Clinical Hemorheology and Microcirculation 59 (2015) 53–65
DOI 10.3233/CH-141816
IOS Press

Cardiometabolic and traditional cardiovascular risk factors and their potential impact on macrovascular and microvascular function: Preliminary data

Mathieu Gayda[a,b,c,*], Martin Juneau[a,b,c], Jean-Claude Tardif[b,c], François Harel[b,c], Sylvie Levesque[d] and Anil Nigam[a,b,c]

[a]*Cardiovascular Prevention and Rehabilitation Centre (Centre ÉPIC), Montreal Heart Institute and Université de Montréal, Montreal, Quebec, Canada*
[b]*Research Center, Montreal Heart Institute and Université de Montréal, Montreal, Quebec, Canada*
[c]*Department of Medicine, Faculty of Medicine, Université de Montréal, Montreal, Quebec, Canada*
[d]*Biostatistics department, Montreal Heart Institute Coordinating Centre and Université de Montréal, Montreal, Quebec, Canada*

Abstract. We studied the relationship between cardiovascular status (CV) and risk factor numbers, macrovascular (flow mediated dilatation: FMD) and microvascular function using near-infrared spectroscopy (NIRS) in adults with different CV status. Seventy adults with different CV status (27 controls, 18 metabolic syndrome (MetS) and 25 coronary heart disease (CHD) patients) underwent a 5-min forearm arterial occlusion in supine position. High-resolution ultrasound examination of the brachial artery was performed during 1 minute at rest and 45 to 120 seconds after cuff release. Oxy, de-oxy and total hemoglobin signals (O_2Hb, HHb and tHb) were measured continuously with NIRS on brachio-radialis muscle. FMD was reduced in CHD patients ($P < 0.05$) compared to controls. Max. amplitude of O_2Hb and Hmax of tHb were reduced ($P < 0.05$) in MetS patients vs. controls. Post-deflation area under the curve (A.U.C) of O_2Hb was lower in CHD ($P < 0.01$) patients vs. controls and MetS patients. Independent predictors of microvascular function (A.U.C of O_2Hb) were abdominal obesity and LDL-cholesterol whereas macrovascular function (FMD) was predicted by CV status. Only A.U.C of O_2Hb related to CV risk numbers whereas FMD was not. Although macro and microvascular function were impaired in MetS and CHD patients, microvascular function was more strongly related to CV risk factors.

Keywords: Macrovascular function, microvascular reactivity, cardiovascular risk factors

*Corresponding author: Mathieu Gayda (Ph.D), Cardiovascular Prevention and Rehabilitation Centre (Centre ÉPIC), Montreal Heart Institute and Université de Montréal, 5055 St-Zotique Street East, Montreal, Quebec H1T 1N6, Canada. Tel.: +1 514 374 1480/Ext 268; Fax: +1 514 374 2445; E-mail: mathieu.gayda@icm-mhi.org.

1. Introduction

Endothelial dysfunction occurs early in the process of atherogenesis and contributes to the formation, progression, and complications of atherosclerotic plaques [26]. Measurement of flow mediated dilatation (FMD) during reactive hyperemia is the non-invasive method of reference for the assessment of macrovascular function *in vivo* and is a predictor of future cardiovascular events in asymptomatic adults [27], patients with stable coronary heart disease (CHD) [6, 10] and following acute coronary syndromes [11]. However, the clinical utility of this evaluation remains under question and variable results have been observed with respect to the association between FMD and cardiovascular (CV) risk factors [4, 33, 34]. Previous studies have demonstrated poor or no association between FMD and CV risk factors [2, 4, 23, 24]. Additionally, the assessment of macrovascular function using FMD is time-consuming and requires a high level of technical expertise, expensive ultrasound equipment, and high-quality ultrasound images for accurate analysis. Furthermore, measurements may be influenced by intra and inter-observer variability [8]. Taken together, these factors limit the usefulness of the FMD in the clinical and prevention setting.

Recently, an alternative microvascular approach has been proposed using near-infra red spectroscopy (NIRS) during reactive hyperemia in healthy adults [19–21, 29], peripheral arterial disease [19, 20] or chronic heart failure patients [1, 22]. Microvascular dysfunction assessed by NIRS was found to be present in patients with CV disease [1, 22] and was suggested to be linked to CV risk factors such as hypertension, obesity, insulin resistance and diabetes [16]. The reactive hyperemia test using NIRS could be a potential interesting non-invasive, inexpensive, non-operator dependent technique for the assessment of global CV risk in primary [20, 21, 32] and secondary prevention clinical settings [1, 17, 19, 22] but has never been compared to more established vascular assessment methods such as FMD. To date, little attention has been focused on the CV risk profile and its impact on microvascular function as assessed by NIRS compared to macrovascular function (FMD). We hypothesized that microvascular function assessed by NIRS would be more related to CV risk factors and the global CV profile compared to macrovascular function assessed by FMD. Our objectives were therefore: 1) To measure the effects of CV status on macrovascular (assessed by FMD) and microvascular function (assessed by NIRS) in patients with different CV status and risk factors; and 2) To study the effect and relationship of the CV risk factors and their absolute number on macro and microvascular function.

2. Materials and methods

2.1. Patients

Seventy adults with one of three CV status were enrolled from the CV prevention and rehabilitation centre of the Montreal Heart Institute, including 27 healthy controls, 18 patients with metabolic syndrome (MetS) and 25 patients with stable coronary heart disease (CHD). Traditional CV risk factors considered were diabetes, hypertension, active smoking, dyslipidemia and obesity. Diabetes was defined as a prior diagnosis of diabetes along with a fasting serum glucose >7.1 mmol/L and a hemoglobin A1c level >0.060 or a treatment with an hypoglycemic agent. Hypertension was defined as a prior diagnosis of hypertension with blood pressure >130/85 mm Hg or antihypertensive treatment. Active smoking was defined as smoking $\geq$1 cigarette, cigar, or pipe per day. Dyslipidemia was defined as total cholesterol $\geq$6.2 mmol/L, low-density lipoprotein cholesterol $\geq$4.2 mmol/L, or a total/high-density lipoprotein cholesterol ratio

≥4.7 or statin treatment. Obesity was defined by a BMI over 30 kg/m^2. For healthy controls, inclusion criteria were: no evidence of CHD or MetS and no more than 2 CV risk factors [14]. Metabolic syndrome was defined according to current recommendations [14]: presence of ≥3 of 5 criteria, namely abdominal obesity (waist circumference ≥94 cm in men and ≥80 cm in women), triglycerides >1.70 mmol/L, decreased HDL-cholesterol (<1.03 mmol/L in men and <1.29 mmol/L in women), systolic blood pressure ≥130 mmHg or diastolic blood pressure ≥85 mmHg, and fasting plasma glucose ≥5.6 mmol/L. For the third group, CHD was defined as the presence of documented prior myocardial infarction (by history, electrocardiogram, and/or enzyme criteria), prior coronary revascularization, angiographic evidence of atherosclerosis (history of ≥70% arterial diameter narrowing of at least one major coronary artery) or documented myocardial ischemia on myocardial scintigraphy [13]. For all patients, exclusion criteria were: recent unstable coronary syndrome, left ventricular dysfunction (ejection fraction <50%), blood and muscle diseases, contraindication of the use of nitroglycerin spray (severe anemia, elevated intraocular pression, intracranial hypertension, arterial hypotension, other vasodilator treatment such as sidenafil). All patients underwent a baseline evaluation that included a medical history, a physical examination with measurement of height and weight, body composition with bio-electrical impedance (Tanita, model BC418, Japan), an initial symptom-limited exercise stress test, and fasting bloodwork (glucose and lipid profile). Macrovascular (FMD) and microvascular function (NIRS) were simultaneously measured before, during and after a 5 min brachial arterial occlusion, while patients were resting in a supine position in a quiet, dark, air-conditioned room (22–25 ºC). Measurements were performed at the end of the morning (11–12 h A.M) for all subjects, 4 h after breakfast. Subjects were instructed to abstain from smoking, ingesting alcohol, caffeine, antioxidant vitamins or performing vigorous exercise for at least 12 h prior to measurements. The study was approved by the Montreal Heart Institute Ethics Committee and written informed consent was obtained from all subjects.

2.2. *Macrovascular function measurement with FMD*

High-resolution ultrasound examination of the brachial artery was performed after a 10-minute rest period in the supine position using a 7.5-MHz transducer connected to an HP Sonos 5500 (Hewlett-Packard, Palo Alto, California) echocardiography machine. Images were recorded on S-VHS tape. A non-tortuous segment of the brachial artery, above the antecubital fossa, was identified. Baseline imaging before cuff inflation was performed by scanning the artery in a longitudinal fashion. After optimization of depth and gain settings, images were magnified in a 20 × 20-mm viewing window [9, 25]. Because of simultaneous measurement of NIRS signals at the forearm level, the automated pneumatic cuff inflator (Hokanson, model E20, USA) was positioned proximally above the elbow and inflated to 100 mmHg above the resting SBP for 5 minutes. The cuff was then deflated and the artery continuously imaged for 5 minutes. After 10 min of recovery, new baseline imaging were obtained followed by the administration of 0.4 mg of sublingual nitroglycerin spray with continuous imaging for an additional 5 minutes [9, 25]. Percent flow-mediated dilatation (FMD), measured after cuff deflation, was used as an index of endothelium-dependent maximal dilatation; percent dilatation obtained 3 minutes after the administration of nitroglycerin (NMD) represented endothelium-independent dilatation [9, 25]. Brachial arterial blood flow (mL/min) was measured by echo-Doppler during the first 15 seconds after cuff release [15]. Maximal dilatation diameter was assessed between 45 to 120 seconds after cuff release with 2D ultrasonography for each patient [15]. Additionally, time elapsed to have the maximal percent dilatation was also recorded [15]. Maximal shear stress was calculated with the following equation: $SS_{max} = 8 \times \mu \times V_{max}/D_{BL}$, were

Vmax indicated hyperemia velocity, μ was the blood viscosity assumed to be 0.035 dynes $\times$ s/cm^2 and D_{BL} is the baseline diameter [23]. Mean diameter of the 20-mm brachial artery segment was quantified by 2 technicians using proprietary software [9, 25]. Frames from 3 consecutive cardiac cycles were taken at the peak of the R wave and results were averaged. Using this method, the intra- and inter-observer variability in our laboratory for brachial artery diameter determinations was shown to be 0.056 ± 0.024 and 0.073 ± 0.031 mm, respectively [9, 25]. The variability of FMD measurements performed on 2 separate days was $1.05 \pm 0.35\%$ [9, 25]. Endothelial dysfunction was defined as FMD <7.5 % [28].

2.3. Microvascular function measurement with NIRS

Microvascular function during reactive hyperemia was assessed using previously described methodology [21]. NIRS optodes (Oxymon, Artinis Medical Systems, Netherlands) were placed on top of the brachio-radialis muscle with an interoptode distance of 45 mm [21, 31, 32]. In order to prevent variations in placement of the optodes and to avoid operator errors, the angle and place of the optodes were kept constant during the test using a special support that was attached to the skin with adhesive stickers [21]. To correct for scattering of photons in the tissue, a differential path-length factor of 4.0 was used for the calculation of absolute concentration changes [21, 31, 32]. Data were sampled at 10 Hz, displayed in real time, and stored on disk for off-line analysis [21]. NIRS signals were sampled before (2 min rest period), during and after cuff inflation (5 minutes post cuff deflation) [21]. Muscle oxygen consumption (mVO_2) was measured by NIRS evaluating the rate by evaluating the rate of decrease in [O_2Hb] during arterial occlusion [–d(O_2Hb)/dt] [21, 31, 32]. Concentration changes of O_2Hb were expressed in μM/s and converted to ml O_2/min/100 g [31, 32]. A value of 1.04 kg/L was used for muscle density [21, 31, 32]. The following NIRS parameters were used during arterial occlusion test [19, 21]: 1) Muscle VO_2 (ml O_2/min/100 g), 2) ½ time recovery of the O_2 Hb (s): interval time after release of the cuff until the initial pre-occlusion values of the O_2 Hb are reached, 3) time to maximal O_2 Hb (s): interval time between the release of the cuff and the moment of the maximum value of O_2 Hb signal, 4) max. amplitude of O_2 Hb (μM): maximal amplitude of O_2 Hb signal after the release of the cuff until maximal O_2 Hb was reached, 5) HRmax of tHb (μM): maximal value the THb signal after the release of the cuff, 6) time to Hmax of tHb (s): interval time between the release of the cuff and the moment of the maximum value for tHb signal, 7) Increase rate to max O_2 Hb (μM/s) was calculated by dividing max. amplitude of O_2 Hb with time to maximal O_2 Hb, 8) Increase rate to max tHb (μM/s) was calculated by dividing HRmax of tHb with time to Hmax of tHb, 9) post-deflation area under the curve (A.U.C) of O_2 Hb (arbitrary unit; a.u) was the area under the O_2 Hb curve calculated 5 minutes after cuff deflation, 10) post-deflation A.U.C of HHb (a.u) was the area under the HHb curve calculated 5 minutes after cuff deflation, 11) post-deflation A.U.C of tHb (a.u) was the area under the tHb curve calculated 5 minutes after cuff deflation.

2.4. Statistical analysis

According to the distribution, descriptive statistics for continuous variables are presented as mean $\pm$ standard deviation or median (minimum, maximum) and difference between groups were evaluated by a one-way analysis of variance or by an analysis of variance on ranked data. For categorical variables, number and percentage were presented and differences between groups were evaluated by chi-square test. Two by two groups comparisons were presented when the global p-value were signifi-

Table 1
Clinical characteristics of adults groups with different cardiovascular status

	Healthy subjects ($n=27$)	MetS patients ($n=18$)	Patients with CHD ($n=25$)
Age (years)	$54 \pm 14^{a\dagger,b\ddagger}$	64 ± 8	66 ± 10
Height (cm)	166 ± 9	169 ± 7	170 ± 7
Female sex	16 (59%)	3 (17%)	2 (8%)
10 years Framingham CHD Risk (%)	6.2 ± 4.5 [a§]	18.7 ± 10.2	–
Smoking	0 (0%)	2 (11%)	2 (8%)
Hypertension [1]	1 (4%)	15 (83%)	16 (64%)
Diabetes [2]	0 (0%)	11 (61%)	8 (32%)
History of dyslipidemia	6 (22%)	17 (94%)	22 (88%)
Obesity [3]	2 (7%)	15 (83%)	10 (40%)
Prior MI	0 (0%)	0 (0%)	11 (44%)
Prior PCI	0 (0%)	0 (0%)	17 (68%)
Prior CABG	0 (0%)	0 (0%)	8 (32%)
Medication			
Beta–blockers	3 (11%)	4 (22%)	15 (60%)
ACE inhibitors	0 (0%)	4 (22%)	8 (32%)
Antiplatelet agents	3 (11%)	8 (44%)	23 (92%)
Angiotensin receptor blockers	1 (4%)	7 (39%)	4 (16%)
Statin	3 (11%)	13 (72%)	25 (100%)
Calcium channel blockers	0 (0%)	4 (22%)	9 (36%)
Diuretics	0 (0%)	0 (0%)	3 (12%)
Nitrates	0 (0%)	0 (0%)	2 (8%)
Hypoglycemic agents	0 (0%)	3 (17%)	5 (20%)
Cardiometabolic Risk Factors			
Exercise capacity (METs)	10.8 (6.6; 16.2) [a∗,b†]	8 (5.9, 12.9)	8.65 (4.9, 17.1)
Body mass (kg)	$70 \pm 13^{a\S,b\ddagger}$	$96 \pm 17^{c\dagger}$	84 ± 11
BMI (kg/m^2)	$25 \pm 4^{a\S,b\dagger}$	$33 \pm 4^{c\ddagger}$	29 ± 3
Fat mass percentage (%)	$27 \pm 8^{a\dagger}$	$34 \pm 5^{c\dagger}$	27 ± 7
Trunk fat mass percentage (%)	$26 \pm 8^{a\S}$	$35 \pm 4^{c\dagger}$	29 ± 7
Waist circumference (cm)	$88 \pm 13^{a\S,b\S}$	$113 \pm 10^{c\dagger}$	101 ± 9
Rest SBP (mmHg)	124 (100, 160) [a†,b∗]	134 (120, 160)	130 (104, 170)
Rest DBP (mmHg)	$76 \pm 8^{a*}$	80 ± 7	77 ± 7
Fasting glucose (mmol/l)	$4.76 \pm 0.39^{a\S,b\S}$	6.33 ± 1.10	5.98 ± 1.17
Total cholesterol (mmol/l)	$4.87 \pm 0.86^{a\dagger,b\S}$	4.08 ± 0.75	3.71 ± 0.66
HDL–cholesterol (mmol/l)	1.47 (1.15, 2.27)[a§,b§]	0.98 (0.64, 2.46) [c∗]	1.19 (0.80, 2.01)
LDL–cholesterol (mmol/l)	$2.92 \pm 0.75^{a\dagger,b\S}$	2.31 ± 0.83	1.93 ± 0.56
Triglycerides (mmol/l)	0.69 (0.31, 2.12)[a§,b†]	1.60 (0.77, 4.08)[c∗]	1.23 (0.51, 2.72)
Total Chol/HDL-cholesterol	$3.22 \pm 0.87^{a\dagger}$	$4.00 \pm 1.03^{c\ddagger}$	3.10 ± 0.52
Triglycerides/HDL	0.43 (0.18, 1.78)[a§,b‡]	1.51 (0.75, 5.88) [c∗]	1.00 (0.25, 2.33)

[1]Rest SBP $\geq$130 mmHg; [2]glucose $\geq$7 mmol/l; [3]BMI > 30 kg/m^2. Group effects: a = Healthy vs. MetS patients, b = Healthy vs. CHD patients, c = MetS patients vs. CHD patients. * = $P<0.05$, † = $P<0.01$, ‡ = $P<0.001$, § = $P<0.0001$. MetS: metabolic syndrome, ACE, angiotensin–converting enzyme; CABG, coronary artery bypass grafting surgery; CHD, coronary heart disease; MI, myocardial infarction; PCI, percutaneous coronary intervention. BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure.

cant. Univariate logistic regression analysis was used to identify which baseline CV risks factors were significantly associated with macro (FMD) and microvascular (A.U.C. of O_2 Hb) functions. The most significative parameters were included in a backward multivariate logistic regression model in order to determine independent predictors of macro and microvascular functions. CV status were forced into the multivariate regression models. In any case, basic assumptions were checked prior to analysis. *P*-values <0.05 were considered to be statistically significant. Analyses were performed with SAS 9.2 (SAS Institute Inc, Cary, NC, USA) and with Statview software 5.0.

3. Results

3.1. Clinical characteristics

Table 1 describes the clinical characteristics of each group. Healthy subjects were significantly younger and included more females ($P < 0.05$) compared to MetS and CHD patients. The prevalence of traditional risk factors was higher in MetS patients, particularly for hypertension, diabetes, dyslipidemia and obesity. For CHD patients, hypertension and dyslipidemia were the most prevalent risk factors and 40% were obese. Compared to healthy patients, MetS and CHD patients had a lower exercise capacity ($P < 0.05$). Compared to the 2 other groups, MetS subjects had higher body mass, BMI, total and trunk fat mass %, waist circumference, serum triglyceride level, and triglycerides/HDL ratio ($P < 0.05$) and worse lipid profile compared to healthy subjects ($P < 0.05$). Compared to the 2 other groups, healthy patients had a lower SBP and fasting glycaemia. Compared to healthy subjects, CHD patients had a higher body mass, BMI, waist circumference, SBP, triglycerides and triglycerides/HDL ratio ($P < 0.05$) and lower total, HDL and LDL cholesterol levels ($P < 0.05$).

Table 2
Echographic parameters during hyperhemia of adults groups with different cardiovascular profile

Parameters	Healthy subjects ($n = 27$)	MetS patients ($n = 18$)	Patients with CHD ($n = 25$)	*P* value
Baseline diameter (mm)	$3.51 \pm 0.65^{a‡,b†}$	4.17 ± 0.60	4.06 ± 0.55	0.0006
Maximal diameter (mm)	$3.91 \pm 0.70^{a‡,b†}$	4.56 ± 0.60	4.39 ± 0.51	0.0015
FMD (%)	$11.6 \pm 3.5^{b†}$	9.6 ± 3.6	8.4 ± 4.4	0.0162
NMD (%)	14.0 (6.0–21.7) ^{a*,b‡}	11.0 (7.4–23.3)	10.6 (4.5–34.4)	0.0010
Time to maximal dilatation (s)	$61 \pm 8^{a§,b§}$	86 ± 15	89 ± 7	<0.0001
Baseline blood velocity (cm/s)	39 ± 11	36 ± 10	40 ± 10	0.6112
Maximal blood velocity (cm/s)	71 ± 18	78 ± 18	72 ± 20	0.4856
Maximal shear stress (dynes/cm^2)	60 ± 23	53 ± 15	50 ± 14	0.1720
FMD/MSS	0.19 (0.10–0.38)	0.19 (0.06–0.38)	0.14 (0.05–1.12)	0.1118
Endothelial dysfunction *	3 (11.1 %)^{b†}	4 (22%)	12 (48%)	0.0099

MetS: metabolic syndrome, FMD: flow mediated dilatation of the brachial artery, NMD: nitroglycerin-mediated dilatation of the brachial artery. MSS: maximal shear stress, Endothelial dysfunction *: defined by a FMD <7.5% (29). Group effects: a = Healthy vs. MS patients, b = Healthy vs. CHD patients, c = MS patients vs. CHD patients, * = $P < 0.05$, † = $P < 0.01$, ‡ = $P < 0.001$, § = $P < 0.0001$.

Table 3
Near-infra red spectroscopy parameters during arterial occlusion test in adults groups with different cardiovascular profile

Parameters ($n=25$)	Healthy subjects ($n=27$) ($n=18$)	MetS patients	Patients with CHD ($n=25$)	P value
Muscle VO_2 (ml O_2/min/100 g)	$0.081 \pm 0.024^{a*}$	$0.062 \pm 0.022^{c\dagger}$	0.083 ± 0.026	0.0180
½ time recovery of O_2 Hb (s)	14 ± 4	16 ± 5	16 ± 6	0.2776
Time to Max. O_2 Hb (s)	34 ± 8	41 ± 11	35 ± 12	0.1221
Max. amplitude of O_2 Hb (μM)	$36.4 \pm 10.9^{a\dagger}$	$26.5 \pm 9.1^{c*}$	34.3 ± 12.3	0.0197
Hmax of tHb (μM)	$13.6 \pm 4.3^{a*}$	9.9 ± 3.5	12.4 ± 4.7	0.0495
Time to Hmax of tHb (s)	19 ± 5	19 ± 9	22 ± 10	0.4130
Increase rate to max O_2 Hb (μM/s)	1.07 (0.43–2.01) ^{a‡}	0.61 (0.31–1.43) ^{c*}	0.95 (0.47–2.09)	0.0029
Increase rate to max tHb (μM/s)	0.76 (0.24–1.10)	0.50 (0.24–1.66)	0.68 (0.16–1.04)	0.0502
Post-deflation A.U.C of O_2 Hb (a.u)	$1305 \pm 698^{b\dagger}$	997 ± 599	631 ± 417	0.0052
Post-deflation A.U.C of HHb (a.u)	$-171 \pm 337^{b*}$	-32 ± 168	71 ± 299	0.0390
Post-deflation A.U.C of tHb (a.u)	$1146 \pm 539^{b*}$	943 ± 537	718 ± 485	0.0409

MetS: metabolic syndrome, O_2 Hb = oxyhemoglobin, tHb: total hemoglobin, HHb: deoxyhemoglobin, A.U.C: area under the curve, a.u: arbitrary unit, Group effects: a = Healthy vs. MS patients, b = Healthy vs. CHD patients, c = MS patients vs. CHD patients. * = $P<0.05$, † = $P<0.01$, ‡ = $P<0.001$.

3.2. Ultrasound parameters and macrovascular function

Table 2 describes echographic parameters and macrovascular function for each group. Compared to the 2 other groups, healthy patients had a lower baseline and maximal diameter, time to maximal dilatation ($P<0.01$). Healthy patients had a higher FMD ($P<0.05$) compared to CHD patients. CHD and MetS patients had lower nitroglycerin-mediated dilatation compared to healthy subjects ($P<0.01$ and $P<0.05$ respectively).

3.3. NIRS parameters and microvascular function

Table 3 describes NIRS parameters and microvascular function for all groups. Compared to MetS patients, healthy subjects had a higher muscle VO_2, maximal amplitude of O_2Hb and increase rate to max O_2Hb($P<0.05$). Compared to CHD patients, healthy subjects had a higher post-deflation A.U.C of O_2Hb ($P<0.01$) and a lower post-deflation A.U.C of HHb ($P<0.05$). Compared to CHD patients, MetS patients had a lower muscle VO_2, maximal amplitude of O_2Hb and increase rate to max. O_2Hb ($P<0.05$).

3.4. Relationship between CV risk factors, macro and microvascular function

In univariate analyses, microvascular function (NIRS) was more strongly related to CV risks factors than macrovascular function (FMD) (Table 4A). In multivariate models, the independent predictor of macrovascular function (FMD) was the CV status ($P=0.0162$) whereas predictors for microvascular function (A.U.C of O_2Hb) were CV status ($P=0.094$), abdominal obesity ($P<0.0001$) and LDL-cholesterol ($P=0.0016$) (Table 4B). CV status was forced into the multivariate models. No correlations were found between FMD and NIRS parameters with the exception of FMD and time to max. O_2Hb which where modestly correlated (R = 0.32, $P=0.008$).

Table 4A
Independent predictors of macro and microvascular function in all patients – Univariate analyses

Parameters	Macrovascular function (FMD)	Microvascular function (A.U.C of O_2Hb)
CV status	$R^2 = 0.1157$, $p = 0.0162$	$R^2 = 0.1863$, $p = 0.0052$
Age	$R^2 = 0.0134$, $p = 0.3408$	$R^2 = 0.0845$, $p = 0.0329$
CV risk number	$R^2 = 0.0311$, $p = 0.1445$	$R^2 = 0.2169$, $p = 0.0004$
Abdominal obesity *	$R^2 = 0.0254$, $p = 0.1880$	$R^2 = 0.2772$, $p < 0.0001$
Body mass	$R^2 = 0.0181$, $p = 0.2663$	$R^2 = 0.1405$, $p = 0.0052$
BMI	$R^2 = 0.0084$, $p = 0.4498$	$R^2 = 0.1941$, $p = 0.0009$
Fat mass %	$R^2 = 0.0073$, $p = 0.4841$	$R^2 = 0.1218$, $p = 0.0104$
Wais circumference	$R^2 = 0.0105$, $p = 0.4245$	$R^2 = 0.1518$, $p = 0.0062$
Trunk fat mass	$R^2 = 0.0000$, $p = 0.9560$	$R^2 = 0.1765$, $p = 0.0017$
VO_2 peak	$R^2 = 0.0001$, $p = 0.9230$	$R^2 = 0.1594$, $p = 0.0041$
Beta Blockers	$R^2 = 0.0277$, $p = 0.1688$	$R^2 = 0.1104$, $p = 0.0141$
Ca^{2+} channel blockers	$R^2 = 0.0515$, $p = 0.0589$	$R^2 = 0.1398$, $p = 0.0053$
Antiplatelets	$R^2 = 0.0568$, $p = 0.0470$	$R^2 = 0.0844$, $p = 0.0330$
Statins	$R^2 = 0.0464$, $p = 0.0732$	$R^2 = 0.1308$, $p = 0.0072$
Total cholesterol	$R^2 = 0.0486$, $p = 0.0707$	$R^2 = 0.1403$, $p = 0.0057$
LDL cholesterol	$R^2 = 0.0170$, $p = 0.2898$	$R^2 = 0.1853$, $p = 0.0013$
Peak HR	$R^2 = 0.0122$, $p = 0.3782$	$R^2 = 0.1080$, $p = 0.0198$
HRR 1 min	$R^2 = 0.0042$, $p = 0.6093$	$R^2 = 0.0998$, $p = 0.0270$
Max HR predicted	$R^2 = 0.0633$, $p = 0.0357$	$R^2 = 0.1715$, $p = 0.0019$

* Abdominal obesity: categorical defined as waist circumference ≥94 cm for men and ≥80 cm for women. HR : heart rate, HRR: heart rate recovery. Gender, smoking, hypertension, diabetes, dyslepidemia, height, ace inhibitor, hypoglycaemic agent, diuretic, nitrate, fasting glucose, HDL, triglyceride, cholesterol/HDL, triglyceride/HDL, resting HR, resting SBP, resting DBP, peak SBP, peak DBP, HR 1 minute, percentage of HR reserve were also tested in univariate models but there are not significant at 0.05 for macro and macrovascular functions.

Table 4B
Independent predictors of macro and microvascular function in all patients – Multivariate analyses

Parameters	Macrovascular function (FMD)	Microvascular function (A.U.C of O_2Hb)
CV status	$p = 0.0162$	$p = 0.09435$
Abdominal obesity *		$p = <0.0001$
LDL cholesterol		$p = 0.0016$

* Abdominal obesity: categorical defined as waist circumference ≥94 cm for men and ≥80 cm for women. FMD : $R^2 = 0.12$, variables included in the multivariate model CV status, gender, diabete, Ca^{2+} channel blocker, antiplatelet, statin and total cholesterol. A.U.C of O_2Hb : $R^2 = 0.47$, variables included in the multivariate model CV status, obesity abdominal, CV risk number, BMI, Peak HR and LDL.

3.5. Relation between cardiovascular risk factors numbers, macro and microvascular function

Figure 1 describes the relationship between absolute number of CV risk factors, FMD and NIRS parameters. FMD was not related to the number of CV risks factors ($P = 0.21$), whereas max. amplitude of O_2Hb, Hmax of tHb, increased rate to max tHb and post-deflation A.U.C of O_2Hb were, the latter parameter being the most strongly related.

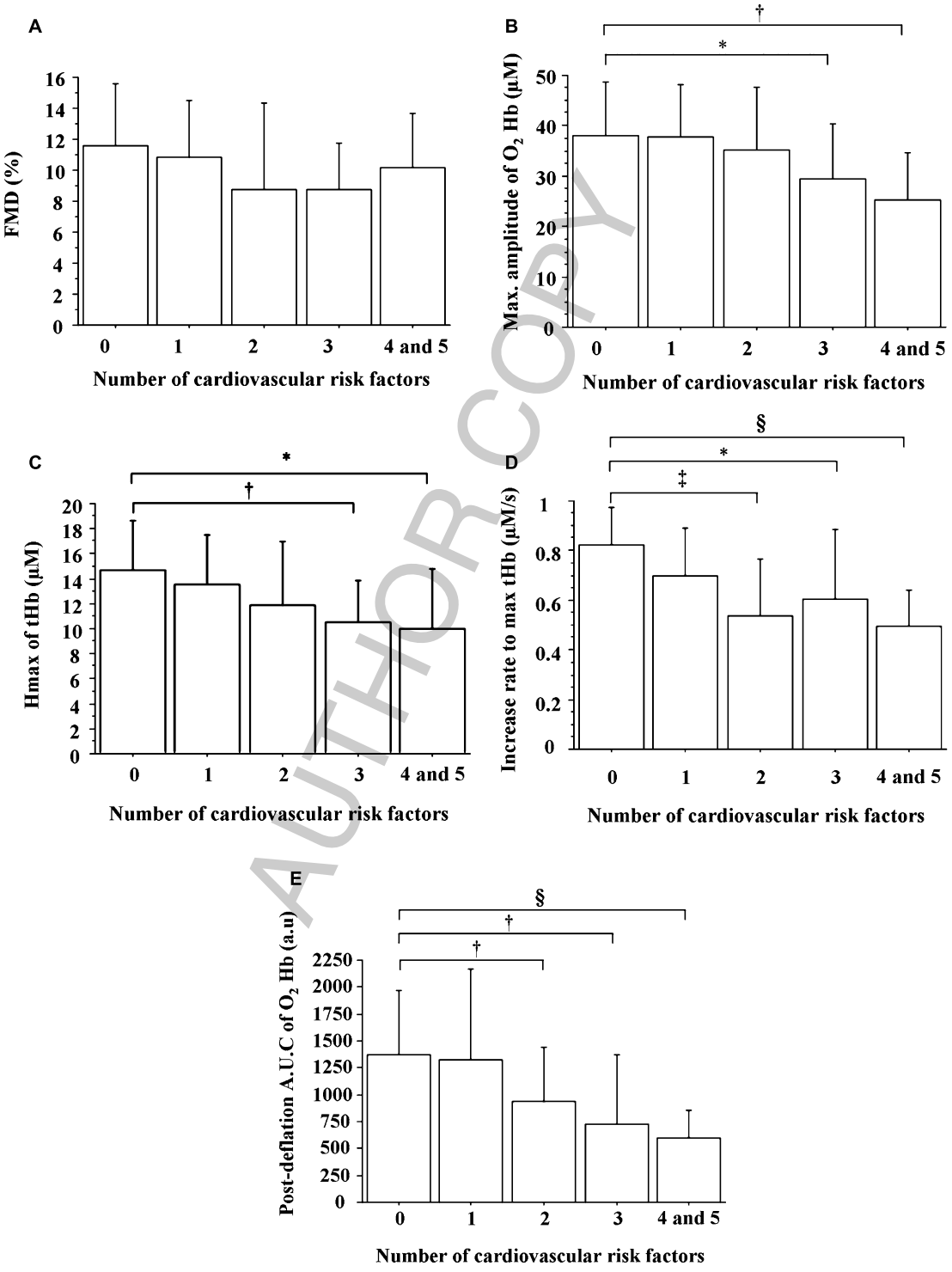

Fig. 1. (A,B,C,D,E): Relation between FMD, NIRS parameters and number of cardiovascular risk factors.

4. Discussion

The principal new findings of this study were that 1) macro (FMD) and microvascular function (NIRS) were impaired in MetS and CHD patients compared to controls; 2) microvascular function assessed with NIRS was more strongly correlated with CV risks factors compared to macrovascular function (FMD); 3) independent determinants of macro and microvascular function were characterized (NIRS: abdominal obesity and dyslipidemia (LDL-cholesterol), FMD: CV status) 4) finally, only microvascular function assessed with NIRS was related to the absolute number of risks factors. The greater the number of CV risk factors, the worse the forearm microvascular function.

To our knowledge, this study is the first to assess simultaneously macrovascular (FMD) and microvascular function using NIRS technique in healthy control subjects, MetS and CHD patients. We also demonstrated for the first time that microvascular function assessed by NIRS was more strongly related to CV risk factors and their numbers compared to macrovascular function (FMD). Additionally, among NIRS reactive hyperemia parameters, we have characterized the one that was better related to CV risk factors, absolute numbers and also their independent predictors. Our results underlie the potential clinical utility of microvascular function measurement with NIRS in patients' CV risk assessment (primary and secondary prevention) and to follow the effects of therapeutic interventions.

Compared to healthy controls, patients with MetS and CHD demonstrated impaired brachial artery FMD in association with higher vascular diameter at baseline and time to maximal dilatation (Table 2), in agreement with previous studies [4, 5]. MetS and CHD subjects had similar age, exercise capacity and risk factor burden (Tables 1 to 3) as well as a similar degree (mild) of endothelial dysfunction. These results may be explained by the fact that patients enrolled in our study were habitual exerciser at our CV prevention centre, and were receiving optimal medical therapy in addition to nutritional counseling [13]. However, as expected, the prevalence of endothelial dysfunction increased in MetS patients (21%), being the highest among CHD patients (46%).

Compared to healthy controls, patients with MetS and those with CHD demonstrated impaired microvascular function as assessed by NIRS. Firstly, we observed impaired resting muscle VO_2 in patients with MetS compared to healthy controls and CHD patients. These data are consistent with previous reports [1, 19, 20, 32], and are potentially due to a reduction of skeletal mitochondrial oxidative capacity and/or skeletal muscle blood flow [3] as demonstrated previously in patients after acute smoking [29], peripheral vascular disease [7] or with heart failure [1, 22]. With respect to post-occlusive NIRS parameters (Max. amplitude and increase rate of O_2Hb, Hmax of tHb), MetS patients had reduced values illustrating the presence of microvascular dysfunction. Maximal amplitude of O_2Hb is a parameter related to oxygen delivery and blood flow delivery to tissue soon after cuff release, whereas Hmax of tHb is more related to blood volume change after cuff release [19]. These results are in agreement with previous studies performed in patients with peripheral vascular disease [17, 19], and in obese and MetS patients using videocapillaroscopy, [12, 18], a method which allows to measure the circulatory response of different provocation manoeuvres directly in human skin capillaries. Reduced NIRS parameters in MetS patients may be related to several alterations of the microcirculation including capillary rarefaction, inability to dilate precapillary vessels, increased stiffness and thickness of capillary walls and well as endothelial dysfunction [19, 30]. It has been demonstrated in obese and MetS patients that skin capillaries at rest are already highly recruited and poor functional capillary reserve is available during post-occlusive reactive hyperemia [12, 18]. The degree of microvascular impairment in MetS/obese patients after reactive hyperemia has been related to the degree of obesity, MetS presence, hypertension and/or insulin resistance [12, 18]. Cardiometabolic risk factors were more prevalent in our MetS group relative to CHD patients,

which may explain at least in part why microvascular function was better among the CHD group. Another potential explanation for a more preserved microvascular function in CHD patients could be due in part to the higher usage of certain medications, (Table 1), particularly statins, that are well known to have beneficial effects on endothelial function both at macro and microvascular levels [16]. Angiotensin–converting enzyme inhibitors and angiotensin receptor blockers have also been shown to have beneficial effects on microcirculation [16], but the prevalence of these agents was similar between the MetS and CHD groups (Table 1).

A novel interesting finding was that microvascular function assessed by NIRS was more strongly related to CV risk factors than macrovascular function (FMD) was (Table 4). Furthermore, microvascular function (NIRS parameters) was principally related to CV status, abdominal obesity and LDL-cholesterol, in contrast to macrovascular function (FMD) which was related to CV status (Table 4). Additionally, only microvascular function (NIRS) was related to the number of CV risk factors when all patients were analyzed independently of CV status (Fig. 1). Our results are in agreement with previous studies [4, 23, 24] where poor or no relationship was demonstrated between FMD and numbers of CV in patients with or without CHD. Again, these results presumably relate to the fact that subjects included in our analysis were habitual exercisers receiving optimal medical therapy in addition to nutritional counseling [13]. Our study has limitations, including the enrolment of stable selected patients recruited in a single centre, hence a potential recruitment bias. Patients with MetS and CHD were also primarily male, undergoing optimal or near-optimal medical therapy and following an exercise training program. Results may differ in women and in other individuals in the real-world setting.

5. Conclusion

In this study, we demonstrate that both micro and macrovascular function are impaired in subjects with increased CV risk relative to healthy controls, namely individuals with MetS and CHD. However, microvascular function assessed by NIRS was more strongly related to CV risks factors and was only related to their absolute number. While brachial artery FMD was worst among CHD patients, microvascular function as assessed by NIRS was worst among MetS subjects. This would appear to be due to the higher prevalence of cardiometabolic risk factors among this group (MetS), including hypertension, diabetes and abdominal obesity, which may be more linked to micro vs. macrovascular dysfunction. Further studies with a larger sample size and including more women are necessary to evaluate the prognostic value of microvascular dysfunction assessed using NIRS, and to determine whether aggressive treatment of CV risk factors can reverse any impairment of microvascular function observed.

Acknowledgments

Cardiovascular Prevention and Rehabilitation Centre Foundation and Montreal Heart Institute Foundation.

Conflict of interest

The authors declare no conflict of interest.

References

[1] K. Abozguia, T.T. Phan, G.N. Shivu, A.R. Maher, I. Ahmed, A. Wagenmakers and M.P. Frenneaux, Reduced *in vivo* skeletal muscle oxygen consumption in patients with chronic heart failure–a study using Near Infrared Spectrophotometry (NIRS), *Eur J Heart Fail* **10** (2008), 652–657.

[2] T.J. Anderson, F. Charbonneau, L.M. Title, J Buithieu, et al., Microvascular function predicts cardiovascular events in primary prevention: Long-term results from the Firefighters and Their Endothelium (FATE) study, *Circulation* **123** (2011), 163–169.

[3] L. Bakkman, M. Fernstrom, P. Loogna, O. Rooyackers, L. Brandt and Y.T. Lagerros, Reduced respiratory capacity in muscle mitochondria of obese subjects, *Obes Facts* **3** (2010), 371–375.

[4] D.S. Celermajer, K.E. Sorensen, C. Bull, J. Robinson and J.E. Deanfield, Endothelium-dependent dilation in the systemic arteries of asymptomatic subjects relates to coronary risk factors and their interaction, *J Am Coll Cardiol* **24** (1994), 1468–1474.

[5] D.S. Celermajer, K.E. Sorensen, V.M. Gooch, D.J. Spiegelhalter, et al., Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis, *Lancet* **340** (1992), 1111–1115.

[6] S.Y. Chan, G.B. Mancini, L. Kuramoto, M. Schulzer, J. Frohlich and A. Ignaszewski, The prognostic importance of endothelial dysfunction and carotid atheroma burden in patients with coronary artery disease, *J Am Coll Cardiol* **42** (2003), 1037–1043.

[7] T.R. Cheatle, L.A. Potter, M. Cope, D.T. Delpy, P.D. Coleridge Smith and J.H. Scurr, Near-infrared spectroscopy in peripheral vascular disease, *Br J Surg* **78** (1991), 405–408.

[8] M.C. Corretti, T.J. Anderson, E.J. Benjamin and D. Celermajer, et al., Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: A report of the International Brachial Artery Reactivity Task Force, *J Am Coll Cardiol* **39** (2002), 257–265.

[9] J. Dupuis, J.C. Tardif, P. Cernacek and P. Theroux, Cholesterol reduction rapidly improves endothelial function after acute coronary syndromes. The RECIFE (reduction of cholesterol in ischemia and function of the endothelium) trial, *Circulation* **99** (1999), 3227–3233.

[10] R. Fathi, B. Haluska, N. Isbel, L. Short and T.H. Marwick, The relative importance of vascular structure and function in predicting cardiovascular events, *J Am Coll Cardiol* **43** (2004), 616–623.

[11] S. Fichtlscherer, S. Breuer and A.M. Zeiher, Prognostic value of systemic endothelial dysfunction in patients with acute coronary syndromes: Further evidence for the existence of the "vulnerable" patient, *Circulation* **110** (2004), 1926–1932.

[12] E.A. Francischetti, E. Tibirica, E.G. da Silva, E. Rodrigues, B.M. Celoria and V.G. de Abreu, Skin capillary density and microvascular reactivity in obese subjects with and without metabolic syndrome, *Microvasc Res* **81** (2011), 325–330.

[13] M. Gayda, C. Brun, M. Juneau, S. Levesque and A. Nigam, Long-term cardiac rehabilitation and exercise training programs improve metabolic parameters in metabolic syndrome patients with and without coronary heart disease, *Nutr Metab Cardiovasc Dis* **18** (2008), 142–151.

[14] S.M. Grundy, J.I. Cleeman, S.R. Daniels, K.A. Donato, et al., Diagnosis and management of the metabolic syndrome: An American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement, *Circulation* **112** (2005), 2735–2752.

[15] M. Hashimoto, K. Kozaki, M. Eto, M. Akishita, et al., Association of coronary risk factors and endothelium-dependent flow-mediated dilatation of the brachial artery, *Hypertens Res* **23** (2000), 233–238.

[16] A.M. Jonk, A.J. Houben, R.T. de Jongh, E.H. Serne, N.C. Schaper and C.D. Stehouwer, Microvascular dysfunction in obesity: A potential mechanism in the pathogenesis of obesity-associated insulin resistance and hypertension, *Physiology (Bethesda)* **22** (2007), 252–260.

[17] H.M. Kooijman, M.T. Hopman, W.N. Colier, J.A. van der Vliet and B. Oeseburg, Near infrared spectroscopy for noninvasive assessment of claudication, *J Surg Res* **72** (1997), 1–7.

[18] L.G. Kraemer-Aguiar, C.M. Laflor and E. Bouskela, Skin microcirculatory dysfunction is already present in normoglycemic subjects with metabolic syndrome, *Metabolism* **57** (2008), 1740–1746.

[19] R. Kragelj, T. Jarm, T. Erjavec, M. Presern-Strukelj and D. Miklavcic, Parameters of postocclusive reactive hyperemia measured by near infrared spectroscopy in patients with peripheral vascular disease and in healthy volunteers, *Ann Biomed Eng* **29** (2001), 311–320.

[20] R. Kragelj, T. Jarm and D. Miklavcic, Reproducibility of parameters of postocclusive reactive hyperemia measured by near infrared spectroscopy and transcutaneous oximetry, *Ann Biomed Eng* **28** (2000), 168–173.

[21] S. Lacroix, M. Gayda, V. Gremeaux, M. Juneau, J.-C. Tardif and A. Nigam, Reproducibility of near-infrared spectroscopy parameters measured during brachial artery occlusion and reactive hyperemia in healthy men, *Journal of Biomedical Optics* **17** (2012), 077010–077015.
[22] C. Manetos, S. Dimopoulos, G. Tzanis, S. Vakrou, et al., Skeletal muscle microcirculatory abnormalities are associated with exercise intolerance, ventilatory inefficiency, and impaired autonomic control in heart failure, *J Heart Lung Transplant* **30** (2011), 1403–1408.
[23] G.F. Mitchell, H. Parise, J.A. Vita, M.G. Larson, et al., Local shear stress and brachial artery flow-mediated dilation: The Framingham Heart Study, *Hypertension* **44** (2004), 134–139.
[24] G.F. Mitchell, J.A. Vita, M.G. Larson, H. Parise, et al., Cross-sectional relations of peripheral microvascular function, cardiovascular disease risk factors, and aortic stiffness: The Framingham Heart Study, *Circulation* **112** (2005), 3722–3728.
[25] A. Nigam, G.F. Mitchell, J. Lambert and J.C. Tardif, Relation between conduit vessel stiffness (assessed by tonometry) and endothelial function (assessed by flow-mediated dilatation) in patients with and without coronary heart disease, *Am J Cardiol* **92** (2003), 395–399.
[26] R. Ross, Atherosclerosis–an inflammatory disease, *N Engl J Med* **340** (1999), 115–126.
[27] M. Shechter, A. Issachar, I. Marai, N. Koren-Morag, et al., Long-term association of brachial artery flow-mediated vasodilation and cardiovascular events in middle-aged subjects with no apparent heart disease, *Int J Cardiol* **134** (2009), 52–58.
[28] D. Shimbo, C. Grahame-Clarke, Y. Miyake, C. Rodriguez, et al., The association between endothelial dysfunction and cardiovascular outcomes in a population-based multi-ethnic cohort, *Atherosclerosis* **192** (2007), 197–203.
[29] A. Siafaka, E. Angelopoulos, K. Kritikos, M. Poriazi, et al., Acute effects of smoking on skeletal muscle microcirculation monitored by near-infrared spectroscopy, *Chest* **131** (2007), 1479–1485.
[30] P.A. Stapleton, M.E. James, A.G. Goodwill and J.C. Frisbee, Obesity and vascular dysfunction, *Pathophysiology* **15** (2008), 79–89.
[31] M.C. Van Beekvelt, W.N. Colier, R.A. Wevers and B.G. Van, Engelen, Performance of near-infrared spectroscopy in measuring local O(2) consumption and blood flow in skeletal muscle, *J Appl Physiol* **90** (2001), 511–519.
[32] M.C. van Beekvelt, B.G. van Engelen, R.A. Wevers and W.N. Colier, *In vivo* quantitative near-infrared spectroscopy in skeletal muscle during incremental isometric handgrip exercise, *Clin Physiol Funct Imaging* **22** (2002), 210–217.
[33] D.R. Witte, J. Westerink, E.J. de Koning, Y. van der Graaf, D.E. Grobbee and M.L. Bots, Is the association between flow-mediated dilation and cardiovascular risk limited to low-risk populations? *J Am Coll Cardiol* **45** (2005), 1987–1993.
[34] R.T. Yan, T.J. Anderson, F. Charbonneau, L. Title, S. Verma and E. Lonn, Relationship between carotid artery intima-media thickness and brachial artery flow-mediated dilation in middle-aged healthy men, *J Am Coll Cardiol* **45** (2005), 1980–1986.