgical Congress, February 2–4, 2016, Jacksonville, FL.

Accepted for publication August 23, 2016.

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0039-6060/$ - see front matter

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http://dx.doi.org/10.1016/j.surg.2016.08.057

Cardiopulmonary bypass (CPB) is often associated with increased vascular permeability, microvascular endothelial cell injury/dysfunction, and decreased peripheral vasomotor tone, manifested as postoperative systemic hypotension and tissue edema.[1-5] In particular, these disturbances are more pronounced in the uncontrolled diabetic patients.[1-5] These effects can be attributed to CPB-induced systemic inflammation and increased expression of vascular endothelial growth factor (VEGF) and vascular permeability factor.[1,6,7] These alterations may contribute to an increased duration of stay and worse outcomes in uncontrolled diabetic patients after cardiac operation.[1,6-8]

The mechanisms responsible for CPB-initiated peripheral vascular permeability need to be defined further. Recently, we demonstrated that the increased permeability after cardioplegic ischemia/reperfusion is associated with changes in the expression/phosphorylation of adherens junction proteins of the coronary microvasculature in uncontrolled diabetic patients.[8] Therefore, we hypothesized that diabetes may also cause downregulation of adherence-junction-proteins in peripheral tissues such as the arterioles of skeletal muscle early after CPB. Thus, the aims of the present study were to determine the role of diabetes in expression of selected proteins (vascular endothelial [VE]-cadherin and β-catenin) in adherens-junctions in human skeletal muscle and peripheral microvasculature and to investigate the effects of diabetes on arteriolar endothelial function in the setting of CPB and cardiac operation.

METHODS

Human subjects and tissue harvesting. Samples of skeletal muscle from the left intercostal muscle bed were harvested pre- and post-CPB from 100 patients undergoing coronary artery bypass grafting. Hemoglobin A1C (HgbA1c) was measured in all patients. The patients were divided into the following 3 groups: (1) patients with a normal HgbA1c and no history or treatment for diabetes were considered nondiabetic (ND); (2) patients with a history of diabetes with a HgbA1c >5.5 but ≤7 were considered well controlled (CDM); and (3) diabetic patients with a HgbA1c ≥8.5 were considered uncontrolled (UDM). Patients who also underwent valve operation were excluded from the study. Although there is no definitive HgbA1c level that is universally accepted as a marker for poorly controlled diabetes, an HgbA1C of ≤7 is regarded generally as a marker of well-controlled diabetes, and HgbA1C >7 is generally regarded as less well controlled; an HgbA1C of 8.5 is indicative of poorly controlled diabetes.

Eight randomly chosen patients in each of 3 groups from 100 cases were included for analysis in this study. The first sample of skeletal muscle in the left intercostal muscle bed was harvested after cannulation before CPB (pre-CPB), and the second sample of skeletal muscle was obtained after removal of the aortic cross-clamp and weaning from CPB (post-CPB).[2] Tissue samples were frozen immediately in liquid nitrogen or stored immediately in 10% formalin for immunoblot analysis and immunofluorescent staining. Tissue samples for microvascular reactivity were stored immediately in cold (5–10°C) Krebs buffer solution.[2] All procedures were approved by the Institutional Review Board of Rhode Island Hospital, Alpert Medical School of Brown University, and informed consent was obtained from all enrolled patients.

Immunoblot. The methods for tissue protein purification, Western blotting, and imaging quantification have been described previously.[2-5,8] Membranes were incubated overnight at 4°C with primary antibodies against VE-cadherin (Cell Signaling, Danvers, MA), phospho-VE-cadherin (Y685), and β-catenin (ABCAM, Cambridge, MA). After washing with TBST, membranes were incubated with the appropriate secondary antibody conjugated to horseradish peroxidase. All membranes were also incubated with GAPDH (glyceraldehyde-3-phosphate, Cell Signaling) for loading controls.

Immunofluorescence microscopy. The detailed methods have been described previously.[2-4,8] After PBS wash, tissue sections of skeletal muscle were incubated overnight with anti-phospho-VE-cadherin antibody (LifeSpan BioScience, Inc, Seattle, WA) and/or anti-smooth muscle α-actin antibody at 4°C (Cell Signaling). The tissue sections were finally mounted with VECTASHIELD Mounting Medium with DAPI (4′,6-diamidino-2-phenylindole; Vector Laboratories, Burlingame, CA).

Microvessel reactivity. Arterial microvessels (100–180 μm internal diameters, $n = 8$ per group) were dissected from skeletal muscle samples taken pre- and post-CPB. Microvessel studies were performed in vitro in a pressurized (40 mm Hg) no-flow state using video-microscopy as previously described.[2-5,8] The vessel was precontracted with thromboxane analog U46619 (3×10^{-6}M to 10^{-7}M) to achieve 30–40% of baseline diameter. Substance P (10^{-12}M to 10^{-7}M) was added to the organ bath and diameter measurements were

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