

2D-Spatially-Selective Real-Time Magnetic Resonance Imaging for the Assessment of Microvascular Function and Its Relation to the Cardiovascular Risk Profile

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ABSTRACT

Background: While local endothelial dysfunction of conduit arteries is well recognized as an early step in atherogenesis, contradictory observations are reported with regard to alterations in the microcirculation and their association with cardiovascular risk factors (RFs). A real-time MR approach was developed to investigate the relationship between the RFs profile and microcirculatory alterations assessed as impairment of reactive hyperemic flow in the leg circulation. **Methods:** The MR technique was applied to patients ($n = 17$, Pats1) with 1.8 ± 0.8 RFs but without peripheral arterial occlusive disease (PAD), to age-matched healthy controls ($n = 13$, Con1), to young controls ($n = 12$, 23 ± 4 y), and to patients with RFs and PAD ($n = 8$, Pats2). **Results:** Superficial femoral artery (SFA) peak hyperemic flow in Pats1 was reduced vs Con1 (24.6 ± 4.2 vs 30.4 ± 7.3 mL min⁻¹ 100 mL⁻¹ calf tissue, $p < 0.02$), and minimal vascular resistance increased incrementally with the number of RFs and with Framingham and Procam risk scores. Flow-mediated vasodilation (FMD) of the SFA was blunted in both Pats1 and Con1 ($-0.5 \pm 3.4\%$ and $+0.6 \pm 3.2\%$, respectively, both ns vs 0). In young controls, peak hyperemic flow (30.1 ± 3.3 mL min⁻¹ 100 mL⁻¹) and endothelium-independent vasodilation ($9.2 \pm 10.0\%$) were preserved, while FMD was minimal ($2.0 \pm 5.9\%$, $p < 0.02$ vs endothelium-independent vasodilation). In Pats2, peak hyperemic flow was severely reduced (12.2 ± 3.6 mL min⁻¹ 100 mL⁻¹, $p < 0.0003$ vs Con1 and Pats1), and both FMD and endothelium-independent vasodilation were absent. **Conclusions:** Reactive hyperemic flow in the SFA, reflecting microcirculatory function of the lower limb, gradually decreases with increasing cardiovascular risk suggesting a role for microvascular dysfunction in atherogenesis. The presented MR approach might become a valuable tool to study (micro)-vascular pathophysiology.

Keywords: real-time phase-contrast flow measurements, spatially selective 2D rf excitations, microcirculation, cardiovascular risk factors, reactive hyperemic response, superficial femoral artery. Received 28 October 2005; accepted 8 March 2006.

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INTRODUCTION

Atherosclerotic vascular disease is the leading cause of death in the Western world, and major efforts are undertaken to impede or delay its development, e.g., by risk factor (RF) modification. In experimental hyperlipidemia, leukocyte recruitment in the microcirculation was increased (1), and micro-computed tomography revealed anatomical changes in the microvasculature of the vessel walls, i.e., neoformations of vasa vasorum of the conduit arteries, which regressed with lipid lowering therapy (2). While a few studies demonstrated a reduced reactive hyperemic flow by strain-gauge plethysmography (SGP) in patients with hypertension and hypercholesterolemia (3–6), the majority of clinical studies did not find a relationship between reactive

hyperemic flow, i.e., microcirculatory alteration, and the presence of RFs such as hypertension (7, 8), diabetes (9), hypercholesterolemia (10, 11), or smoking (10, 12–14). Considering these contradictory results, the involvement of the microcirculation in atherogenesis remains unresolved. SGP appears more sensitive to detect disturbances in hyperemic flow (3, 4) than Doppler ultrasound (15). We hypothesized that highly accurate measurements of hyperemic flow in patients with various RFs could answer the question, whether microcirculatory changes are associated with the presence and the degree of RFs. To this aim, a novel real-time MR technique was developed, which automatically quantifies hyperemic flow in the superficial femoral artery (SFA) with high precision. Mitchel et al. showed that the hyperemic flow stimulus induces flow-mediated dilation (FMD) through reduced local shear stress (15, 16), thus demonstrating that FMD is not only reflecting local endothelial function of conduit arteries. Therefore, FMD was calculated from the MR data to answer the question to what extent FMD is correlated with the amount of reactive hyperemic flow.

MATERIALS AND METHODS

Phantom studies

A standardized pulsatile flow pump (Shelley Limited, London, Ontario, Canada) (17) was connected to a conical bore within a block of agarose gel encompassing cross-sectional areas from 50 to 80 mm² over a bore length of 150 mm. Pulsatile flow (water doped with 0.005% Manganese(II)chloride for T₁-correction) with stroke volumes of 1.2–3.6 mL was delivered by the flow pump into the conical bore. Various cross-sectional areas were measured at 27 different positions along the conical bore generating areas differing by 1.1 mm². The real-time spatially selective MR technique (ssMR) technique is based on a 2D-spatially selective rf-excitation and measures with a spatial/temporal resolution of 1 × 1 mm²/48 ms at a slice thickness of 2.5 cm and a field of view of 6.4 × 1.6 cm² (Fig. 1). Lumen areas determined by ssMR were compared with those obtained by caliper gauge measurements of the conical bore. Flow volumes were determined by stopwatch and graduated cylinder. In order to determine whether precision of lumen area measurements is flow-dependent, lumen area was measured at a constant bore position (corresponding to 47.8 mm² matching SFA dimensions) at various peak velocities of pulsatile flow ranging from 10–30 mL sec⁻¹.

Human Studies

Seventeen patients (Pats1, 49 ± 6 years) with known cardiovascular RFs (according Adult Treatment Panel III) (18) and without peripheral arterial occlusive disease (PAD), 10 patients (Pats2, 68 ± 11 years) with RFs and PAD, 13 healthy subjects without RFs (Con1, 45 ± 8 years, age-matched with Pats1), and 12 younger healthy subjects without RFs (Con2, 23 ± 4 years) were recruited for this study. All Pats1 and controls were free of symptoms for PAD, and Doppler ultrasound, pulse volume recordings, and ankle-brachial index measurements (1.0–1.2 in

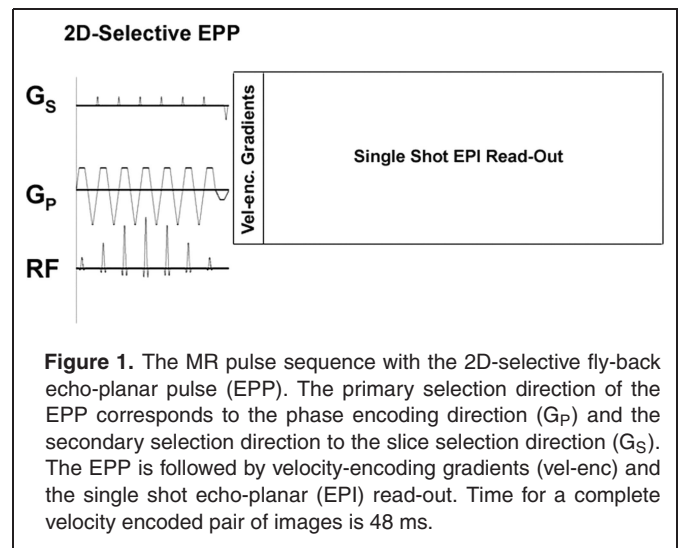


Figure 1. The MR pulse sequence with the 2D-selective fly-back echo-planar pulse (EPP). The primary selection direction of the EPP corresponds to the phase encoding direction (G_p) and the secondary selection direction to the slice selection direction (G_s). The EPP is followed by velocity-encoding gradients (vel-enc) and the single shot echo-planar (EPI) read-out. Time for a complete velocity encoded pair of images is 48 ms.

all subjects) were performed to exclude PAD. All Pats2 had 3–4 RFs, had PAD with percutaneous interventions in the iliac and/or femoral arteries within 2 weeks prior to the MR study, and had stenosis in the lower limb circulation by conventional angiography. The study protocol was approved by the local ethics committee and all subjects gave written informed consent.

Flow measurements by real-time ssMR

All MR studies (74 sessions in 52 subjects) were performed in the evenings at a constant room temperature of 20°C. In order to assess vascular pathophysiology in relation to the actual cardiovascular risk, all patients were kept on their regular medication during the MR studies. All subjects were fasting for ≥4 hours, and smoking was not allowed for 12 hours prior to the MR examination. From the 10 subjects of the Pats2 group, 2 MR examinations could not be performed (one patient did not tolerate cuff inflation, one patient felt claustrophobic). For determination of reproducibility, the first 22 subjects (9 Pats1 and 13 Con1) were studied twice at 2 different days (Fig. 2). Following immobilization of the leg by vacuum cushions, measurements in the SFA were planned on a 3D-localizer (placed at 6.7 ± 3.4 cm proximal to the cranial border of the patella which was used as a reference for repeat studies). Baseline flow measurements were started after a resting period of at least 20 minutes and episodes of ischemia were induced as shown in Fig. 2 with a cuff inflated directly distal to the knee. Following 4 minutes of cuff inflation (at 280 mmHg), the cuff was instantaneously deflated by vacuum and the hyperemic response was monitored using the same real-time ssMR sequence and parameters as in the flow phantom. Since FMD in the age-matched healthy controls (Con1) was absent, young controls (Con2) were also studied during hyperemia as well as 3 minutes after sublingual administration of 2.5 mg isosorbide-dinitrate to demonstrate that ssMR is sensitive for small lumen area changes in-vivo.

As a standard of reference for the in-vivo validation, a high-resolution ECG-gated phase-contrast pulse sequence was used

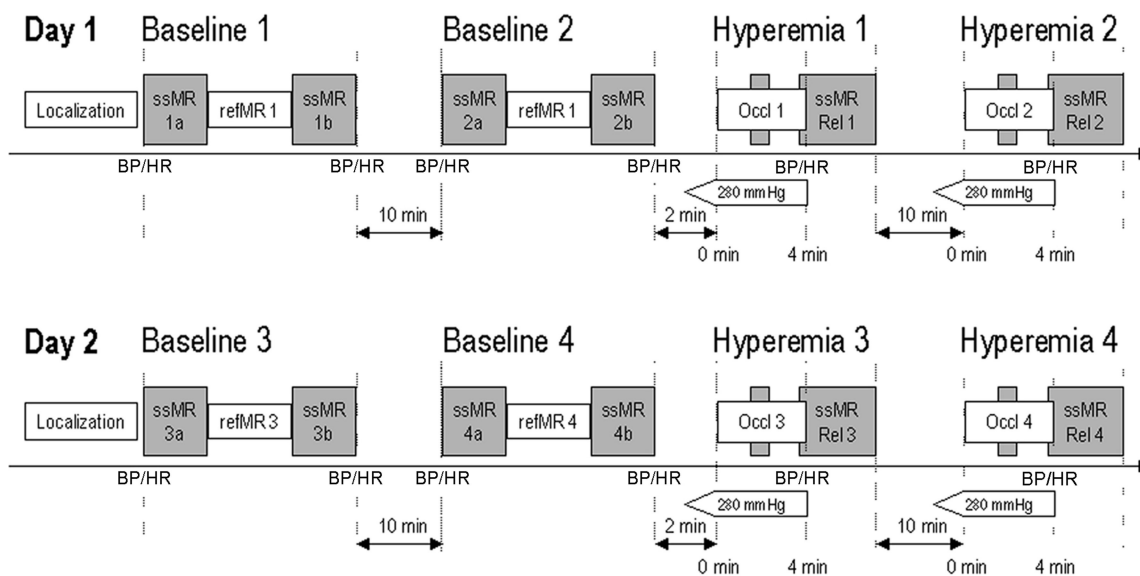


Figure 2. Protocol of MR examinations performed on 2 separate sessions (day 1 and 2, mean interval 9 ± 13 days). ssMR and refMR refer to real-time spatially selective MR measurements (1024 images acquired over 50 seconds) and reference MR measurements using a high-resolution ECG-triggered phase-contrast acquisition (temporal/spatial resolution: $0.7 \text{ mm}/22 \text{ ms}$). BP/HR indicate time points of measurements of blood pressure (by sphygmomanometry) and heart rate, respectively.

under resting conditions to achieve a spatial/temporal resolution of $0.7 \times 0.7 \text{ mm}^2/22 \text{ ms}$ (19).

Image analysis and calculation of indexes of hyperemic response

The automatic analysis algorithm is initiated by a single click into the vessel lumen and calculates pixel-wise time-velocity correlations in a rectangular region of interest (ROI, 1024 pixels) encompassing the vessel lumen and surrounding stationary tissue (20). When correlations are calculated for a region growing from a seed pixel within the vessel lumen, high correlations are maintained as long as the area is located within the vessel lumen and the correlation drops when neighbouring “stationary” pixels are included. On an area versus correlation plot the inflection point then identifies the vessel border. The zero offset in the velocity data was corrected for by subtracting the mean value measured in stationary tissue (adjacent to the ROI).

While flow was measured in the SFA, cuff occlusion was performed directly distal to the knee to avoid ischemia and motion of the SFA during occlusion and cuff deflation, respectively. Therefore, all indexes of hyperemic response were based on $\text{Flow}_{\text{hyperemic}}$ ($= \text{flow during hyperemia} - \text{flow during occlusion}$). Peak hyperemic flow ($\text{Flow}_{\text{peak}}$) was the mean of 5 consecutive beats with highest flow normalized to calf volume, which was measured by water displacement. Minimal vascular resistance (MVR , $\text{mmHg} \cdot \text{mL}^{-1} \cdot \text{min}^{-1} \cdot 100 \text{ mL}^{-1}$) was mean blood pressure (mean BP = diastolic BP plus 1/3 of the BP amplitude) divided by $\text{Flow}_{\text{peak}}$. Time to $\text{Flow}_{\text{peak}}$ was defined as time from first heart beat after release to heart beat with highest blood flow. The area under the hyperemic response curve

(AUC) was the integral of $\text{Flow}_{\text{hyperemic}}$ starting at cuff release up to 35 seconds. Half time of hyperemic response was the time from $\text{Flow}_{\text{peak}}$ to 50% of $\text{Flow}_{\text{peak}}$. FMD during hyperemia was monitored over 50 seconds in Con1, Pats1, and Pats2, and over 2 min 30 sec in Con2. FMD was calculated as maximum change in diameter (mean of 15 heart beats) divided by baseline diameter times 100%. Vessel diameter was calculated from lumen area assuming a circular vessel shape. The reference flow data obtained by triggered high-resolution MR were analyzed manually (19).

Statistics

Values are given as mean $\pm$ SD. For comparisons between phantom, ssMR, and ECG-triggered high-resolution MR measurements as well as for the reproducibility assessment of ssMR, the mean $\pm$ SD of paired measurements ($= \text{SD}_{\text{Diff}}$), and corresponding 95% confidence intervals (CI) are given (21). The effect of number of RFs and annual risk rates on MVR and comparisons in Fig. 3 and Table 1 were examined using a one-way ANOVA with post Hoc Bonferroni testing (StatView v5.0.1, SAS Institute Inc., USA). The assessment of hemodynamics, baseline flows and hyperemic responses between controls and patients (Table 2) involved a 2-way repeated measures ANOVA with post Hoc Bonferroni testing. Reported p values are Bonferroni-corrected in case of multiple comparisons with corrected $p < 0.05$ considered statistically significant. To identify explanatory variables for MVR, a stepwise regression analysis was performed (StatView v5.0.1, SAS Institute Inc.).

For an expected difference in $\text{Flow}_{\text{peak}}$ between patients and controls of approximately 20% (3, 5) with an inter-study reproducibility of 15.5% ($= \text{SD}_{\text{Diff}}$) by ssMR yields a power of 85%

Table 1. Characteristics of patients and controls

	Controls 1 (age-matched)	Patients 1	Patients 2
n	13	17	8
Gender (male/female)	10/3	16/1	7/1
Age (years)	45 ± 8	49 ± 6	68 ± 11*
Body mass index (kg/m ²)	22.4 ± 2.3	30.0 ± 4.9 [†]	25.1 ± 1.9
Risk factors (ATP III)	−0.2 ± 0.6	1.8 ± 0.8 [†]	3.3 ± 0.7*
Systemic hypertension	—	8 (47)	7 (88)
Systolic BP	127 ± 13	132 ± 13	149 ± 10 [‡]
Diastolic BP	72 ± 9	75 ± 11	74 ± 10
Hypercholesterolemia	—	5 (29)	4 (50)
Total cholesterol (mmol/L)	5.3 ± 1.3	5.3 ± 1.2	5.2 ± 1.6
HDL-cholesterol (mmol/L)	1.7 ± 0.4	1.2 ± 0.2 [†]	1.4 ± 0.3
LDL-cholesterol (mmol/L)	2.8 ± 1.0	3.2 ± 1.1	3.0 ± 1.8
Total cholesterol/HDL ratio	3.2 ± 0.8	4.6 ± 1.2	3.9 ± 1.4
Triglycerides (mg/dL)	146 ± 86	209 ± 106	235 ± 185
Diabetes mellitus	—	1 (6)	1 (13)
HbA _{1c}	0.054 ± 0.004	0.059 ± 0.007	0.063 ± 0.004 [§]
Smoking	—	5 (29)	5 (63)
Positive family history	—	3 (18)	1 (13)
Medication			
Antihypertensives	—	8 (47)	4 (50)
Beta-blockers	—	—	3 (38)
Nitrates	—	—	1 (13)
Calcium channel blocker	—	—	3 (38)
Statines	—	14 (82)	7 (88)
Lipidapheresis	—	4 (24)	—

Controls 1 are age-matched with patients 1. Patients 1 and 2: without and with peripheral arterial occlusive disease, respectively. Data are mean ± SD or number of patients (%). BP = blood pressure. *p < 0.0003 vs controls and patients 1; [†]p < 0.0003 vs controls. [‡]p < 0.01 vs controls and patients 1, [§]p < 0.05 vs controls, ^{||}p < 0.003 vs controls (all p values Bonferroni-corrected).

to detect differences at a p value of 0.01 in a sample size of 30 subjects. With the same sample size, FMD measurements with an inter-study reproducibility of 4.3% (= SD_{Diff}) would detect a difference of 5% with a power of 80% at a p value of 0.05.

RESULTS

Hyperemic response and cardiovascular risk

In the subjects without PAD (Pats1 and age-matched Con1; n = 30), overall annual event rates were 0.55 ± 0.46% and 0.31 ± 0.29% for Framingham (22) and Procam (23) risk scores, respectively. Demographics of the study population are given in Table 1. Despite this low cardiovascular risk profile in Pats1, Flow_{peak} during established medical therapy was reduced in Pats1 versus Con1 (24.6 ± 4.2 vs 30.4 ± 7.3 mL · min^{−1} · 100 mL^{−1}, p < 0.02, n = 30, 1-way ANOVA, Fig. 3), and MVR during hyperemia was elevated in Pats1 versus Con1 (3.9 ± 0.9 vs 2.9 ± 0.7 mmHg · mL^{−1} · min^{−1} · 100 mL^{−1}, respectively, p < 0.03). An example is given in Fig. 4. Figure 5 illustrates the increase in MVR under hyperemic conditions with increasing number of RFs (18), and increasing risk categories calculated with different models (22, 23) in subjects without PAD (Pats1 and Con1). In this population, stepwise regression analysis revealed HDL-cholesterol and smoking status (pack-years of active smokers) as explanatory vari-

ables for MVR (MVR = −1.1 HDL-cholesterol + 0.06 pack-years + 4.87, r = 0.73, p < 0.0045), while mean BP, age, physical activity (hours/week), LDL-cholesterol and HbA_{1c} did not enter the model. Flow_{peak} in the diseased Pats2 group was severely reduced with 12.2 ± 3.6 mL · min^{−1} · 100 mL^{−1} (p < 0.0003 vs both, Con1 and Pats1) and MVR was elevated (8.1 ± 1.9 mmHg · mL^{−1} · min^{−1} · 100 mL^{−1}, p < 0.0003 vs both, Pats1 and Con1).

FMD of the SFA in Con1, Con2, Pats1, and Pats2 was small (+0.6 ± 3.2%, +2.0 ± 5.9%, −0.5 ± 3.4%, and −3.4 ± 7.0%, respectively, none significant vs 0), and differences between groups did not reach statistical significance. In Con2 (n = 12, 6 female, mean age: 23 ± 4 years, BMI: 21.2 ± 2.5 kg/m²) with an FMD of only +2.0 ± 5.9% (at a Flow_{peak} of 30.1 ± 3.3 mL · min^{−1} · 100 mL^{−1} at SBP and DBP of 116 ± 11 and 65 ± 8 mmHg, respectively), isosorbide-dinitrate-induced vasodilation of the SFA was 9.2 ± 10.0% (p < 0.02 vs FMD in Con2). For comparison, in Pats2 neither reactive hyperemia nor isosorbide-dinitrate administration dilated the SFA (−3.4 ± 7.0% and −0.5 ± 1.7%, respectively). FMD of the SFA was minimal in all groups and did not correlate with Flow_{peak} (r = 0.03, p = 0.86). Baseline lumen areas of the SFA were not different between Con1, Con2, Pats1, and Pats2 (48.6 ± 8.8, 35.7 ± 9.1, 42.5 ± 14.7, and 30.7 ± 14.6 mm², respectively), also lumen area normalized for calf volume did not differ.

Table 2. Hemodynamics during ssMR measurements

	Day 1				Day 2				Contr1 vs Pat 1	p value Day1 vs Day2	Meas1 vs Meas2
	Measurement 1		Measurement 2		Measurement 1		Measurement 2				
	Controls 1	Patients 1	Controls 1	Patients 1	Controls 1	Patients 1	Controls 1	Patients 1			
n	13	9	13	9	13	9	13	9			
Resting heart rate (bpm)	62 ± 5	63 ± 4	63 ± 4	62 ± 3	60 ± 7	63 ± 4	60 ± 5	62 ± 3	ns	<0.02	ns
Resting systolic BP (mmHg)	130 ± 10	136 ± 14	129 ± 6	137 ± 15	125 ± 10	126 ± 17	126 ± 7	133 ± 14	ns	<0.001	ns
Resting diastolic BP (mmHg)	74 ± 5	74 ± 10	78 ± 7	79 ± 10	71 ± 5	77 ± 12	74 ± 6	77 ± 14	ns	ns	<0.03
Baseline flow (mL · min ⁻¹ · 100 mL ⁻¹)	4.4 ± 1.3	4.1 ± 1.8	4.8 ± 1.6	4.2 ± 1.9	4.3 ± 1.1	3.6 ± 1.5	4.9 ± 1.7	3.8 ± 1.4	ns	ns	<0.01
Peak flow (mL · min ⁻¹ · 100 mL ⁻¹)	30.4 ± 7.3	26.2 ± 6.6	32.7 ± 8.8	26.7 ± 6.2	30.1 ± 6.6	22.8 ± 4.3	32.6 ± 7.7	23.5 ± 4.7	<0.03	ns	<0.01
AUC (mL · 100 mL ⁻¹)	11.2 ± 2.7	11.2 ± 2.3	10.8 ± 2.5	10.8 ± 2.2	11.5 ± 3.1	10.0 ± 1.7	11.7 ± 3.4	10.0 ± 1.7	ns	ns	ns
Half time (sec)	29.0 ± 5.2	26.1 ± .3	23.3 ± 3.7	23.6 ± 5.6	28.2 ± 5.0	28.8 ± 5.7	24.3 ± 4.1	23.9 ± 4.5	ns	ns	<0.0001

Contr1 = Controls 1 (= age-matched). Pat1 = Patients 1. Meas 1 vs 2: Measurement 1 is first baseline and first release of examinations at day 1 and day 2, meas 2 is second baseline and second release of examinations of day 1 and day 2, respectively. bpm = beat per minute. BP = blood pressure. AUC: Area under the flow response curve (flow curve integral over 35 seconds starting after cuff release). Half time = time from peak flow to 50% of peak flow. Resting heart rate, and resting systolic and diastolic BP are the average values of measurements performed immediately before and after the baseline MR flow measurements. Statistics involve a two-way analysis of variance for repeated measurements.

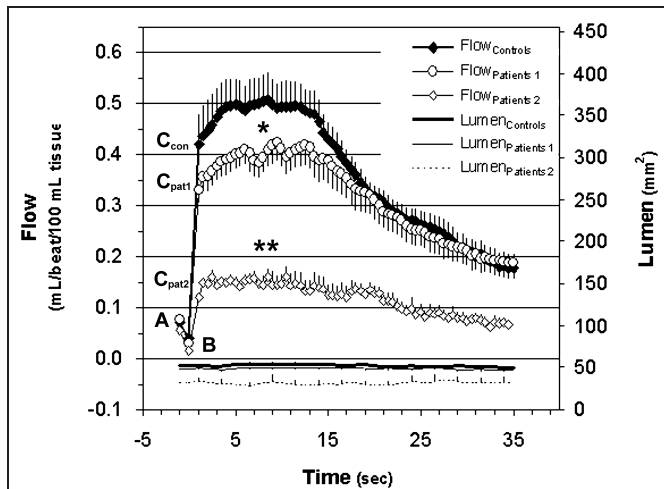


Figure 3. Hyperemic response in patients with risk factors (RFs) and without peripheral arterial occlusive disease (Pats1, $n = 17$), in patients with peripheral arterial occlusive disease and RFs (Pats2, $n = 8$), and in age-matched healthy controls without RFs ($n = 13$) acquired with real-time ssMR. A and B represent flow in the SFA at rest and during occlusion (cuff directly distal to knee at 280 mmHg), respectively. C represents the first heart beat after instantaneous vacuum-induced release of the cuff (suffix_{con} and _{pat} relate to controls and patients, respectively). Pats1 demonstrate a reduced peak flow vs controls (* $p < 0.011$, ANOVA). A biphasic flow response is observed in controls and Pats1 with an instantaneous increase in flow (reaching C_{con} and C_{pat1}) and a second phase of slower flow increase reaching peak flow (in controls: peak flow $p < 0.01$ vs C_{con} ; in Pats1: $p < 0.001$ vs C_{pat1}). In Pats2 peak flow was most severely reduced (** $p < 0.0005$ vs Pats1 and controls, ANOVA) and a biphasic flow response was absent. Error bars represent standard error of the mean.

In-vitro validation

In the phantom with pulsatile flow of varying stroke volumes, the mean difference for ssMR measurements of flow vs phantom flow was $6 \pm 1\%$ (95% CI: 4–8%; Fig. 6A). In this phantom with lumen areas differing by 1.1 mm^2 , mean difference for ssMR diameter measurements vs phantom diameter was $1.7 \pm 0.9\%$ (95% CI: 0 to -3.4% ; Fig. 6B) at a stroke volume of 2.9 mL. Diameter measurements were not dependent on flow and differed by only $\pm 0.5\%$ ($= \text{SD}_{\text{Diff}}$; $-0.8 \pm 0.5\%$, 95% CI -1.8 to 0.2%) when performed over the full flow range.

In-vivo validation

For the in-vivo validation a total of 291, 456 images from 22 subjects were analyzed (mean interval between repeated studies: 9 ± 13 days). The reproducibility of the real-time ssMR measurements were 12.5% and 15.5% ($= \text{SD}_{\text{Diff}}$) for resting and $\text{Flow}_{\text{peak}}$, respectively, and 1.4% and 4.3% (absolute percentage) for lumen diameter and FMD, respectively (Fig. 7). Regarding precision, the mean difference $\pm \text{SD}$ between the reference and the real-time ssMR measurements was $0.10 \pm 0.40 \text{ mL/beat}$ ($2.3 \pm 13.3\%$) for flow and $2.04 \pm 3.92 \text{ mm}^2$ ($5.3 \pm 10.4\%$) for lumen area. These differences are within the reproducibility of the reference measurements themselves ($7.8 \pm 13.9\%$ for flow; $0.8 \pm 9.5\%$ for lumen area). In comparison with the real-

time ssMR measurements, the reproducibility of the reference method ($= \text{SD}_{\text{Diff}}$) was 13.9% for resting flow (12.5% for ssMR) and 9.5% for lumen area (1.4% for ssMR). The fully automatic analysis of the ssMR data eliminated any intra- and interobserver variability for flow, lumen area, and FMD quantification.

In the 22 subjects of the reproducibility assessment, $\text{Flow}_{\text{peak}}$ was higher in Con1 than in Pats1 ($p < 0.03$, Table 2). In comparison with first release, $\text{Flow}_{\text{peak}}$ of second release (mean of Pats1 and Con1) was 6.3% higher ($p < 0.01$) and shorter (half time: $p < 0.0001$).

DISCUSSION

Reactive hyperemic response and cardiovascular risk

This study demonstrates a gradual reduction of peak reactive hyperemic flow with increasing cardiovascular risk, i.e., an increasing number of RFs or an increasing risk score calculated according Framingham (22) and Procam (23) models. This finding is in agreement with a recent report by Mitchell et al., which demonstrated in 2045 patients an inverse correlation between reactive hyperemic flow in the brachial artery and RFs (15). It is noteworthy that in the present study this finding is established in 30 patients only. In many studies with smaller sample sizes, a reduction in hyperemic flow could not be demonstrated, e.g., in patients with hypertension (7, 8), diabetes (9), hypercholesterolemia (10, 11), or in smokers (10, 13, 14). Interestingly, however, the studies which did demonstrate a reduced hyperemic flow in the presence of RFs mainly utilized SGP (3, 4) and/or involved large sample sizes (3, 15). These mixed results can be simply explained by the fact that heterogeneous patient populations were studied but favor the possibility that methodological aspects and/or too small sample sizes did not allow detection of small differences in hyperemic flow. Accordingly, the current finding of an impaired hyperemic flow in the SFA in patients with RFs is in line with hyperemic brachial artery data acquired in large studies (3, 15), which suggest that similar mechanisms might be involved in the regulation of the microcirculatory response to ischemia in both the forearm and leg circulation. Ischemia-induced hyperemic flow is a complex event in the microcirculation involving metabolic, neurogenic, and myogenic mechanisms (24). It also depends at least in part on shear stress-induced and thus, endothelium-dependent production of vasodilators including NO (11, 24–27). The fact that NO, a “vasoprotective” compound, is partly involved in the reactive hyperemic response may explain a relationship between reactive hyperemic flow, i.e., the response of the microcirculation to ischemia, and prognosis. In experimental studies, statins could reverse structural alterations of the vessel wall microcirculation, i.e., of adventitial vasa vasorum of atherosclerotic vessels (2). Furthermore, in clinical studies, statins (5, 6) and other drugs (3) which are known to improve prognosis also improved reactive hyperemic flow in the forearm. Together with these findings the results of the current study supports the idea that microcirculatory disturbances may play a role in atherogenesis,

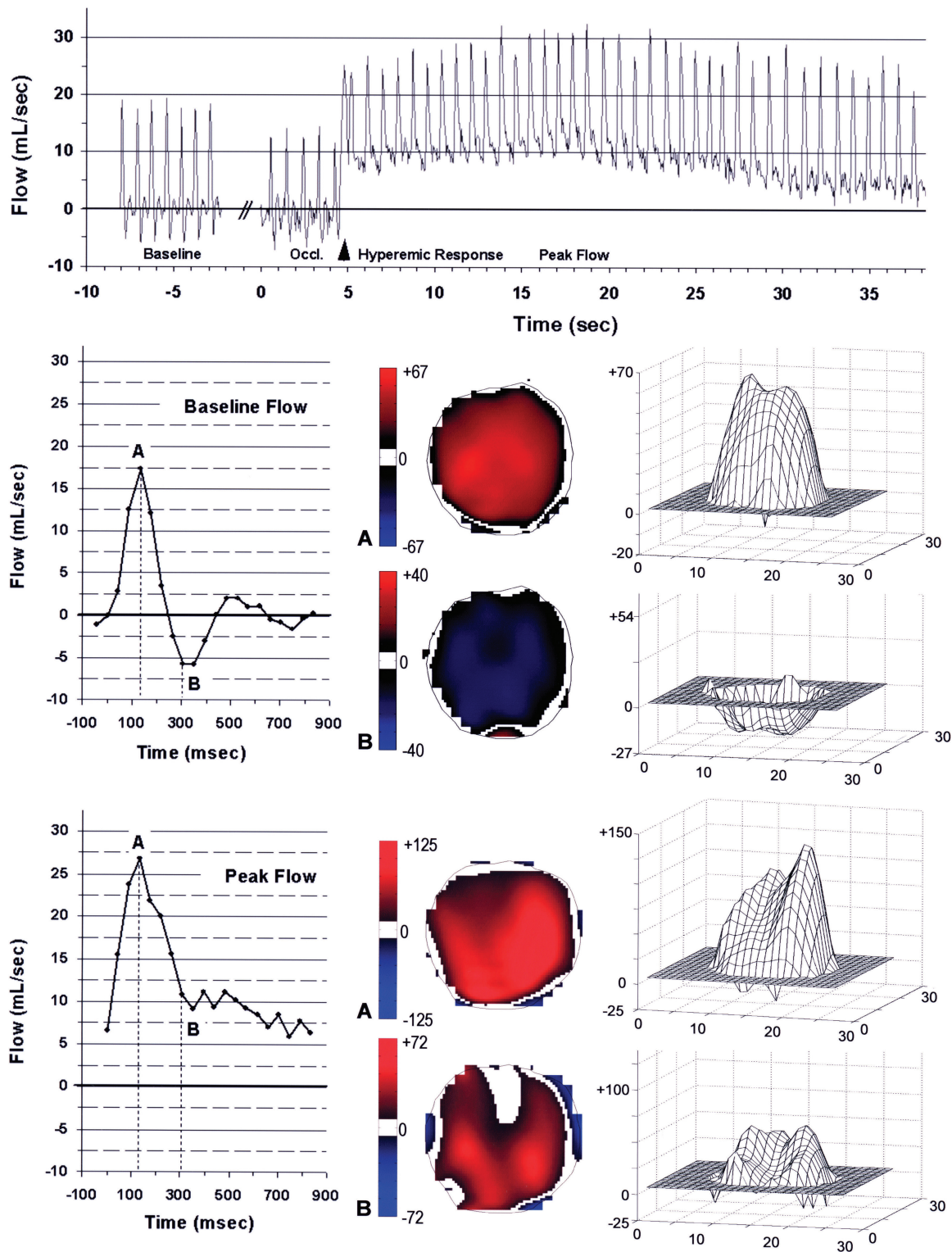


Figure 4. Assessment of hyperemic response by real-time ssMR. On top, a hyperemic response over 40 seconds is shown in a healthy volunteer, for comparison baseline flow is shown on the left. During occlusion flow is low (supplying the tissue between site of measurement, i.e. distal SFA, and site of cuff occlusion distal to the knee). Note high flow at the time of cuff release during diastole (arrow head), which is followed by a further increase in flow reaching peak flow after approximately 13 seconds in this case. On the left, two examples of flow curves at baseline (middle row) and after first beat following release (bottom row) are given acquired with ssMR (temporal/spatial resolution: 48 ms/1 mm²). A and B refer to peak systole and early diastole, respectively. In the center, color-coded phase-contrast images are shown (red: forward flow; blue retrograde flow), and to the right corresponding flow profiles are given. Note the symmetrical flow profile at baseline during systole, while profiles are asymmetric during hyperemic response during both, peak systole and early diastole.

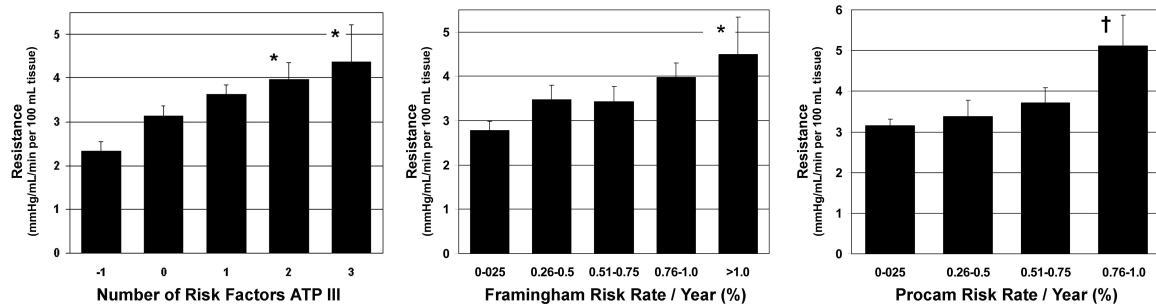


Figure 5. Bar graphs demonstrating the relationship between subgroups of patients at various risks and minimal vascular resistance during ischemia-induced hyperemic response as assessed by real-time ssMR ($n = 30$). Minimal vascular resistance increases with increasing number of ATP III (18) cardiovascular risk factors (Fig. 5A), and annual event rates as calculated from Framingham (22) (Fig. 5B) or Procaml studies (23) (Fig. 5C). † $p < 0.01$ and * $p < 0.05$ vs lowest risk group (one-way ANOVA, Bonferroni-corrected p values). Error bars represent standard error of the mean.

and thus, reactive hyperemic flow in the microcirculation may be linked to prognosis. In order to study the mechanisms involved in the regulation of microvascular function and its relation to RFs, the ssMR technique can be easily combined with the intra-arterial administration of specific substances such as acetylcholine and others.

While hyperemic flow in the SFA decreases with an increasing burden of cardiovascular risk, the current study also shows that reactive hyperemic flow induces only minimal FMD even in young controls without RFs, while endothelium-independent vasodilation is preserved in these subjects. Conversely, in the small group of patients with established PAD, neither hyperemic flow (which was severely reduced) nor isosorbide-dinitrate administration could dilate the SFA. A minimal or absent FMD of the SFA was also reported for young subjects in a study utilizing ultrasound (10). In the brachial artery, longer durations of ischemia and a larger mass of ischemic tissue (upper arm occlusions) induce a more pronounced FMD (28). In analogy, an ischemic period of ≥ 4 minutes and/or upper leg occlusion might have induced a sufficient hyperemic flow stimulus in the SFA to provoke a measurable FMD.

The results obtained with the current protocol suggest that peak reactive hyperemic flow in the SFA is a suitable measure for risk stratification of patient groups, e.g., the multiple regression model derived from the current data applied to smokers with 10 pack-years would predict an increase in MVR of $0.60 \text{ mmHg} \cdot \text{mL}^{-1} \cdot \text{min}^{-1} \cdot 100 \text{ mL}^{-1}$, which closely matches results of a study (29) performed in approximately 100 young non-smokers and smokers (mean age 29 years) which showed an increase in MVR in smokers of $0.70 \text{ mmHg} \cdot \text{mL}^{-1} \cdot \text{min}^{-1} \cdot 100 \text{ mL}^{-1}$ measured by SGP. The proposed MR approach may also allow to quantify positive effects of new treatment regimens designed for RFs modification.

2D-spatially selective MR measurements for assessment of hyperemic flow and FMD

ECG-triggered high-resolution MR techniques (encoding 12 pixels/brachial artery diameter) require 15–25 seconds for ac-

quisition (16). To study the transient hyperemic flow response, real-time sequences would be ideal. Up to now, real-time sequences yielded a temporal resolution of 78 ms (at 4 pixels/brachial artery diameter) (30) and 124 ms (at 3 pixels/carotid and iliac artery diameter) (31), thus, allowing for peak velocity but not for peak flow measurements (32). Recently, parallel imaging techniques (33) yielded a temporal resolution of 39 ms, however, at the costs of a spatial resolution 7-times lower than with the presented technique. With a temporal resolution of 48 ms (at 7–8 pixels/SFA diameter) the proposed real-time ssMR approach is several times more efficient in data acquisition than the fastest techniques currently available. As a result, the ssMR method was highly accurate in-vitro and in-vivo (at rest) and showed a high reproducibility for measurements of hyperemic response. Consequently, this ssMR approach was accurate enough to demonstrate a correlation between reactive hyperemic flow and individual risk scores in a small sample size of 30 subjects, while SGP and Doppler ultrasound required up to 296 (3) and 2045 (15) subjects, respectively, to detect such correlations.

Validation

The flow phantom measurements proved ssMR to be highly precise with stroke volumes differing by only $6 \pm 1\%$ and diameters by $1.7 \pm 0.9\%$. In-vivo reproducibility was also high with 15.5% ($= \text{SD}_{\text{Diff}}$) for repetitive $\text{Flow}_{\text{peak}}$ measurements and 4.3% for FMD. Furthermore, resting flow of $4.4 \text{ mL} \cdot \text{min}^{-1} \cdot 100 \text{ mL}^{-1}$, calf tissue measured by ssMR is well within the range of reported forearm and calf data of 4.3 to $4.9 \text{ mL} \cdot \text{min}^{-1} \cdot 100 \text{ mL}^{-1}$ (3, 5, 27, 34) measured by SGP. Also $\text{Flow}_{\text{peak}}$ of $30.4 \text{ mL} \cdot \text{min}^{-1} \cdot 100 \text{ mL}^{-1}$ measured by ssMR is close to reported SGP data of the forearm (27) and calf (5) ranging from 32.3 to $33.0 \text{ mL} \cdot \text{min}^{-1} \cdot 100 \text{ mL}^{-1}$. The important features of this novel ssMR approach are the standardized acquisition protocol, which shortens the examination time and reduces costs, the high precision, and the observer-independent data analysis. Standardization of acquisition and automatic analysis are particular helpful for studies in large patient populations or for multicenter trials.

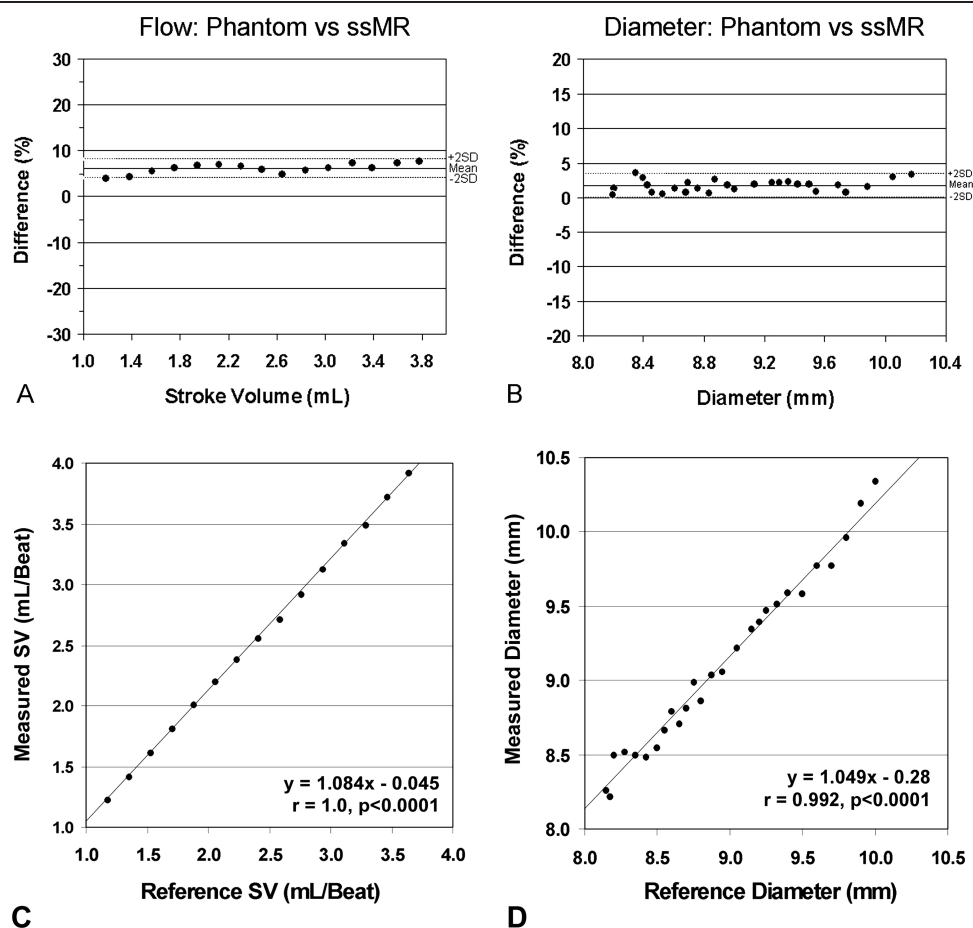


Figure 6. Bland-Altman plots demonstrate in-vitro validation experiments for flow (6A) and diameter (6B) measurements. The overestimation of flow and diameter in comparison with the true phantom values is small, i.e. 6.1% and 1.7%, respectively. The 95% confidence intervals for repeated flow and diameter measurements are narrow, i.e. 4 to 8% and 0 to 3.4%, respectively. In Fig. 6C and 6D, corresponding linear regression plots are given for flow and diameter measurements, respectively.

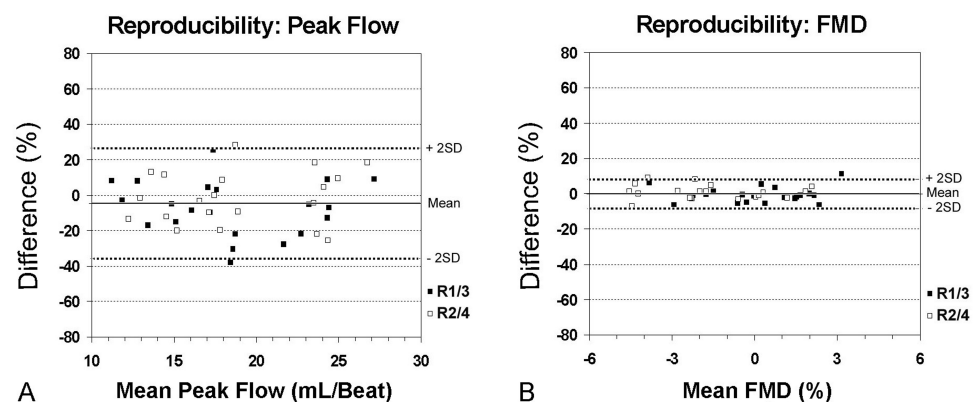


Figure 7. ssMR measurements performed in 22 subjects show high inter-session reproducibility for peak hyperemic flow (a) and FMD (b) determined in 291'456 images (mean interval between sessions: 9 ± 3 days). R1/3 refer to first releases on day 1 and day 2, respectively, R2/4 to second releases of day 1 and day 2, respectively (as shown in Fig. 2).

LIMITATIONS

This real-time ssMR technique was compared in-vivo with ECG-triggered MR for the resting condition only. This comparison yielded an inter-session reproducibility of 15.5% ($= SD_{Diff}$). Since the reproducibility of SGP is in the range of 4–28% SD_{Diff} (3, 35–37), a comparison of the real-time ssMR technique with SGP would not provide useful information with respect to the source of variability of hyperemic MR measurements and was therefore not performed. Similar considerations apply for Doppler measurements since reproducibility of this method is reported somewhat lower than for SGP (35).

The presented normalization of $Flow_{peak}$ by calf volume does not consider the composition of the lower leg (muscle, fat, bone etc.). Covering the lower leg with a 3D-acquisition would allow to measure tissue components and would solve this current limitation. In order to estimate vascular resistance of the lower leg, brachial artery BP was obtained assuming that BP in the arm and leg are closely correlated. This may not be correct for all patients. However, the technique is designed primarily for subjects not yet suffering from morphologically overt atherosclerosis, in which case our assumption may be justified. Furthermore, in this study patients with RFs (Pats1) and healthy controls (Con1) did not differ in BP (measurements were performed during drug treatment, Table 1).

While a minimum protocol (one baseline measurement, one release, one calf volume measurement) can be as short as 15 minutes, the technique is still costly and not a bed-side modality. Therefore, its main application, in our view, is for research purposes, where any increase in precision and reproducibility reduces the sample size required and thus, translates into reduced costs.

CONCLUSIONS

This study shows that reactive hyperemic flow in the SFA, reflecting microcirculatory response to ischemia in the lower limb, gradually decreases with increasing cardiovascular risk. These findings underscore the potential role of microcirculatory alterations in the early stages of atherosclerosis. Due to the high precision of this novel MR approach and its fully automatic analysis, these findings could be established in a small study population, indicating the potential value of the presented MR approach for studying the influence of RFs and their modification on (micro)-vascular pathophysiology.

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