Title: Age-related differences in left ventricular structure and function between healthy men and women

Running title: Ageing and LV mechanics

Authors: Amanda Q.X. Nio, Eric J. Stöhr, Rob E. Shave

Affiliation: Department of Physiology and Health, Cardiff School of Sport, Cardiff Metropolitan University, Cardiff, United Kingdom

Corresponding author: Amanda Nio
Department of Biomedical Engineering, King's College London, St Thomas' Hospital, London SE1 7EH, United Kingdom
Email: nio@aqxn.info

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Abstract

Objectives: Cardiovascular function generally decreases with age, but whether this decrease differs between men and women is unclear. Our aims were twofold: (i) to investigate age-related sex differences in left ventricular (LV) structure, function and mechanics, and (ii) to compare these measures between pre- and post-menopausal women in the middle-aged group.

Methods: Resting echocardiography was performed in a cross-sectional sample of 82 healthy adults (14 young men, 19 middle-aged men, 15 young women, 34 middle-aged women: 15 pre-menopausal and 19 post-menopausal). Two-way ANOVAs were used to examine sex $\times$ age interactions, and t-tests to compare pre- and post-menopausal women ($\alpha < 0.1$).

Results: Normalised LV mass, stroke volume and end-diastolic volume were significantly lower in middle-aged than young men, but this difference was smaller between middle-aged and young women. Peak systolic apical mechanics were significantly greater in middle-aged men than middle-aged women, but not between young men and women. Post-menopausal women had significantly lower LV relaxation and mechanics (torsion, twisting velocity and apical circumferential strain rates) compared with middle-aged pre-menopausal women.

Conclusion: Our cross-sectional findings suggest that the hearts of men and women may age differently, with men displaying greater differences in LV volumes accompanied by differences in apical mechanics.

Introduction

Ageing is associated with a general decline in cardiovascular function[1,2]. Whilst recent reviews have suggested a different pattern of age-related changes in men compared with women[1,2], conflicting data in the literature—such as that on left ventricular (LV) mass and diastolic function[3–11]—highlight the need for more empirical evidence. Owing to the chronic exposure to different levels of testosterone, oestrogen, progesterone and epinephrine, it seems reasonable to expect that age may have a differential impact on LV structure and function between men and women[12–14]. Specifically, these hormones have been implicated in myocardial apoptosis[7], contractility[7,15–17] and stiffness[18–21]. The drop in endogenous oestrogen and progesterone concentrations following the menopause[22] likely contributes to the reduced systolic and diastolic function[23–25] observed in post-menopausal women, yet menopausal status has rarely been accounted for within large-scale ageing studies. In studies focused on comparing pre- and post-menopausal women alone, study groups have been limited by large age differences (e.g. a mean age difference of 24 years[26]), or by a lack of distinction between women of natural and surgically-induced menopause[23]. Accordingly, a detailed characterisation of the impact of the natural menopause on LV function and mechanics in a relatively age-matched cohort will help clarify the age-related decline in cardiac function in men and women.

To first investigate sex differences in early cardiac ageing, we examined the interaction between age and sex on LV structure, function and mechanics (rotation and deformation of the LV base and apex) in a cross-sectional sample of young and middle-aged men and women. Additionally, in the middle-aged female cohort, we hypothesised that indicators of systolic and diastolic function, as well as measures of LV mechanics, would be lower in post-menopausal women.

Methods

Ethical approval

All experimental procedures were approved by the Cardiff Metropolitan University's School of Sport Research Ethics Committee and conformed to the ethical principles in the Declaration of Helsinki. Prior to the start of any experimental procedures all participants provided written and verbal informed consent.

Study design

Young adult (age 19–32 years) and middle-aged (age 45–58 years) men and women were recruited from the university population and the general community for a cross-sectional study examining the interaction of sex and age on LV structure, function and in particular, mechanics (15 young women, 34 middle-aged women, 14 young men, 19 middle-aged men; Table S1). Only non-smoking, non-diabetic (self-reported) and normotensive healthy volunteers not taking any cardiovascular or lipid-lowering medications were recruited. In addition, to examine the impact of menopausal status on LV structure, function and mechanics, our recruitment of middle-aged women was targeted to include only distinctly pre- or post-menopausal women (15 pre-menopausal, 19 post-menopausal; Table S2; Figure S1); by design we did not recruit peri-menopausal women. The middle-aged pre-menopausal women were characterised as having regular menstrual cycles ranging from 21–35 days in length without a persistent difference of more than seven days between consecutive cycles[22,27], and had not used oral contraceptives in the preceding four months. Post-menopausal women were identified by at least 12 consecutive months of amenorrhoea[22], which had not been induced by surgery (e.g. hysterectomy). None of the post-menopausal women had used hormone replacement therapy (HRT) in the preceding six months.

Aerobic capacity test

To ensure that participants were euhydrated and well-rested before any measurements, they were asked to abstain from caffeine, alcohol and strenuous exercise for 24 h, and to drink 500 ml of water 90 min before arrival at the laboratory. Participants' height and body mass (Model 770, Seca, Hamburg, Germany) were assessed (Table S1; Table S2), and skinfolds measured at the biceps, triceps, subscapular and suprailiac (Harpenden Skinfold Calliper, Baty International, West Sussex, UK) in order to estimate percentage body fat and fat-free mass (FFM)[28,29]. All participants completed a continuous ramp test to volitional exhaustion on an upright cycle ergometer (Corival, Lode, Groningen, The Netherlands) to determine peak aerobic capacity ($\dot{V}O_{2peak}$). Each test started at zero Watts, and the subsequent increase in intensity was individualised using age, height and body mass[30] to achieve peak power output in approximately 10 min. Respiratory gas exchange (Oxycon Pro, Viasys Healthcare, Basingstoke, UK) and heart rate (RS400, Polar Electro, Kempele, Finland) were monitored and recorded throughout the test. Measured $\dot{V}O_{2peak}$ was not statistically different from predicted maximal oxygen uptake[31] within each age-sex group ($P > 0.05$ with Holm-Bonferroni correction).

Resting cardiovascular function

Resting cardiovascular function was assessed either prior to the exercise test, or on a separate day. Following 10 minutes of rest, blood pressure (FinometerPRO, FMS, Finapres Measurement Systems, Arnhem, Netherlands) and echocardiographic images were recorded with the participant lying supine at a 30–45° left lateral tilt (Angio 2003, Lode, Groningen, Netherlands). In accordance with current guidelines, echocardiographic images were acquired at end-expiration by the same trained sonographer[21,32]. Phased array transducers (M4S-RS, 1.5–3.6 MHz; 4V 1.7–3.3 MHz) were used on commercially available ultrasound systems (Vivid q, GE Medical Systems, Israel Ltd, Israel; Vivid E9, GE Vingmed Ultrasound AS, Horten, Norway, respectively), and images were analysed offline for LV structure, function and mechanics (EchoPAC, Version 112, GE Healthcare, Horten,

Norway). Three consecutive cardiac cycles were analysed for each variable and the mean was used for statistical analyses.

Left ventricular structure and function. Left ventricular dimensions were determined directly from 2D parasternal long-axis images[32]. Left ventricular mass was estimated according to the American Society of Echocardiography recommendations[32]. End-diastolic and end-systolic volumes (EDV and ESV, respectively) were determined using the biplane method of discs ("modified Simpson's rule")[32]. Left ventricular length was calculated as the mean of the diastolic LV lengths from the biplane images. Left ventricular mass, dimensions, volumes and cardiac output were allometrically-scaled to FFM to enable cross-sectional comparisons independent of body size, as recommended[33]. A "best compromise" scaling exponent was calculated and applied to each measure of LV size[34]. Heart rate was determined from the ECG inherent to the ultrasound. Stroke volume (SV = EDV − ESV), ejection fraction ([SV/EDV] × 100), cardiac output (heart rate × SV) and systemic vascular resistance (mean arterial pressure/cardiac output) were then calculated. Trans-mitral peak filling velocities were measured using pulsed-wave Doppler in the apical four-chamber view[21]. Isovolumic relaxation time (IVRT) and peak septal wall velocities at the level of the mitral annulus were assessed using pulsed-wave tissue Doppler imaging (TDI) in the apical four-chamber view[9,21].

Left ventricular mechanics. Left ventricular mechanics were assessed using 2D speckle tracking of the myocardium in the parasternal short-axis images at the LV base and apex, in line with previous methodology[35]. Circumferential strain and strain rate, rotation and rotational velocity at the base and apex of the LV were analysed offline using commercial software (EchoPAC). Longitudinal strain was out of the scope of this study, as we were primarily interested in basal and apical mechanics[17,36,37], and our group has previously found this measure to underestimate apical contribution[35]. To account for differences in heart rate, raw data were normalised to the percentage of systole and diastole (2D Strain Analysis Tool 1.0β14, Stuttgart, Germany)[35]. Twist and twisting velocity curves were

calculated by subtracting time-aligned basal data from apical data, and peak values for all parameters were extracted from interpolated curves. Similarly, time to peak untwisting velocity, and to peak diastolic basal and apical rotational velocities were derived from interpolated curves[38]. Torsion was calculated as LV twist/end-diastolic LV length. Due to poor image quality, data on LV mechanics could not be obtained from one middle-aged male participant.

Statistical analysis

Statistical analyses were performed with R[39]. Reasonable normality of residuals was confirmed with the Shapiro-Francia test for normality and Normal quantile-quantile (Q-Q) plots. As Levene's test for homogeneity of variances revealed unequal variances in some of our parameters, the two-way analysis of variance (ANOVA; factors: sex and age) with White-adjusted p-values for heteroscedasticity was used to compare all variables between young adult and middle-aged men and women. For variables where the sex $\times$ age interaction effect was significant, Student's t-test for independent samples was used *post-hoc* to identify differences between groups. In our secondary analysis, Student's t-test for independent samples was used to compare all variables between middle-aged pre- and post-menopausal women, and age was added as a covariate to verify our findings. Alpha was set at 0.1 *a priori* for the best possible trade-off between false positives and negatives (based on power calculations[40] using published data[9,36,37,41–43] and the available sample size for this study). Data are presented as mean and standard deviation (SD) unless stated otherwise.

Results

Sex differences in left ventricular structure, function and mechanics

Left ventricular mass, wall thicknesses, volumes and cardiac output were all smaller in women than men ($P < 0.01$; Table 1). Once scaled to FFM, however, these parameters were no longer significantly different between the sexes ($P > 0.1$). Diastolic function was greater in women than men, as indicated by greater early diastolic velocities (E, E/A and E′; $P < 0.1$) and peak diastolic basal circumferential strain rate ($P < 0.001$; Table 2).

Age-related differences in left ventricular structure, function and mechanics

Left ventricular volumes, mass and cardiac output were smaller in middle-aged participants compared with the young adults ($P < 0.05$; Table 1). After normalising for differences in FFM, LV mass was no longer statistically different between young and middle-aged adults ($P = 0.23$), while the effect of age on LV volumes and cardiac output was still statistically significant ($P < 0.05$).

Peak LV twist, torsion, twisting velocity, and basal and apical rotation were greater in middle-aged participants compared with the young adults ($P < 0.1$; Table 2; Figure 1). Diastolic function was lower in middle-aged participants, as evidenced by longer isovolumic relaxation times and slower early diastolic velocities (E and E′), with faster late diastolic velocities (A and A′) to compensate (lower E/A; all $P < 0.01$; Table 1). In addition, middle-aged participants achieved peak untwisting velocities later in the cardiac cycle, and had lower peak diastolic apical circumferential strain rates compared with the young adults ($P < 0.001$; Table 2; Figure 1).

Sex differences with age in left ventricular structure, function and mechanics

Normalised LV mass, SV and EDV were smaller in middle-aged than young men; but LV mass and SV were similar in middle-aged and young women, and the difference in EDV between female groups was smaller than that between male groups ($P < 0.06$; Table 1). Measures of peak systolic apical mechanics were similar in young men and women, but yet were all larger in middle-aged men than middle-aged women ($P < 0.1$; Table 2; Figure 1). Middle-aged men achieved peak diastolic apical and basal rotational velocities, and untwisting velocity later in the cardiac cycle than the young men, but this age-related difference was smaller in females ($P < 0.05$).

Impact of menopausal status on general haemodynamics, and left ventricular structure, function and mechanics

General haemodynamics and LV mass, dimensions and volumes were all similar in middle-aged pre- and post-menopausal women (Table 3). Normalising LV structure and volumes to FFM, however, revealed a greater relative LV mass, ESV and EDV in post-menopausal women ($P < 0.1$). Peak LV torsion, twisting velocity and systolic apical circumferential strain rate were lower in post-menopausal women compared with the pre-menopausal women ($P < 0.1$; Table 4; Figure S2). In line with their slower early diastolic myocardial velocity (E'; $P = 0.06$), post-menopausal women also had lower peak diastolic apical circumferential strain rates ($P = 0.01$). Our findings did not change when age was added as a covariate.

Discussion

In this study, we assessed LV structure, function and mechanics in a cross-sectional sample of young adult and middle-aged men and women. We found a greater age-related difference in LV mass, SV and EDV in men compared with women, coincident with greater peak

systolic apical mechanics and later peak diastolic rotational velocities over the cardiac cycle in middle-aged men compared with middle-aged women. These findings suggest that sex differences in early cardiac ageing may be related to changes at the apex. In addition, we observed that post-menopausal women had impaired LV relaxation—as indicated by E′—and lower peak LV mechanics (torsion, twisting velocity and apical circumferential strain rates) compared with their middle-aged pre-menopausal counterparts. This may indicate an initial reduction in myocardial function after the menopause.

Age-related differences in left ventricular structure and function between men and women

We found that LV mass and SV were lower in middle-aged men than young adult men, but were similar in middle-aged and young adult women. In addition, EDV was lower in the middle-aged groups relative to the young adult groups, but this difference was greater among men than women. This supports previous work showing that a significant loss of cardiomyocytes in response to early ageing occurs only in men[10]. The associated lower EDV in middle-aged men could be underpinned, at least in part, by a greater sub-clinical impairment in LV relaxation in men than women with early ageing, as suggested by longer times to peak diastolic rotational velocities[38] in our study. Although we did not measure hormone concentrations in this study, it is helpful to consider our findings in the context of previous research. It is unclear if differences in oestrogen and progesterone concentrations contribute to the age-related differences in LV mass and volumes observed here, as these parameters have been found to be similar in pre- and post-menopausal women who typically experience contrasting levels of oestrogen and progesterone[10,22,25]. Higher levels of testosterone and/or epinephrine in men compared with women[12,14] may however explain the age-related differences in LV structure and volumes, as these hormones have been shown to stimulate apoptosis and fibrosis, which could thus decrease LV mass and increase LV stiffness[7,18,20,21,44]. Notwithstanding, it is important to acknowledge that our structural data conflict with a number of previous studies. Contradictory findings

to ours—such as an age-related increase in LV mass[4,6,11]—may have arisen from the inclusion of individuals with cardiovascular risk factors[3], overlapping but different levels of cardiorespiratory fitness and age groups perused[11], and/or different scaling methods in previous studies[33].

Sex differences in apical mechanics with early ageing

Interestingly, we found that the differences in peak LV mechanics between young and middle-aged men compared with women were localised at the apex, with males showing a greater systolic rotation and rotational velocity. Beyond the previously discussed loss of functional myocytes in men, a potential explanation for these differences is their higher epinephrine concentrations compared with women[14], which coupled with a greater β-adrenergic receptor density in males[45] may influence LV mechanics. Epinephrine has been shown to exert a dominant effect on the LV apex compared with the base[15], while catecholamine administration in animal studies has been shown to induce myocardial fibrosis especially at the apex[18]. We thus speculate that men may experience—sub-clinically—a greater sub-endocardial fibrosis[46] at the apex with ageing compared with women, induced by higher circulating epinephrine concentrations[18]. If true, this could explain the higher peak apical rotation and rotational velocity that we observed in the middle-aged men compared with women due to a more dominant apical sub-epicardium[36,37,47].

Sex differences in arterial stiffness with ageing could further explain the localised apical differences that we observed[11,48]. In the Multi-Ethnic Study of Atherosclerosis (MESA), regression analyses detected a significant relationship between arterial stiffness and circumferential strain rate at the apex but not the base[48]. The lower peak apical circumferential strain and strain rate that we observed in middle-aged women in our cross-sectional study could thus reflect a greater increase in arterial stiffness with early ageing in women than men[48]. Whilst our measures of brachial blood pressure and calculated systemic vascular resistance did not indicate sex differences with age, these are poor surrogates for central pressure and arterial stiffness[49]. Future investigations focused on

delineating age-related differences in vascular properties between men and women, and on the influence of differing levels of epinephrine on regional LV function would help further interpretation of our findings.

Impact of the menopause on left ventricular structure, function and mechanics

Given that the menopause has been associated with decreases in vascular function[27], it is important to also consider this influence within the context of ageing studies examining the heart. Counter to previous reports of early concentric remodelling in women following the menopause[25,32,50], here we observed similar LV mass, dimensions and volumes in pre- and post-menopausal women. This discrepancy may be due to the inclusion of women with surgically-induced menopause in earlier work[25], and/or different cardiovascular risk factors and cardiorespiratory fitness levels relative to our study[50]. The greater relative LV mass that we observed in post-menopausal women may, in fact, reflect a maintenance of LV mass despite the known menopausal-related decline in FFM. A longitudinal study following middle-aged pre-menopausal women through the menopause is needed to clarify our findings.

Irrespective of LV structure, and in line with previous studies[23,24] and our hypothesis, our results do indicate lower LV diastolic function in post-menopausal middle-aged women, as evidenced by slower early diastolic wall velocity (E'). In addition, while lower longitudinal systolic strain and diastolic strain rate have been shown in post-menopausal women previously[42], we have further identified lower torsion, twisting velocity and circumferential strain rates in post-menopausal women. These lower LV mechanics—albeit from a cross-sectional study—may reflect an initial reduction in myocardial function following the menopause, due to withdrawal of the positive effects of oestrogen and progesterone on apoptosis[7], contractility[7,17] and/or stiffness[19]. It is possible that these changes in the underpinning cardiac mechanics occur prior to differences in global measures of function, such as cardiac output or ejection fraction[42]. Interestingly, our findings do not indicate

a localised effect of menopausal status on the either the LV base or apex. This suggests that menopausal status is unlikely to explain the age-related apical sex differences discussed earlier, and additionally, appears to contradict the effects of oestrogen specific to the LV base that have been identified through animal research[17]. Further work is clearly necessary to understand cardiovascular ageing in women.

Limitations, implications and future directions

A limitation of this study was that circulating concentrations of catecholamines and sex hormones were not measured. Pre- and post-menopausal women were, however, carefully recruited based on menstrual history to ensure that circulating female sex hormone concentrations were chronically lower in the post-menopausal group. Future work delineating the effects of cyclical variations in female sex hormone concentrations (e.g. comparing early-follicular, late-follicular and mid-luteal menstrual cycle phases) from chronically lower concentrations (e.g. after the menopause) would provide further insight into the potent effects of female sex hormones on the heart.

Additional limitations of this study are its relatively small sample size and cross-sectional design. The small sample size reflects our limited resources, but also our primary focus on LV mechanics, which have been suggested to be more sensitive than global indicators of cardiac function (e.g. heart rate and cardiac output). To reduce the likelihood of committing a type II error due a small sample size, we set our level of statistical significance *a priori* at 0.1 and accepted the resultant trade-off between type I and type II errors in this study. Our findings, nonetheless, highlight mechanical differences localised to the apical region of the LV, which could inform future studies investigating sex differences with ageing. We and others have previously discussed the difficulties in separating the effects of the menopause from those of ageing on the female heart[1,2], and the present study is another example of this. Despite including only middle-aged women in our secondary analysis, naturally post-menopausal women were, on average, six years older than the pre-menopausal women. Including age as a covariate, however, did not change our findings,

and accordingly confirmed a significant impact of menopause on the LV. Notwithstanding, longitudinal ageing studies from young adulthood and through the menopausal transition will provide further insight into female cardiovascular ageing. Of particular relevance to women's health in mid-life, we recommend future work into whether lifestyle interventions (e.g. exercise training and dietary modifications) may mitigate the decline in myocardial function associated with the menopause.

Conclusions

In conclusion, the findings of our cross-sectional study suggest that changes in LV structure and function from young adulthood to middle-age differ between men and women: normalised LV mass, SV and EDV are lower in middle-aged men compared with their younger counterparts, but this difference is markedly less in women. Peak systolic apical mechanics are greater in middle-aged men than middle-aged women, but not between young men and women or at the base. During middle-age, post-menopausal women have reduced LV relaxation (as indicated by E′) and altered LV mechanics (lower peak torsion, twisting velocity and apical circumferential strain rates) compared with pre-menopausal women. Our findings provide new insight into the regional cardiac changes that may occur with healthy ageing, and set the foundation for future longitudinal studies investigating this life stage.

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TABLE 1: General haemodynamics, and left ventricular (LV) structure and function in young adult and middle-aged (older) men and women at rest.

	Female		Male		*P*		
Parameter	Younger	Older	Younger	Older	Sex	Age	Sex × Age
General haemodynamics							
SBP (mmHg)	114 (7)	131 (14) ‡	121 (12)	128 (13)	0.33	**<0.01**	**0.08**
SVR (mmHg·min/L)	26.0 (4.0)	32.3 (5.2)	19.4 (5.0)	25.3 (5.5)	**<0.01**	**<0.01**	0.84
Heart rate (beats/min)	57 (6)	56 (7)	54 (11)	55 (7)	0.40	0.93	0.57
$\dot{Q}$ (L/min)	3.25 (0.45)	2.89 (0.45)	4.51 (1.05)	3.69 (0.79)	**<0.01**	**<0.01**	0.23
$\dot{Q}$ (L/min/kg$^{0.68}$)	0.25 (0.03)	0.23 (0.04)	0.26 (0.06)	0.22 (0.04)	0.67	**0.02**	0.21
LV structure							
IVSd (cm)	0.8 (0.1)	0.8 (0.1)	0.9 (0.1)	0.9 (0.1)	**<0.01**	0.25	0.83
LVPWd (cm)	0.8 (0.1)	0.8 (0.1)	0.9 (0.1)	0.8 (0.1)	**<0.01**	0.60	0.92
LV mass (g)	114 (24)	107 (18)	173 (30)	148 (31)	**<0.01**	**0.01**	0.18
SV (mL)	58 (8) †	52 (9) †‡	84 (12)	67 (11) ‡	**<0.01**	**<0.01**	**0.02**
EDV (mL)	97 (14) †	78 (13) †‡	146 (17)	109 (15) ‡	**<0.01**	**<0.01**	**0.02**
ESV (mL)	39 (7) †	26 (7) †‡	62 (8)	43 (7) ‡	**<0.01**	**<0.01**	**0.08**
LVLd (cm)	8.0 (0.5)	7.5 (0.7)	9.3 (0.5)	8.7 (0.5)	**<0.01**	**<0.01**	0.75
Allometrically-scaled							
IVSd (cm/kg$^{0.26}$)	0.30 (0.03)	0.30 (0.04)	0.30 (0.02)	0.30 (0.02)	0.92	0.69	0.96
LVPWd (cm/kg$^{0.30}$)	0.25 (0.03)	0.25 (0.03)	0.25 (0.03)	0.25 (0.02)	0.78	0.80	0.77
LV mass (g/kg$^{0.90}$)	3.71 (0.49)	3.81 (0.66)	4.03 (0.58)	3.60 (0.53)‡	0.70	0.23	**0.05**
SV (mL/kg$^{0.74}$)	3.34 (0.36) †	3.27 (0.59)	3.68 (0.51)	3.06 (0.43)‡	0.58	**<0.01**	**0.02**
EDV (mL/kg$^{0.92}$)	2.89 (0.31) †	2.57 (0.45) ‡	3.10 (0.34)	2.46 (0.31)‡	0.56	**<0.01**	**0.05**
ESV (mL/kg$^{1.21}$)	0.38 (0.05)	0.29 (0.08)	0.39 (0.05)	0.29 (0.06)	0.91	**<0.01**	0.81
Systolic function							
Ejection fraction (%)	60 (2) †	67 (6) †‡	57 (4)	61 (5) ‡	**<0.01**	**<0.01**	**0.05**
S′ (m/s)	0.07 (0.01)	0.07 (0.01)	0.08 (0.01)	0.08 (0.01)	**0.02**	0.52	0.51
Diastolic function							
IVRT (ms)	75 (8)	92 (16)	78 (14)	93 (14)	0.59	**<0.01**	0.70
IVRT (%)	12 (3)	14 (3)	11 (4)	14 (3)	0.73	**<0.01**	0.69
E (m/s)	0.80 (0.07)	0.70 (0.13)	0.74 (0.10)	0.59 (0.10)	**<0.01**	**<0.01**	0.25
E′ (m/s)	0.14 (0.02)	0.10 (0.02)	0.14 (0.02)	0.09 (0.02)	**0.06**	**<0.01**	0.40
A (m/s)	0.38 (0.07)	0.54 (0.10) ‡	0.42 (0.08)	0.51 (0.08)‡	0.93	**<0.01**	**0.10**
A′ (m/s)	0.07 (0.01)	0.09 (0.01)	0.07 (0.01)	0.09 (0.01)	0.17	**<0.01**	0.63
E/A	2.14 (0.39)	1.33 (0.27)	1.82 (0.32)	1.19 (0.26)	**<0.01**	**<0.01**	0.26

SBP: systolic blood pressure. SVR: systemic vascular resistance. $\dot{Q}$: cardiac output. FFM: fat-free mass. IVSd: inter-ventricular septum thickness during diastole. LVPWd: LV posterior wall thickness during diastole. SV: stroke volume. EDV: end-diastolic volume. ESV: end-systolic volume. LVLd: LV length during diastole. Peak septal wall velocity at the level of the mitral annulus during systole (S′), and early (E′) and late diastole (A′). IVRT: isovolumic relaxation time in ms and in % diastole, where the time at end-systole is defined as 100% and end-diastole is 200%. Peak trans-mitral filling velocity during early (E) and late diastole (A). †$P < 0.1$ compared with age-matched men. ‡$P < 0.1$ compared with younger counterparts. ANOVA effects with $P < 0.1$ (White-adjusted for heteroscedasticity) are in **bold**.

TABLE 2: Peak left ventricular (LV) mechanics during systole and diastole in young adult and middle-aged (older) men and women at rest.

	Female		Male		*P*		
Parameter	Younger	Older	Younger	Older	Sex	Age	Sex × Age
Systolic peaks							
Twist (deg)	12.7 (4.0)	16.8 (4.6)	12.4 (3.3)	18.7 (5.2)	0.43	**<0.01**	0.31
Torsion (deg/cm)	1.6 (0.5)	2.3 (0.6)	1.3 (0.3)	2.2 (0.7)	0.16	**<0.01**	0.54
Twisting vel (deg/s)	85 (14)	91 (16)	91 (10)	104 (32)	**0.06**	**0.04**	0.44
LV base							
Rotation (deg)	-3.5 (2.5)	-5.6 (3.1)	-4.4 (2.1)	-4.7 (2.6)	0.95	**0.06**	0.16
Rotational vel (deg/s)	-55 (11)	-49 (15)	-55 (13)	-45 (16)	0.51	**0.01**	0.65
Circ strain (%)	-18 (3)	-18 (4)	-16 (2)	-15 (3)	**<0.01**	0.82	0.40
Circ strain rate (1/s)	-1.0 (0.2)	-1.0 (0.2)	-1.0 (0.2)	-0.9 (0.1)	0.82	0.51	0.22
LV apex							
Rotation (deg)	9.7 (2.7)	11.8 (3.8) †‡	8.7 (2.4)	14.9 (4.2) ‡	0.18	**<0.01**	**0.01**
Rotational vel (deg/s)	56 (21)	53 (14) †	53 (16)	70 (23) ‡	0.15	0.13	**0.03**
Circ strain (%)	-22 (4)	-20 (4) †‡	-22 (4)	-24 (5)	0.12	0.67	**0.10**
Circ strain rate (1/s)	-1.4 (0.2)	-1.1 (0.2) †‡	-1.4 (0.3)	-1.4 (0.3)	**<0.01**	**0.01**	**0.02**
Diastolic peaks							
Untwisting vel (deg/s)	-104 (33)	-93 (28)	-101 (27)	-91 (25)	0.70	0.13	0.94
Time to untwisting vel (%)	105 (6)	109 (7) †‡	106 (4)	116 (8) ‡	**<0.01**	**<0.01**	**0.03**
LV base							
Rotational vel (deg/s)	55 (20)	50 (16)	49 (22)	45 (13)	0.24	0.35	0.88
Time to rotational vel (%)	105 (7)	104 (7) †	104 (5)	111 (10) ‡	0.17	**0.10**	**0.03**
Circ strain rate (1/s)	1.6 (0.3)	1.4 (0.4)	1.2 (0.3)	1.1 (0.3)	**<0.01**	0.10	0.55
LV apex							
Rotational vel (deg/s)	-69 (29)	-58 (22)	-62 (22)	-68 (21)	0.84	0.71	0.14
Time to rotational vel (%)	110 (8)	113 (10) †	107 (6)	120 (10) ‡	0.33	**<0.01**	**0.01**
Circ strain rate (1/s)	2.2 (0.6)	1.6 (0.5)	2.1 (0.7)	1.7 (0.6)	0.98	**<0.01**	0.47

Vel: velocity. Circ: circumferential. Time to peak untwisting vel and rotational vel in % diastole, where the time at end-systole is defined as 100% and end-diastole is 200%. †$P < 0.1$ compared with age-matched men. ‡$P < 0.1$ compared with younger counterparts. ANOVA effects with $P < 0.1$ (White-adjusted for heteroscedasticity) are in **bold**.

TABLE 3: General haemodynamics, and left ventricular (LV) structure and function in middle-aged pre- and post-menopausal women at rest.

	Middle-aged female		P
Parameter	Pre-menopausal	Post-menopausal	Menopause
General haemodynamics			
Systolic blood pressure (mmHg)	128 (14)	132 (15)	0.42
SVR (mmHg·min/L)	32.3 (6.9)	32.3 (3.6)	0.99
Heart rate (beats/min)	57 (7)	55 (7)	0.63
$\dot{Q}$ (L/min)	2.89 (0.57)	2.89 (0.35)	0.99
$\dot{Q}$ (L/min/kg FFM$^{0.68}$)	0.23 (0.04)	0.24 (0.03)	0.30
LV structure			
IVSd (cm)	0.8 (0.1)	0.8 (0.1)	0.51
LVPWd (cm)	0.8 (0.1)	0.8 (0.1)	0.45
LV mass (g)	104 (17)	109 (19)	0.44
SV (mL)	52 (11)	53 (9)	0.79
EDV (mL)	76 (14)	80 (13)	0.36
ESV (mL)	24 (6)	28 (7)	0.13
LVLd (cm)	7.4 (0.5)	7.5 (0.8)	0.45
Allometrically-scaled			
IVSd (cm/kg FFM$^{0.26}$)	0.29 (0.04)	0.31 (0.03)	0.27
LVPWd (cm/kg FFM$^{0.30}$)	0.25 (0.03)	0.25 (0.03)	0.94
LV mass (g/kg FFM$^{0.90}$)	3.55 (0.50)	4.02 (0.71)	**0.04**
SV (mL/kg FFM$^{0.74}$)	3.12 (0.60)	3.39 (0.58)	0.19
EDV (mL/kg FFM$^{0.92}$)	2.38 (0.39)	2.72 (0.45)	**0.03**
ESV (mL/kg FFM$^{1.21}$)	0.25 (0.06)	0.32 (0.07)	**0.01**
Systolic function			
Ejection fraction (%)	68 (6)	66 (5)	0.27
S′ (m/s)	0.08 (0.01)	0.07 (0.01)	0.73
Diastolic function			
IVRT (ms)	89 (17)	95 (15)	0.25
IVRT (%)	114 (3)	115 (4)	0.47
E (m/s)	0.72 (0.12)	0.68 (0.13)	0.40
E′ (m/s)	0.11 (0.02)	0.09 (0.02)	**0.06**
A (m/s)	0.54 (0.09)	0.54 (0.11)	0.88
A′ (m/s)	0.09 (0.02)	0.09 (0.01)	0.47
E/A	1.37 (0.28)	1.29 (0.26)	0.41

SVR: systemic vascular resistance. $\dot{Q}$: cardiac output. FFM: fat-free mass. IVSd: interventricular septum thickness during diastole. LVPWd: LV posterior wall thickness during diastole. SV: stroke volume. EDV: end-diastolic volume. ESV: end-systolic volume. LVLd: LV length during diastole. Peak septal wall velocity at the level of the mitral annulus during systole (S′), and early (E′) and late diastole (A′). IVRT: isovolumic relaxation time in ms and in % diastole, where the time at end-systole is defined as 100% and end-diastole is 200%. Peak trans-mitral filling velocity during early (E) and late diastole (A). T-tests with $P < 0.1$ are in **bold**.

TABLE 4: Peak left ventricular (LV) mechanics in middle-aged pre- and post-menopausal women at rest.

	Middle-aged female		P
Parameter	Pre-menopausal	Post-menopausal	Menopause
Systolic peaks			
Twist (deg)	18.1 (3.6)	15.8 (5.2)	0.15
Torsion (deg/cm)	2.5 (0.5)	2.1 (0.6)	**0.07**
Twisting vel (deg/s)	98 (13)	86 (17)	**0.03**
LV base			
Rotation (deg)	-6.2 (3.2)	-5.1 (2.9)	0.32
Rotational vel (deg/s)	-51 (16)	-47 (15)	0.43
Circ strain (%)	-19 (4)	-17 (4)	0.33
Circ strain rate (1/s)	-1.0 (0.2)	-1.0 (0.2)	0.19
LV apex			
Rotation (deg)	12.3 (3.6)	11.4 (4.0)	0.53
Rotational vel (deg/s)	55 (14)	51 (14)	0.39
Circ strain (%)	-21 (3)	-19 (4)	0.13
Circ strain rate (1/s)	-1.1 (0.2)	-1.0 (0.2)	**0.05**
Diastolic peaks			
Untwisting vel (deg/s)	-98 (26)	-89 (29)	0.37
Time to untwisting vel (%)	108 (6)	109 (7)	0.80
LV base			
Rotational vel (deg/s)	54 (14)	46 (17)	0.17
Time to rotational vel (%)	105 (5)	104 (8)	0.82
Circ strain rate (1/s)	1.5 (0.4)	1.4 (0.5)	0.58
LV apex			
Rotational vel (deg/s)	-60 (24)	-57 (21)	0.67
Time to rotational vel (%)	114 (11)	112 (9)	0.56
Circ strain rate (1/s)	1.8 (0.6)	1.4 (0.3)	**0.01**

Vel: velocity. Circ: circumferential. Time to peak untwisting vel and rotational vel in % diastole, where the time at end-systole is defined as 100% and end-diastole is 200%. T-tests with $P < 0.1$ are in **bold**.

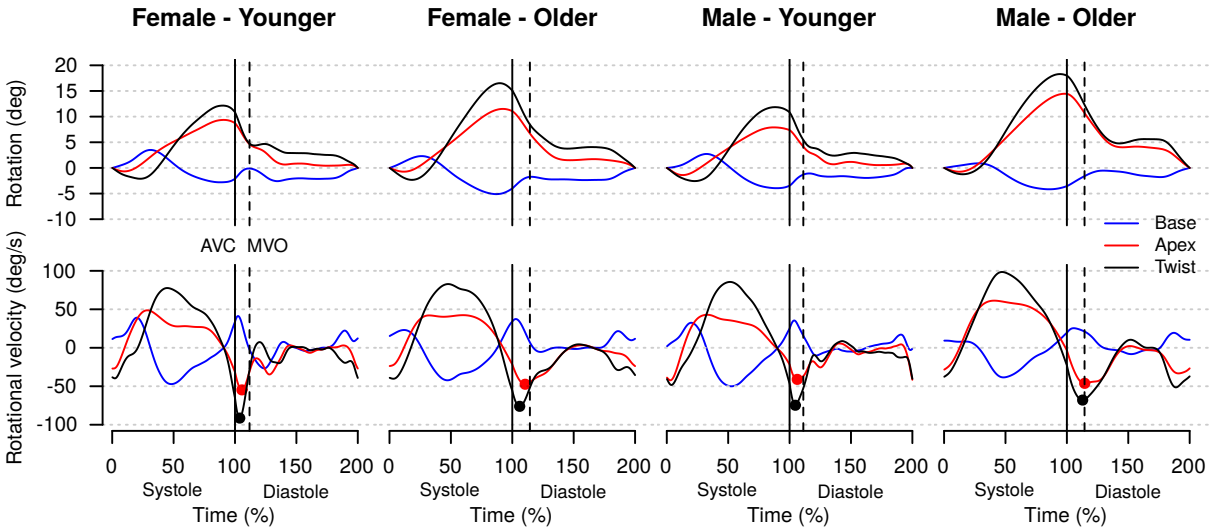

FIGURE 1: Interpolated rotation (top) and rotational velocity (bottom) curves at the base (blue) and apex (red), and the resultant twist/twisting velocity (black) across the cardiac cycle. Time at end-systole is defined as 100%, and end-diastole is 200%. AVC: aortic valve closure (solid vertical line). MVO: mitral valve opening (dashed vertical line). • peak untwisting velocity. • peak apical rotational velocity during diastole.

TABLE S1: Demographics and aerobic capacity of young adult and middle-aged (older) men and women.

	Female		Male		*P*		
Parameter	Younger	Older	Younger	Older	Sex	Age	Sex × Age
Age (years)	23 (4)	52 (4)	24 (4)	52 (4)	0.60	**<0.01**	0.70
Height (cm)	165.9 (5.7)	163.5 (5.8)	179.0 (6.6)	178.4 (7.8)	**<0.01**	0.36	0.58
Body mass (kg)	65.9 (9.1)	64.0 (9.2)	81.0 (8.5)	83.2 (12.6)	**<0.01**	0.93	0.39
Body fat (%)	30 (4)	34 (5)	17 (5)	24 (4)	**<0.01**	**<0.01**	0.24
FFM (kg)	45.9 (5.8)	41.8 (5.0)	66.7 (6.6)	63.2 (8.9)	**<0.01**	**0.02**	0.87
Upright peak power test							
W_{peak} (W)	191 (34)	146 (26)	297 (31)	254 (46)	**<0.01**	**<0.01**	0.85
$\dot{V}O_{2peak}$ (mL/min/kg)	36 (6)	29 (5)	44 (7)	36 (8)	**<0.01**	**<0.01**	0.97
Predicted $\dot{V}O_{2max}$ (mL/min/kg)	39 (3)	28 (3)	48 (2)	36 (4)	**<0.01**	**<0.01**	0.53
HR_{max} (beats/min)	181 (8)	169 (11)	181 (5)	166 (9)	0.47	**<0.01**	0.47
Test duration (min)	8.45 (1.16)	8.11 (1.21)	8.64 (0.75)	8.83 (1.14)	**0.08**	0.77	0.30

Values are in mean (SD). FFM: fat-free mass. W_{peak}: Peak power output. $\dot{V}O_{2peak}$: Peak oxygen uptake. Predicted $\dot{V}O_{2max}$: Maximal oxygen uptake predicted using the FRIEND equation[31]. HR_{max}: Maximum heart rate. ANOVA effects with $P < 0.1$ (White-adjusted for heteroscedasticity) are in **bold**.

TABLE S2: Demographics and aerobic capacity of middle-aged pre- and post-menopausal women.

	Middle-aged female		*P*
Parameter	Pre-menopausal	Post-menopausal	Menopause
Height (cm)	162.3 (6.8)	164.5 (4.8)	0.27
Body mass (kg)	65.3 (10.5)	63.0 (8.3)	0.49
Body fat (%)	32 (4)	36 (5)	**0.03**
FFM (kg)	43.8 (5.9)	40.1 (3.4)	**0.03**
Upright peak power test			
W_{peak} (W)	150 (27)	142 (25)	0.40
$\dot{V}O_{2peak}$ (mL/min/kg)	29 (4)	29 (5)	0.74
Predicted $\dot{V}O_{2max}$ (mL/min/kg)	29 (3)	27 (3)	0.10
HR_{max} (beats/min)	169 (10)	168 (11)	0.70
Test duration (min)	8.17 (1.11)	8.07 (1.32)	0.81

FFM: fat-free mass. W_{peak}: Peak power output. $\dot{V}O_{2peak}$: Peak oxygen uptake. Predicted $\dot{V}O_{2max}$: Maximal oxygen uptake predicted using the FRIEND equation[31]. HR_{max}: Maximum heart rate. T-tests with $P < 0.1$ are in **bold**.

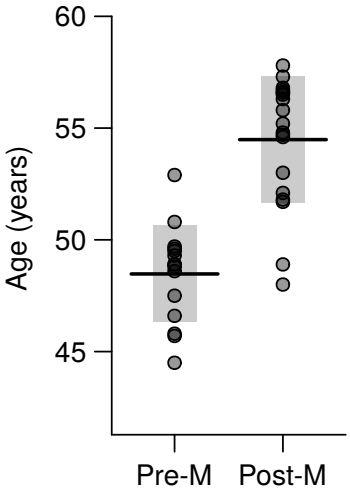

FIGURE S1: Age distribution of pre- (Pre-M) and post-menopausal (Post-M) women. —— Mean and ■ standard deviation within each group.

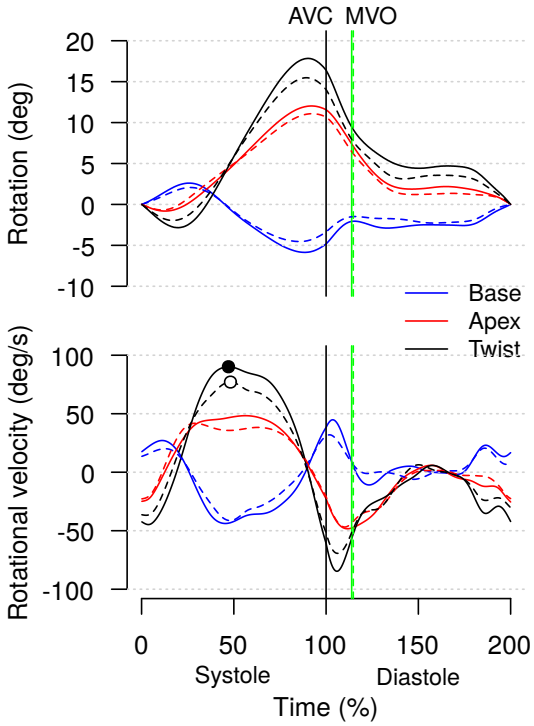

FIGURE S2: Interpolated rotation (top) and rotational velocity (bottom) curves at the base (blue) and apex (red), and the resultant twist/twisting velocity (black) across the cardiac cycle in middle-aged pre- (solid lines) and post-menopausal (dashed lines) women. Time at end-systole is defined as 100%, and end-diastole is 200%. Peak twisting velocity in • pre-menopausal and ○ post-menopausal women. AVC: aortic valve closure (solid black vertical line). MVO: mitral valve opening (green vertical line).