Microvascular Research 101 (2015) 1–7

Contents lists available at ScienceDirect

Microvascular Research

journal homepage: www.elsevier.com/locate/ymvre

Impaired coronary and retinal vasomotor function to hyperoxia in Individuals with Type 2 diabetes

Mary E. Lott [a,*], Julia E. Slocomb [b], Zhaohui Gao [a], Robert A. Gabbay [c], David Quillen [d], Thomas W. Gardner [e], Kerstin Bettermann [f]

[a] Penn State Hershey Medical Center, Heart and Vascular Institute, Hershey, PA 17033, USA
[b] John Hopkins University, Department of Biology, Baltimore, MD 21218, USA
[c] Joslin Diabetes Center, Harvard Medical School, Boston, MA 02116, USA
[d] Penn State Hershey Medical Center, Department of Ophthalmology, Hershey, PA 17033, USA
[e] University of Michigan, Kellogg Eye Center, Ann Arbor, MI 48105, USA
[f] Penn State Hershey Medical Center, Department of Neurology, Hershey, PA 17033, USA

ARTICLE INFO

Article history:
Accepted 12 May 2015
Available online 20 May 2015

Keywords:
Coronary reactivity
Retinal reactivity
Diabetes
Vasoconstriction
Hyperoxia

ABSTRACT

Purpose: Adults with diabetes are at a high risk of developing coronary heart disease. The purpose of this study was to assess coronary artery vascular function non-invasively in individuals with and without Type 2 diabetes and to compare these coronary responses to another microvascular bed (i.e. retina). We hypothesized that individuals with diabetes would have impaired coronary reactivity and that these impairments would be associated with impairments in retinal reactivity.
Methods: Coronary blood velocity (Transthoracic Doppler Echocardiography) and retinal diameters (Dynamic Vessel Analyzer) were measured continuously during five minutes of breathing 100% oxygen (i.e. hyperoxia) in 15 persons with Type 2 diabetes and 15 age-matched control subjects. Using fundus photographs, retinal vascular calibers were also measured (central retinal arteriole and venule equivalents).
Results: Individuals with diabetes compared to controls had impaired coronary ($-2.34 \pm 16.64\%$ vs. $-14.27 \pm 10.58\%$, $P = 0.03$) and retinal (arteriole: $-0.04 \pm 3.34\%$ vs. $-3.65 \pm 5.07\%$, $P = 0.03$; venule: $-1.65 \pm 3.68\%$ vs. $-5.23 \pm 5.47\%$, $P = 0.05$) vasoconstrictor responses to hyperoxia, and smaller central arteriole–venule equivalent ratios (0.83 ± 0.07 vs. 0.90 ± 0.07, $P = 0.014$). Coronary reactivity was associated with central retinal arteriole equivalents ($r = -0.516$, $P = 0.005$) and retinal venular reactivity ($r = 0.387$, $P = 0.034$).
Conclusion: Diabetes impairs coronary and retinal microvascular function to hyperoxia. Impaired vasoconstrictor responses may be part of a systemic diabetic vasculopathy, which may contribute to adverse cardiovascular events in individuals with diabetes.

Introduction

Adults with diabetes are two to four times more likely to have heart disease than adults without diabetes (Centers for Disease Control and Prevention (CDC), 2014). Endothelial vascular dysfunction is the initiating process in the development of heart disease (Caballero, 2003). Abnormalities of coronary vascular vasodilation using pharmacological methods or sympathetic stimulation (cold pressor test) have been reported in patients with diabetes even in the presence of normal coronary arteries (Akasaka et al., 1997; Di Carli et al., 2003; Nahser et al., 1995; Nitenberg et al., 1993; Prior et al., 2005; Yokoyama et al., 1997). However, these techniques involve methodologies, which were invasive (i.e. angiograms) and/or expensive (i.e. pharmacological adenosine and positron emission tomography (PET)). Yet vascular function in the coronary arteries can be studied in other ways. Transthoracic Doppler echocardiography (TTDE) may be a good non-invasive and low cost alternative for examining coronary reactivity to a variety of stimuli in individuals who are healthy as well as those with diabetes (Atar et al., 2012). We were interested in using a robust systemic stimulus in which the coronary vascular bed and another microvascular bed could be examined under similar conditions.

The administration of 100% oxygen (i.e. hyperoxia) has been shown to be a potent non-invasive stimulus to measure vascular function (i.e. reactivity) in the coronary vascular bed during angiograms in individuals with coronary artery disease (McNulty et al., 2005, 2007). The underlying mechanism of hyperoxia-induced vasoconstriction is believed to be due in part to an increased oxidative degradation of endothelial-derived nitric oxide (Jamieson et al., 1986; Rubanyi

* Corresponding author at: Penn State Heart and Vascular Institute, The Milton S. Hershey Medical Center, Campus Box H047, 500 University Drive, Hershey, PA 17033-2390, USA. Fax: +1 717 531 1792.
E-mail address: mlott@hmc.psu.edu (M.E. Lott).

http://dx.doi.org/10.1016/j.mvr.2015.05.002
0026-2862/

and Vanhoutte, 1986b); however, animal studies suggest that other factors such as increases in arachidonic acid and the endothelin-1 may also play a role in hyperoxic vasoconstriction (Zhu et al., 1998). Thus, regardless of the mechanism, hyperoxia can be used as potent vascular vasoconstrictor. Our laboratory has used this stimulus to measure changes in peak coronary blood velocity with TTDE in healthy subjects (Gao et al., 2012; Momen et al., 2009). However, there has been a lack of studies using hyperoxia to examine the impact of diabetes on coronary blood flow dynamics measured by TTDE and to examine whether vascular functions in other microvascular beds such as retinal vascular bed are equally affected.

The purpose of this study was to assess the effect of diabetes on epicardial coronary blood velocity responses to hyperoxia measured by TTDE. We hypothesized that individuals with Type 2 diabetes would have impaired coronary reactivity. In addition, we compared the magnitude of change of coronary blood velocity responses to a change in another microvascular bed (i.e. the retinal blood vessels) to hyperoxia. We hypothesized that the magnitude of change to hyperoxia would be similar between the coronary and retinal vascular beds.

Material and methods

Subjects

Thirty of 44 enrolled subjects recruited by study research flyers had complete retinal and coronary studies. Individuals with Type 2 diabetes ($n = 15$) ranged in age from 38 to 73 years and were non-smokers. Control subjects ($n = 15$) were age and body mass index (BMI) matched similar to the diabetic group. The diagnosis of diabetes was based on hemoglobin $A1_C$ ($HbA1_C$) levels using the American Diabetes Association criteria of diabetes to be $HbA1_C \geq 6.5\%$ and non-diabetic to be $HbA1_C < 5.7\%$ (American Diabetes Association, 2010). All participants signed an informed consent that was institutionally approved by the Penn State College of Medicine Institutional Review Board prior to testing under the Declaration of Helsinki. All individuals had a physical examination and eye screening and were excluded if they had any history of cardiac, cerebrovascular, or pulmonary disease, uncontrolled hypertension, intraocular pressures greater than 21 mm Hg, diabetic retinopathy or other eye diseases, seizures, were currently pregnant or breastfeeding, had a BMI greater than 45 kg/m^2 or were unable to fixate using the Dynamic Vessel Analyzer (DVA).

Study design & protocol

This explorative cross-sectional design assessed coronary and retinal reactivity to hyperoxia in individuals with Type 2 diabetes compared to healthy controls. Twenty-four hours prior to testing, subjects avoided exercise, caffeine or alcohol containing products, and non-steroidal anti-inflammatory agents. Individuals with diabetes held all their medications except for antihypertensive medications on the morning of the study. Measurements were performed in a dimly lit room at room temperature (21 °C). Subjects arrived in a fasted state and had blood samples of glucose, insulin, lipid panel and HbA1c drawn from the antecubital vein. The eye with the best visual acuity was dilated with tropicamide (1%) and if needed, phenylephrine (2.5%) was added to obtain optimal dilation. Subjects were seated upright for the DVA examination and were positioned supine for the coronary vessel part of the study. After a 15 minute rest period, fundus photographs were initially taken using the DVA. Using a standardized hyperoxia protocol (Lott et al., 2012), coronary blood velocity was measured by TTDE (Momen et al., 2009) and retinal diameters by the DVA (Garhofer et al., 2010). While the study participants inhaled room air, continuous measurements of the retinal diameters were taken to establish baseline data. Oxygen (100%) was then administered via a mouthpiece and a Douglas bag setup for 5 min followed by inhalation of room air. After a 30 minute rest period, the hyperoxia protocol was repeated for coronary blood velocity measurements. Heart rate, blood pressure, end tidal carbon dioxide (CO_{2ET}) and systemic oxygen saturation were measured continuously (see Fig. 1 for study schematic).

Coronary blood velocity

We externally insonated the left descending coronary artery using pulsed ultrasound TTDE (S8-3 MHz probe, IE33, Phillips Healthcare, Andover, MA). For imaging over the apical region of the heart, the probe was manually adjusted and color enhancement was used to locate the artery. Coronary blood velocity was measured continuously (Fig. 2A) and post analysis of peak coronary diastolic velocity was calculated using Prosolve software (Momen et al., 2009). It has been reported that changes in coronary blood velocity reflect changes in absolute coronary blood flow, thus it is a surrogate for coronary blood flow (Doucette et al., 1992).

Retinal arteriole and venule reactivity

Dynamic measurements were obtained using the DVA (Imedos Inc., Germany), which consists of a fundus camera (FF450 Zeiss, Jena, Germany), a charge-coupled device (CCD)-measuring video camera, and a computer (Seifertl and Vilser, 2002). The DVA analyzes the brightness profile of the retinal blood vessels using two optical pathways, and light reflected by the retina back to the CCD video camera. This optical display is viewed on a computer system and, using specialized software, vessel diameters can be measured. On the computerized screen, two regions of interest are marked over a superior or inferior temporal retinal arteriole and venule between one to two optic disc diameters from the optic nerve disc margin (Fig. 2B). Eye-tracking technology in the DVA compensates for small eye movements. All images were stored on a videotape recorder for off line measurements by one observer using the DVA software. The coefficient of variability for measuring retinal blood vessels ranges between 1.5% and 2.8% (Seifertl and Vilser, 2002) and we have similar standards established in our laboratory.

Static fundus photographs

The diameters of all the arterioles and venules traversing a specified area of rings positioned 1 to 2 disc distances from the optic disc were measured using Imedos Visualis software (see Fig. 2C). This software program derives two indices that are estimates of the averaged retinal arteriole and venule calibers taking into consideration branching patterns: central retinal arteriole equivalents and central retinal venular equivalents. A ratio of these two measurements known as the central arteriole–venule equivalent ratio is also calculated (Hubbard et al., 1999; Ikram et al., 2006; Liew et al., 2008; Wong et al., 2002). Smaller arterioles, larger venules, and lower ratio are associated with chronic diseases (Kifley et al., 2008; Nguyen et al., 2008; Wong et al., 2002). Reproducibility for these measurements has been previously reported to be between 0.78 and 0.99 (Hubbard et al., 1999; Wong et al., 2006).

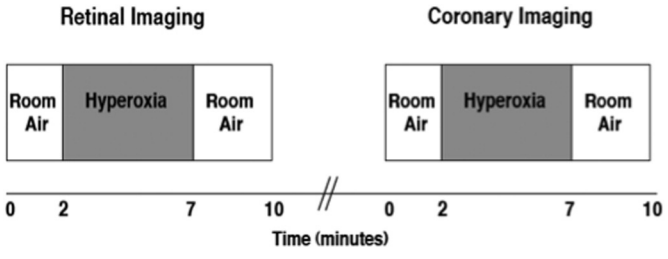

Fig. 1. Experimental protocol. After baseline measurements (1 min) at room air, hyperoxia stimulus was applied for 5 min and then returned back to room air for 3 min of recovery. Retinal imaging with hyperoxia occurred first and then after 30 min of rest, coronary imaging during the hyperoxia was performed.