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NEW RESEARCH PAPER

Red Blood Cells in Type 2 Diabetes Impair Cardiac Post-Ischemic Recovery Through an Arginase-Dependent Modulation of Nitric Oxide Synthase and Reactive Oxygen Species

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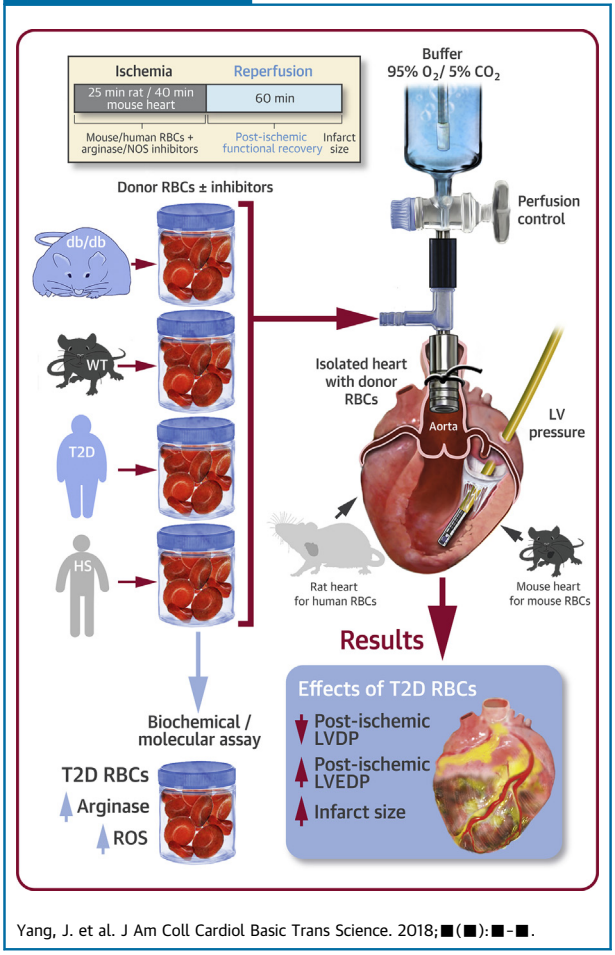
VISUAL ABSTRACT



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HIGHLIGHTS

- **RBCs from mice and patients with type 2 diabetes have increased arginase activity and production of reactive oxygen species.**
- **RBCs from mice and patients with type 2 diabetes aggravate myocardial ischemia-reperfusion injury.**
- **Inhibition of arginase in RBCs from mice and patients with type 2 diabetes improves post-ischemic myocardial recovery via reduced oxidative stress.**
- **Inhibition of nitric oxide synthase in RBC reduces oxidative stress and restores post-ischemic myocardial functional recovery.**
- **These data demonstrate a novel disease mechanism by which RBC drive post-ischemic cardiac dysfunction in type 2 diabetes.**

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ABBREVIATIONS AND ACRONYMS

ABH = 2 (S)-amino-6-boronohexanoic acid

dP/dt = the first derivative of left ventricular pressure

eNOS = endothelial nitric oxide synthase

iNOS = inducible isoform of nitric oxide synthase

KH = Krebs-Henseleit

L-NAME = N^G-nitro-L-arginine methyl ester

LVDP = left ventricular developed pressure

LVEDP = left ventricular end-diastolic pressure

NAC = N-acetylcysteine

NO = nitric oxide

nor-NOHA = N^{ω}-hydroxy-nor-L-arginine

NOS = nitric oxide synthase

RBC = red blood cell

ROS = reactive oxygen species

WT = wild type

SUMMARY

This study tested the hypothesis that red blood cell (RBC) arginase represents a potential therapeutic target in ischemia-reperfusion in type 2 diabetes. Post-ischemic cardiac recovery was impaired in hearts from db/db mice compared with wild-type hearts. RBCs from mice and patients with type 2 diabetes attenuated post-ischemic cardiac recovery of nondiabetic hearts. This impaired cardiac recovery was reversed by inhibition of RBCs arginase or nitric oxide synthase. The results suggest that RBCs from type 2 diabetics impair cardiac tolerance to ischemia-reperfusion via a pathway involving arginase activity and nitric oxide synthase-dependent oxidative stress. (J Am Coll Cardiol Basic Trans Science 2018;■:■-■) © 2018 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

It is well established that type 2 diabetes is an important risk factor for development of myocardial infarction (1,2) and for poor outcome following an acute coronary event (3). A key component contributing to cardiovascular complications including ischemic heart disease in type 2 diabetes is an altered vascular homeostasis involving reduced bioavailability of nitric oxide (NO) and increased oxidative stress (1,2,4-7). The mechanisms behind reduced bioavailability of NO are complex and involve both reduced production by endothelial nitric oxide synthase (eNOS) and increased inactivation of NO by up-regulation of reactive oxygen species (ROS). Previously it has been demonstrated that an important regulator of NO production is arginase, which competes with eNOS for their common substrate L-arginine (8,9). Arginase may also trigger formation of ROS by inducing uncoupling of NOS, a condition in which eNOS produces superoxide instead of NO (4,9-11). By these mechanisms increased arginase activity is suggested to contribute to cardiovascular dysfunction.

The source of these actions of arginase in the cardiovascular system has until recently been considered to be the endothelium (8,12). Of importance, we have recently demonstrated that arginase 1 expressed in red blood cells (RBCs) serves as a critical regulator of the formation and export of cardioprotective NO-like bioactivity produced by RBCs eNOS during ischemia-reperfusion (13). This novel source and effect of arginase was shown to be of importance for cardiac function. Thus, inhibition of RBCs arginase protects the heart from ischemia-reperfusion injury via an eNOS-dependent mechanism. Additionally, in vivo observations support a role of RBC eNOS during myocardial ischemia-reperfusion (14). However, the pathophysiological role of arginase as a regulator of NO bioavailability and ROS production in RBCs in the setting of myocardial ischemia-reperfusion under conditions with increased arginase activity remains unknown. Interestingly, increased arginase activity has emerged as an important regulator of NO formation and ROS production in diabetes (15,16). Furthermore, a previous study has suggested that arginase is up-regulated in RBCs from patients with diabetes (17). Therefore, it is conceivable to assume that up-regulation of RBC arginase in diabetes is of functional importance for the susceptibility to myocardial ischemia-reperfusion injury. Based on these considerations, we tested the hypothesis that up-regulation of RBC arginase is a key

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All authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the *JACC: Basic to Translational Science* author instructions page.

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