Relationship between endothelial function and the eliciting shear stress stimulus in women:

Changes across the lifespan differ to men

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Short title: Sex impacts the relationship between FMD and shear rate.

ABSTRACT

Background. Pre-menopausal women have a lower incidence of cardiovascular disease, which may partly be due to a protective effect of estrogen on endothelial function. Animal studies suggest that estrogen may also improve the relationship between shear rate (SR) and endothelial function. We aimed to explore the relation between endothelial function (i.e. flow-mediated dilation (FMD)) and SR (i.e. SR area-under-the-curve (SRAUC)) in women *versus* men, and between pre- *versus* post-menopausal women.

Methods and Results. Brachial artery FMD and SRAUC were measured in accordance with expert-consensus guidelines in 932 healthy participants who were stratified into young adults (18-40yrs, 389 men, 144 women) and older adults (>40yrs, 260 men, 139 women). Secondly, we compared pre-menopausal (*n*=173) and post-menopausal women (*n*=110). There was evidence of a weak correlation between SRAUC and FMD in all groups but older men, although there was variation in strength of outcomes. Further exploration using interaction terms (age-sex*SRAUC) in linear regression revealed differential relationships with FMD (young women *versus* young men (β=-5.8^{-4}, *P*=0.017) and older women (β=-5.9^{-4}, *P*=0.049)). The correlation between SRAUC and FMD in pre-menopausal women (r^2=0.097) was not statistically different to post-menopausal women (r^2=0.025; Fisher: *P*=0.30). Subgroup analysis using stringent inclusion criteria for health markers (n=505) confirmed a stronger FMD-SRAUC correlation in young women compared to young men and older women.

Conclusions. Evidence for a stronger relationship between endothelial function and the eliciting SR stimulus is present in young women compared to men. Estrogen

may contribute to this finding, but larger healthy cohorts are required for conclusive outcomes.

Key words: flow-mediated dilation, endothelial function, sex differences, shear rate, cardiovascular disease

CLINICAL PERSPECTIVE

What is new? In a sample of 932individuals, we have shown the correlation between brachial artery flow-mediated dilation (FMD) and its eliciting shear rate stimulus were not statistically different between sexes or age groups. Systolic blood pressure was an important factor that influenced FMD. After repeated analysis using stringent inclusion criteria for blood pressure (n=505), sex and age-related differences were apparent in the relationship between FMD and shear rate.

What are the clinical implications? Shear stress as a hemodynamic stimulus for acute artery vasodilation as well as chronic adaptation. It promotes anti-atherogenic properties for protection against the development/progression of atherosclerosis. Pre-menopausal women benefit from the cardio-protective effects of estrogen, which may play a role in increasing sensitivity to a given shear stress stimulus. A stronger relationship between shear stress and artery vasodilation may contribute to the lower incidence of cardiovascular disease observed in pre-menopausal women, compared to men of similar age and post-menopausal women.

INTRODUCTION

Cardiovascular diseases (CVD) remain the world's leading causes of morbidity and mortality in women. The vascular endothelium is responsive to hormonal and hemodynamic stimuli and plays a pivotal role in the development and progression of atherosclerosis[1]. Consequently, endothelial dysfunction has been identified as an early biomarker of CVD[2, 3] and predictor of future CVD[4-6]. Although the incidence of CVD in women is lower compared to age-matched men, an increase in CVD-related mortality in women coincides with the onset of menopause[7]. These sex-related differences in CVD may, at least partly, relate to differences in endothelial function[8]. Interestingly, pre-menopausal women exhibit enhanced endothelial function, assessed using the flow-mediated dilation (FMD), compared to men[8-11].

An important physiological characteristic explaining sex differences in endothelial function relates to the sex hormone estrogen. FMD declines markedly in women after menopause[8, 12] and some studies show that FMD follows the fluctuating levels of estrogen across the menstrual cycle[13-15]. The direct vasodilator effects of estrogen may contribute to the larger FMD in pre-menopausal women. An alternative explanation for sex differences in endothelial function relates to observations in animal studies, which suggest that estrogen improves the vascular responsiveness to changes in shear stress. For example, Huang and colleagues found that female and ovariectomized rats with estrogen replacement show significantly greater dilation in response to a given shear stress, compared to male and ovariectomized rats[16]. A stronger relationship between endothelial function and shear stress may therefore contribute to the enhanced endothelial function observed in pre-menopausal women,

compared to post-menopausal women and age-matched men. To date, no study has examined this hypothesis in humans.

The purpose of this study was to explore the relationship between endothelial function (measured as FMD) and arterial shear rate (SR; i.e. SR area-under-the-curve (SRAUC)) between healthy men and women across the lifespan, and also between pre- *versus* post-menopausal women. We hypothesized that the relationship between FMD and its eliciting SR stimulus would be stronger in younger women, compared to men, and that this relationship would be attenuated with older age and post-menopausal status.

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Participants. This study utilised a retrospective design, including studies performed previously in our laboratories From these studies, we have identified 932 healthy individuals who were stratified into young adults (18-40 years; 389 men, 144 women) and older adults (>40 years; 260 men, 139 women) (Table 1). The cut-off level of 40 years was chosen based on the increase in CVD incidence after this age[17] and is in line with previous research[18]. Secondly, based on pre-screening of menopausal status (post-menopause was defined as at least one year without a menstrual cycle/spotting[12]), sub-analysis was performed between pre-menopausal (*n*=173) and post-menopausal women (*n*=110) (Table 2). All participants were non-smokers, not taking any medication, and free of risk factors and signs or symptoms of

cardiovascular or metabolic disease. Pre-menopausal women were not on any hormone-based contraception and post-menopausal women were not on hormone replacement therapy. All participants gave informed consent and all studies were ethically approved by the local ethics committees of Liverpool John Moores School of Sport and Exercise Science Research Ethics Committee, Radboud University Medical Center or The University of Western Australia. All work adhered to the Declaration of Helsinki.

Brachial artery FMD

Participants reported to the temperature-controlled (20-22°C) laboratory on one occasion for FMD assessment. In preparation, participants abstained from strenuous exercise for 24 hours and alcohol for 8 hours, as well as any food/caffeine/stimulants 6 hours prior to reporting to the laboratory.

Following 20 minutes of supine rest, brachial artery diameter was assessed via high-resolution duplex ultrasound (Terason t3000/u-smart 3300, Teratech or Aspen, Acuson) with a 7.5-12 MHz linear array probe. B-mode images were obtained and optimized, and Doppler velocity was recorded simultaneously. Expert-consensus protocol guidelines were followed for the performance of the FMD[19]. Briefly, after 1 minute of baseline diameter and flow measurement, an occlusion cuff, connected to a rapid inflator (Hokanson, Bellevue, WA), placed distal to the olecranon process, was inflated to a suprasystolic pressure (>200mmHg) for 5 minutes. Brachial artery diameter and flow recordings were resumed 30 seconds before cuff deflation, and FMD was recorded for a further 3 minutes post cuff deflation.

All FMD data were analysed using a specialised custom-designed edge-detection and wall-tracking software, the reproducibility and validity of which have been previously reported [20]. This software tracks the vessel walls and blood velocity trace in B-mode frames via pixel density and frequency distribution algorithm. An optimal region of interest to be analysed was selected by the sonographer, chosen on the basis of image quality, with a clear distinction between the artery walls and lumen. The FMD was defined as the percentage change in artery diameter from baseline to the peak captured during the 3 minutes post cuff release. The software automatically calculated the relative diameter change, time to peak (following cuff release) and SRAUC[21]. SRAUC was calculated as the area under the SR curve between the points of cuff release (manually selected by the sonographer) to peak diameter (determined by the software)[19]. Despite the initial region of interest selection being operator-determined, the remaining analysis was automated and independent of operator bias.

Statistical analysis

Statistical analyses were performed using SPSS (Version 24, SPSS, Chicago, Illinois). Pearson's correlation coefficient was used to calculate the correlation between FMD and SRAUC across age groups in men and women. This analysis was repeated using the allometrically scaled FMD to correct for baseline artery diameter[22]. Fisher r-to-z transformation was used to compare the difference between two correlation coefficients in the independent groups (i.e. sex, age, menopause status). Linear regression analysis was performed to examine the interaction between age-sex group and SRAUC with FMD as the dependent outcome. Other variables (e.g.

age, sex, BMI, blood pressure) that have been purported to influence SR and/or FMD were also considered in the model. Two-way analysis of variance (ANOVA) was also used to examine the differences between sex and age. Independent t-tests examined the differences between pre- and post-menopausal women. All data were presented as mean ± standard deviation (SD), unless stated otherwise. Statistical significance was assumed at $P<0.05$.

RESULTS

Impact of sex and age. Older age was associated with lower FMD, and higher body mass, body mass index (BMI), systolic, diastolic and mean blood pressure, alongside higher baseline and peak brachial artery diameters (all $P<0.05$). There was a significant main effect for sex, with women demonstrating a lower height, body mass, systolic, diastolic and mean blood pressure, baseline diameter, peak diameter, but a higher FMD response and SRAUC ($P<0.05$; Table 1). A significant interaction effect between age and sex was observed for height, body mass, BMI, systolic blood pressure, FMD response and time to peak ($P<0.05$, Table 1).

A significant positive correlation between FMD response and SRAUC was evident in young men ($r^2=0.042$, $P<0.001$; Figure 1A). Young women also demonstrated a significant correlation between FMD and SRAUC (young women $r^2=0.112$, $P<0.001$), which did not significantly differ compared to young men (Fisher: $P=0.15$). The correlation between FMD and SRAUC was non-significant in older men ($r^2=0.011$, $P=0.098$), whilst older women presented a very weak, but significant correlation

(r^2=0.029, *P*=0.047, Figure 1B). Using the allometrically scaled FMD, we confirmed presence of a correlation in young women (r^2=0.108, *P*<0.001), and a lower correlation in older women (r^2=0.029, *P*=0.045), although this difference did not reach statistical significance (Fisher: *P*=0.15). Young and older men did not demonstrate a significant correlation between the allometrically scaled FMD and SRAUC (r^2<0.001 and *P*=0.662, r^2<0.001 and *P*=0.779, respectively).

The impact of age, sex and SRAUC on FMD was further investigated using interaction terms in linear regression. This approach revealed evidence of a differential relationship between sex and age status and SRAUC on subsequent FMD outcomes. More specifically, young women demonstrated a significantly stronger relationship between SRAUC and FMD compared to young men (β=-5.8^{-4}, *P*=0.017) and older women (β=-5.9^{-4}, *P*=0.049). Age did not significantly alter the relation between SRAUC and FMD in men (β=-2.5^{-4}, *P*=0.30).

Other variables that might contribute to FMD response were also explored in the linear regression model. In addition to age-sex-SRAUC interactions, FMD is influenced by systolic blood pressure (β=-0.035, *P*=0.001), but not diastolic blood pressure (β=0.006, *P*=0.60) or BMI (β=0.033, *P*=0.26). Given the systolic blood pressure outcome, we repeated the bivariate correlations in a subset of n=505 who all fell within strict cut-off values for normal blood pressure (systolic <130 mmHg, diastolic <80 mmHg), BMI (<25 kg/m2) and, when available, glucose (<5.6 mmol/L) and cholesterol levels (<4.9 mmol/L). Young men show evidence of a correlation between FMD response and SRAUC (r^2=0.02, *P*=0.024), but this response was significantly stronger in young women (r^2=0.124, *P*<0.001, Fisher: *P*=0.05). Older

men and women did not show a correlation between FMD and SRAUC (r^2=0.006 and 0.002, respectively, both *P*>0.05).

Impact of menopausal status. Compared to pre-menopausal women, post-menopausal women demonstrated a higher BMI and blood pressure, but lower height and FMD (all *P*<0.05, Table 2). Pre-menopausal women demonstrated a significant correlation between FMD and SRAUC (r^2=0.097, *P*<0.001), whilst this correlation was not significant post-menopause (r^2=0.025, *P*=0.100, Figure 2, Fisher: *P*=0.19). Using the allometrically scaled FMD, we confirmed these findings as the correlation with SRAUC in pre-menopausal women (r^2=0.095, *P*<0.001), disappeared post-menopause (r^2=0.025, *P*=0.099, Fisher: *P*=0.20). Re-analysis of the correlation coefficients within the subgroup of healthy participants (n=505) confirmed the presence of a correlation between FMD and SRAUC in pre-menopausal women (r^2=0.09, *P*=0.001), which is absent in post-menopausal women (r^2=0.006, *P*=0.73, Fisher: *P*=0.30).

DISCUSSION

Our initial analyses were suggestive of sex differences in conduit artery flow mediated dilation across the lifespan. However, given the impact of systolic blood pressure on FMD, we repeated our analysis on a subset of participants following the American Heart Association guidelines for blood pressure[23]. This analysis revealed a significantly stronger relationship between FMD and SRAUC in young women compared to young men, and this was attenuated with advancing age. The sex-related difference and the impact of menopausal status on the relationship between

FMD and its eliciting shear stress stimulus suggests that estrogen may play a role in mediating the higher FMD in pre-menopausal women and, consequently, the reduced risk of CVD in comparison to young men[7].

Our work in a large population of 932 healthy individuals confirms previous work on the association between FMD and SR, in that a statistically significant correlation is present between endothelial function and the magnitude of the shear stress stimulus. This correlation remained present after correcting the FMD for individual differences in baseline diameter and when performed in a subset of healthy individuals (n=505). Given that SRAUC is the eliciting stimulus of the FMD response[24], one would expect to observe a moderate-strong correlation between FMD and SR. However, our data shows a somewhat weaker correlation, in general, compared to previous work, especially in men[18]. This finding could be attributed to a number of participant characteristics, which may lead to a weaker or even absent relation between FMD and SRAUC (e.g. age, CVD risk factors)[18, 25]. Indeed, the sub-analysis performed within individuals with no risk factors revealed a slightly higher r-value. In addition, other factors that impact upon the FMD response must be acknowledged, such as the response of the vascular smooth muscle cells to dilator signals (we did not assess endothelium-independent dilation in our studies) and the structural properties of the artery (i.e., wall thickness, stiffness and diameter)[26-28]. Also, numerous studies have shown that baseline diameter is a stronger predictor of the FMD response than SRAUC[18, 24, 25, 29, 30] and our scaling of FMD responses to baseline diameter attempted to account for this.

In line with some previous observations, we observed sex-related differences in the relationship between FMD and the eliciting SRAUC stimulus. More specifically, we found that young healthy women demonstrate a stronger correlation between FMD and SRAUC, compared with their male peers, especially in the healthy subgroup. To examine the potential role of estrogen, we performed a sub-analysis based on menopausal status and found that the relationship between FMD and SRAUC was absent in post-menopausal women. The potential cardio-protective properties of estrogen have been described before, and may relate to upregulated endothelial nitric oxide (NO) synthase (eNOS) activity[31], vasodilator prostacyclin synthase, expression of vascular endothelial growth factor, inhibition of endothelial cell apoptosis, vascular smooth muscle cell migration and/or proliferation[32, 33]. These adaptations likely contribute to changes in vascular health, especially since some studies have shown that the cyclical estrogen levels across the menstrual cycle are mirrored by fluctuations in arterial stiffness[34, 35] and endothelial function[13-15, 34]. Some of this work used intra-brachial infusions to examine forearm blood flow responses, an endothelial assessment independent of SR, and confirmed that endothelial function *per se* fluctuates across the menstrual cycle[14]. Studies that utilised FMD found that fluctuations in this variable across the menstrual cycle were independent of changes in the SR stimulus[13-15, 34]. This suggests that these larger FMD responses are explained, at least partly, by enhanced sensitivity of the endothelium to SR.

Distinction between levels of estrogen receptors (ERα and ERβ respectively) may contribute to the relationship between FMD and the SR stimulus in pre-menopausal women. Estrogen receptors are located within endothelial cells, and play an

important role in the vasodilator effects of estrogen[36]. In animal models, abundance of ERα is linked to higher circulating estrogen levels[37-39], which is consequently linked to increased NO bioavailability[38, 40]. In humans, ERα expression was lower in the early follicular phase (i.e. low estrogen) and also in post-menopausal women, and was positively associated with (phosphorylated) eNOS protein expression and brachial artery FMD[41]. Indeed, the binding of estrogen to a receptor upregulates NO release and since shear-independent dilation also mirrors the menstrual cycle[14], this implies a greater release of NO with higher estrogen abundance. NO possesses a myriad of anti-atherogenic properties to protect against the development of CVD[42], and is negatively associated with traditional CVD risk factors[43]. Given the above evidence, it could be suggested that estrogen receptors mediate the relationship between FMD and shear stress, resulting in greater dilator responses to a given shear stress stimulus. More research is required to explore the mechanisms underlying the FMD-SRAUC relationships we observed.

When exploring the effects of age, we found an attenuated FMD-SRAUC relationship with advancing age in both men and women, which confirms previous findings[18]. Notably, we observed a weak, but significant correlation in older women. However, this observation may be attributable to the inclusion of 29 (21%) pre-menopausal women in the older (over 40yrs) group. Our findings therefore provide further evidence that older age impairs the FMD-SRAUC relationship. Various components of vascular ageing, including alterations in blood vessel structure[44, 45], shear patterns[46-49] and attenuated NO bioavailability[50, 51] may potentially contribute to the age-related attenuation in the FMD-SRAUC relationship. Since these processes are

also present in women, one may question the relative importance of age (*versus* estrogen) in the loss of the relationship between FMD and SRAUC in post-menopausal women. Given the more gradual impact of age on these factors compared with the relatively rapid alterations in estrogen, one may hypothesise that the loss of estrogen may represent a stronger factor than age in explaining the loss of the relationship between FMD and SRAUC. Future studies are required to untangle the effects of age and sex on this relationship.

A potential lifestyle factor underlying the age- and sex-related differences in the FMD-SRAUC relationship relates to fitness and/or physical activity levels. It is well established that physical activity and subsequent fitness is associated with enhanced endothelial function[52-54] amongst a myriad of other health markers, mediated by the activity-induced exposure to increases in cyclical shear stress[55]. Since studies highlight a trend for declining physical activity levels with advancing age[56, 57], age-related differences in physical activity may represent a confounding variable in the relationship between FMD and SRAUC. Future studies are warranted to better understand this potential link.

The clinical relevance of our findings relate to the importance of changes in shear stress as an important hemodynamic stimulus for acute[58, 59] and chronic[60, 61] adaptation in vascular function and structure[62]. High levels of shear stress have also been linked to the upregulation of anti-atherogenic proteins and down-regulation of pro-atherogenic substances[62-64] to provide further protection against the development/progression of atherosclerosis. Accordingly, enhanced sensitivity of the

endothelium to increases in shear stress (e.g. induced by physical activity) in younger women may contribute to relatively lower risk for CVD events in this cohort. In addition, such changes may also contribute to impaired ability for remodelling of arteries in response to prolonged periods of changes in shear stress in older women. Importantly, shear stress-mediated changes in endothelial function, for example by exercise training, lead to clinically important improvements in vascular health. Notably, meta-analyses have concluded that a 1% increase in brachial FMD is associated with 8-13% reduction in CVD risk[4, 6, 65].

Limitations. Firstly, we do not have data available on estrogen levels, which makes it difficult to directly link our observations to menstrual status and/or estrogen. Furthermore, we must acknowledge that the timing/duration of menopause may also play a role in mediating the FMD-SR relationship. However, vigorous eligibility screening for the respective study established menopause status. Furthermore, markers of endothelial activation/damage were not available, which may have helped to better understand the age-related changes in endothelial function and/or the role of shear stress. Another limitation is that data were collected in different laboratories, which may contribute to some variation. Nonetheless, all labs strictly followed expert-consensus guidelines[19] and utilised identical data collection and validated software analysis procedures which result in high reproducibility of FMD[66].

In conclusion, a stronger relationship between endothelial function and the eliciting SR stimulus was found in women, compared to men, with this sex difference being attenuated with advancing age in the healthy subgroup. We suggest that endogenous estrogen may play a role in mediating the relationship between SRAUC

and FMD. Therefore, the stronger relationship between endothelial function and shear stress (compared to men) may contribute to the cardio-protection of young women and subsequent lower prevalence of CVD.

DISCLOSURES: None

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TABLE 1: Subject characteristics of participants divided based on sex and age into young men and women (aged 18-40yrs) and older men and women (>40yrs). Values are mean ± SD. Comparisons between groups was made using a 2-way ANOVA with sex and age as factors.

	Young Adults (18-40yrs)		Older Adults (>40yrs)		ANOVA		
	Women	*Men*	*Women*	*Men*	*Sex*	*Age*	*Sex*Age*
n	144	389	139	260			
Age (years)	27±6	25±5	56±10	59±10	0.535	<0.001	<0.001
Height (m)	1.69±0.08	1.80±0.07	1.63±0.07	1.77±0.06	<0.001	<0.001	0.003
Body mass (kg)	69.6±14.0	76.3±10.3	69.7±14.0	82.9±14.1	<0.001	<0.001	<0.001
BMI (kg/m^2)	24.6±5.2	23.6±2.8	25.5±4.5	26.1±4.8	0.130	<0.001	0.030
SBP (mmHg)	113±10	120±11	124±15	127±14	<0.001	<0.001	0.010
DBP (mmHg)	68±8	72±14	74±9	77±9	<0.001	<0.001	0.907
MAP (mmHg)	86±11	87±11	92±10	94±10	<0.001	<0.001	0.524
Diameter (mm, rest)	3.3±0.5	4.1±0.6	3.5±0.5	4.4±0.6	<0.001	<0.001	0.218
Diameter (mm, peak)	3.6±0.5	4.3±0.6	3.7±0.5	4.6±0.6	<0.001	<0.001	0.103
FMD%	7.9±3.9	6.4±2.7	5.3±3.3	4.8±2.3	<0.001	<0.001	0.021
SRAUC (s^{-1}, $x10^3$)	23.0±12.0	20.4±10.7	21.6±11.0	19.7±9.0	0.003	0.175	0.662
Time to peak (secs)	51±25	59±30	64±30	58±28	0.575	0.006	0.002

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; FMD, flow-mediated dilation; SRAUC, shear rate area-under-the-curve.

TABLE 2: Subject characteristics of women divided based on menopausal status. Values are mean ± SD. P-value refers to an independent t-test.

	Pre-menopause	Post-menopause	*P* value
n	173	110	
Age (years)	30±8	59±9	<0.001
Height (m)	1.69±0.08	1.62±0.07	<0.001
Body mass (kg)	69.6±13.7	70.0±15.1	0.938
BMI (kg/m^2)	24.7±5.1	26.4±5.0	0.007
SBP (mmHg)	113±10	126±14	<0.001
DBP (mmHg)	74±9	74±9	<0.001
MAP (mmHg)	82±8	90±10	<0.001
Baseline diameter (mm)	3.3±0.5	3.6±0.5	<0.001
Peak diameter (mm)	3.6±0.5	3.8±0.6	0.018
FMD%	7.8±3.9	4.9±3.1	<0.001
SRAUC (s^{-1}, $x10^3$)	23.0±11.6	21.3±11.4	0.213
Time to peak (secs)	51±24	68±32	<0.001

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; FMD, flow-mediated dilation; SRAUC, shear rate area-under-the-curve

Figure Legends

FIGURE 1: Brachial artery flow-mediated dilation (FMD; % from baseline) and the eliciting shear rate area-under-the curve (SRAUC) stimulus (in s^{-1}) in healthy younger (A, total n=533) and older (B, total n=399) adults. In these figures, data were presented and analysed separately for younger men (open circles, n=389) and women (solid circles, n=144), but also for older men (open triangles, n=260) and women (solid triangles, n=139). Pearson's correlation coefficient was used to examine the relation between the FMD and SRAUC in younger and older women (dotted line) and men (solid line).

FIGURE 2: Brachial artery flow-mediated dilation (FMD; % from baseline) and the eliciting shear rate area-under-the-curve (SRAUC) stimulus (in s^{-1}) in healthy pre-menopausal women (solid circles, n=173) and post-menopausal women (open circles, n=110). Pearson's correlation coefficient was used to examine the relation between the FMD and SRAUC in pre- (solid line) and post-menopausal women (dotted line).

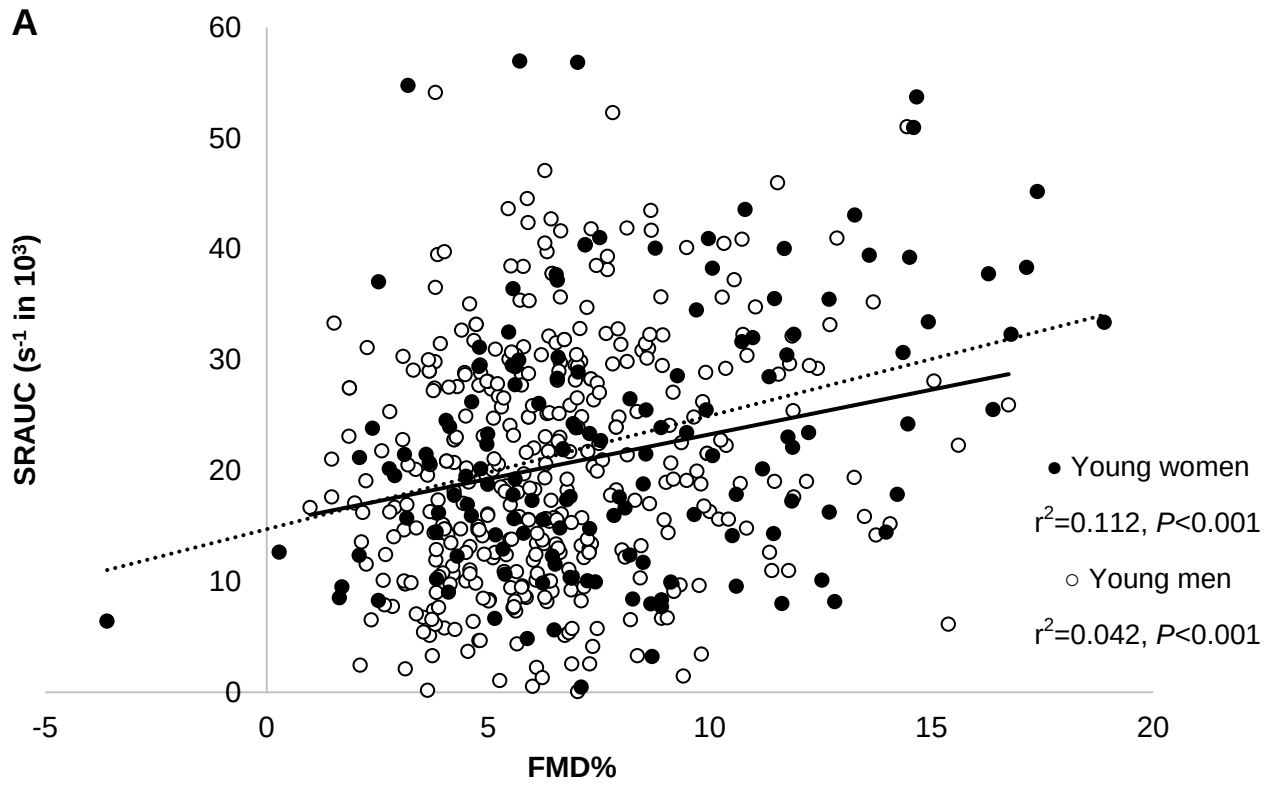

A
SRAUC (s-1 in 10³)
60
50
40
30
20
10
0
-5
0
5
10
15
20
FMD%
● Young women
r²=0.112, P<0.001
○ Young men
r²=0.042, P<0.001

Figure 1

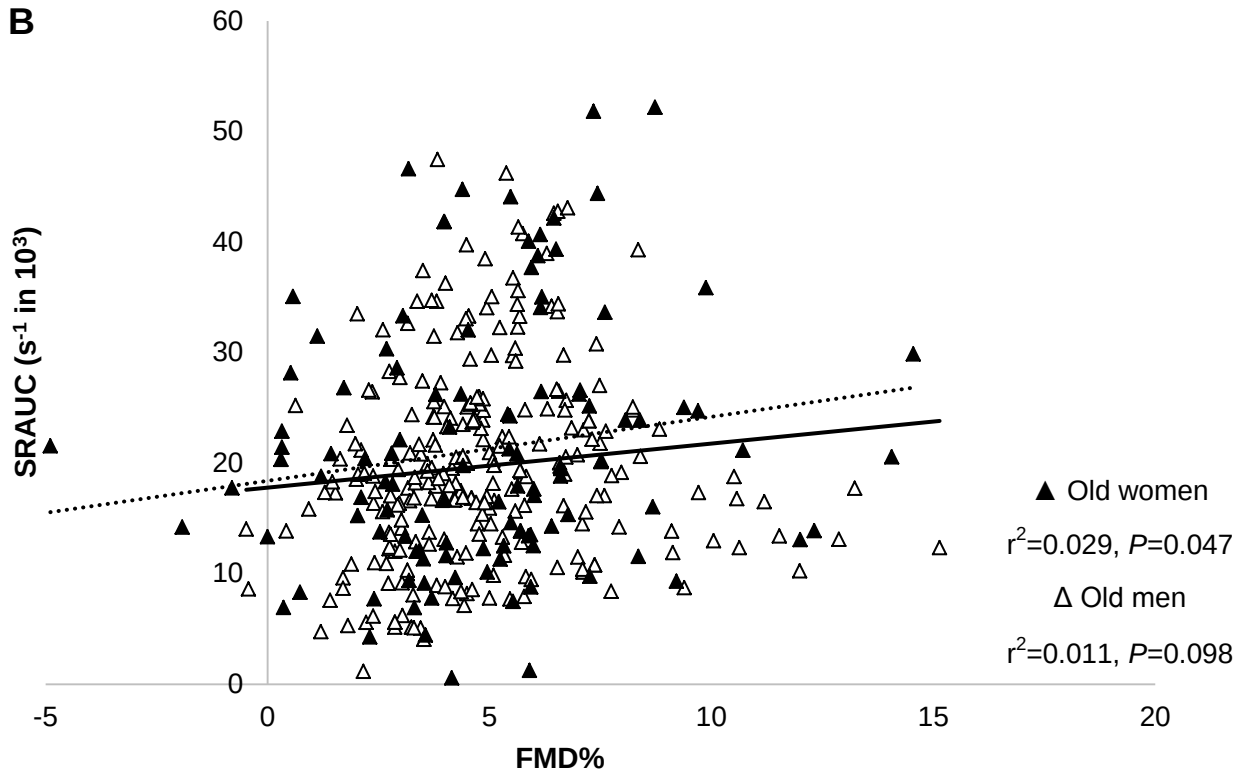
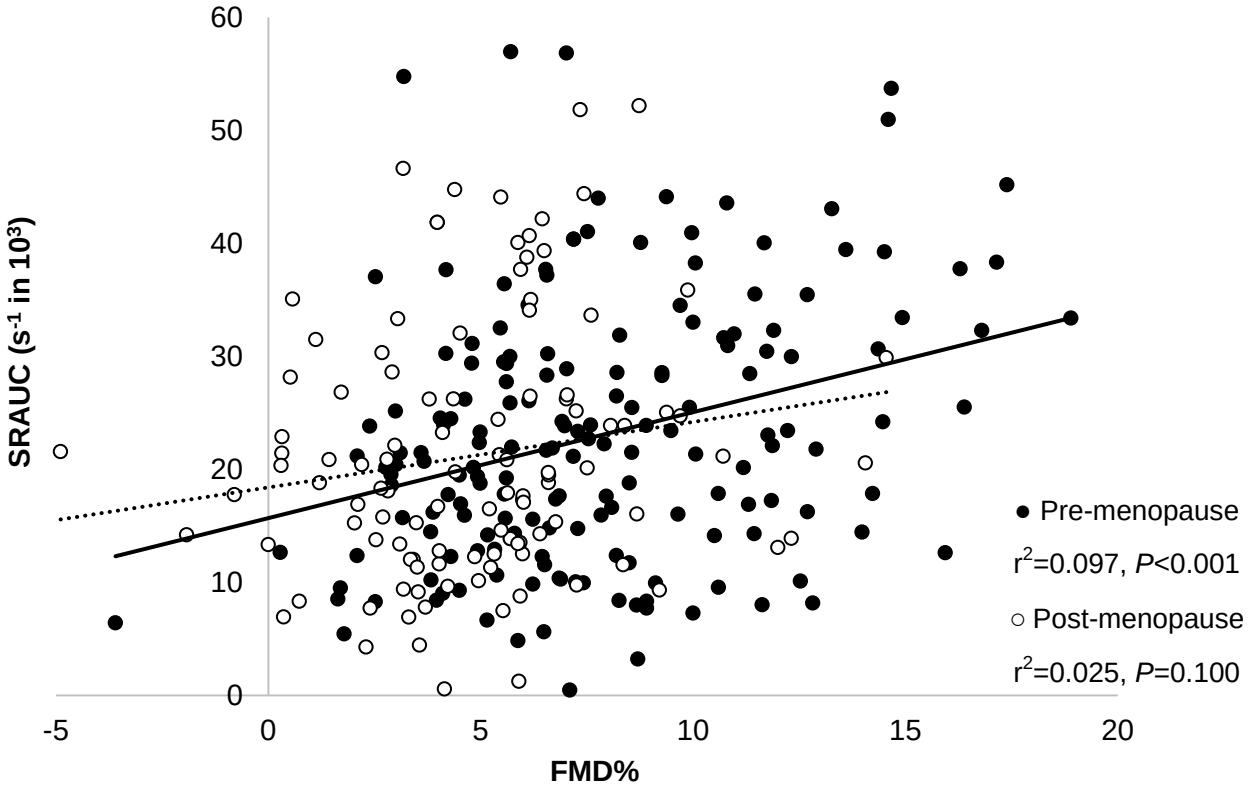

B
60
50
40
30
20
10
0
SRAUC (s-1 in 103)
-5
0
5
10
15
20
FMD%
▲ Old women
r2=0.029, P=0.047
Δ Old men
r2=0.011, P=0.098

Figure 2


60
50
40
30
20
10
0
SRAUC (s-1 in 103)
-5
0
5
10
15
20
FMD%
● Pre-menopause
r2=0.097, P<0.001
○ Post-menopause
r2=0.025, P=0.100