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Reproducibility of four frequently used local heating protocols to assess cutaneous microvascular function

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ABSTRACT

Background: Skin microvascular responses to local heating are frequently used to assess microvascular function. Several local heating protocols have been developed, all varying slightly in execution. The aim of this study was to determine the inter-day reproducibility of the four most commonly used local heating protocols in healthy young subjects.

Methods: Fifteen, healthy males (28 ± 5 yrs, BMI 25 ± 2 kg/m^2) attended two experimental trials 2–7 days apart. During each trial, baseline and maximal thermally stimulated forearm skin responses were examined simultaneously at four sites on the dominant forearm using laser Doppler flowmetry (LDF). The following heating protocols were adopted: 1. *Rapid 39 °C* (0.5 °C/5 s), 2. *Rapid 42 °C* (0.5 °C/5 s) 3. *Gradual 42 °C* (0.5 °C/2 min 30 s) and 4. *Slow 42 °C* (0.5 °C/5 min). The coefficient of variation (CV) was calculated for absolute flux, cutaneous vascular conductance (CVC; flux/mean arterial pressure, MAP) and CVC expressed as a percentage of maximal CVC at 44 °C (%CVC$_{max}$) at three different time points; baseline (33 °C), plateau (39/42 °C) and maximal (44 °C).

Results: Reproducibility of baseline flux, CVC and %CVC$_{max}$ was 17–29% across all protocols. During the plateau, *Rapid*, *Gradual* and *Slow 42 °C* demonstrated a reproducibility of 13–18% for flux and CVC and 5–11% for %CVC$_{max}$. However, *Rapid 39 °C* demonstrated a lower reproducibility for flux, CVC and %CVC$_{max}$ (all 21%). Reproducibility at 44 °C was 12–15% for flux and CVC across all protocols.

Conclusion: This is the first study examining inter-day reproducibility across four local heating protocols. The good-to-moderate reproducibility of the *Rapid*, *Gradual* and *Slow 42 °C* protocols support their (simultaneous) use to assess microvascular function. Using *Rapid 39 °C* may require a greater number of subjects to detect differences within subjects.

1. Introduction

Microvascular dysfunction may predict the manifestation of future cardiovascular disease, preceding abnormalities in larger conduit arteries and arterioles (Holowatz et al., 2007; Bonetti et al., 2003; Sena et al., 2013; Minson, 2010; Ijzerman et al., 2003; Levy et al., 2001). The skin provides an easily accessible site to assess microvascular integrity through non-invasive methods, which can be used as an index of overall systemic vascular function. Control of the cutaneous microcirculation involves both neural and non-neural pathways (Johnson et al., 2014). Neurogenic reflexes and local chemical mediators, such as nitric oxide (NO), contribute towards the vasodilatory effect mediated by the vascular endothelium during local skin heating (Black et al., 2008; Houghton et al., 2006). Protocols that locally heat the skin are increasingly used in conjunction with laser Doppler flowmetry (LDF) to evaluate skin blood flow responses and microvascular function, particularly for comparing between healthy and diseased individuals and/or assessing responses to interventions.

There are currently several local heating protocols that are widely used to assess cutaneous microvascular function (Black et al., 2008; Minson et al., 2001; Choi et al., 2014). These protocols all aim to increase skin blood flow to maximal/near-maximal levels (39–42 °C), but they vary in the rate at which the skin is heated (0.5 °C per 5 s, 2 min 30 s or 5 min) and/or the plateau at which the temperature is set (39 °C vs 42 °C) (Black et al., 2008; Minson et al., 2001; Choi et al., 2014; Dawson et al., 2015; Pugh et al., 2013; Sprung et al., 2013). Due to the differences in the plateau and the rate of skin heating, a different contribution of the vasodilator pathways to the local heating response is

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present. Rapid local heating (0.5 °C per 5 s) induces a transient axon-reflex (~5–10 min), produced via activation of heat sensitive sensory nerves and adrenergic nerves, followed by a more gradual, sustained vasodilatory response (20–30 min) that is partly (60–70%) NO-mediated (Minson et al., 2001). A modification of this protocol, by maintaining the plateau phase at 39 °C, is believed to lead to a larger contribution of NO to the plateau phase (Choi et al., 2014). Alternatively, gradually heating the skin (0.5 °C per 2 min 30 s or 5 min) evokes a largely NO-mediated vasodilatory response, without producing an axon-reflex (Black et al., 2008; Dawson et al., 2015).

Previous work found moderate to good inter-day reproducibility for all local heating protocols (Dawson et al., 2015; Roustit et al., 2010; Huang et al., 2013; Agarwal et al., 2010; Tew et al., 2011), especially when data were expressed relative to maximal values (Dawson et al., 2015; Roustit et al., 2010; Tew et al., 2011). However, no previous study examined the reproducibility of these local heating protocols within the same subjects and/or simultaneously. This latter aspect is of special importance, since simultaneous assessment of distinct heating protocols may achieve better insight due to the distinct dilator pathways involved. Therefore, the aim of this study was to simultaneously determine the inter-day reproducibility of four commonly used local heating protocols for assessing cutaneous microvascular function. We expect comparable reproducibility of all four protocols, which would facilitate simultaneous use of multiple local heating protocols within the same study.

2. Methods

2.1. Participants

Fifteen healthy, male participants were recruited through local advertisement. All participants were healthy and non-smokers (28 ± 5 yrs, height 1.79 ± 0.10 m, weight 78.3 ± 8.5 kg, BMI 25 ± 2 kg/m^2, mean arterial pressure (MAP) 79 ± 5 mm Hg). Individuals with a medical history of hypercholesterolaemia (total cholesterol > 6.5 mmol/l), (Reiner et al., 2011) cardiovascular disease and/or hypertension (systolic blood pressure ≥ 140 mm Hg, diastolic blood pressure ≥ 90 mm Hg) (NICE, 2011; Yarmolinsky et al., 2015) were excluded. Participants were not taking any vasoactive medications or supplements. After being fully informed of the methods, written informed consent was obtained from all participants. The study conformed to the Declaration of Helsinki and was approved by the Research Ethics Committee of Liverpool John Moores University.

2.2. Experimental design

All participants attended two experimental trials which were 2–7 days apart. During each trial, baseline and maximal thermally stimulated forearm cutaneous blood flow was examined simultaneously at four different sites on the dominant forearm using LDF. At each site, separated by ~5 cm, a different local heating protocol was adopted: 1. *Rapid 39 °C* (Choi et al., 2014), 2. *Rapid 42 °C* (Minson et al., 2001), 3. *Gradual 42 °C* (Black et al., 2008), and 4. *Slow 42 °C* (Black et al., 2008). The sites at the forearm were kept the same within subjects between the two testing days.

2.3. Experimental measures

All participants fasted for at least 6 h and refrained from alcohol, food products high in polyphenols (dark chocolate, red wine), caffeine and exercise for 24 h prior to testing (Thijssen et al., 2011). Sips of water were permitted prior to testing to ensure that participants were well hydrated. All trials were conducted in a quiet, temperature controlled environment (23.0 ± 0.4 °C) (Thijssen et al., 2011; Cracowski et al., 2006) and at the same time of day to reduce any circadian influences on vascular function (Thijssen et al., 2011; Jones et al., 2010). Stature (seca 217 stadiometer, seca UK, Birmingham, UK) and body mass (seca 767 calibrated electronic scales, Germany) were recorded using standardised protocols. Body mass index was calculated (BMI) as the body weight (kg) divided by the height squared (m^2).

Following a 20-minute stabilisation period, the LDF equipment was calibrated using two generic points, 0 and 250 PU, a zeroing disc and motility standard, according to manufacturer's guidelines (Perimed AB, Järfälla, Stockholm, Sweden). Participants assumed a comfortable, supine position on a bed, with the head slightly elevated and the dominant arm relaxed, supinated and supported by a vacuum cushion to minimise microcirculatory fluctuations resulting from motion artefact (Thijssen et al., 2011; Cracowski et al., 2006). Four measurement sites on the volar aspect of the dominant forearm were randomly chosen ≥2.5 cm from the antecubital fossa and ≥2.5 cm from the distal radioulnar joint at the wrist, avoiding visible veins, hair follicles and dermatological lesions (Cracowski et al., 2006). If necessary, the measurement sites were shaved 24 h prior to testing to avoid any inflammatory response that may affect cutaneous blood flow. Following a 20-minute rest period, participants were instrumented for LDF measurements; four heating discs (Perimed 355, Perimed AB, Järfälla, Stockholm, Sweden) were placed ~5 cm apart on the dominant forearm, with a 7-laser array probe (PF 413, Perimed AB, Järfälla, Stockholm, Sweden) placed into each heater and firmly attached to the skin using adhesive stickers. To ensure accuracy of measurement sites between trials, the relevant areas were marked on the skin following the first experimental trial. In addition, we took digital photographs and recorded measurements to the nearest millimetre using anatomical and skin-surface landmarks for reference.

Cutaneous blood flow was measured as a signal of red blood cell flux (RBCF) using the non-invasive technique of LDF (Periflux system 5000, Perimed AB, Järfälla, Stockholm, Sweden). The four local heating discs were connected to a heating unit (Peritemp 4005 heater, Perimed AB, Järfälla, Stockholm, Sweden) which was used to induce thermal hyperaemia and was manually controlled during the local heating protocols. Baseline skin RBCF was recorded with the local heating disc temperature set at 33 °C for 10-minutes for each measurement site. Subsequently, local skin temperature was heated according to four distinct protocols:

Rapid 39 °C (Choi et al., 2014). This recently introduced protocol (0.5 °C per 5 s, 30-min at 39 °C, 20-min at 44 °C) induces an axon-reflex and gradual plateau following local heating. By stopping the heating protocol at 39 °C, the plateau phase is largely NO-mediated and causes dilation that is equivalent to 50% of the maximal response (Choi et al., 2014).

Rapid 42 °C (Minson et al., 2001). This classic local heating protocol (0.5 °C per 5 s, 30-min at 42 °C, 20-min at 44 °C) induces a rapid, transient axon-reflex which is followed by a more gradual, but sustained, heating response. The plateau phase represents 80–90% of the maximal response, and is partly (60–70%) NO-mediated (Minson et al., 2001; Kellogg et al., 1999).

Gradual 42 °C (Black et al., 2008). This adapted, shortened version of the *Slow 42 °C* local heating protocol increases temperature to *42 °C* (0.5 °C per 2-min 30 s, 30-min at 42 °C, 20-min at 44 °C), and induces a slow heating response that is largely NO-mediated and reflects 80–90% of the maximal response (Black et al., 2008).

Slow 42 °C (Black et al., 2008). This validated, longer version of the former heating protocol induces a gradual, slow heating response (0.5 °C per 5-min, 30-min at 42 °C, 20-min at 44 °C) that is largely NO-mediated, reflecting 80–90% of the maximal response.

2.3.1. Haemodynamics

Heart rate (HR) and blood pressure (BP) were recorded at the beginning and at the end of the 20-minute acclimation period using an automated sphygmomanometer (Dinamap V100, GE Healthcare, UK) positioned on the contralateral upper arm. Thereafter, MAP (mm Hg) and HR (bpm) were recorded at 5-minute intervals throughout the