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Association Between Cardiorespiratory Fitness and Non-invasively Assessed Macrovascular Reactivity Across the Cardiovascular Risk Spectrum

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Abstract

Background Flow-mediated slowing (FMS) is a user-friendly, non-invasive measure of macrovascular reactivity based on the decline in brachial–radial pulse wave velocity (PWV) during reactive hyperaemia. To evaluate the clinical utility of FMS in cardiovascular (CV) risk stratification we examined its association with cardiorespiratory fitness (CRF) across the CV risk spectrum.

Methods This cross-sectional analysis included 284 adults with or without CV risk factors. FMS was analysed continuously as relative PWV changes at 30-second intervals, and categorically as abnormal peak FMS and response. CRF was quantified as peak oxygen uptake (VO_2peak), including percentage of predicted VO_2peak (pp VO_2peak). Associations were explored using correlation and multivariable regression, stratified by CV risk status and sex.

Results Of 284 participants (61 ± 11 years; 44% women), 237 had ≥ 1 CV risk factor. In apparently healthy individuals, greater PWV change correlated with higher VO_2peak ($p < 0.05$), a pattern not observed in at-risk participants. Multivariable models showed that greater PWV reductions were independently associated with higher pp VO_2peak in the full cohort ($p < 0.01$) and at-risk subgroup ($p < 0.05$), with the strongest associations with both VO_2peak and pp VO_2peak seen in healthy individuals ($p < 0.01$ for most intervals). An abnormally low FMS response was associated with lower $\text{VO}_2\text{peak}/\text{kg}$ and pp VO_2peak ($p < 0.05$). Associations appeared stronger in women ($p < 0.01$ for most intervals), though sex interactions were not significant.

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Conclusions Better macrovascular reactivity, as assessed by FMS, was associated with higher CRF, particularly in women and apparently healthy individuals. These findings highlight FMS as a promising, scalable tool for CV risk stratification.

Keywords Vascular reactivity, Cardiorespiratory fitness, Oxygen consumption, Pulse wave analysis, Endothelium, Vascular/physiology, Cardiovascular diseases/ Prevention and control, Risk factors, Sex factors, Cross-sectional studies

1 Background

Cardiovascular disease (CVD) remains the leading cause of mortality in Europe, responsible for approximately 3.9 million deaths annually, representing nearly 45% of all deaths [1]. With increasing life expectancy and the rising prevalence of cardiovascular (CV) risk factors, the societal burden of CVD is expected to keep rising over the next decades [2]. Effective long-term preventive strategies and early detection methods are needed to mitigate the decline in both lifespan and health-related quality of life in individuals at-risk for CVD [1].

Endothelial dysfunction is a hallmark of the early development of CVD, including conditions such as atherosclerosis and heart failure with preserved ejection fraction (HFpEF) and thus represents a key target for CVD prevention [3, 4]. The vascular endothelium, as the inner cellular lining of arteries, veins, and capillaries, functions as an endocrine organ, playing a key role in regulating regional blood flow and pressure by controlling the vascular tone, inflammation, immune response, angiogenesis and vascular permeability [5].

Several tests aim to non-invasively quantify vasoreactivity as a proxy of endothelial function by inducing shear stress through reactive hyperaemia [6]. Following transient occlusion of blood flow, an increase in shear stress stimulates nitric oxide-mediated vasodilation in healthy endothelial cells, whereas endothelial dysfunction manifests as a blunted vasodilatory response [7]. Brachial artery flow-mediated dilation (FMD), measured by ultrasound tracking of changes in brachial artery diameter during reactive hyperaemia, is widely regarded as the gold standard for non-invasive assessment of macrovascular endothelial function [8]. However, its reliability is highly dependent on operator expertise, limiting widespread clinical application [8].

In recent years, flow-mediated slowing (FMS) has emerged as an automated operator-independent, user-friendly and non-invasive alternative for assessing macrovascular reactivity [9]. FMS evaluates macrovascular reactivity by tracking changes in pulse wave velocity (PWV), such as brachial-radial PWV, during reactive hyperaemia, which inversely correlates with vasodilation [10]. In healthy individuals, PWV decreases during reactive hyperaemia due to vessel dilation (i.e., an increase in internal radius). So while FMD directly measures the changes in vessel diameter, FMS captures the

corresponding slowing in PWV. This slowing forms the physiological basis of FMS: a lower, less negative FMS value (i.e., less slowing in PWV) may indicate impaired vasodilation and, indirectly, poorer endothelial function [10].

Cardiorespiratory fitness (CRF), commonly expressed as peak oxygen uptake (VO_{2peak}), is a powerful predictor of CV outcomes and all-cause mortality. Extensive evidence shows that low CRF is associated with a higher risk of CV events and death, independent of traditional risk factors such as hypertension, diabetes, and smoking [11–13]. Physiologically, CRF reflects the integrated function of the CV, pulmonary, and muscular systems to deliver and utilize oxygen during sustained exercise [14]. The primary determinants of CRF include cardiac output, arterial-venous oxygen difference, ventilatory capacity, and the oxidative function of skeletal muscle [14]. As key regulator of vascular tone, endothelial function is a critical component of this oxygen delivery system. Impaired endothelial responsiveness may limit blood flow to working tissues during exercise, contributing to reduced oxygen delivery, slower VO_2 kinetics, and diminished aerobic capacity [15]. Previous studies have explored the relationship between CRF and macrovascular endothelial function, observing modest associations between CRF and FMD [16]. However, the association between CRF and a more user-friendly and potentially more granular vasoreactivity test, such as FMS remains uninvestigated.

To evaluate the clinical relevance of FMS in CV risk stratification, this study aimed to investigate its association with VO_{2peak} as a key measure of CRF. To strengthen the generalizability of FMS, the association was investigated across the CV risk spectrum, from apparently healthy subjects to individuals at high risk for CVD. We hypothesized that lower FMS, indicating impaired vascular reactivity, would be associated with lower CRF across the CV risk spectrum.

2 Methods

2.1 Study Design and Population

Baseline data from the ‘Personalized remotely guided preventive exercise for a healthy heart’ (PRIORITY) and data from the ‘Flemish Study on Environment, Genes and Health Outcomes’ (FLEMENGHO) were pooled. The study designs of both studies have been described elsewhere [17, 18]. In short, PRIORITY (NCT04745013)

is a randomized controlled trial on the feasibility, clinical efficacy and cost-effectiveness of a hybrid centre and home-based personalized exercise and physical activity intervention for patients along the HFpEF continuum. FLEMENGHO, a longitudinal population study, consists of a family-based population sample that has been randomly recruited in northeastern Belgium between 1985 and 2005. For our analysis we considered examinations performed between December 2019 and March 2023. Both studies were approved by the Ethics Committee Research UZ Leuven/KU Leuven (S65155 and S64406). Participants provided written informed consent prior to their participation.

Within these two available cohorts, 387 participants were identified who underwent both a cardiopulmonary exercise test (CPET) and FMS. Twenty-seven participants were excluded from analyses, due to factors compromising the FMS assessment, such as an incomplete FMS test ($n=4$), arrhythmias or extrasystole interfering with the FMS tracing ($n=8$), or overall low test quality ($n=15$). Additionally, we excluded participants with symptomatic heart failure ($n=35$), those with an office systolic blood pressure at rest exceeding 180 mmHg ($n=4$), and those with submaximal CPET as indicated by a respiratory exchange ratio (RER) below 1.06 ($n=37$) [19]. The total analysis sample thus included 284 individuals.

2.2 Apparently Healthy Versus At-Risk

Individuals were further stratified based on the presence of clinical CV risk factors. While information on both clinical and behavioural risk factors (e.g. smoking, excessive alcohol consumption) were collected, the current analysis focused solely on clinical CV risk factors. Participants classified as 'at-risk' were asymptomatic for CVD but had at least one of the following confirmed clinical CV risk factors based on medical history and medication use: history of treated or untreated hypertension (blood pressure 130/80–159/99 mmHg) AND/OR prediabetes (impaired fasting glucose and/or insulin resistance with either fasting plasma glucose: 100–125 mg/dL (5.6–6.9 mmol/L) or HbA1c: 5.7–6.4% or Homeostatic Model Assessment of Insulin Resistance index >2.0) AND/OR obesity ($30 \text{ kg/m}^2 \geq \text{BMI} \leq 42 \text{ kg/m}^2$) AND/OR diabetes treated with anti-diabetic medications AND/OR current use of lipid-lowering medication. Participants were considered apparently healthy if they had none of the aforementioned CV risk factors and no history of CVD, as confirmed by their medical history and medication use.

2.3 Data collection

2.3.1 Cardiorespiratory Fitness

Each patient performed a symptom-limited incremental exercise protocol on a cycle ergometer (JAEGER Vyntus CPX, Vyair medical; Mettawa, USA), aiming

to reach maximum volitional exertion within 8–12 min ($20 \text{ W} \pm 20 \text{ W/min}$; $10 \text{ W} \pm 10 \text{ W/min}$). Exercise testing was performed under continuous ECG monitoring (CardioSoft ECG, Cardiosoft, USA) with breath-by-breath analysis of inspired and expired gas (VO_2 , VCO_2) and minute ventilation. The blood pressure was measured every other minute using an automated monitor. The test was stopped upon volitional exertion (i.e. when the patient was unable to keep the pedalling rate above 60 rpm) or when any AHA termination criterion occurred [20]. After the test, the patient rated the maximal level of perceived exertion on the 6 to 20 Borg scale. The CPET was considered maximal by the assessor when both the second ventilatory threshold was reached and the respiratory exchange ratio (RER) was ≥ 1.06 . $\text{VO}_{2\text{peak}}$ was defined as the highest achieved oxygen uptake measured over an average duration of 30 s of exercise. $\text{VO}_{2\text{peak}}$ was further normalized for body weight and expressed as relative oxygen uptake ($\text{VO}_{2\text{peak/kg}}$, in $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$). Additionally, the percent of the predicted $\text{VO}_{2\text{peak}}$ ($\text{ppVO}_{2\text{peak}}$) was calculated based on reference equations by Wasserman and Gläser, adjusted for age, sex, height, and body weight [21, 22].

2.3.2 Brachial-Radial FMS

Participants were instructed to refrain from exercise for at least 24 hours prior to the measurement. Subjects were asked to come in a fasted state and to not ingest substances that might affect endothelial function, such as caffeine, tobacco and alcohol. FMS measurements were subsequently conducted in a temperature-controlled, dimly lit room around 22 °C after a five-minute rest in supine position. An automated protocol to measure brachial-radial FMS was applied using the Vicorder™ device (80 Beats Medical, Berlin, Germany) [10]. The oscillometric assessment of brachial-radial FMS was found highly reproducible, with a coefficient of variation (CoV) of 7.3% for between-day measurements [9]. Its reproducibility is therefore comparable with FMD (CoV: 7.1%) and superior to other non-invasive vasoreactivity tests [23, 24].

During the procedure, participants lay supine with their left arm positioned at an angle of 70–80° from their body. Two oscillometric cuffs connected to the Vicorder™ were placed around the upper arm and wrist. Brachial-radial distance (D) was determined as the distance between the midst of both cuffs. The FMS procedure comprised a three-minute baseline period, an occlusion period of five minutes during which the brachial cuff was inflated to 200 mmHg to block blood flow, and a four-minute post-occlusion period. During pre- and post-occlusion periods, both cuffs were mildly inflated ($\leq 65 \text{ mm Hg}$) to simultaneously record brachial and radial pulse wave tracings.

The onset-time of upstroke was derived from both the brachial (t_{brachial}) and radial pulse tracings (t_{radial}) in real-time. Brachial-radial PWV was calculated from pulse tracings every three to four seconds during the baseline and post-occlusion period as the brachial-radial distance D divided by the transit time ΔT ($\text{PWV} = D / [t_{\text{radial}} - t_{\text{brachial}}] = D / \Delta T$). Mean baseline PWV (PWV_{BL}) and mean post-occlusion PWV were calculated for each 30 s interval starting from the first measurement after occlusion (recorded about 15 s after brachial cuff release). FMS was analysed as a continuous variable, defined as the relative change (Δ) in PWV following hyperaemia (e.g. $\text{FMS}_{0-30} = [\text{PWV}_{0-30} - \text{PWV}_{\text{BL}}] / \text{PWV}_{\text{BL}} \times 100\%$). In addition, a categorical classification was applied, where an abnormal FMS peak was defined as a peak slowing less than 9.4% (if < 60 years) or less than 4.6% (if ≥ 60 years) [25]. For each participant, the FMS trajectory was further characterised and labelled as a low (abnormal) or moderate-to-high FMS response using a previously validated group-based trajectory modelling algorithm [25].

2.3.3 Other Measurements

Medical history and medication were obtained by self-report and adjudicated and supplemented by hospital records. Anthropometric measurements included height, weight and BMI (calculated as $\text{weight} / \text{height}^2$). Office blood pressure was measured in triplicate following a 10 minutes seated rest using an OMRON M3 monitor (Omron Healthcare, Japan) per ESH guidelines [26]. Biochemical analyses on fasting venous blood samples, assessed HbA1c and lipids (total cholesterol, LDL-C, HDL-C and triglycerides).

2.4 Statistical Analysis

Study data were managed using REDCap (Research Electronic Data Capture) tools hosted at KU Leuven [27, 28]. For statistical analysis, Python v3.13 was used [29]. Normality of distributions was evaluated by skewness statistic and visual inspection of histograms. Clinical characteristics of both groups were compared by means of a Z-test or chi-squared test based on the characteristics of the data. For continuous variables, a Z-test was applied to compare means. Categorical variables were compared using the chi-squared test.

Univariate Pearson correlations and multivariable regression models were applied to investigate the relation between the FMS and the VO_2peak metrics. Analyses were conducted for the whole cohort, by CV risk group (at-risk vs. apparently healthy) and by sex. The FMS response was the independent variable, expressed either on a continuous scale as the relative change in PWV at each 30-s post-occlusion interval, and on a categorical scale, as abnormal FMS peak or response. Multivariable-adjusted models accounted for differences in age, sex,

height, body weight, systolic and diastolic blood pressure, current smoking status, resting heart rate, use of beta-blocker medication, use of lipid-lowering medication and diabetes diagnosis. An interaction term between sex and FMS was introduced and evaluated. For all tests, statistical significance was set at a two-sided $P < 0.05$.

3 Results

3.1 Cohort Characteristics

Characteristics for the overall cohort, and by CV risk group are summarized in Table 1. The mean age of the participants was 61 ± 11 years old, with women comprising 44% of the study sample. Of the 284 participants, 237 ($n = 103$ women, 43.5%) presented with at least one CV risk factor (187 with hypertension [138 on treatment], 57 with diabetes mellitus, and 102 on lipid-lowering drugs) and 47 ($n = 22$ women, 46.8%) were considered as apparently healthy. Compared to the at-risk group, apparently healthy individuals were significantly younger, had lower BMI and blood pressure, and showed a distinct lipid profile (higher total cholesterol, LDL and HDL, and lower triglycerides), along with lower HbA1c, higher estimated Glomerular Filtration Rate (eGFR), and higher VO_2peak (per kg and ppVO_2peak Gläser). No statistically significant differences were found in the PWV change between the two CV risk groups.

3.2 Unadjusted Correlations Between VO_2peak Metrics and Continuous FMS Response

Figure 1 shows the correlation matrix between different VO_2peak metrics and the PWV change at 30 s intervals. In the apparently healthy group, a greater reduction in PWV in the 0–60 s post-occlusion interval was significantly correlated with VO_2peak (VO_2peak : $r = -0.43$ to -0.34 ; $\text{VO}_2\text{peak}/\text{kg}$: $r = -0.56$ to -0.45 ; ppVO_2peak (Wasserman): $r = -0.38$ to -0.37 ; ppVO_2peak (Gläser): $r = -0.54$ to -0.48) whereas for the 60 to 180 s post-occlusion intervals, ppVO_2peak (Wassermann) and ppVO_2peak (Gläser) correlated inversely with the PWV change. In contrast, in the at-risk group, no significant correlations between the VO_2peak metrics and PWV change were observed. In the total cohort, we observed significant correlations between $\text{VO}_2\text{peak}/\text{kg}$ and the PWV change within the initial 30 s post-occlusion, and between ppVO_2peak (Gläser) and the immediate post-occlusion PWV change (Figure S1).

3.3 Adjusted Associations Between VO_2peak and FMS Response

Table S1 and Table 2 summarize the multivariable-adjusted associations between VO_2peak metrics and the PWV change in the full cohort and by CV risk group, respectively. In both the overall sample (Table S1) and the at-risk group (Table 2), a greater reduction

Table 1 Cohort characteristics

	Overall cohort (n=284)	At-risk (n=237)	Apparently healthy (n=47)
<i>General characteristics</i>			
Age, years	61 ± 11	62 ± 10	56 ± 12*
Females, n(%)	125 (44.0)	103 (43.5)	22 (46.8)
BMI, kg/m ²	28.3 ± 4.8	29.1 ± 4.8	24.7 ± 2.5*
SBP, mmHg	131 ± 16	134 ± 15	119 ± 11*
DBP, mmHg	80 ± 11	81 ± 11	74 ± 7.6*
Regular alcohol use, n(%)	117 (41.2)	94 (39.7)	23 (48.9)
Current smoker, n(%)	16 (5.6)	11 (4.6)	5 (10.6)
<i>CV risk factors</i>			
Hypertension, n(%)	187 (65.9)	187 (78.9)	–
Treated for hypertension, n(%)	138 (48.6)	138 (58.2)	–
Beta-blockers, n(%)	69 (24.3)	69 (29.1)	–
ACEi/ARBs, n(%)	93 (32.8)	93 (39.2)	–
CCB, n(%)	52 (18.3)	52 (21.9)	–
Diuretics, n(%)	51 (18.0)	51 (21.5)	–
Diabetes, n(%)	57 (20.1)	57 (24.1)	–
Lipid-lowering drugs, n(%)	102 (35.9)	102 (43.0)	–
<i>Biochemical parameters</i>			
Total cholesterol, mmol/L	4.6 ± 1.0	4.5 ± 1.0	5.2 ± 0.9*
Triglycerides, mmol/L	1.29 ± 0.67	1.34 ± 0.69	1.01 ± 0.41*
HDL cholesterol, mmol/L	1.4 ± 0.4	1.4 ± 0.4	1.7 ± 0.5*
LDL cholesterol, mmol/L	2.6 ± 0.9	2.5 ± 0.9	3.1 ± 0.7*
Hemoglobin A1c, %	5.8 ± 0.7	5.8 ± 0.7	5.5 ± 0.2*
eGFR (CKD-EPI), mL/min/1.73m ²	88.4 ± 14.5	87.6 ± 14.8	92.4 ± 11.8*
<i>Cardiopulmonary exercise test data</i>			
VO ₂ peak, mL·min ⁻¹	1933 ± 609	1911 ± 596	2046 ± 661
VO ₂ peak/kg, mL·kg ⁻¹ ·min ⁻¹	23.2 ± 6.5	22.4 ± 5.8	27.7 ± 7.5*
ppVO ₂ peak (Wasserman)	102 ± 19	102 ± 19	106 ± 17
ppVO ₂ peak (Gläser)	98 ± 18	97 ± 18	102 ± 15*
HRpeak, bpm	152 ± 22	149 ± 23	165 ± 14*
SBPpeak, mmHg	194 ± 29	196 ± 29	186 ± 23*
RERpeak	1.19 ± 0.07	1.18 ± 0.07	1.22 ± 0.07*
<i>PWV change: raw output per 30 s interval</i>			
Baseline PWV, m/s	7.4 ± 1.1	7.5 ± 1.1	7.2 ± 1.0
PWV change 0 s, %	−15.3 ± 10.1	−14.8 ± 10.2	−17.7 ± 8.8
PWV change 0-30s, %	−11.6 ± 10.2	−11.0 ± 10.4	−14.2 ± 8.4
PWV change 30-60s, %	−8.8 ± 10.9	−8.4 ± 11.2	−10.8 ± 9.3
PWV change 60-90s, %	−8.0 ± 10.8	−7.8 ± 10.9	−8.7 ± 10.0
PWV change 90-120s, %	−7.5 ± 10.7	−7.2 ± 10.6	−8.9 ± 10.6
PWV change 120-150s, %	−7.4 ± 10.3	−7.0 ± 10.3	−9.0 ± 10.1
PWV change 150-180s, %	−6.7 ± 10.0	−6.4 ± 10.0	−8.2 ± 10.0

Table 1 (continued)

	Overall cohort (n=284)	At-risk (n=237)	Apparently healthy (n=47)
PWV change 180-210s, %	−3.1 ± 7.8	−3.1 ± 7.6	−2.8 ± 8.8
PWV change 210-240s, %	−1.1 ± 7.8	−1.0 ± 7.5	−1.6 ± 8.8

Values are displayed as mean ± standard deviation or as number of subjects (n) and %. Regular alcohol use was defined as a self-reported weekly alcohol consumption corresponding to an estimated daily intake of more than five grams of pure alcohol

*Significant at the 0.05 level

BMI body mass index; SBP systolic blood pressure; DBP diastolic blood pressure; ACEi/ARBs angiotensin-converting enzyme inhibitors/angiotensin receptor blockers; CCB calcium channel blockers, HDL high-density lipoprotein; LDL low-density lipoprotein; eGFR (CKD-EPI) estimated glomerular filtration rate according to CKD-EPI equations; VO₂peak peak oxygen consumption; ppVO₂peak % of the predicted VO₂peak; HRpeak heart rate during peak exercise; SBPpeak systolic blood pressure during peak exercise; RERpeak respiratory exchange ratio during peak exercise; PWV pulse wave velocity

in PWV within the post-occlusion interval was independently associated with higher VO₂peak, when assessed as ppVO₂peak. In the total cohort, this association was significant across 0–180 s for both the Gläser and Wasserman methods, whereas in the at-risk group it was significant across 0–180 s for Gläser and from 30–180 s for Wasserman ($p < 0.05$ for all). The strongest associations between PWV change and all VO₂peak metrics were observed in the apparently healthy group. For instance, in the apparently healthy group, a one standard deviation decrease in PWV change (corresponding to a stronger response) was associated with a 249.0 mL·min⁻¹ increase in VO₂peak ($p < 0.01$), a 3.2 mL·kg⁻¹·min⁻¹ increase in VO₂peak/kg ($p < 0.01$), and 9% increase in ppVO₂peak ($p < 0.01$) (Table 2).

In categorical analyses, an abnormally low FMS response was independently associated with lower VO₂peak/kg and ppVO₂peak (Wasserman and Gläser) in the full cohort (Table S1). After full adjustment, a ± 6% lower ppVO₂peak for Wasserman ($p < 0.05$) for Gläser ($p < 0.01$) was observed in those with abnormal low FMS response.

Subgroup analysis showed similar trends for VO₂peak metrics and abnormal (peak) FMS response, but these associations did not reach statistical significance with the exception of ppVO₂peak (Gläser) and abnormal FMS response in the at-risk and apparently healthy subgroup (Table 2).

3.4 Sex-Specific Differences

Sex-specific heatmaps of the unadjusted correlations between VO₂peak metrics and continuous PWV change at 30-s intervals are displayed in Fig. 2. In the at-risk group, women showed stronger correlations than men, whereas in the apparently healthy group both men and women showed strong associations. The strongest correlations ($p < 0.01$) were observed in apparently healthy

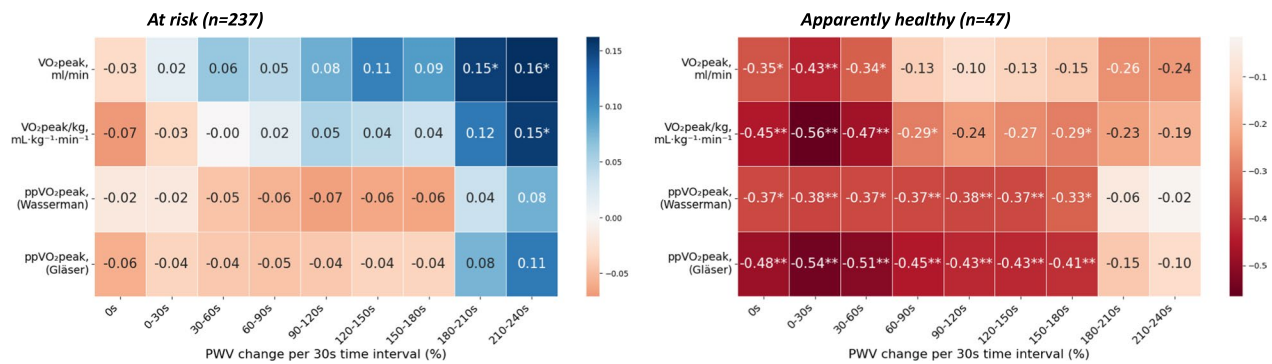


Fig. 1 Unadjusted correlations between VO_2 peak metrics and continuous PWV change by CV risk group. The heatmap displays Pearson correlation coefficients (r) between different VO_2 peak metrics and continuous changes in pulse wave velocity (PWV) measured at 30 s intervals, stratified by cardiovascular (CV) risk group. Positive correlations are shown in blue, and negative correlations in red. Statistically significant correlations are indicated by $p < 0.05$ (*) and $p < 0.01$ (**). VO_2 peak peak oxygen consumption; $ppVO_2$ peak % of the predicted VO_2 peak; PWV pulse wave velocity

Table 2 Adjusted associations between VO_2 peak metrics and PWV change by CV risk group

	VO_2 peak, mL·min ⁻¹		VO_2 peak/kg, mL·kg ⁻¹ ·min ⁻¹		pp VO_2 peak (Wasserman)		pp VO_2 peak (Gläser)	
	At-risk (237)	Healthy (47)	At-risk (237)	Healthy (47)	At-risk (237)	Healthy (47)	At-risk (237)	Healthy (47)
	Estimate ± SE	Estimate ± SE	Estimate ± SE	Estimate ± SE	Estimate ± SE	Estimate ± SE	Estimate ± SE	Estimate ± SE
<i>Continuous PWV change</i>								
First measurement	28.3 ± 33.8	223.3 ± 60.4**	0.7 ± 0.5	2.5 ± 1.1*	2.0 ± 1.3	6.3 ± 2.4*	2.7 ± 1.2*	6.7 ± 2.0**
0-30s -1SD, %	10.6 ± 32.4	249.0 ± 57.5**	0.4 ± 0.5	3.2 ± 1.0**	2.4 ± 1.3	7.6 ± 2.5**	3.0 ± 1.2*	8.7 ± 2.0**
30-60s -1SD, %	20.2 ± 32.2	185.2 ± 65.0**	0.4 ± 0.5	2.1 ± 1.1	3.0 ± 1.3*	6.8 ± 2.5*	3.1 ± 1.2*	7.7 ± 2.1**
60-90s -1SD, %	36.7 ± 32.0	157.2 ± 62.7*	0.3 ± 0.5	1.5 ± 1.1	3.5 ± 1.3**	7.6 ± 2.5**	3.4 ± 1.2**	7.5 ± 2.2**
90-120s -1SD, %	26.4 ± 32.2	141.8 ± 66.5*	0.3 ± 0.5	1.1 ± 1.1	3.7 ± 1.3**	7.4 ± 2.5**	3.3 ± 1.2**	6.8 ± 2.2**
120-150s -1SD, %	30.2 ± 32.2	211.2 ± 67.8**	0.5 ± 0.5	2.0 ± 1.2	3.5 ± 1.3**	8.0 ± 2.4**	3.0 ± 1.2*	7.3 ± 2.1**
150-180s -1SD, %	29.8 ± 31.0	202.0 ± 66.3**	0.3 ± 0.4	1.7 ± 1.1	3.4 ± 1.3*	7.4 ± 2.5**	2.8 ± 1.2*	7.2 ± 2.2**
180-210s -1SD, %	-19.9 ± 31.7	81.0 ± 68.9	0.0 ± 0.5	0.1 ± 1.1	-0.1 ± 1.2	2.4 ± 2.5	-0.8 ± 1.1	3.2 ± 2.2
210-240s -1SD, %	-25.5 ± 31.6	-21.6 ± 74.6	-0.3 ± 0.5	-1.4 ± 1.2	-0.9 ± 1.2	0.9 ± 2.4	-1.3 ± 1.1	2.0 ± 2.1
<i>Categorical PWV change</i>								
Abnormal FMS peak	-36.5 ± 61.8	-77.1 ± 151.5	-1.3 ± 0.9	-0.5 ± 2.4	-4.2 ± 3.2	-6.3 ± 7.9	-4.4 ± 2.9	-6.8 ± 6.9
Abnormal FMS response	-67.7 ± 64.1	-264.3 ± 149.3	-1.6 ± 0.9	-2.5 ± 2.4	-5.0 ± 2.7	-9.7 ± 5.6	-5.3 ± 2.4*	-11.2 ± 4.8*

The multivariable-adjusted model included the following predictors: age, sex, length, weight, systolic and diastolic blood pressure, current smoking status, resting heart rate, use of beta-blocker medication, diabetes diagnosis and use of lipid-lowering drugs. An interaction term was added for sex but this was not significant for all models. Values represent regression coefficients (Estimate) with standard errors (SE). Continuous variables were analysed per 30-s intervals and displayed as change in VO_2 peak metric per SD improvement ($=-1SD$) in PWV change. An abnormal FMS peak was defined as a peak slowing less than 9.4% (if < 60 years) or less than 4.6% (if ≥ 60 years). An abnormal FMS response was determined based on FMS trajectory, with low responses classified as abnormal, and moderate or high responses classified as normal

*Significant effect size at the 0.05 level

**Significant effect size at the 0.01 level

men and women, while men in the at-risk group exhibited the weakest associations. These findings were largely confirmed for women, whereas in men the multivariable-adjusted analyses showed no consistent significant associations between VO_2 peak metrics and PWV change by sex (Table 3 and Tables S2 and S3). Although associations in women appeared generally stronger and more likely to be significant than in men, an interaction term added for sex was not significant. All associations in women between PWV change in the 0 and 180 s post-occlusion intervals across all VO_2 peak metrics were significant ($p < 0.05$ for all, $p < 0.01$ for most). Abnormal FMS response was associated with lower VO_2 peak in women,

lower VO_2 peak/kg in both sexes, and lower pp VO_2 peak according to Gläser for women.

4 Discussion

We found that higher CRE, across various VO_2 peak metrics, was independently associated with better macrovascular reactivity as assessed via the user-friendly FMS method. This association was strongest in apparently healthy individuals and in women. These findings are in line with previous research indicating that better endothelial function associates with higher levels of CRE, and add to the growing evidence that FMS may serve as a promising tool for non-invasive vascular assessment [30]. In addition, our findings suggest that the presence of CV

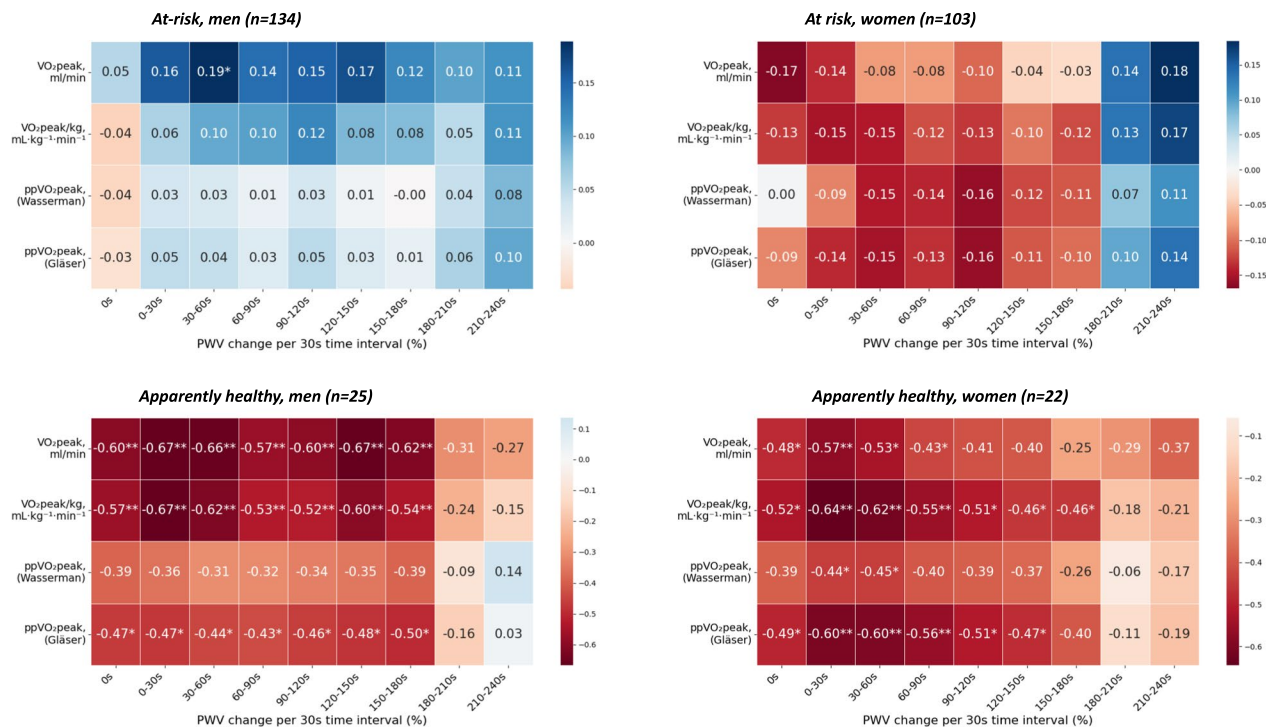


Fig. 2 Unadjusted correlations between VO_2 peak metrics and continuous PWV change by CV risk group and sex. The heatmap displays Pearson correlation coefficients (r) between different VO_2 peak metrics and continuous changes in pulse wave velocity (PWV) measured at 30 s intervals, stratified by cardiovascular (CV) risk group. Positive correlations are shown in blue, and negative correlations in red. Statistically significant correlations are indicated by $p < 0.05$ (*) and $p < 0.01$ (**). VO_2 peak peak oxygen consumption; $ppVO_2$ peak % of the predicted VO_2 peak; PWV pulse wave velocity

Table 3 Adjusted associations between VO_2 peak metrics and PWV change by sex

	VO_2 peak, mL·min ⁻¹		VO_2 peak/kg, mL·kg ⁻¹ ·min ⁻¹		$ppVO_2$ peak (Wasserman)		$ppVO_2$ peak (Gläser)	
	Men (159)	Women (125)	Men (159)	Women (125)	Men (159)	Women (125)	Men (159)	Women (125)
	Estimate ± SE	Estimate ± SE	Estimate ± SE	Estimate ± SE	Estimate ± SE	Estimate ± SE	Estimate ± SE	Estimate ± SE
<i>Continuous PWV change</i>								
First measurement	51.7 ± 31.8	64.1 ± 26.9*	1.0 ± 0.5*	1.2 ± 0.4**	2.6 ± 1.4	3.2 ± 2.0	2.6 ± 1.4	4.0 ± 1.7*
0-30s -1SD, %	47.5 ± 33.7	83.5 ± 25.8**	0.9 ± 0.5	1.4 ± 0.4**	2.4 ± 1.5	5.0 ± 1.9**	2.5 ± 1.5	5.3 ± 1.7**
30-60s -1SD, %	53.3 ± 33.6	83.6 ± 25.0**	0.8 ± 0.5	1.3 ± 0.4**	2.6 ± 1.5	5.4 ± 1.8**	2.6 ± 1.5	5.4 ± 1.6**
60-90s -1SD, %	67.8 ± 32.6*	85.0 ± 25.3**	0.6 ± 0.5	1.4 ± 0.4**	2.8 ± 1.5	5.8 ± 1.9**	2.7 ± 1.4	5.6 ± 1.6**
90-120s -1SD, %	56.6 ± 32.8	78.6 ± 25.4**	0.7 ± 0.5	1.3 ± 0.4**	2.8 ± 1.5	5.5 ± 1.8**	2.8 ± 1.4	5.1 ± 1.6**
120-150s -1SD, %	62.4 ± 32.4	72.3 ± 25.4**	0.9 ± 0.5	1.1 ± 0.4**	3.0 ± 1.4*	4.7 ± 1.9*	2.9 ± 1.4*	4.5 ± 1.6**
150-180s -1SD, %	64.7 ± 31.3*	77.1 ± 26.0**	0.7 ± 0.5	1.2 ± 0.4**	3.0 ± 1.4*	4.5 ± 1.9*	2.9 ± 1.4*	4.4 ± 1.7*
180-210s -1SD, %	0.2 ± 30.2	-4.6 ± 24.5	0.2 ± 0.4	-0.3 ± 0.4	0.0 ± 1.4	-0.7 ± 1.8	-0.1 ± 1.3	-0.6 ± 1.6
210-240s -1SD, %	-14.6 ± 30.3	-8.5 ± 24.2	-0.1 ± 0.4	-0.3 ± 0.4	-0.7 ± 1.4	-0.9 ± 1.8	-0.6 ± 1.3	-0.9 ± 1.6
<i>Categorical PWV change</i>								
Abnormal FMS peak	-60.8 ± 81.9	-75.3 ± 66.6	-1.8 ± 1.2	-1.6 ± 1.1	-3.7 ± 3.6	-3.0 ± 4.8	-3.5 ± 3.5	-4.5 ± 4.3
Abnormal FMS response	-101.2 ± 66.4	-108.4 ± 54.2*	-1.9 ± 1.0*	-1.8 ± 0.9*	-5.2 ± 2.9	-6.5 ± 4.0	-5.5 ± 2.8	-7.2 ± 3.5*

The multivariable-adjusted model included the following predictors: age, length, weight, systolic and diastolic blood pressure, current smoking status, resting heart rate, use of beta-blocker medication, diabetes diagnosis and use of lipid-lowering drugs. Values represent regression coefficients (Estimate) with standard errors (SE). Continuous variables were analysed per 30 s intervals and displayed as change in VO_2 peak metric per SD improvement (= -1SD) in PWV change. An abnormal FMS peak was defined as a peak slowing less than 9.4% (if < 60 years) or less than 4.6% (if ≥ 60 years). An abnormal FMS response was determined based on FMS trajectory, with low responses classified as abnormal, and moderate or high responses classified as normal

*Significant effect size at the 0.05 level

**Significant effect size at the 0.01 level

risk factors may attenuate the strength of the association between CRF and macrovascular health, highlighting the importance of preserving CRF and vascular health before CV risk factors develop.

4.1 Key Findings

In our cohort, better FMS responses during the early post-occlusion phase (0–60s) were significantly correlated with higher VO_2peak , particularly in healthy participants. This aligns with previous findings showing VO_2peak as a strong correlate of endothelial function, when measured as FMD [31]. Similarly, a large cross-sectional study in healthy adults and patients with CVD found that CRF is positively associated with endothelial function, independent of traditional CV risk factors [16].

Multivariable-adjusted analysis in the total cohort and stratified per CV risk group, accounting for important covariates such as age, sex, smoking, lipid-lowering medication and beta-blocker use, indicated that a better FMS response was independently associated with higher ppVO_2peak and thus CRF regardless of CV risk. These findings reinforce the previously established relationship between FMD and VO_2peak and the predictive value of endothelial function for long-term CV outcomes [30]. Notably the associations across all VO_2peak metrics were consistently stronger and more statistically robust in the apparently healthy group than in the at-risk group. This pattern suggests that existing CV risk factors may dampen the relationship between CRF and macrovascular function, underscoring the importance of maintaining CRF and vascular health before such risk factors emerge. Additionally, we also investigated the FMS response in a categorical way based on definitions recently suggested by our group. When the FMS response was categorized as a whole (using trajectory-based modeling), an abnormally low FMS response remained significantly associated with reduced $\text{VO}_2\text{peak}/\text{kg}$ and ppVO_2peak , reinforcing its clinical utility when used as a categorical risk marker.

Interestingly, despite these associations, we observed no statistically significant difference in FMS response between individuals with and without CV risk factors. While unexpected at first sight, this may be explained by the high proportion of the at-risk group receiving antihypertensive and lipid-lowering therapies. Namely, around 60% of the participants in the at-risk group was treated for hypertension and 30% were on beta-blockers, which may confound FMS results. Beta-blockers lower blood pressure and thereby mitigate the hemodynamic factors contributing to impaired vasoreactivity in hypertension. Third-generation beta-adrenergic antagonists additionally exhibit vasodilatory effects, mediated through enhanced nitric oxide bioavailability, alpha-1 receptor blockade, or beta-3 receptor activation [32]. Agents

such as nebivolol and carvedilol have been shown to preserve endothelial nitric oxide synthase (eNOS) function and exert antioxidant and antiproliferative actions [32]. A meta-analysis of 16 randomized controlled trials demonstrated that macrovascular function, measured as FMD was significantly improved by beta-blockers [33]. Similarly, a previous study by our research group demonstrated a strong association between the intake of beta-blockers and an enhanced brachial-radial FMS response [25]. Likewise, 40% of the at-risk group were prescribed statins, which are known to improve FMD independently of its lipid-lowering effects, possibly by upregulating eNOS and reducing oxidative stress [34]. Therefore, the absence of between-group differences in FMS, along with the lack of unadjusted correlations between FMS and CRF in the at-risk group—but the emergence of significant associations after adjusting for factors including medication use—likely reflects the protective vascular effects of pharmacological treatment, rather than indicating true physiological similarity between groups.

4.2 A Bidirectional Relationship Between CRF and Endothelial Function

Although our primary focus was on how macrovascular reactivity impacts CRF as a dependent variable, the relationship between CRF and macrovascular reactivity might be physiologically bidirectional, as described for endothelial function [30]. On the one hand, preserved endothelial function supports CRF by maintaining adequate vasodilatory reserve and oxygen delivery during exercise. Nitric oxide, generated by endothelial nitric oxide synthase, facilitates dynamic blood flow adjustments to active muscle tissue during activity. Impaired NO signalling in the context of endothelial dysfunction results in suboptimal tissue perfusion, delayed VO_2 kinetics, reduced exercise tolerance and early-onset fatigue [30, 35].

On the other hand, higher CRF promotes vascular health through chronic shear stress stimulation, which upregulates eNOS and enhances NO bioavailability, key to maintaining vasodilatory capacity [35]. Individuals with greater CRF also demonstrate lower levels of mitochondrial-derived reactive oxygen species (mtROS), preserving endothelial function by minimizing oxidative damage to NO pathways [36]. Additionally, individuals with high CRF show attenuated declines in endothelial function during stress or inflammation, suggesting a protective vascular phenotype [37].

4.3 Sex-Specific Differences

Women in our study demonstrated stronger and more consistent associations and correlations between FMS response and CRF compared to men, especially in the apparently healthy subgroup. Although several

physiological and molecular mechanisms, such as oestrogen-enhanced eNOS activity and increased nitric oxide availability, are often proposed to explain sex differences, these acute hormonal influences are unlikely to account for our findings because most women in our cohort were postmenopausal [38, 39]. A more plausible interpretation is that long-term oestrogen exposure before menopause may confer a vascular advantage that persists into early postmenopausal years [39]. However, intrinsic sex differences in endothelial biology also exist independent of hormonal status [40], and current evidence does not clearly support the idea that prior oestrogen exposure alone would produce a stronger CRF–endothelial function relationship later in life. Recent findings from Königstein et al. showed that VO_2peak -stratified differences in FMD begin to diverge earlier in women (around age 30) than in men (around age 50), suggesting that improvements in exercise capacity may exert more favourable effects on macrovascular function in women [16]. In addition, because women generally have lower absolute CRF than men, it might be possible that they experience proportionally greater vascular benefits for a given increase in CRF [41–43]. Incorporating sex-specific measures and thresholds into CV risk assessments, such as with FMS, may improve early detection of subclinical vascular dysfunction and enable more personalized, effective CV risk stratification strategies [41].

4.4 Clinical Relevance

These findings suggest FMS could be a valuable, user-friendly and non-invasive marker for CV risk stratification in clinical and preventive settings. The strong association between FMS and CRF, especially in healthy individuals and women may enable the identification of early, subclinical CVD stages. Furthermore, given prior evidence that exercise interventions can improve both macrovascular reactivity and CRF [44], FMS may also serve as a responsive outcome measure in lifestyle intervention trials. The use of FMS-response categories based on reference limits or the full trajectory may facilitate straightforward evaluation of the FMS-response, especially since the association with CRF remained intact after categorizing. As individuals with a low FMS response demonstrated approximately 5–11% lower percentage-predicted VO_2peak , our findings further support the potential clinical utility of CRF as a modifiable target to preserve endothelial health and overall mitigate vascular dysfunction in populations at substantial CV risk. Future studies should examine the longitudinal interaction between CRF, CV risk factors and macrovascular health, clarify underlying mechanisms, and determine whether CRF-enhancing interventions can prevent or reverse early impairments in macrovascular reactivity. Larger sex-stratified cohorts are essential to further

elucidate sex-specific determinants of macrovascular responsiveness.

4.5 Limitations

The study's cross-sectional design limits causal inference. Although multivariable models were used, residual confounding by unmeasured factors (e.g., diet, medication adherence) remains possible. While our cohort spanned a broad CV risk spectrum by integrating data from an observational (FLEMENGHO) and an interventional (PRIORITY) trial, it was predominantly composed of white Europeans. Furthermore, although our study included a substantial number of individuals with CV risk factors, the apparently healthy subgroup was relatively small, which may have limited the statistical power to detect significant associations within this group when FMS was analysed categorically. Additionally, selection bias may have occurred, particularly within the PRIORITY trial, where participants with CV risk factors and a relatively more active lifestyle may have been more likely to participate than the general at-risk population. Finally, while brachial-radial FMS offers technical advantages, its reproducibility and standardization across various clinical settings require further validation compared to established markers like FMD.

5 Conclusions

In conclusion, our study supports a strong association between macrovascular reactivity, as assessed by brachial-radial FMS and CRF across the CV risk spectrum. These associations were most pronounced in women and apparently healthy individuals without CV risk factors, emphasizing the importance of primary prevention to preserve CRF and macrovascular health before CV risk factors emerge. These findings contribute to the concept of a potentially bidirectional, modifiable relationship between FMS and CRF, and highlight FMS as a potentially promising, scalable tool for CV risk stratification in primary prevention.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1007/s44200-026-00112-3>.

Supplementary material 1.

Author contributions

MR, VC, and NC jointly conceived and designed the concept for this analysis. MR performed the data analysis and drafted the initial version of the manuscript. VC and NC provided supervision and feedback throughout the project. TK, ED, JC, YB, GC, DDS, KG, DH, MM, EN, ES, EVC, NV, NC, and VC contributed to the design, funding acquisition, and data collection of the original studies from which the data were derived. All authors critically reviewed and revised the manuscript for important intellectual content and approved the final version for submission.

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Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee Research UZ Leuven/KU Leuven, reference number S65155 and S64406. Participants provided written informed consent prior to their participation.

Consent for publication

Not applicable.

Competing interests.

The authors declare no competing interests.

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