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Reproducibility of flow mediated skin fluorescence to assess microvascular function

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ABSTRACT

Objective: Recent technical developments enable skin fluorescence to be quantified in vivo in humans. The present study aimed at determining whether flow mediated skin fluorescence was reproducible, sensitive to changes within an individual, and if it could differ between patients with coronary artery disease and healthy volunteers.
Methods: First, forearm flow mediated skin fluorescence recorded during and after brachial artery occlusion was assessed following successive forearm occlusion periods (1, 2, 3 and 5 min) and expressed as ischemic and hyperemic responses (as % of baseline). Secondly, 3 min flow mediated skin fluorescence was assessed before and after 10 min local cooling to 15 °C. In a third protocol, the inter-day reproducibility of ischemic and hyperemic responses to 3 min occlusion was tested at an interval of 7 days, and compared between healthy controls and patients with coronary artery disease (CAD).
Results: In the first protocol, we observed a time dependent increase in the ischemic and hyperemic responses to occlusion. Next, we observed a lower hyperemic response after local cooling (9.8 ± 4.2 versus 17.8 ± 2.5% respectively, $P < 0.001$), while in contrast, the ischemic response was higher and exhibited greater variability (23 ± 15 versus 11.8 ± 6.4%; $P = 0.028$). In the third protocol, the inter-day reproducibility of flow mediated skin fluorescence for a 3 min occlusion period was excellent. The ischemic response was significantly lower in CAD patients than in healthy controls (6.7 ± 4.8% vs 14.7 ± 6.8% respectively, $P < 0.001$). Similarly, the hyperemic response was significantly decreased in the CAD group compared to healthy controls (11.6 ± 3.6% vs 19.5 ± 5.4% respectively, $P < 0.001$).
Conclusion: We show that quantifying the ischemic and hyperemic flow mediated skin fluorescence is feasible, reproducible, sensitive to acute changes in skin blood flow, and distinguishes patients populations. However, more data are needed to evaluate the correlation with other methods or specific biochemical endothelial markers.

1. Introduction

Coronary artery disease is the single largest cause of death in industrialized countries. There is compelling evidence for a link between microvascular dysfunction and the pathogenesis of cardiovascular disease (Hellmann et al., 2015). Great emphasis has also been placed on the involvement of endothelial dysfunction in the pathogenesis of hypertension, coronary artery disease and heart failure (Flammer et al., 2012). Most studies have focused on assessment of endothelial function in conduit arteries. Although less well studied, microvascular dysfunction may precede endothelial impairment in large arteries and clinical manifestations. The skin microcirculation is a readily accessible vascular bed that can be used as a model of generalized microvascular function (Holowatz et al., 2008).

In humans, microvascular function in the skin has been extensively studied using non-invasive laser Doppler flowmetry (LDF) or more recently laser speckle contrast imaging (LSCI), which provides a real-time quantification of relative changes in tissue perfusion (Roustit and Cracowski, 2013). The conventional approach is to couple these techniques with various dynamic tests such as post-occlusive reactive hyperemia (PORH). Indeed, peripheral microvascular dysfunction assessed by LDF and PORH has been shown to be an independent predictor of atherosclerotic damage (Rossi et al., 2013).

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All current microvascular techniques quantify skin blood flow. However, we are now able to measure changes in skin biochemistry, including the NADH redox state (Mayevsky and Rogatsky, 2007). NADH fluorescence has been used in vitro as a test for mitochondrial function since the 50s. Indeed, the difference in the ultraviolet absorption spectra between the two forms of the coenzyme allows us to measure their interconversion using a spectrophotometer. In addition, NADH possesses substantial fluorescence ability, with a fluorescence emission band from 420 to 480 nm, while NAD^+ does not fluoresce (Mayevsky and Chance, 2007). Light emitted by NADH will therefore depend on the redox equilibrium. During ischemia, the redox state will shift towards the reduced form of the coenzyme leading to an increase in NADH fluorescence while during reactive hyperemia the redox equilibrium will shift towards the oxidized form of the coenzyme, decreasing NADH fluorescence. In addition, a second mechanism influences the detectable NADH fluorescence, the volume of blood in the tested area. Inefficient absorption of excited light by blood red cells and other components of the blood; and poor scattering of the excited light will amplify the decrease in NADH fluorescence during reactive hyperemia (Mayevsky and Rogatsky, 2007). Taken together, NADH fluorescence will depend on both the cellular levels of NADH and on skin perfusion. Recent technical developments enable us to go one step further and to quantify skin fluorescence in vivo in humans. Flow mediated skin fluorescence (FMSF) can now be analyzed to measure the changes in cutaneous fluorescence over time in response to brachial artery occlusion (Piotrowski et al., 2016).

The present study aimed at determining whether FMSF was reproducible, sensitive to changes within an individual, and if it can be used to differentiate between patients with coronary artery disease (CAD) and healthy volunteers.

2. Materials and methods

2.1. Study population

This study enrolled healthy subjects and patients with coronary artery disease (CAD) recruited from the Cardiology Outpatient Clinic at the University Hospital in Gdansk, Poland. All the participants were over 18 years of age and were included between September 2016 and January 2017. A diagnosis of CAD was angiographically confirmed and based on European Society of Cardiology guidelines (Task Force Members et al., 2013). The control group consisted of unrelated healthy volunteers from the general population. All had normal electrocardiogram, ambulatory blood pressure and clinical examination. None of the healthy subjects had any chronic disease or ongoing treatment.

The study conforms to the principles outlined in the Declaration of Helsinki. The study protocol was approved in June 2016 by the Independent Ethics Committee at the Medical University of Gdansk (IRB no. 667). All subjects gave written informed consent before participation.

2.2. Study design

This was an open labeled physiology study. On arrival at the laboratory, subjects were placed in a temperature-controlled room (24 ± 1 °C). After a 15-min acclimatization period, baseline fluorescence intensity was recorded for 3 min on the forearm. Then, blood flow in the brachial artery was occluded by inflating a cuff placed on the left upper arm to 50 mm Hg above systolic blood pressure (Fig. 1). During the occlusion period, the fluorescence was continuously measured in the same area of the forearm. The cuff was then released and the decrease in fluorescence was recorded until return to baseline values.

2.3. Flow mediated skin fluorescence measurements

Flow mediated skin fluorescence (FMSF) is a noninvasive optical technique to study microcirculation based on measurements of skin fluorescence intensity. FMSF was quantified using AngioTester (SN-2016-009M, Angionica, Lodz, Poland).

Excitation of the forearm with ultraviolet (UV) light at 340 nm results in the emission of a NADH fluorescence signal from the skin tissue cells. The level of NADH fluorescence corresponds to the balance of mitochondrial oxidation-reduction processes occurring in the tissue, reflected by the balance between the oxidized form of the coenzyme (NAD^+) and its reduced form (NADH). Indeed, NADH fluorescence is the strongest component of the fluorescence emitted from human skin. The intensity of the signal also changes as a function of time in response to blockage and release of blood flow in the brachial artery (Fig. 2). The emitted fluorescence light of NADH at 460 nm is detected by receiver diode and allows one to monitor the activity of skin microcirculation (Piotrowski et al., 2016).

The maximal penetration of the exciting light (340 nm) is about 0.3 to 0.5 mm, but over 90% of the NADH excitation occurs at a depth of 0.1 mm. Therefore, a substantial fraction of the exciting light is absorbed by the epidermidis. To allow for this, in FMSF, the diameter of the probe (detection window) is relatively large, 20 mm, which gives approximately 100 mm^3 volume of the investigated tissue (Piotrowski et al., 2016).

2.4. Technical description of FMSF device

The FMSF device consists of a light source, system of filters, and detector. The UV diode emits light at 340 nm wavelength and a small amount of blue light to show that the diode is working (Marktech Optoelectronics MTE340H21-UV, Peak Wavelength 340 nm, Spectral Line Half Width 9 nm). Blue light is cut through the band pass filter (Hoya U340) that allow to transmit only UV light at 340 nm and block the visible light. Then the light beam passes through a quartz window, which has excellent transmissions to the skin (over 90%).

NADH fluorescence is the strongest component of overall fluorescence which is emitted from the skin. The emitted fluorescence light

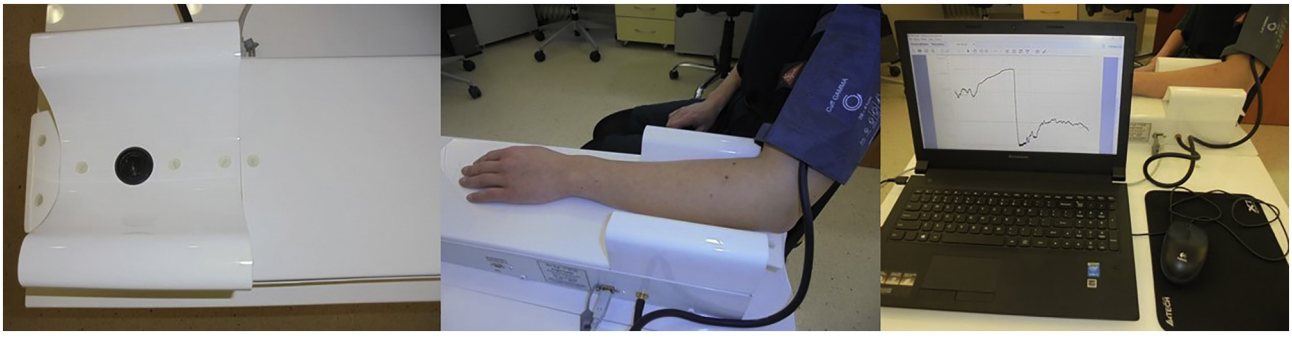

Fig. 1. Experimental setup. NADH fluorescence intensity was recorded on the left forearm of healthy subjects and CAD patients in response to blockage and release of blood flow in the brachial artery.