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The Acute Impact of Changes to Haemodynamic Load on the Left Ventricular Strain-Volume Relationship in Young and Older Men.

THE ACUTE IMPACT OF CHANGES TO HAEMODYNAMIC LOAD ON THE LEFT VENTRICULAR STRAIN-VOLUME RELATIONSHIP IN YOUNG AND OLDER MEN

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Short title: ε-volume loop detects afterload increase

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ABSTRACT

Objectives. Chronic changes in left ventricular (LV) haemodynamics, for example induced by increased afterload (i.e., hypertension), mediate changes in LV function. This study examined the proof-of-concept that: *1*. the LV longitudinal strain (ε)-volume loop is sensitive to detect an acute increase in afterload, and *2*. these effects differ between healthy young *versus* older men.

Methods. Thirty-five healthy male volunteers were recruited including 19 young (24±2 yr) and 16 older participants (67±5 yr). Tests were performed prior to, during and after 10-min recovery from acute manipulation of afterload. Real-time haemodynamic data were obtained and LV longitudinal ε-volume loops were calculated from 4-chamber images using 2D-echocardiography.

Results. Inflation of the anti-G-suit resulted in an immediate increase in heart rate, blood pressure and systemic vascular resistance, and a decrease in stroke volume (all $P<0.05$). This was accompanied by a decrease in LV peak ε, slower slope of the ε-volume relationship during early diastole, increase in uncoupling (i.e., compared to systole; little change in ε per volume decline during early diastole and large changes in ε per volume decline during late diastole) (all $P<0.05$). All values returned to baseline levels after recovery (all $P>0.05$). Manipulation of cardiac haemodynamics caused comparable effects in young *versus* older men (all $P>0.05$).

Conclusions. Acute increases in afterload immediately change the diastolic phase of the LV longitudinal ε-volume loop in young and older men. This supports the potency of the LV longitudinal ε-volume loop to provide novel insights into *dynamic* cardiac function in humans *in vivo.*

KEYWORDS: cardiac adaptation, ε-volume loop, haemodynamics, echocardiography, ultrasound

INTRODUCTION

The ability to provide sufficient cardiac output is constantly challenged by beat-to-beat changes in haemodynamic loading (preload, afterload), contractility and heart rate.(8, 15, 23) Acute changes in left ventricular (LV) haemodynamics and heart rate will alter LV mechanics(1, 4, 5, 14, 17, 24), such as LV peak longitudinal strain (ε)(4, 14, 24), circumferential ε (1, 4, 14, 24), and peak torsion(5), and thus LV function. Chronic exposure to such changes can lead to LV remodeling affecting both LV structure and function.(15) However, current measures for LV function, such as LV peak longitudinal ε, LV ejection fraction (LVEF) or mitral flow velocities all provide only partial insights of the effects on LV function.

In a series of studies, we introduced a novel echocardiographic-based method to provide insight into *dynamic* LV function, using non-invasive simultaneous measures of longitudinal ε and volume (i.e. LV longitudinal ε-volume loop) across a cardiac cycle.(10, 11, 13, 18) Concomitant ε-volume analysis provides insight in the relative contribution of longitudinal ε to volume change during systole and diastole, and thus mechanical contribution to changes LV function. We previously observed that a chronic increase in afterload, induced by aortic stenosis, is associated with less coherence between the systolic and diastolic ε-volume relation, indicating a shift in mechanical (i.e. longitudinal ε) contributions to volume change in either systole or diastole. (10) This highlights the ability of the LV longitudinal ε-volume loop to provide novel and potentially clinically relevant insight into both systolic and diastolic dynamic LV function in response to chronic (patho)physiological stimuli affecting haemodynamics in humans *in vivo*. Whether this technique is also able to detect changes in dynamic LV function upon acute changes in afterload and thus contributes to a better understanding of (patho)physiological effects of various stimuli on the heart is currently unknown.

Ageing is known to affect cardiac structure and function(6, 12) and is associated with increased ventricular stiffness(19) as well as reduced ventricular compliance.(7) Ageing is therefore associated with impaired cardiac responses to physiological stimuli such as exercise.(22) Accordingly, older age may influence cardiac responses to acute changes in LV workload. Whether older age alters the shape of the LV longitudinal ε-volume loop or contributes to a different response of the LV longitudinal ε-volume loop to acute changes in LV haemodynamics is yet to be determined.

First, this study explores the impact of an acute increase in LV afterload on the coherence between systolic and diastolic dynamic LV function (i.e. LV longitudinal ε-volume loop) in healthy volunteers (Aim 1). In line with our previous observation of chronic elevation in afterload(10, 11), we expect that an acute increase in afterload results in less coherence between the systolic and diastolic phase of the LV longitudinal ε-volume loop. Second, we examined the hypothesis that participants of older age will show less coherence between the systolic and diastolic phase of the LV longitudinal ε-volume loop at baseline compared to younger participants, whilst this lack of coherence will not affect the ability to detect an acute increase in LV afterload in older participants (Aim 2).

METHODS

Ethics approval

Ethics approval was obtained from the Radboud University Medical Center ethics committee to perform the proposed work (reference number 2016-3166). This study was registered at the Netherlands Trial Register (NTR6349). This study conforms to the standards set by the latest revision of the Declaration of Helsinki.

Study population

Forty-two healthy male participants were recruited within the social environment of the study team and colleagues, including 20 young participants (24±2 yr) and 22 older participants (67±5 yr). All participants received a standardized screening protocol, consisting of measurements of blood pressure, weight, height, 12-lead ECG and a health questionnaire. Participants were excluded in the presence of diagnosed hypertension (or usage of antihypertensive medication), diabetes mellitus, the use of medication which influences cardiovascular function, ECG abnormalities or in case of a history of or presently existing cardiovascular diseases. A medical doctor cleared all patients prior to inclusion, taking into account the likelihood of present hypertension and a history of, or present cardiovascular diseases. Before final inclusion all participants provided written informed consent. Additional data regarding the included study population can be found in Table 1.

Study design

A within-subject design was adopted to explore the impact of changes in LV afterload upon dynamic LV function (Aim 1). In addition, a between-subject design was adopted to explore the impact of age upon dynamic LV function at baseline as well as after acute increase in afterload (Aim 2). Assessment was performed prior to, during and after recovery from an acute increase in afterload. To increase LV afterload, an anti-gravity suit (ANTI-G Garment Cutaway CSU-13B/P; a garment fitted with inflatable bladders, capable of applying pressure on the abdomen and lower extremities) was used.(16) The anti-G suit predominantly increases afterload at inflation pressure >˜70 mmHg. Nonetheless secondary adaptive mechanisms may also simultaneously influence preload and cardiac contractility(16), as often observed when manipulating cardiac haemodynamics.

Due to instability after inflation of the anti-G suit in left-lateral supine position, all measurements were performed supine position. Participants were zipped into the anti-G-suit, placed at an echo table and instructed to relax for approximately 10 minutes to allow the haemodynamic system to adjust to the supine position. Hereafter a series of baseline measurements were performed (Phase 1). Subsequently, the anti-G suit was inflated to a pressure 10 mmHg above systolic blood pressure (SBP). After 1-minute a second series of measurements was performed (Phase 2). The anti-G suit was then cautiously deflated and a second resting period of 10 minutes was applied to allow the haemodynamic system to recover. Subsequently, a third series of measurements was performed (Phase 3) to determine whether haemodynamics and echocardiographic measures normalize after a recovery period and thus relate to inflation of the anti-G suit. The participant was then disconnected from all devices and given the necessary time to recuperate.

Procedures

Central haemodynamics. The Nexfin HD monitor (BMEYE, Amsterdam, The Netherlands) was used to obtain data on the central haemodynamical changes that occurred between the 3 measurement phases. Estimates of blood flow, blood pressure and vascular resistance were obtained, using an inflatable finger cuff connected to the left index finger. The data were stored locally and analysed with MATLAB R2014b (Matworks Inc., Massachusetts, United States). For all 3 phases, periods of 5 seconds (time-matched to the echocardiographic data) were extracted from the Nexfin data. The mean values of these 5 second periods were used to define the heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), systemic vascular resistance (SVR) and an estimate of contractility (dP/dT).

LV volumes and cardiac output. Echocardiographic data were obtained using a Vivid E9 ultrasound machine (GE Medical System, Horton, Norway) with a 1.5-4 MHZ phased array transducer. The data were stored in raw DICOM format in a local archive of the Department of Physiology at the Radboud University Medical Center (Nijmegen). Echocardiographic images were acquired in accordance with the recommendations of the American Society of Echocardiography (ASE) by a single experienced researcher from the RadboudUMC (Nijmegen, the Netherlands) with the patient in the supine position. Standard apical 4, 2 and 3 chamber views were collected as well as a flow velocity measurement in the aortic outflow tract (used to determine valve closure time). Images were optimized using gain, compression and reject to ensure adequate endocardial delineation. Frame-rates were standardized at 56 fps and a focal zone was positioned at mid-cavity to reduce the impact of beam divergence.

Data were analysed using commercially available software (EchoPac version 113.05, GE Medical, Horten, Norway). LV end diastolic volume (LVEDV), LV end systolic volume (LVESV), LV stroke volume (LVSV) and LV ejection fraction (LVEF) were calculated using Simpson's biplane method utilizing both apical four and two chamber orientations.

Dynamic LV function. For the LV longitudinal ε-volume loops an apical four chamber view was used to assess simultaneous LV longitudinal ε and volume. Myocardial ε and volume were assessed offline using the apical 4 chamber view and dedicated software (EchoPac V113.05, GE Healthcare, Horton, Norway). A region of interest (ROI) was placed from the basal septum to the basal lateral wall of the LV enclosing the myocardium. The ROI was divided in six myocardial segments, providing segmental and global longitudinal ε. Global longitudinal ε was used for subsequent analysis.

Temporal global longitudinal ε values were exported to a spreadsheet (Excel, Microsoft Corp, Washington, US). Global temporal longitudinal ε values were divided in 300 ε values for systole and 300 ε values for diastole by cubic spline interpolation. For both systole and diastole, these 300 ε values were split into 5% increments of the cardiac cycle providing longitudinal ε values at 11 time points in systole and 11 time points in diastole. Concomitant time points for the ε values were used in the same image and cardiac cycle to trace monoplane LV volumes to provide simultaneous ε and volume values. For each measurement phase a LV longitudinal ε-volume loop was created for each participant after which a mean LV longitudinal ε-volume loops for each phase was calculated.

Using the individual LV longitudinal ε-volume loops a linear regression line and a polynomial of two orders were applied to both diastolic and systolic parts of the loop. This derived polynomial equation allowed the derivation of ε-values per % increment of LVEDV (i.e. a value for ε at 100% of LVEDV, at 99% of LVEDV etc.), within the working range of the heart. The longitudinal ε-volume relationship was assessed by *1)* linear slope of systolic ε-volume relation during the first 5% of volume change (ESslope), *2)* linear slope of ε-volume relation during systole (Sslope), *3)* End-systolic peak ε (peak ε), *4)* Diastolic uncoupling (i.e. difference systolic vs diastolic ε at the same volume), during early filling (UNCOUP_ED), *5)* during atrial contraction (UNCOUP_LD), *6)* during the entire cardiac cycle (UNCOUP) *7)* linear slope of diastolic ε-volume relation during the first 5% of volume change (EDslope) and *8)* linear slope of diastolic ε-volume relation during the last 5% of volume change (LDslope). Parameters 2 till 5 were collected as previously described.(10) Parameter 1, 7 and 8 were calculated as the gradient of the linear regression line, over the by polynomial equation derived ε-values and matching volumes for the first or last 5% of volume change within the working range of the heart, providing insight in the contribution of early and late contraction and or relaxation to

volume change. Parameter 6 was calculated as the mean difference between all values for systolic and diastolic ε contribution at a certain % of LVEDV within the working range of the heart, presenting a normalized estimation of the area between the curves (Figure 1, adapted from(10)). Previously collected data on the intra-user variability of the loop characteristics presented good to excellent (0.737-0.950) intraclass correlations for our loop characteristics.(10)

Statistical analysis

Data for each time point is expressed as mean ± standard deviation. Normality of data distribution was examined using a Kolmogorov-Smirnov test. In case of non-Gaussian distribution, log-transformation was applied after which the data were re-examined. A two-way repeated measures ANOVA (IBM SPSS statistics version 23) was used to assess the effect of an afterload increase on the LV longitudinal ε-volume loop (time: baseline *versus* anti-G suit *versus* recovery) and whether this effect differed between young and older participants (time*age). In case of significant differences across time, a Bonferonni Post Hoc analysis was applied to establish differences between pairwise time point comparisons. To provide further insight in the temporal changes within the systolic or diastolic ε-volume relation in response to inflation of the anti-G suit, the contribution of ε to volume change during both systole and diastole was assessed by comparison of the ε-values at 10% increments of EDV.

RESULTS

Study population

Five out of the 42 recruited patients were excluded prior to the measurements due to hypertension (n=2), atrial fibrillation (n=2), presence of an old undiagnosed myocardial

infarction (n=1) observed during medical screening. One participant failed to complete the testing session and one participant did not attend, resulting in 35 participants who completed all procedures, consisting of 19 young and 16 older participants.

Aim 1: Acute increase in LV afterload

Central haemodynamics (Nexfin). Immediately after inflation of the anti-G suit an increase in HR, DBP, MAP and SVR was observed (all $P<0.05$, Table 2). No changes in SBP and dP/dT were present (Table 2). Central haemodynamics returned to baseline upon recovery (all $P>0.05$, Table 2).

LV volumes and cardiac output. A small, but significant decrease was present in LVEDV, LVSV and LVEF (all $P<0.05$) immediately after inflation of the anti-G suit, while no changes in LVESV were observed (Table 3). LV characteristics returned to baseline during recovery (all $P>0.05$, Table 3).

Dynamic LV function (ε-volume loop). A marked change in the shape of the LV longitudinal ε-volume loop was present after inflation of the anti-G suit (Figure 2). A significant decrease in peak ε and EDslope was present, whilst a significant increase in UNCOUP_ED, UNCOUP_LD, UNCOUP and LDslope was observed (all $P<0.05$). No changes in Sslope and ESslope were present. (Table 3). All LV longitudinal ε-volume loop parameters returned to baseline during recovery (all $P>0.05$, Table 3). Inflation of the anti-G suit did not change systolic ε contribution to volume change at 90, 80, 70, 60 and 50% of EDV (all $P>0.05$), whilst a significant decrease was found in diastolic ε contribution to volume change at 90, 80, 70 and 60% of EDV after inflation of the Anti-G suit (all $P<0.05$, Table 4).

Aim 2: Effects of age on acute increase in afterload

Central haemodynamics (Nexfin). At baseline, older participant presented with higher SBP, DBP, MAP and SVR (Table 2). Acute manipulation of the afterload induced comparable changes in central haemodynamics compared to young subjects, except for a significantly larger increase in DBP in older participants compared to younger participants (Table 2).

LV volumes and cardiac output. No significant differences in echocardiographic measures of LV structure or function were present at baseline (Table 3). Older participants showed a comparable decrease in measures of LV volumes and cardiac output in response to inflation of the anti-G-suit compared to their younger peers (Table 3, Figure 3).

Dynamic LV function (ε-volume loop). At baseline, older participants presented less coherence between the systolic and diastolic ε-volume relation, and showed significantly higher values for UNCOUP_ED, UNCOUP_LD and UNCOUP as well as LDslope (Table 3). Older subjects also demonstrated a smaller change in diastolic ε contribution to volume change at 90, 80, 70, 60 and 50% of EDV compared to young subjects (Table 4). No significant differences were found between young and older subjects in the changes in dynamic LV function upon inflation of the anti-G suit (Table 3+4, figure 4A and 4B).

DISCUSSION

We present the following novel findings; (1) An acute increase in LV afterload resulted in uncoupling between the systolic and diastolic parts LV longitudinal ε-volume loop, (2) despite a decrease in peak ε, no further changes in the systolic ε-volume relationship were observed, while a shift of the diastolic ε-volume relationship was present (for any given volume, the longitudinal length is shorter during diastole than in systole), highlighting the importance of examining diastolic characteristics, (3) older participants presented with greater uncoupling of the systolic and diastolic ε-volume relation at baseline compared to their younger peers, whilst

no differences were found between groups for any of the traditional measures for LV volumes and cardiac output, and (4) acute manipulation of LV afterload in older men resulted in similar effects on the LV longitudinal ε-volume loop compared to young healthy males. Taken together, these data indicate that an acute increase in LV afterload leads to changes in diastolic LV function that can be detected using the LV longitudinal ε-volume loop, with younger and older humans demonstrating similar changes in LV longitudinal ε-volume loop characteristics in response to acute increases in LV afterload. This highlights the potency of the LV longitudinal ε-volume loop in providing additional information on the effect of (patho)physiological stimuli on dynamic LV function.

Effects of anti-G suit inflation on the systolic ε-volume relation

After inflation of the anti-G suit we observed an increase in MAP and SVR, findings which can be expected in the presence of an increase in afterload. In line with the findings of Donal *et al*.(4), who reported a small decrease in peak ε with an acute increase in LV afterload, we confirmed the presence of a small decrease in peak ε after inflation of the Anti-G suit. Despite the reduction in peak ε, we found no change in the magnitude of LV longitudinal shortening per 10% increment of LV volume change (Table 4). This indicates that an immediate increase in LV afterload does not importantly change the temporal relation between LV volume and longitudinal ε during systole.

Effects of anti-G suit inflation on the diastolic ε-volume relation

In contrast to the systolic phase, the diastolic phase of the ε-volume loop presented marked changes in response to changes in central haemodynamics. First, under unloaded conditions, early diastolic rapid LV relaxation creates an atrioventricular pressure gradient across the mitral valve, causing the LV to suck blood from the left atrium (LA) to the LV (i.e. early filling).(9)

During early filling, LV longitudinal strain relaxation dominates volume displacement, leading to a relatively large decrease in strain for a given decline in LV volume. After the early filling, when the atrioventricular pressure gradient approaches 0 mmHg, the LA contracts to increase atrial pressure and push blood from the LA to the LV (i.e. atrial filling). During this phase, volume displacement by atrial contraction dominates LV longitudinal relaxation. Consequently, the diastolic relation between LV longitudinal ε-volume crosses the systolic relation between LV longitudinal ε-volume, as presented in Figure 2. Our study adds the novel observation that inflation of the Anti-G-suit caused a rightward shift of the diastolic ε-volume relation. This shift suggests a more dominant role for atrial volume displacement compared to LV longitudinal relaxation during LV filling. Indeed, an attenuated EDslope and smaller change in longitudinal ε value across a 10% increment of EDV (Table 4) confirms the reduced contribution of LV longitudinal deformation to LV filling upon an increase in LV afterload, which may explain the observed slightly reduced LVEDV. A possible explanation for these characteristic changes in the diastolic relation between LV longitudinal ε-volume during elevation in afterload is an alteration or delay in LV untwist.(2) Dong *et al.* showed that LV torsion is impaired under the influence of an increase in afterload.(5) Impairment of LV torsion results in less twist and energy storage in the myocardial fibers during the (systolic) ejection phase and, consequently, less energy release during early diastole (i.e. recoil) to facilitate LV suction and thus filling.(2, 21) Taken together, our results indicate that an increase in afterload leads to a characteristic shift in the LV longitudinal ε-volume loop, especially during diastole, that may contribute to less suction and less effective filling of the ventricle. Assessing LV longitudinal ε-volume loop characteristics alongside other ventricular mechanics could potentially provide complemental data and should be subject of future studies.

Effects of age

Ageing is related to an increase in arterial stiffness causing an increase in systemic blood pressure and LV afterload.(3) In line with this we observed higher blood pressure and SVR at baseline in our older participants compared to younger participants. We did not observe any age-related differences in the characteristics of the systolic phase of the LV longitudinal ε-volume loop or contribution of ε to volume change (Table 4). However, young and older subjects demonstrate differences in the diastolic phase of the LV longitudinal ε-volume loop. Specifically, older participants showed a rightward shift of the diastolic ε-volume relation. This rightward shift may reflect an age-related reduction in ventricular compliance, caused by an increase in LV stiffness. Using invasive cardiac catheterisation Fujimoto *et al.*(7) observed preserved systolic LV function in a healthy ageing population, but steepening of the diastolic pressure–volume relationship in healthy older participants (i.e. reduction in ventricular compliance). These findings support the presence of age-related differences in the LV longitudinal ε-volume loop, with specific changes in diastolic LV function.

After inflation of the Anti-G suit, both young and older participants showed a similar increase in uncoupling with comparable declines in LVEDV and SV. This indicates that, although the longitudinal structure-function relationship in older participants is altered under resting conditions, an increase in LV afterload leads to a characteristic change in dynamic cardiac function, especially visible during diastole. This similarity in responsiveness between young and older participants may partially be explained by the inclusion of healthy older individuals, who demonstrated no structural or functional impairment of the heart. Our findings cannot be simply extrapolated to subjects with impaired cardiac function and/or presence of cardiovascular disease.

Limitations. Due to the nature of our intervention, we were not able to perform a full

echocardiographic assessment at all time points. We chose to collect images that were important for our ε-volume loop analysis. As a result, we were unable to determine all standard diastolic echocardiographic parameters, including twist or torsion parameters. In addition, current ε-volume loop analysis has been performed on 2D-4CH images, using monoplane derived volumes. Due to temporal differences between the 4CH and 2CH images, bi-plane volumes cannot be derived for this purpose. Optimizing current techniques through Tri-plane or 3D-imaging is warranted. Another limitation is that besides an increase in afterload the anti-G suit also affects preload and cardiac contractility(16) in order to adapt to the inflated anti-G garments and increased afterload. We indeed observed a slight decrease in LVEDV, suggesting presence of a decline in preload. The effects of a reduction in preload on the ε-volume loop have been previously examined by Schneider *et al*., who observed a decrease in peak ε, but no changes in the shape of the LV longitudinal ε-volume loop.(20) This means that the changes in the LV longitudinal ε-volume loop in our study can be primarily attributed to the alterations in LV afterload. Finally, in current study an normalized estimation of the area between the curves was utilized to measure the coherence between the systolic and diastolic phase of the LV strain-volume loop. Automatizing the current method should allow for calculating a more global area between the curve value and should be subject of future studies.

In conclusion, our findings indicate that an acute increase in LV afterload may lead to immediate and afterload-dependent changes within the diastolic phase of the LV longitudinal ε-volume loop, and thus less coherence between systolic and diastolic phase of the LV longitudinal ε-volume loop. Although older participants demonstrated an age-related decline in longitudinal relaxation at rest, an acute increase in LV afterload results in similar changes in coherence of the LV longitudinal ε-volume loop characteristics as in younger participants. The potential clinical implication of our observations is that the LV longitudinal ε-volume loop may

provide novel insight into dynamic cardiac function, which may help to understand and explain the impact of (patho)physiological processes on the heart *in vivo* in humans.

Perspectives and Significance

With this study we provide novel insights into our understanding of the influence of haemodynamical changes on systolic and diastolic cardiac function by demonstrating that changes in afterload lead to suppression of diastolic relaxation. The ability of our (novel) technique to simultaneously assess systolic and diastolic ventricular function in a non-invasive way provides major advantages over current techniques and provide a novel quick method to assess overall ventricular function in the near future.

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Disclosures

None

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Figure 1 - A schematic view of the methods used to assess the ε-volume loops. The black line represents the ε-volume loop, the thick part represents the systolic phase and the thin line the diastolic phase. We assessed the ε-volume loop by a) ε-volume relation at the first 5% of systole (i.e. ESslope; red dotted line), b) ε-volume relation across the systolic phase (i.e. Sslope, orange dashed line), c) peak ε at end-systole (i.e. peak ε, purple arrow), d) difference in systolic vs diastolic ε during early filling (i.e. UNCOUP_ED, pink arrow), e) difference in systolic vs diastolic ε during atrial contraction (i.e. UNCOUP_LD, dark blue arrow), f) difference in systolic vs diastolic ε during the entire cardiac cycle (ie UNCOUP, light blue area), g) ε-volume relation at the first 5% of diastole (i.e. EDslope, yellow dashed line) and h) ε-volume relation at the last 5% of diastole (i.e. ESslope, green dashed line).

Figure 2 – Mean ε-volume loops prior to and after acute manipulation of afterload. Data represents mean longitudinal ε-volume loops over the entire study population (n=35) at baseline (black lines) and after an increase in afterload (filled grey lines). The thick lines represent the systolic ε-volume relation, whilst the thin lines represent the diastolic ε-volume relation.

Figure 3 – Overview of the influence of an acute afterload manipulation and age on the most important haemodynamic, functional and ε-volume loop parameters. Data represents the mean ± SEM values for the measurements of LV haemodynamics (i.e. MAP), ε-volume loop characteristics (i.e. UNCOUP) and LV function (i.e. LVEF) at baseline, after acute manipulation of afterload and after a recovery period. The white bars represent young participants while the black bars represent older participants.

Figure 4 - Mean ε-volume loops prior to and after acute manipulation of afterload in young and older participants. Data represents mean longitudinal ε-volume loops in A) young

participants (24±2 years of age, n=19) and B) older participants (67±5 years of age, n=16). The solid black lines represent the ε-volume loop at baseline, whilst the grey lines represent the ε-volume loop after an increase in afterload. The thick lines represent the systolic ε-volume relation, whilst the thin lines represent the diastolic ε-volume relation.

Table 1 – Population Characteristics

Characteristics	**Young**	**Older**	P-value
Height (cm)	182±6	180±6	0.28
Weight (kg)	82±12	85±11	0.46
SBP (mmHg)	132±13	144±13	**0.01**
DBP (mmHg)	80±8	85±6	**0.04**
HR (BPM)	68±13	70±12	0.66

Baseline group characteristics as measured manually during the screening procedure.
SBP=Systolic Blood Pressure; DBP=Diastolic Blood Pressure; HR=Heart Rate.

Table 2 – Data represents the mean±SD of central haemodynamic measurements at baseline, after an increase of the afterload and after an recovery period in a group of young participants and older participants.

Characteristics		**Baseline**	**Anti-G suit**	**Recovery**	**Intervention**	**Group**	**Intervention*Group**
Central haemodynamics (n=33)							
HR (bpm)	*Young*	62±14	66±17	62±14	**<0.01***	0.80	0.55
	Older	62±10	65±11	60±9			
SBP (mmHg)	*Young*	133±16	138±16	133±15	0.08	**0.02**	0.69
	Older	146±30	157±25	150±22			
DBP (mmHg)	*Young*	73±9	81±11	74±9	**<0.01***	**<0.01**	**0.02**
	Older	80±13	99±13	85±10			
MAP (mmHg)	*Young*	93±12	99±12	94±12	**<0.01***	**<0.01**	0.07
	Older	105±16	121±15	109±12			
SVR (dyn•s/cm^5)	*Young*	1026±225	1123±192	1055±239	**0.04**	**<0.01**	0.22
	Older	1907±788	2366±914	2145±645			
dP/dT (mmHg/s)	*Young*	1187±262	1158±287	1210±284	0.72	0.98	0.22
	Older	1216±710	1263±791	1117±616			

Symbols denote P<0.05 between ANTI-G-Suit vs. Baseline and Recovery=*, all 3 Phases=† and Baseline vs. ANTI-G-Suit=‡. HR=Heart Rate; SBP=Systolic Blood Pressure; DBP=Diastolic Blood Pressure; MAP=Mean Arterial Pressure; SVR=Systemic Vascular Resistance; dP/dT=Delta Pressure/Delta Time;

Table 3 – Data represents the mean±SD of echocardiographic measurements at baseline, after an increase of the afterload and after an recovery period in a group of young participants and older participants.

Dynamic *LV function (ε-volume loop)*		**Baseline**	**Anti-G suit**	**Recovery**	**Intervention**	**Group**	**Intervention*Group**
ESslope (%/ml)	*Young*	0.31±0.12	0.31±0.11	0.31±0.16	0.40	0.59	0.44
	Older	0.37±0.15	0.31±0.16	0.31±0.17			
Sslope (%/ml)	*Young*	0.35±0.08	0.34±0.07	0.36±0.09	0.24	0.18	0.59
	Older	0.40±0.11	0.38±0.09	0.39±0.16			
Peak ε (%)	*Young*	-20.3±2.4	-19.0±2.2	-20.3±2.6	**<0.01**†	0.18	0.06
	Older	-22.0±2.2	-19.9±2.2	-20.9±2.8			
UNCOUP_ED (AU)	*Young*	-1.32±1.38	0.68±1.54	-0.45±1.79	**<0.01***	**<0.01**	0.68
	Older	-0.23±1.42	2.31±1.35	0.55±1.69			
UNCOUP_LD (AU)	*Young*	-0.32±1.48	1.09±1.65	0.66±1.73	**<0.01**‡	**<0.01**	0.34
	Older	0.91±1.75	2.64±1.34	1.20±1.32			
UNCOUP (AU)	*Young*	-0.90±1.13	0.82±1.53	-0.08±1.70	**<0.01***	**<0.01**	0.32
	Older	0.15±1.45	2.51±1.44	0.56±1.15			
EDslope (%/ml)	*Young*	0.50±0.14	0.30±0.11	0.47±0.20	**<0.01***	0.12	0.68
	Older	0.43±0.15	0.21±0.10	0.43±0.23			
LDslope (%/ml)	*Young*	0.15±0.09	0.33±0.12	0.19±0.16	**<0.01***	**<0.01**	0.54
	Older	0.31±0.13	0.47±0.17	0.29±0.12			
LV volumes and cardiac output (n=30)							
LVEDV (ml)	*Young*	113±18	107±12	109±14	**0.02**	0.15	0.70
	Older	102±15	96±23	101±24			
LVESV (ml)	*Young*	47±9	47±7	47±8	0.81	0.26	0.70
	Older	43±13	42±11	44±13			
LVSV (ml)	*Young*	65±12	60±7	63±7	**<0.01***	0.11	0.74
	Older	58±14	54±14	58±12			
LVEF (%)	*Young*	58±4	56±4	57±4	**0.05**‡	0.98	0.84
	Older	58±4	56±4	57±4			

Symbols denote P<0.05 between ANTI-G-Suit vs. Baseline and Recovery=*, all 3 Phases=† and Baseline vs. ANTI-G-Suit=‡. LVEDV= Left Ventricular End Diastolic Volume; LVESV=Left Ventricular End Systolic Volume; LVEF=Left Ventricular Ejection Fraction

Table 4 – ε-values per 10% increment in volume during systole and diastole

Characteristics		Baseline	Anti-G-suit	Recovery	Intervention	Group	Intervention*Group
Dynamic LV function (n=35)							
Systolic ε at 90% EDV	*Young*	-3.58±1.29	-3.67±1.12	-3.31±1.71	0.50	0.64	0.77
	Older	-3.67±1.47	-3.30±1.12	-3.18±1.59			
Systolic ε at 80% EDV	*Young*	-7.29±1.70	-7.34±1.36	-6.99±2.15	0.33	0.88	0.52
	Older	-7.93±2.24	-6.96±1.86	-6.91±2.31			
Systolic ε at 70% EDV	*Young*	-11.19±1.92	-11.18±1.45	-10.98±2.39	0.26	0.54	0.38
	Older	-12.30±2.72	-10.94±2.23	-10.99±2.66			
Systolic ε at 60% EDV	*Young*	-15.28±2.02	15.21±1.70	-15.23±2.52	0.24	0.29	0.30
	Older	-16.77±2.89	-15.24±2.30	-15.45±2.75			
Systolic ε at 50% EDV	*Young*	-19.56±2.29	-19.42±2.50	-19.78±2.89	0.31	0.18	0.36
	Older	-21.35±2.90	-19.86±2.32	-20.27±2.95			
Diastolic ε at 90% EDV	*Young*	-3.17±1.29	-4.88±1.65	-3.74±1.89	**<0.01***	**<0.01**	0.70
	Older	-4.42±1.35	-6.17±1.42	-4.51±1.41			
Diastolic ε at 80% EDV	*Young*	-5.98±1.55	-8.55±2.00	-6.69±2.52	**<0.01***	**<0.01**	0.57
	Older	-8.22±1.68	-10.44±1.89	-8.04±1.61			
Diastolic ε at 70% EDV	*Young*	-9.63±1.84	-12.17±2.04	-10.29±2.73	**<0.01***	**<0.01**	0.42
	Older	-12.30±1.90	-14.09±2.18	-11.79±1.91			
Diastolic ε at 60% EDV	*Young*	-14.12±2.36	-15.73±1.81	-14.55±2.74	**0.02***	**<0.01**	0.32
	Older	-16.64±2.16	-17.14±2.27	-15.76±2.40			
Diastolic ε at 50% EDV	*Young*	-19.46±3.35	-19.23±1.65	-19.46±3.11	0.24	**<0.01**	0.37
	Older	-21.25±2.83	-19.58±2.36	-19.94±3.27			

Symbols denote P<0.05 between ANTI-G-Suit vs. Baseline and Recovery=*. EDV=End diastolic volume