Clinical Hemorheology and Microcirculation 59 (2015) 177–183
DOI 10.3233/CH-141836
IOS Press

Brief Report

Muscle VO_2 and forearm blood flow repeatability during venous and arterial occlusions in healthy and coronary heart disease subjects

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Abstract. This study aims were: 1) to assess forearm blood flow (FBF) and muscle oxygen consumption (mVO_2) repeatability assessed with near-infra red spectroscopy (NIRS) during venous occlusions (VO) in middle aged healthy subjects and patients with stable coronary heart disease (CHD), 2) to assess the agreement between mVO_2 calculated from NIRS signals during VO and arterial occlusion (AO) in 18 middle aged healthy subjects and 12 patients with CHD. FBF and mVO_2 were measured using NIRS during 2 successive VO (1-min duration), followed by a 5-min AO. Repeatability for FBF and mVO_2 during VO was assessed with intra class correlation (ICC), coefficient of variation (CV %) and agreement between VO and AO mVO_2 was assessed with a Bland and Altman analysis. FBF and mVO_2 during VO were highly reproducible in healthy (FBF: ICC 0.73, CV% 9.75; mVO_2: ICC 0.89, CV% 12.6) and CHD subjects (FBF: ICC 0.95, CV% 10.26; mVO_2: ICC 0.98, CV% 7.92). VO and AO mVO_2 were in agreement in healthy (mean bias: 0.002 mL O_2.min^{-1}.100g^{-1}) and CHD subjects (mean bias: 0.014 mL O_2.min^{-1}.100g^{-1}). FBF and mVO_2 measured with NIRS during VO and/or AO are highly reproducible methods to assess microvascular function in healthy subjects and stable CHD patients.

Keywords: Near-infra red spectroscopy, repeatability, agreement, vascular occlusions, healthy middle aged subjects, patients with coronary heart disease

1. Introduction

Near-infra red spectroscopy (NIRS) is a non-invasive optical method to assess microvascular function during exercise and/or vascular occlusions [1–4]. Microvascular dysfunction assessed by NIRS is present

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in patients with cardiovascular (CV) risk factors and cardiac disease [5–7] and is improved by exercise training [8–10]. Venous occlusions (VO) can simultaneously assess muscle oxygen consumption (mVO_2) and blood flow, with a better patients comfort vs. the arterial occlusion (AO). The AO is a validated and more accurate method [1] to measure mVO_2 and post occlusive reactive hyperhemia variables [3, 6, 7, 11]. However, AO duration is longer and less comfortable for the patient (higher duration and occlusion pressure). Repeatability of mVO_2 and forearm blood flow (FBF) using VO is unknown in patients with coronary heart disease (CHD) (only 1 study in young subjects [12]). Agreement of NIRS mVO_2 during VO and AO have been only studied in patients admitted to intensive unit care [1] or in healthy young subjects [12]. Vascular occlusion tests using NIRS is interesting for the assessment of microvascular function in primary [3, 4, 11] and secondary prevention clinical settings [5, 6, 13, 14]. However, repeatability and agreement of VO/AO techniques are poorly studied in healthy middle aged subjects (HS) and in CHD patients. The objectives of this study were: 1) to assess FBF and mVO_2 repeatability assessed with NIRS during VO in HS and CHD patients, 2) to assess the agreement between mVO_2 calculated from NIRS signals during VO and AO in HS and CHD patients.

2. Methods

Eighteen HS and 12 CHD patients were enrolled at the Montreal Heart Institute (Table 1). Diabetes, hypertension, dyslipidemia and obesity were defined as previously published [15]. For HS, inclusion criteria were: no evidence of CHD and no more than 2 CV risk factors. CHD was defined as previously published [15]. For all patients, exclusion criteria were: recent unstable coronary syndrome, left ventricular dysfunction (ejection fraction <50%), any blood and/or muscle diseases. All patients underwent a medical history, physical examination, body composition (Tanita, BC418 model, Japan) and fasting blood sample. FBF and mVO_2 were measured by NIRS during 2 successive VO of 1 min duration each followed by a 5 min brachial AO at the end of the morning (11-12 h A.M) and 4 h after standardized breakfast. Patients were resting in a supine position in a quiet, dark, air-conditioned room (22–25ºC) [3, 7]. Smoking, alcohol ingestion, caffeine, antioxidant vitamins and vigorous exercise for at least 12 h prior to measurements were not allowed. Patients with CHD took their usual medication in the morning (8-9 h A.M). The study was approved by the Montreal Heart Institute Ethics Committee and written informed consent was obtained.

2.1. NIRS forearm blood flow and mVO_2 measurement during VO

An pneumatic cuff inflator (Hokanson, model E20, U.S.A) was positioned above the right elbow and NIRS optodes (Artinis, Oxymon Mk III system, Netherlands) were placed on top of the brachio-radialis muscle with an interoptode distance of 45 mm (differential path-length factor: 4.0). Optodes were attached to the skin with adhesive stickers as previously published [3, 7]. NIRS signals were sampled during a 2 min rest period, 2 successive venous occlusions and the 5-min duration arterial occlusion and 5 min post cuff deflation period. During VO, the cuff was inflated at 50 mmHg for 1 minute [14]; a 2 minute recovery period was performed between the 2nd VO, thereafter, a 2 minute recovery period was performed before the AO. For AO, the cuff was inflated during 5-minute at 100 mmHg over the systolic blood pressure [3, 7]. Data were sampled at 10 Hz, displayed in real time, and stored on disk for off-line analysis. During VO, FBF using NIRS was calculated as previously described [1, 2] using total haemoglobin (tHb) increase. Results were expressed in μM/L of tissue and converted into $mL.100\,mL^{-1}.min^{-1}$ and

Table 1
Baseline characteristics of the subjects

	Healthy controls ($n = 18$)	CHD patients ($n = 12$)
Age (years)	46 ± 21	72 ± 6 ‡
Sex (male/female)	(13/5)	(10/2)
Body mass (kg)	70 ± 10	75 ± 12
Height (cm)	168 ± 5	168 ± 10
BMI ($kg.m^{-2}$)	24 ± 4	27 ± 3
Body fat (%)	22 ± 9	29 ± 6
SBP (mmHg)	120 ± 14	130 ± 16
DBP (mmHg)	72 ± 8	75 ± 7
Smoking	0 (0)	0 (0)
Hypertension	1 (5)	6 (50)
Diabetes	0 (0)	2 (12)
Dyslipidemia	2 (11)	10 (83)
Abdominal obesity	2 (11)	8 (66)
Prior MI (n and %)	0 (0)	3 (25)
Prior PCI (n and %)	0 (0)	4 (33)
Prior CABG (n and %)	0 (0)	4 (33)
Beta–blockers (n and %)	0	7 (58)
ACE inhibitors (n and %)	0	2 (17)
Antiplatelet agents (n and %)	0	11 (92)
AR blockers (n and %)	0	5 (42)
Statin (n and %)	0	11 (92)
Ca^{2+} channel blockers (n and %)	0	1 (8)

CHD: coronary heart disease, BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, MI: ACE: angiotensin-converting-enzyme, AR:, angiotensin receptor, Myocardial infarction, PCI: percutaneous coronary intervention, CABG: coronary artery bypass surgery, Ca^{2+}: calcium. ‡ = $P < 0.001$.

the molecular weight of Hb was assumed to be 64 g/mol with an arterial blood saturation of 97 % in all subjects [2]. During VO, mVO_2 was calculated from the rate of increase in deoxyhaemoglobin (HHb) [1, 2] and expressed in mL $O_2.min^{-1}.100g^{-1}$ assuming that each molecule of HB binds four O_2 molecules, a molar gas volume of 22.4 L (STPD) and a muscle density of 1.04 kg/L [2, 4, 12]. During AO, mVO_2 (mL $O_2.min^{-1}.100g^{-1}$) was calculated from the rate of decline in oxyhaemoglobin (O_2Hb) during the 5 min occlusion [3, 7].

2.2. Statistical analysis

Data were analyzed using Statview software (version 5.0, S.A.S, U.S.A) and are presented as mean ± standard deviation except where otherwise indicated. For continuous variables, statistical differences between tests were evaluated by a one-way ANOVA with repeated measure. A *post-hoc* test (Bonferoni test) with a p-value<0.05 was considered significant. Intra-subject repeatability was obtained by comparing NIRS FBF and mVO_2 measured during the 2 VO. The relative repeatability was assessed by intraclass correlation coefficient (ICC), absolute repeatability by coefficient of variation (CV %) and

standard error of measurement (SEM) [3]. In addition, correlation and agreement of mVO_2 measured during the 1st VO and AO were performed using a linear regression and a Bland and Altman analysis [16].

3. Results

3.1. Subjects characteristics

Table 1 describes the HS and CHD subjects's characteristics. Compared to HS, CHD patients were older ($P<0.001$) and had more CV risks factors. Right arm adiposity was not different between the 2 groups (healthy: 25 ± 7 % vs. CHD: 20 ± 10 %, $P>0.05$).

3.2. Forearm blood flow and mVO_2 repeatability measured during VO

Table 2 describes FBF and mVO_2 repeatability in HS and CHD patients. FBF and mVO_2 repeatability were highly reproducible in both groups with no difference in means ($P>0.05$) and ICC ranging from good to excellent (ICC: 0.73 to 0.98).

3.3. Agreement of mVO_2 measured during the VO and AO

Table 3 describes the agreement of mVO_2 measured during the VO and AO in both groups. There was no difference in mean mVO_2 obtained from venous and arterial occlusion ($P>0.05$) and both methods were in agreement.

Table 2
Forearm blood flow and mVO_2 repeatability measured during venous occlusions

	1st VO	2nd VO	*P* value	ICC	CV (%)	SEM (%)
Healthy controls						
FBF (mL.100 $mL^{-1}.min^{-1}$)	1.39 ± 1.08	1.24 ± 0.715	0.35	0.73	9.75	36.04
mVO_2 (mL $O_2.min^{-1}.100g^{-1}$)	0.117 ± 0.093	0.103 ± 0.082	0.14	0.89	12.6	25.23
CHD patients						
FBF (mL.100 $mL^{-1}.min^{-1}$)	1.09 ± 0.73	0.99 ± 0.68	0.12	0.95	10.26	13.67
mVO_2 (mL $O_2.min^{-1}.100g^{-1}$)	0.150 ± 0.219	0.139 ± 0.183	0.36	0.98	7.92	19.39

FBF: forearm blood flow, mVO_2: muscle oxygen consumption, VO: venous occlusion

Table 3
Agreement of mVO_2 measured during the 1st venous and arterial occlusion

	1st VO	Arterial Occlusion	ANOVA P value	R	Mean Bias (95% LOA)
Healthy controls					
mVO_2 (mL $O_2.min^{-1}.100g^{-1}$)	0.117 ± 0.093	0.120 ± 0.107	0.87	0.78	0.002 (0.108)
CHD patients					
mVO_2 (mL $O_2.min^{-1}.100g^{-1}$)	0.150 ± 0.219	0.098 ± 0.065	0.53	0.62	0.014 (0.220)

mVO_2: muscle oxygen consumption, VO: venous occlusion, LOA: limit of agreement.

4. Discussion

4.1. Main findings

The principal new findings of this study were that: 1) FBF and mVO_2 measured during successive VO were highly reproducible in healthy subjects and CHD patients. 2) There was a good agreement between mVO_2 measured by VO and AO occlusion in healthy subjects and CHD patients. This study is the first to assess the FBF and mVO_2 repeatability during VO and the agreement with AO mVO_2 measured in HS and CHD patients. Microvascular function is of particular interest since its has been showed to be significantly reduced in patients with CV risks [7] or cardiac disease [5–7]. The VO method has several advantage over AO in clinical research settings: 1) VO is more comfortable for the patients [1, 17], 2) VO protocol is shorter and allows simultaneous measure of FBF and mVO_2, [1, 17] 3) VO can be repeated more easily in time when needed.

4.2. Relative and absolute intra subject FBF and mVO$_2$ repeatability measured during VO

Relative repeatability (ICC) of FBF and mVO_2 ranged from good (healthy subjects) to excellent (CHD patients) during the successive VO. Relative repeatability for FBF has not been reported previously, it is therefore difficult to compare our results with previous ones. However, FBF reported in our older healthy subjects agreed with to those reported in young subjects [2, 12]. Muscle VO_2 during VO for our healthy subjects agreed with two previous studies [12, 18]. Our results in CHD patients for VO are difficult to compare, but mVO_2 ICC found (VO) were in agreement with one previous study (AO) from our group [3]. Our CVs (healthy and CHD patients) agreed with a previous study [2] and were lower to a second one [12]. Potential differences may be explained by differences in subjects and methodology used.

4.3. Agreement between mVO$_2$ measured during VO and AO

A good agreement between mVO_2 measured during VO and AO (Table 3) were found in HS and CHD patients, in agreement with previous studies [1, 12]. Our mVO_2 values (VO and AO) also agreed with previous studies [1, 2, 4, 7, 12]. Additionally, our correlation coefficients were slightly lower to that previously reported [1]. Our results are clinically important, AO can cause an important discomfort to the patients [17] and be less usable for consecutive measure [1]. The VO method is more comfortable [3, 6, 7, 11, 17] and more easily repeatable in time for simultaneous measure of FBF and mVO_2 [1]. Our limitation included a low sample of men (healthy and CHD), our results may not be applicable for other groups.

5. Conclusions

In conclusion, FBF and mVO_2 measured during VO were highly reproducible in healthy and CHD subjects, and a good agreement between mVO_2 measured by VO and AO was found. VO method using NIRS can be advantageous to assess microvascular function in clinical settings.

Acknowledgments

ÉPIC Foundation and Montreal Heart Institute Foundation.

Conflict of interest

There is no conflict of interest.

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