

Heart Strain During Different Loading Conditions

in Health and Cardiac Illness

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Till min underbara fru

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Cover image: Strain curves of the left atrium and left ventricle during the same cardiac cycle, obtained from the author’s heart.

ABSTRACT

Circulatory dysfunction and shock in critical illness has a high mortality. Hypovolemia can contribute to inadequate cardiac output in critical illness. One clinical challenge is that hypovolemia can be difficult to identify. Many hypotensive critically ill patients are hypovolemic, thus need fluid resuscitation, though not all hypotensive patients are hypovolemic. Fluid management is thought to influence patient outcome in critical illness. There is a very common clinical diagnostic dilemma: does the critically ill patient in question need additional fluid administration or have enough or too much already been given. Cardiac 2-dimensional strain offers new parameters for assessment of chamber mechanical function and possibly of left ventricular filling. These novel measurements could be used in the assessment of hypovolemia and cardiac function.

Objectives

The aim of this thesis is assessment of load dependence of left heart chamber wall strain and the interrelation between left atrial and left ventricular strain, with a particular focus on left atrial contraction strain, in healthy individuals and in patients with cardiac illness (cardiac amyloidosis).

Methods

Papers 1 and 2 were conducted in a cohort of healthy volunteers to evaluate the effects of varying loading conditions and sympathetic activation on left atrial contraction strain. Changes in preload were induced through controlled alterations of airway pressure using a CPAP manoeuvre to decrease preload, a passive leg raise to increase preload, and by a Valsalva manoeuvre to decrease preload with a simultaneous increase in sympathetic tone. Paper 3 comprised a retrospective analysis of a study-cohort with cardiac amyloidosis, in which a passive leg raise was used to assess load-dependent changes with a particular focus on left atrial contraction strain. Paper 4 examined the relationship between left atrial and ventricular strain in relation to preload. Atrial and ventricular strain were measured within the same cardiac cycle, and linear regression analysis was used to assess a left atrial/left ventricular strain curve, describing their interdependence under varying loading conditions.

Results

Across the four papers included in this thesis, acute alterations in preload did not result in measurable changes in left atrial contraction strain. This finding was consistent across different preload-modifying interventions, including passive leg raise, CPAP, the Valsalva manoeuvre; and this was observed in both healthy

individuals and patients with cardiac amyloidosis. Furthermore, the relationship between left atrial and left ventricular strain during the atrial contraction phase was not affected by changes in preload.

Conclusion

In this thesis, left atrial contraction strain demonstrated stability in response to acute preload alterations in both healthy individuals of different ages and patients with cardiac amyloidosis. These findings support the concept that left atrial contraction strain is largely preload independent within clinically relevant loading ranges and therefore may serve as a robust marker of intrinsic left atrial contractile function.

ENKEL SAMMANFATTNING PÅ SVENSKA

Cirkulationssvikt och chock hos svårt sjuka patienter är ett tillstånd som har en hög dödlighet. En bidragande orsak kan vara en för liten cirkulerande blodvolym. Med tillgängliga metoder kan det vara svårt att bedöma om en patient har en för liten blodvolym eller inte. Behandling med intravenös vätska är av stort värde, men både för lite och för mycket vätska kan vara skadligt. Detta skapar ett vanligt kliniskt dilemma: behöver patienten med chock mer vätska, eller har patienten redan fått tillräckligt – eller kanske för mycket?

Nya ultraljudsmetoder för att undersöka hjärtat har förbättrat möjligheterna att bedöma hjärtats funktion. En sådan metod är analys av hjärtmuskelnens rörelse under ett hjärtslag, så kallad cardiac strain. Tekniken gör det möjligt att mer detaljerat studera hur hjärtats väggar rör sig och deformeras under hjärtats arbete, och kan därmed bidra till en mer noggrann bedömning av både hjärtfunktion och blodvolym.

Syftet med denna avhandling var att undersöka hur väggrörelsen (strain) i hjärtats vänstra förmak och vänstra kammare påverkas av förändringar i hjärtats fyllnad (mängden blodvolym). Särskilt fokus låg på rörelsen i vänster förmak under dess kontraktionsfas. Studierna genomfördes både på friska personer och på personer med hjärtamyloidos (en sjukdom där proteiner lagras in i hjärtvävnaden och gör hjärtmuskeln stelare).

För att framkalla förändringar i hjärtats fyllnad användes olika fysiologiska manövrar:

- Lyft av benen då personen ligger på rygg vilket får mängden blod som återvänder till hjärtat att öka.
- Ta ett långt djupt andetag med ett kontinuerligt övertryck (via en andningsmask) vilket minskar mängden blod i hjärtat
- Andas ut kraftigt mot motstånd, en så kallad Valsalva-manöver vilket också minskar mängden blod i hjärtat samtidigt som pulsen stiger och blodtrycket sjunker.

Ultraljudsundersökningar av hjärtat utfördes både före och under dessa manövrar.

Resultaten visade att väggrörelsen i vänster förmak under dess kontraktionsfas inte påverkades av förändringar i hjärtats fyllnad. Detta gällde oavsett vilken metod som användes för att förändra fyllnaden och sågs både hos friska personer och hos patienter med hjärtamyloidos. Inte heller sambandet mellan väggrörelsen i vänster förmak och vänster kammare under förmakets kontraktionsfas påverkades av förändringar i fyllnaden.

Sammanfattningsvis tyder resultaten på att väggrörelsen i vänster förmak under dess kontraktionsfas i stor utsträckning speglar förmakets egen kontraktila funktion, snarare än förändringar i hjärtats fyllnad eller blodvolymen. Metoden kan därför vara en robust markör för vänster förmaks funktion och potentiellt ett värdefullt verktyg vid klinisk bedömning av hjärtfunktion.

PAPERS INCLUDED IN THE THESIS

Gottfridsson, P., et al., Left atrial contraction strain and controlled preload alterations, a study in healthy individuals. *Cardiovasc Ultrasound*, 2022. 20(1): p. 8.

Gottfridsson, P., et al., Left atrial contraction strain during a Valsalva manoeuvre, a study in healthy humans. *Clin Physiol Funct Imaging*, 2022.

Gottfridsson, P., et al., Left atrial contraction strain during a passive leg raise in cardiac amyloidosis, a retrospective cohort analysis. Submitted to peer reviewed journal.

Gottfridsson, P, et al., Preload change affects left atrial and left ventricular strain relation, a study in healthy individuals. Submitted to peer reviewed journal.

ABBREVIATIONS

2D	Two-dimensional
A	Late trans mitral maximum flow velocity
a	Late diastolic maximal lateral mitral annular tissue velocity with atrial contraction
ATTR	The inherited and acquired transthyretin protein form of amyloidosis
AVS-slope	Left atrial and left ventricular strain curve
BMI	Body Mass Index
CA	Cardiac amyloidosis
CPAP	Continuous positive airway pressure
dB	Decibel
E	Early trans mitral maximum flow velocity
e	Early diastolic maximal lateral mitral annular tissue velocity
HFpEF	Heart failure with preserved ejection fraction
HFrEF	Heart failure with reduced ejection fraction
HR	Heart rate
LA	Left atrium
LAS	Left atrial strain
LASR	Left atrial strain rate
LAScd	Left atrial conduction strain
LASct	Left atrial contraction strain
LASr	Left atrial reservoir strain
LV	Left ventricle
LV EDV	Left ventricular end-diastolic volume
LV GLS	Left ventricular global longitudinal strain
LV GLSR	Left ventricular global longitudinal strain rate
LVOT VTI	Left ventricular outflow tract velocity time integral
MHz	Megahertz
m/s	Meters per second
PLR	Passive leg raise manoeuvre
PLR15	Passive leg raise performed with trunk 15 degrees up and then simultaneously lowering of the trunk, and raising the legs by tilting the bed
PLR45	Passive leg raise performed supine while passively raising the legs to 45 degrees angle
pLASRct	Peak left atrial contraction strain rate
pLASRr	Peak left atrial reservoir strain rate
PVR	Pulmonary vascular resistance
ROI	Region of interest
s	Maximal systolic lateral mitral annular tissue
VM	Valsalva manoeuvre

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PREFACE

Imagine a hypothetical critically ill patient where there has been major abdominal surgery with an intestinal resection, and where the circulation has been suboptimal. There is suspected post-operative peritonitis and concern about viability of the intestinal anastomosis. The patient is hypotensive, has metabolic acidosis, and is oliguric despite aggressive fluid resuscitation. The responsible surgeon is concerned that more fluid administration will further jeopardize a problematical intestinal anastomosis and also may lead to pulmonary oedema. The question arises if the patient should be given more intravenous resuscitation fluid, or if too much already have been given. A passive leg raise is painful for the patient and hence unreliable. An echocardiographic examination is performed, to try to help guide optimization of the resuscitation treatments. Heart chamber sizes and movement are judged by eyeballing as unremarkable, left ventricular ejection fraction is 55% but atrial and ventricular strain measurements show a left ventricular global longitudinal strain of 15%, a left atrial global longitudinal strain of 29% and a left atrial global longitudinal contraction strain of 17%. Are these measurements useful, and how could one interpret the strain data when guiding the fluid management of the patient? Better understanding of this is the central focus of the clinical question for my thesis.

BACKGROUND

Circulatory dysfunction and shock in critical illness has a high mortality [1]. Hypovolemia, defined as a combination of reduced blood volume and suboptimal venous return to the heart can contribute to inadequate cardiac output and delivery of substrates and oxygen to vital organs in critical illness. One clinical challenge is that hypovolemia can be difficult to identify with certainty [2-4]. Many hypotensive critically ill patients can have fluid 'losses' and are hypovolemic, thus need fluid resuscitation, though not all hypotensive patients are hypovolemic. Fluid management is thought to influence patient outcome in critical illness [5-8], although there is conflicting evidence [9-11].

Intravascular fluid administration which leads to hypervolemia can contribute to excessive heart strain and contribute to vital organ oedema formation. Organ oedema can negatively influence the microcirculation, oxygen and other substrate delivery to parenchymal cells, and so potentially also increase morbidity and mortality.

The clinical approach to suspected hypovolemia in critical illness is still often empirical or presumptive, and we lack a solid pathophysiological scientific foundation on which to base the amount of intravascular infusion to give to patients for circulatory support. Hypovolemia needs to be quantified in order to support optimal resuscitation interventions, both with intravascular fluid replacement and with vasoactive drugs.[12] The aim of fluid therapy is to replace fluid losses, or support adequate venous return to the heart, and this often means treating hypovolemia where fluid losses have occurred. The aim of vasoactive (potent vasopressor) drugs is to restore demolished peripheral vascular resistance and restore venous capacitance vessel tone (to promote an increase in venous return to the heart).

Optimal hemodynamic stability and tissue perfusion should be monitored and tailored using a synthesis of thorough clinical examination, integrated interpretation of diagnostic and monitoring information, and laboratory results to support understanding of the pathophysiological derangement. Currently, the circulatory status of many critically ill patients is still not appropriately assessed and monitored.[13]

In order to identify a patient where additional fluid administration would be beneficial, dynamic methods are recommended over static measurements.[14] This means administering a fluid bolus or performing a passive leg raise (PLR) and comparing the cardiac output (or a surrogate estimate of this) before and after the bolus or PLR. Tools recommended for guiding fluid management are either invasive or non-invasive. [15] Examples of invasive tools are using an arterial catheter to directly assess and analyse the blood pressure curve, a central venous catheter to assess central venous pressure or a with pulmonary artery catheter to assess pulmonary artery occlusion pressure. There are thermodilution flow measurement methods which require both arterial and central venous access. Oesophageal Doppler measuring velocity, which together with an assumption of cross-section area, can estimate flow in aorta. Echocardiography is an example of a non-invasive method.[16]

Echocardiographic measurements have the advantage of being immediately available, non-invasive and inexpensive (once the quite expensive device is acquired). It is easily employed for serial measurements. It can detect cardiac, vascular, and lung pathology even with a non-expert user.[17]. Flow in the aortic outflow tract can be measured, as well as the end-diastolic and end-systolic heart chamber volumes to estimate stroke volume, which together with the heart frequency, allows an estimate of cardiac output. Also, assessment of flow in the portal, hepatic and renal veins can be used to guide fluid management. [18] In patients treated with controlled ventilatory support with large tidal volumes, the variation of vena cava inferior (or vena cava superior) cross-sectional dimensions

seen with ultrasound, assessed during breathing can guide fluid treatment.[19] However, the quality of evidence for guiding fluid management is low, and to calibrate treatment of hypovolemia in critical illness remains a clinical challenge where better bedside assessment tools are needed. [16] There is a very common clinical diagnostic dilemma: does the critically ill patient in question need additional fluid administration, or have enough or even too much already been given.

Heart chamber diastolic and systolic strain, which are a regionalized assessment of change of myocardial fibre length during the different phases of the cardiac cycle, are new parameters for assessment of chamber mechanical function and possibly even of left ventricular filling [20]. These novel measurements could be used in the assessment of hypovolemia and cardiac function.

HEART STRAIN

Cardiac mechanics refers to the relationship between the forces acting on the heart tissue and the resulting deformation of the tissue. Mechanical stress quantifies the intensity of these forces, while mechanical strain describes the extent of deformation of the tissue. Stress is measured in force per unit area, whereas strain is a dimensionless quantity. [21] Strain is used as a term in assessment of cardiac mechanics and in this context, means the amount to which heart muscle fibers shorten or lengthen compared to resting length (the deformation of the heart muscle fibers during different parts of the heart cycle). In ultrasound terms, strain is estimated by following the distance between several selected (in a region of interest in the heart wall) highly acoustically reflective tissue points (speckles) during the heart cycle.

The 3-dimensional movements of the heart muscle are in the longitudinal, radial and circumferential direction. Hence, the strain measurement is referenced as longitudinal, circumferential and radial strain. The longitudinal strain is the most studied [22] and it is presumed that it measures mostly the movement caused by the endocardial layer, which is sensitive to both ischemia and increased wall stress. However, there are multiple layers of myocardial fibers with different directional orientation, helical oriented fibers in the mid-myocardium and in the epi-myocardium (with the helical fibers in the two layers going in opposite directions). There are longitudinally oriented fibers in the endo-myocardium. The movements of the chamber wall during a heart cycle are the result of all these fiber layer movements. [23] The assessment of the wall movements in the different directions is recognized, but these multi-directional aspects are not clinically assessed independently.[24] Cardiac mechanics are influenced by both

intracellular components, particularly in the myocytes, and the extracellular matrix. Titin is large elastic protein in the myocytes that affects the passive mechanical properties of healthy heart tissue and has a function to provide stiffness in order to prevent overextension of the sarcomeres. Variations in titin isoforms can affect stiffness and contribute to diastolic dysfunction. The active contractile force in the myocytes is generated by the interaction of actin and myosin filaments. Myosin activity is regulated by calcium levels, phosphorylation, and sarcomere length (preload stretch). The extracellular matrix is composed mostly of structural proteins (collagen and elastin) that also influence heart mechanics. Excessive collagen deposition, for example due to fibrosis, can reduce heart wall compliance and lead to diastolic dysfunction. [21] The deformation of the myocardium is dependent on factors which influence contractility, ventricular geometry, extrinsic influences on loading conditions, and the elastic properties of the heart tissue mentioned above.

Heart strain, assessed at bedside, can be measured both with tissue Doppler and with speckle tracking analysis. The focus of most published reports is speckle tracking, presumably since it is semiautomatic, angle-independent and less user dependent [25] Where the time of the strain change is measured, it is possible to calculate the speed at which the strain change occurs, and this is called strain rate.

LEFT VENTRICULAR STRAIN

Longitudinal left ventricular strain measurements can provide information of both regional and global ventricular function. Longitudinal strain is more robust when it is used to assess global strain. Compared with other methods for measuring left ventricular (LV) function, global longitudinal strain has been shown in some reports to have a relatively low intra- and inter-observer variability. [26]. Guidelines for measurement of left ventricular global longitudinal strain (LV GLS) has been published. [22] LV GLS is used for providing information in cardiomyopathy, ischemic heart disease, LV dysfunction, and detecting subclinical dysfunction in patients receiving chemotherapy [27], as well as in sepsis [28, 29]. When comparing LV GLS with ventricular ejection fraction, LV GLS has increased sensitivity for systolic dysfunction and for diastolic dysfunction [Heart failure with preserved ejection fraction (HFpEF)], if HFpEF is the only pathology. Also, reduced LV GLS has been linked to mortality [27] and has been shown, in animal models as well as in humans, to be preload-dependent and it needs to be cautiously interpreted. [30-33]

LEFT ATRIAL FUNCTION

The normal or healthy function of the left atrium (LA) is to contribute to (or be a conduit for) LV filling and cardiovascular performance. This is done by accommodating blood arriving from the pulmonary veins during ventricular systole and then to eject blood into the ventricle during diastole. LA function is dependent on preload, afterload and intrinsic contractility, the same as ventricular function. LA function is closely inter-dependent with LV performance throughout both systole and diastole, reflecting the functional continuity between the two chambers. The left atrium and ventricle are mechanically connected through the mitral ring (atrioventricular plane), which undergoes a piston-like displacement during the cardiac cycle. In addition, both chambers are enclosed within the relatively noncompliant pericardium, such that the combined atrial and ventricular volume remains nearly constant throughout the cardiac cycle. This structural and functional coupling underlies the reciprocal interaction between left ventricular and atrial deformation, and this is fundamental to the interpretation when measuring atrial parameters. Atrial function can be divided into three phases: the reservoir phase when the mitral valve is closed, the conduction phase when then mitral valves open until the pressure gradient is equalized and/or the third phase starts with active contraction of the atria. Atrial reservoir phase can be divided into early and late reservoir phase, where the early phase is affected by atrial relaxation and the late phase by left atrial chamber stiffness and systolic shortening of the left ventricle.[34] Conduct phase in early diastole is affected by ventricular diastolic pressures (ventricular relaxation and compliance) and to a lesser degree atrial compliance. The contraction phase is affected by ventricular compliance and end-diastolic pressure as well as intrinsic atrial contractility. [35] The LA may, in pathological conditions, be influenced by increased LV filling pressures which will be in some continuity to LA pressure. LA remodelling has an interrelation with LV remodelling. [36, 37] The LA has an important role for maintaining optimal cardiac output despite impaired LV function. Understanding how each phase of the atrial cycle is affected by ventricular function, and how each atrial phase contributes to maintain an optimal stroke volume in normal and pathological conditions, is of importance when assessing data from LA function.[38]

LEFT ATRIAL STRAIN

When studying atrial mechanics using strain with these methods, atrial strain analysis can be divided into the same phases as mentioned above: reservoir, conduction (or recoil [39]) and contraction strain. Since blood passes through the LA during the conduct phase without causing any correlated deformation in the

LA chamber, this phase is less informative when studying atrial strain. [40] Guidelines for measurement of LA strain (LAS) have been published. [41, 42] LA reservoir strain (LASr) is related to several factors including heart rate, body size, age and in some studies sex [43]. LASr is independently correlated with LV stroke volume, LV GLS, E/A, e' . [35, 44] It has been shown that LASr is affected by afterload, and it can provide indirect information about central vascular pressures [20, 45]. It is suggested that it can be used when assessing LV diastolic dysfunction. [46, 47]. In HFpEF and in heart failure with reduced ejection fraction (HFrEF), LASr has been shown to be predictive of mortality and hospitalization [48, 49]. Prognostic value for LASr after adjustment for LV GLS, has been presented [50, 51] However, there are conflicting results from other studies, after adjusting for LV GLS, E/ e' or LA-area, LASr was no longer predictive. [52, 53] It must be used with caution since LASr is preload dependent. [54, 55] Left atrial reservoir and conduction strain are affected by the simultaneous active movements in the ventricle [56].

LA contractile strain (LASct) is dependent on the same factors as reservoir strain, although relatively less dependent on LV function since there are no active movements in the ventricle during late diastole when LA contraction occurs. [43, 57]. LASct is also affected by increased afterload and could be used for assessing LV end-diastolic pressures. [57]. LASct has been described in the setting of controlled preload alterations, although studies show conflicting findings concerning strain changes related to preload changes. [55, 58-60] LASct also reflects the intrinsic contractility of the atria. The understanding of the relationship between LA volume, LA pressure loading and LA contractile function in regard to altered loading conditions is not yet fully understood. There is also no clarity about how the interaction between left atrial and ventricular movement is affected by changes in preload, that is, if atrial strain is more sensitive to preload changes than the ventricular strain. To use LAS analysis in critically ill patients for guiding treatment, more knowledge of how LAS is affected by cardiac illness and loading conditions is needed, since both myocardial function and venous return to the heart can be highly dynamic and disturbed during critical illness.

CARDIAC AMYLOIDOSIS

Systemic amyloidosis is a rare group of illnesses which are caused by the extracellular deposition of a group of proteins consisting of amyloid fibrils and has a heterogenous expression and can affect many organs. Different variants are hereditary amyloidosis, acquired immunoglobulin light-chain amyloidosis, reactive systemic amyloidosis, β 2-microglobulin dialysis-related amyloidosis,

and in association with the ageing process wild-type transthyretin amyloidosis and atrial natriuretic peptide amyloidosis. [61] More than 30 different proteins have been shown to be amyloid precursors. [62] The most common in high income countries are the light chain amyloidosis. Different types have different organ impact and disease manifestations. Frequently occurring illness manifestations with heart engagement involve the acquired systemic light chain amyloidosis and the inherited and acquired transthyretin protein form (ATTR). Heart engagement, or cardiac amyloidosis (CA), is associated with a worse prognosis and can be assessed with cardiac ultrasound and magnetic resonance imaging. [61] When amyloid proteins are deposited in the myocardial interstitium, both structural and functional changes occur. The structural change is characterized by the deposition of amyloid fibrils within both the myocardial interstitial compartment and vessels. This can lead to myocardial atrophy and fibrosis[63]. The main functional change is reduced heart chamber compliance which negatively affects heart diastolic function. The degree of reduced compliance is related to the amount of amyloid fibrils deposited in the myocardium. When there are large amounts of fibrils in the myocardium the systolic function can also be affected and increases in intraventricular septal mass can be observed.[64]

CARDIAC AMYLOIDOSIS AND STRAIN

Longitudinal cardiac strain analysis can be used to assess how heart mechanics are affected by amyloidosis.[65] Basal systolic strain and strain rate are impaired in CA even in the absence of overt heart failure. [66, 67] Moreover, dissociation between LV ejection fraction and global longitudinal strain, as well as reductions in LASr, have been proposed as features distinguishing CA from other hypertrophic phenotypes. [68, 69] A relative apical sparing pattern is observed in 75% of patients with histologically confirmed cardiac amyloid infiltration, while a pronounced base-to-apex longitudinal strain gradient has been reported as typical. [70] No significant differences in strain patterns have been identified among the various amyloidosis subtypes. [71] The diagnostic relevance of strain imaging has been further acknowledged in the 2023 cardiomyopathy guidelines. [72] In amyloid cardiomyopathy, LV GLS has been shown to be independently associated with mortality [73, 74] Also, atrial function has been shown to be independently related to mortality in CA [75-77].

In CA, the LA is affected by an increased afterload due to LV diastolic dysfunction, and through amyloid fibril infiltration affecting the LA myocardium. This can impair atrial compliance and contractility. At rest, echocardiographic indices of diastolic function are not predictable in ATTR-CA, reported as ranging from only

mild diastolic dysfunction to restrictive filling. [78] A CA cohort with reduced atrial compliance and atrial systolic dysfunction with corresponding histological changes in the atria due to transthyretin amyloid deposition has been presented [79]. However, less is known how diastolic function and especially how LASct is affected by preload in CA.

AIM OF THE PROJECT

The aim of this thesis is assessment of load dependence of left heart chamber wall strain and the interrelation between left atrial and left ventricular strain, with a particular focus on left atrial contraction strain, in healthy individuals and in patients with cardiac illness (cardiac amyloidosis).

SPECIFIC AIMS

The purpose and aims of Papers 1-4:

In the first paper we hypothesized that in healthy individuals, LASct and peak left atrial contraction strain (pLASRct) are increased or decreased by increasing or decreasing preload, respectively. The project aimed to investigate this relationship in healthy study participants using 2 controlled interventions, designed to alter preload. A PLR, intended to increase preload, and a continuous positive airway pressure manoeuvre (CPAP), intended to decrease preload in the left atrium.

The second paper focused on the combination of both controlled preload reduction and sympathetic nervous system activation, and their effects of them on LASct. Our aim was to determine if there was a predictable change in LASct in response to an experimentally controlled reduction in preload with a simultaneous increase in sympathetic nervous system activity achieved via the Valsalva manoeuvre (VM) in healthy volunteers. We hypothesized that the controlled preload reduction and the sympathetic nervous system activation that occurs during a standardized VM would bring about a change in LASct.

The third paper focused on LASct preload dependency in infiltrative cardiac disease (cardiac amyloidosis). We hypothesized that LASct remains unchanged during a PLR in cardiac amyloidosis and may increase in healthy age matched individuals.

The fourth paper assessed the interaction between atrial and ventricular strain during different loading conditions in healthy volunteers. The project constituted a secondary analysis of the same dataset as project 1, with a specific aim to characterize the relationship between LAS and LV-GLS throughout the cardiac cycle, and to explore potential phase-specific patterns of atrioventricular interaction. Our hypothesis was that a mild controlled preload increase would cause a change in the LA-LV strain relation during the whole cardiac cycle, as well as the LA contraction phase. Then, a preload reduction would also cause a change in the LAS-LVS relation during the whole cardiac cycle, as well as the LA contraction phase.

METHODS

Papers 1 and 2 were conducted in a cohort of healthy volunteers (medical students) to evaluate the effects of varying loading conditions and sympathetic activation on LASct. Changes in preload were induced through controlled alterations of airway pressure using the CPAP, a PLR and by a VM which also increases sympathetic tone. Paper 3 comprised a retrospective analysis of a cohort with CA, in which a PLR was used to assess load-dependent changes with a particular focus on LASct. Finally, Paper 4 examined the relationship between LA and LV strain in relation to preload. Atrial and ventricular strain were measured within the same cardiac cycle, and linear regression analysis was used to construct an LA/LV strain curve (AVS-slope) describing their interdependence under varying loading conditions.

PRELOAD ALTERATIONS

Different experimental and clinical methods have been developed to modify cardiac preload in order to study cardiac function and hemodynamic responses. Common approaches include volume loading, venous occlusion, and the use of pharmacological agents that alter venous return. Non-invasive techniques, such as increasing intrathoracic pressure and positional changes, have also proven valuable in controlled preload manipulation. Accurate adjustment and measurement of preload are essential for interpreting cardiac performance and understanding pathophysiological mechanisms. In this thesis, a PLR was chosen for preload increase, CPAP for preload decrease and a Valsalva manoeuvre for preload decrease with simultaneous sympathetic nervous system activation. We evaluated different types of PLR.

PASSIVE LEG RAISE

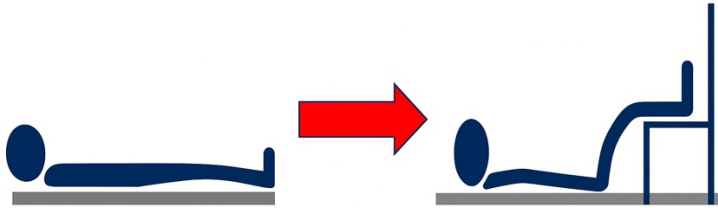
The PLR is used to assess cardiac response to increased venous return. It is minimal invasive and easily reversible. By elevating the lower limbs to approximately 45 degrees above the horizontal plane, gravitational force facilitates the displacement of venous blood from the capacitance vessels of the lower extremities toward the central circulation. This manoeuvre increases the mean venous pressure and venous flow towards the right atrium, transiently increases right atrial filling and, consequently, cardiac preload. [80] [81] The hemodynamic effects of the manoeuvre are influenced by cardiac function and variations in venous compliance, total blood volume and autonomic tone. The timing of the cardiac assessment since the start of the manoeuvre is of importance since the increase in preload is dynamic, it occurs after about 30-60s, and can be transient.[82, 83] Alteration of autonomic nervous system output during a PLR has been described.[84] The PLR has been validated as a diagnostic adjunct in cardiovascular care to unmask cardiac dysfunction and in critical care to determine if a patient is a fluid 'responder' (where cardiac output increases significantly with the manoeuvre).

There is no standardized way to perform a PLR [80, 85-87], although reviews of the method and recommendations how to perform a PLR have been published in the field of intensive care medicine. [88-90] In the recommendations, it is stressed that it is of importance to minimize provocation of the autonomous nervous system when performing the manoeuvre or avoid as much of sensory stimulation as possible. The simultaneous lowering of the trunk from a semi recumbent position to supine can increase the venous return allowing blood to return centrally. However, to perform a PLR as described by Jacobs et al[88] in a modern ICU-bed, is only possibly with a modest angle (15 degrees), since they are complex devices focused on supporting hygiene, safety, durability and comfort. [91] To perform a PLR with a larger angle requires flexion in the hip, thus increasing the risk for autonomic nervous system provocation.

We performed 2 different types of PLR in healthy volunteers, one with simultaneous lowering of the trunk while raising the legs to 15 degrees without any active movement of the volunteer (PLR15). The other PLR was performed with the trunk in 0 degrees, passively raising the legs (a passively flexion of the hip) to 45 degrees (PLR45) (Figure 1).

Figure 1

Panel A



Panel B

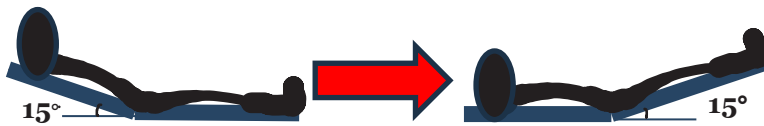


Figure 1. Passive leg raise.

Panel A illustrates a passive leg raise (PLR₄₅) performed by elevating the legs from the supine position to 45°. **Panel B** illustrates a passive leg raise initiated from a 15° head-up tilt of the trunk (PLR₁₅), followed by elevation of the legs while simultaneously lowering the trunk by tilting the entire bed head-down.

CONTINUOUS POSITIVE AIRWAY PRESSURE

The thorax cavity is comprised of several compartments through which pressure can spread. Increased airway pressure induced by CPAP will increase intrathoracic pressure generally, depending on lung- and chest wall compliance and airway resistance. The increase will translate into increased pleural pressure, pericardial pressure, and increased intracavity (intracardial) pressure for the heart chambers.[92] The relation between the pressures is not always predictable, and can vary to a large extent. [93] The increased airway pressure has also been shown to increase the mean systemic venous pressure. [94] An increased thoracic pressure will affect the cardio-respiratory interaction through several inter-related physiological deterrents: venous return, preload, afterload and pulmonary vascular resistance (PVR).

Venous return is dynamic and is influenced by many factors. Reduced blood flow from the periphery to the thoracic cavity occurs due to a decreased pressure

gradient from the mean venous filling pressure compartment to the right atrium, where the heart's function is to keep right atrial pressure low. However, increased intrathoracic pressure has also been reported with reduced venous return despite an unchanged pressure gradient between mean venous filling pressures and right atrial pressure, with a proposed mechanism involving increased venous resistance[92].

Preload is the pre-systolic stretch in the myofibrils, though this is translated to pre-systolic chamber filling. Decreased load or stretch of the myocytes at end-diastole occurs if there is reduced venous return.

Left ventricular afterload may also be called left ventricular wall stress, and is formally defined as the force in the chamber wall (corresponding to pull it apart) which occurs during systole/contraction/ejection, and is determined by the chamber size and shape, wall thickness, as well as force generated by myocyte contraction or shortening, along with the pressure against which the ventricle is ejecting.[95] The amount of pressure the left ventricular myocytes generate in the chamber (and chamber wall as experienced by the cardiomyocytes) through systole is related to arterial ejection flow which also occurs based on a pressure gradient in the arterial tree. An increase in airway pressure, which raises the intracardiac pressure, thereby decreasing the myocardial workload necessary to generate the required pressure for effective blood ejection.

Right ventricle afterload has the same physical characteristics as for the left ventricle, though for a lower pressure and more normally thin-walled chamber which ejects into a normally lower pressure and resistance arterial system. The amount of force the myocytes generate in the right ventricle generate blood flow through the pulmonary vessels. Increased airway pressure during CPAP can be transmitted to the pulmonary capillaries, leading to elevated intra-alveolar vascular resistance. This occurs because higher airway pressures compress the intra-alveolar vessels, reducing their luminal diameter. In contrast, the resistance in the inter-alveolar pulmonary vessels may decrease, due to the mechanical stretching of the lung parenchyma during lung expansion (due to increased airway pressure), which enlarges the vessel lumen. The net effect of these two mechanisms is an initial decrease of PVR, whereafter the PVR will increase with further increase of the airway pressure.[96, 97] Additionally, CPAP may influence the ventilation/perfusion ratio, thereby modulating pulmonary capillary resistance, which is primarily regulated by local oxygen and carbon dioxide tensions.

In health, the increased intra-thoracic pressure will successively lower the intrathoracic blood-volume, through decreased venous return to the right atrium as mentioned above. Also, with a meaningful increase in airway pressure there will be an initial increase in pulmonary capillary emptying and pulmonary venous

return to the left atrium, from the lung's capacitance vessels. When assessing the cardiac output of the left ventricle there will be an initial increase of stroke volume during 3-5 heartbeats, followed by a decrease that will persist when the intra-thoracic pressure is raised. [98]

In our paper, we chose a CPAP manoeuvre as it induces a preload reduction similar to that observed in critically ill patients receiving positive pressure ventilation. Additionally, the assessment of heart function during positive pressure ventilation is of importance.

VALSALVA MANOEUVRE

The VM is a standardized physiological test involving a voluntary forced expiratory effort against a closed airway. [99] The participant inhales normally and then exhale against resistance while maintaining an intra-oral pressure of 40 mmHg for 15–20 seconds, monitored with a manometer connected to a mouthpiece. This pressure approximates intrathoracic pressure and requires the glottis to remain open. Small air leaks must be permitted to prevent inadvertent glottic closure. The VM produces a characteristic haemodynamic pattern divided into four phases:

Phase I – a rise in arterial pressure with reflex bradycardia,

Early Phase II – reduced venous return and falling arterial pressure,

Late Phase II – baroreflex-mediated tachycardia and vasoconstriction producing partial arterial pressure recovery

Phase III – abrupt arterial pressure drop upon strain release

Phase IV – arterial pressure overshoot with reflex bradycardia.

These responses reflect both mechanical effects of increased intrathoracic pressure and autonomic reflex activity. Variations in strain level, duration, lung volume at onset, body position, hydration status, and prior inspiration influence the magnitude of the cardiovascular response. Autonomic evaluation uses indices derived from R-R interval changes, including the Valsalva ratio (maximal tachycardia divided by maximal bradycardia), phase-specific tachycardia and bradycardia amplitudes, and measures of pressure recovery such as blood pressure recovery time and derived adrenergic baroreflex sensitivity indices. Multiple repeated manoeuvres (typically 3–4) separated by 5 minutes are recommended for reliable quantification. Baseline heart rate and blood pressure are recorded after stabilization prior to initiating the manoeuvre. The VM is

generally safe, though rare adverse events such as syncope, arrhythmias, or excessive blood pressure fluctuations have been reported, particularly in individuals with cardiac or cerebrovascular disease.

In our paper, we chose a VM as it induces a preload reduction with simultaneous autonomous nervous system activation, which in theory could be similar to what is observed in critically ill patients under stress, receiving positive pressure ventilation.

ECHOCARDIOGRAPHY

Sound is a form of energy and requires a media to be transferred. It has areas of high pressure and low pressure that propagate through the media. It can be described by its wavelength and frequency. Wavelength is the length between two high-pressure areas and frequency is defined as how many oscillations of pressure that occur at one fixed point during, for example, one second. The speed at which the pressure changes propagate through the media is defined by the media's impedance and density, or by the product of wavelength and frequency. The energy of the sound wave is described by its amplitude (the graphical difference between maximal and average pressure) or the power used to generate the amplitude. The intensity (power/area) can be used to describe the energy in the sound wave. Attenuation occurs when the sound travels through a media and energy is "lost" to the media it travels through. Attenuation in ultrasound occur in three major different ways: absorption of energy (transfers into heat) in the media, reflection of soundwaves, and scattering of soundwaves. Absorption of energy is measured in decibels, and different medias can be compared through how much is absorbed per distance, dB/cm. Water has an attenuation coefficient of 0.002dB/cm, fat 0.6 dB/cm, muscle 1 dB/cm, and bone 20dB/cm. Reflection occurs at an interface between two media with different impedance. How much reflection occurs is dependent on the relation between the impedance of the two media, the angle of the interface to the direction of the wavelength, and how the intermedial surface is shaped (smooth or rough). Scattering occurs when an object has a diameter smaller than the wavelength of the sound and reflection of the sound in several direction occurs. Higher-frequency sound scatters more because the shorter wavelength is comparable to the size of medium inhomogeneities, causing a larger redirection of wave energy. [100] The principle of the Doppler effect is when a sound source is moving toward or away from the recording site and appears to have a higher or lower pitch respectively. This difference in observed frequency is the Doppler effect and can be used to calculate the relative speed of the moving object. Sound has both a magnitude and a direction, hence the Doppler effect is angle-dependent, and only the speed vector parallel to the sound wave can be calculated.

Ultrasound with a frequency of 1–20 MHz is emitted by a combined transmitter and receiver placed against the skin. A sound pulse is emitted by a piezoelectric crystal that transforms electric energy into acoustic energy and generates sound waves. These crystals then also convert the returning sound waves into electric energy. The sound waves travel in an organized way from the transducer, initially becoming narrower until they reach a focus point, whereafter the beam becomes wider. When the sound wave encounters a tissue interface, some of the wave is reflected toward the transducer. This reflection is determined by the sound wave physics as mentioned above. The returning sound waves are analysed in terms of their travel time and intensity, and then can be displayed, for example as a two-dimensional (2D) image.

The 2D-images provide a possibility to accurately display the structural details (anatomy) of the media that the waves have travelled through. This is called the image resolution. Resolution may be defined as accurately displaying objects in space (axial or lateral resolution), in shades of grey (contrast), and in time (temporal resolution).[101]

Axial resolution is the image ability to distinguish 2 objects parallel to the direction of the sound wave. The minimum distance is equal to half of the spatial pulse length. Since the length of the sound pulse is dependent on the sound wavelength, shorter wavelength gives a better resolution. Lateral resolution is the image ability to distinguish 2 objects placed perpendicular to the direction of the sound wave and is best at the focus point, where the sound wave is most narrow. Contrast resolution is the image ability to distinguish variations in the reflected echo amplitude between adjacent anatomical structures. Contrast resolution can be improved at multiple stages of the imaging process, including through signal compression, image memory, and the application of contrast-enhancing agents. Time resolution (or temporal resolution) is inversely related to how much time is required to generate one frame and is dependent on the depth and width of the image.

Doppler measurements can be carried out using short, emitted pulses, known as pulsed-wave Doppler. Pulsed Doppler measures the time until the echo returns, thereby determining the depth of the reflecting structure. It is commonly used to display flow patterns as colour superimposed on the 2D image. When sound is emitted continuously—continuous-wave Doppler—the system measures only the velocity of the reflecting target, without determining its depth, but with known direction based on the insonation angle. The advantage of continuous-wave Doppler is the ability to capture the highest velocities along the beam path and achieve superior temporal resolution.

To analyse the returned sound wave, the ultrasound machine makes four assumptions; that the sound waves travel along a straight path, that they return

to the machine after just one reflection, that the tissues that the sound wave passes through attenuate the wave uniformly with 0.5 dB/cm/MHz and that the speed of sound is 1540 m/s. Since these assumptions are a simplification of the behaviour of soundwaves in human tissues, artifacts can occur. [102]

Ultrasound artifacts that commonly occur can be divided into three types: axial direction, lateral direction, and due to equipment artifacts. Axial artifacts are located below the created image of the object and consist of mirror image artifacts, reverberations, and acoustic shadowing/enhancement. In the lateral direction they are located to the side of the image of the object, as are side-lobe artifacts, refraction artifacts and beam width artifacts. Artifacts due to equipment include artifacts from unshielded electrical equipment, cauterization artifacts, aliasing, and click artifacts.

Ultrasound waves are generally considered safe for diagnostic purposes. However, their use should be approached with caution, and regulations exist to govern their application [103]. The harmful bioeffects of ultrasound are primarily driven by two mechanisms: thermal and mechanical effects. [104] Thermal effects result from the absorption of ultrasound energy by tissue, leading to localized heating, which may pose a risk during diagnostic ultrasound procedures when applied to small, sensitive organs such as the eye. Mechanical effects are theoretically described as resulting from the interaction between ultrasound waves and small gas pockets, known as inertial acoustic cavitation, and from the direct shear forces generated when the sound wave propagates through the tissue. Pericapillary haemorrhage due to mechanical effects has been described in animal models exposed to diagnostic ultrasound waves. [105] It is recommended that diagnostic ultrasound users apply the ALARA (As Low As Reasonably Achievable) principle when exposing tissues to ultrasound waves. [106] The Mechanical Index and Thermal Index are numerical values displayed on ultrasound machines, as appropriate, and serve as indicators of the potential risk of tissue injury associated with the current settings.

Ultrasound is used for evaluation of cardiac anatomy and function, usually called echocardiography, and guidelines exist. [107-109] Use of echocardiography for diagnostics in hospital patients has been shown to lead to active change in medical care [110], and has been shown to be associated with lower risk for mortality in certain diagnosis, including for example sepsis and myocardial infarction. [111]

The disadvantage of echocardiography is that it is user-dependent, only suitable for repeated single timepoint measurements (not monitoring), and that it requires an adequate acoustic window which not always is available. Factors associated with difficulty of acquiring an acoustic window are common in critically ill patients. These include high body mass index, mechanical ventilation,

bandages, subcutaneous emphysema and supine position. Air in lungs or other tissues interrupts sound wave transmission and presents a barrier for interrogating organs underneath. Reports of not being able to acquire adequate imaging have described in up to 3/10 critically ill patients[4, 112]

Knowledge of the variability of an echocardiographic examination and its ability to support a reliable assessment of heart anatomy and function is crucial to interpret the results of imaging results. It can be difficult to know if the results from an examination are reliable owing to intrinsic biological variability, variability in measurement techniques and interpretation among operators. Variability in this context, which can also be called measurement error, can be described as reproducibility, repeatability and reliability. Reproducibility is how well the same or two different operators assess the same variable from the same patient or study participant, repeatability is the variability in the same variable measured during different timepoints/conditions, and reliability is the magnitude between two repeated measurements. This can be presented using statistical methods. [113]

2-DIMENSIONAL CARDIAC STRAIN

The general change in myocardial fiber length from diastole to systole is divided by the initial length (Lagrangian strain) and then expressed as a dimensionless parameter in percent (%). This value is theoretical, since there is not a measure of one 'muscle-fiber' going in one direction, but rather the sum of movement from multiple fibers. Strain is positive or negative depending on if the length increases (diastole, for instance) or decreases (systole), and the measured result depends on the reference point. Strain has been validated with MRI tagging techniques and by sonomicrometry, where absolute distance between specific markers in the chamber wall is measured. [25, 114, 115] The deformation of the myocardium during contraction is related to the shortening of the myocardial fibers, many of which are in a helical orientation in the heart wall from the perspective of the whole heart, or wrapping, though fiber orientation is still more complex. From a cross-sectional perspective, the ventricle can be described as 3 layers, with epicardial, middle or myocardial (thickest), and endocardial layers, each with own orientation for fibers. During systole, the myocardial fibers shorten, and the summary result is that the wall moves longitudinally (shortening in the long axis), inwards towards the chamber centre, and twists or double twists along its long axis. Since the myocardium is incompressible, the wall thickens during systole when the fibers become shorter.[23]

Speckle tracking is derived from 2D ultrasound images, where analysis software recognizes small specific high intensity tissue parts of the images (also called

speckles) and then follows their movements during a heart cycle. Speckles are not tissue structures but rather interference patterns that move together with the tissue for a limited time and distance, and so they can be used for following how the tissues move. [116] The change in distance between the speckles during a heart cycle is used to calculate the deformation of the myocardium, expressed as a dimensionless parameter in percent (%). The software uses several smaller regions and spline interpolation averages their motion. After this a regional curve can be presented.[117] It requires high image quality and frame rate in order to reliably track the speckles. Tracing requires a placement of the region of interest (ROI) and then traceable speckles within it.

Some software uses a thin ROI in the subendocardial region, and sometimes also in addition, the midwall and the subepicardial myocardium. Despite a ROI of one-third of the wall thickness, the software often displays it as only a thin line. There are also software solutions that use full wall tracking with all available speckles inside the myocardial wall. These two methods will get different strain values from the same wall. When analysing the atrial wall, which is about 3mm thick, this will result in a less robust strain analysis compared to analysing the left ventricular wall that is 10mm thick. [41] Due to ultrasound physics, axial resolution of the speckles is higher compared to the lateral resolution. Apical echocardiographic views are optimal for assessment of longitudinal myocardial strain, since the movement is in the ultrasound's axial direction, but provides limited reliability for the evaluation of radial strain.

A field close to the probe has a better resolution compared to a field deeper in the tissue due to the spread of the ultrasound beam. Tracking quality should not be evaluated solely based on derived strain curves, as pathological conditions may also produce abnormal curve morphologies. Visual verification of the tracking process by overlaying tracked speckles on the grayscale images, and directly comparing them with the underlying myocardial motion, is important.[116] Out-of-plane speckle motion represents an additional source of tracking error, and affects short-axis views to a greater extent than apical views. [118]

When studying LAS, care should be taken to correctly track the myocardial wall movement. Since the atrial wall is only approximately 3mm thick in health, and there are anatomical "gaps" in the wall due to the atrial appendage and pulmonary veins, there is a challenge to include the whole atrial wall in one image for some cases. Atrial strain analysis is used as a global measure, rather than regional atrial wall analysis, due to the reasons mentioned above. The only currently recommended parameter when studying the left atria is global longitudinal strain. A correct view without foreshortening of the atria is important, and image window should be aimed specifically for the LA, since the long axis of the LA and the LV do not lie in the same plane. In one study, a two-

chamber view of the LA was only feasible in 70% of 244 study persons, compared to 94% with four-chamber view. [43] LAS mean values obtained from either four-chamber, two and four-chamber or two, three and four-chamber views, assessed in a meta-analysis are not expected to differ.[119] Several software packages exist to support 2D strain-image analysis. Since their algorithms of calculating heart strain are proprietary, or have not been published and are presumable different, values derived from one software probably cannot be assumed to generate the exact same values from the same images with a different software. Inter-, intra-observer, and inter-vendor variability has been assessed. [120]

STRAIN RATE

It is possible to calculate the rate at which the strain change occurs, and this is called strain rate and is expressed as a dimensionless parameter (%) per second (% / second). Strain rate may provide a more load-independent index of myocardial contractility than strain, as it is less influenced by changes in cardiac loading conditions and chamber geometry. [121] However, the assessment of strain rate is limited by the temporal resolution of conventional speckle-tracking echocardiography, which typically operates at frame rates of 40–80 frames/second and therefore does not allow reliable quantification of rapid deformation events. [122, 123] Experimental data suggest that substantial atrial and ventricular deformation occurs during the isovolumic phases of contraction and relaxation, which cannot be adequately captured with currently available frame rates. Tissue Doppler imaging with its higher temporal resolution (up to 300–400 frames/s), may serve as an alternative for strain rate analysis. Recent advances in high-frame rate echocardiography have enabled frame rates exceeding those of tissue Doppler imaging. [124] Whether these very high sampling rates translate into clinically meaningful improvements in the assessment of myocardial strain rate remains to be established.[40]

STATISTICAL METHODS

Statistical analyses in this thesis were conducted using quantitative methods with the aim of evaluating within-subject physiological responses under different loading conditions.

For measure of the central tendency, mean and median have been used. The variability is described by standard deviation, and interquartile range. For all continuous variables, normality was assessed. A formal test was favoured over graphical analysis. [125] Considering the small data samples ($n < 50$) in the

studies, a Shapiro-Wilk test was used. Grouped comparisons were made using a paired T-test, independent T-test, or if the data were considered not normally distributed, non-parametric tests were used (Mann-Whitney U or Wilcoxon signed rank test as appropriate). All inferential tests were two-sided. Statistical analyses were conducted using SPSS (IBM Corp. 28.0.1.1). Descriptive statistics are presented as means with 95% confidence intervals unless otherwise specified.

DATA MANAGEMENT

In all papers we managed the data with anonymized data and a separate and locked 'key', according to our data management plan.

ETHICAL CONSIDERATIONS

All papers included in this thesis were conducted in accordance with the principles outlined in the Declaration of Helsinki [126] and were approved by the Swedish national Human Research Ethics Review Board. Written informed consent was obtained from all participants prior to inclusion in Papers 1, 2, 3 and 4. Papers 1 and 2 involved healthy volunteers and were designed to investigate the effects of controlled alterations in loading conditions and sympathetic activation on left atrial contraction strain. The applied interventions, including CPAP, PLR and the VM are non-invasive and commonly used in physiological research. Participants were closely monitored throughout the protocols, and all procedures were well tolerated. From an ethical perspective, there was little risk to the participants, and little expected gain for them personally for participating in the studies. The expected gain from the study was new knowledge, though also, if there was an unexpected finding on the ultrasound examination that could potentially have health consequences, then this would have been referred to an appropriate medical specialist not involved in the study.

Paper 3 was a retrospective analysis of already collected data from patients with cardiac amyloidosis who were participants in an observational cohort study. Data were derived or measured from previously collected echocardiographic examinations, including PLR, performed as part of routine assessment.

Paper 4 investigated the relationship between left atrial and left ventricular strain under varying preload conditions. This was a secondary analysis of the data collected in Paper 1.

All data were handled in compliance with applicable data protection regulations. Imaging study results were pseudo anonymized, and participant confidentiality was maintained throughout all stages of the research process.

RESULTS

The results of the different leg raise manoeuvres, PLR15 and PLR45 in healthy medical students, are presented in Table 1. The observed differences in outcome between the two preload alteration manoeuvres (PLR15 and PLR45) may be caused by the initial position of the volunteers. In the PLR45, the volunteers at baseline were positioned in a strict supine posture which may result in a larger venous return and presystolic volume compared to the PLR15, where they had their trunk tilted upwards by 15 degrees. The hearts in the PRL15 are likely positioned on a steeper part of the Frank Starling curve [127]., where the myocardial response to preload changes is more pronounced. In our prospective study with healthy volunteers, we choose PLR15, since it is in theory less influenced by dynamic autonomic nerve system reactions, and is easily performed in modern ICU-beds which will be used in future studies. In our retrospective study with amyloidosis patients, a PLR45 had been performed.

Table 1. Doppler and area results from 2 different passive leg raise manoeuvres performed in healthy medical students.

n=33	PLR 15 Baseline	PLR 15 During ICU	p value	PLR 45 Baseline	PLR 45 During	p value
LA Area (cm ⁻²)	14.8 (13.4 to 16.2)*	16.0 (14.8 to 17.2)*	0.009	15.3 (14.3 to 16.3)**	16.1 (15.0 to 17.3)**	0.006
LVOT VTI (cm)	20.4 (19.2 to 21.7)	21.9 (20.8 to 23.0)	<0.001	21.5 (20.2 to 22.8)	21.7 (20.3 to 23.1)	0.651
E (m/s)	0.78 (0.74 to 0.83)	0.80 (0.76 to 0.85)	0.183	0.79 (0.74 to 0.84)	0.85 (0.80 to 0.90)	<0.001
A (m/s)	0.38 (0.36 to 0.41)	0.39 (0.36 to 0.41)	0.810	0.37 (0.35 to 0.40)	0.42 (0.40 to 0.45)	<0.001
e (m/s)	0.14 (0.13 to 0.15)	0.15 (0.14 to 0.15)	0.034	0.15 (0.14 to 0.16)	0.15 (0.14 to 0.15)	0.923
a (m/s)	0.052 (0.048 to 0.057)	0.059 (0.054 to 0.063)	0.020	0.057 (0.053 to 0.062)	0.058 (0.053 to 0.063)	0.629
s (m/s)	0.089 (0.082 to 0.095)	0.091 (0.085 to 0.097)	0.351	0.094 (0.089 to 0.99)	0.092 (0.086 to 0.97)	0.141
EDV (ml)	104 (93 to 114)	106 (96 to 117)	0.215	101 (92 to 110)	104 (96 to 113)	0.234

Results presented as mean values with 95% confidence intervals. P-values from a paired T-test. A, late trans mitral maximum flow velocity; a, late diastolic maximal lateral mitral annular tissue velocity with atrial contraction; E, early trans mitral maximum flow velocity; e, early diastolic maximal lateral mitral annular tissue velocity; LA area, left atrial area from 4-chamber view; LV EDV, left ventricular end-diastolic volume from 4-chamber view only; LVOT VTI, left ventricular outflow tract velocity time integral; PLR15, passive leg raise performed with trunk 15 degrees up and the simultaneously lowering the trunk and raising the legs by tilting the bed; PLR45, passive leg raise performed supine while passively raising the legs to 45 degrees angle; s, maximal systolic lateral mitral annular tissue. *) n=23, **) n=27.

Paper 1. Thirty-eight healthy volunteer participants (18 women and 20 men, mean age 25.3 ± 3.6 years, mean BMI 23.8 ± 1.9 kg/m²) were examined. There were 23 complete studies included for the CPAP intervention and 27 for the PLR15. The primary finding was that neither CPAP nor PLR15 caused a significant change in LASct. CPAP resulted in lower atrial loading, as indicated by reduced LA area, LASr, HF, a, e, s, LVOT-VTI (stroke volume), LV EDV, and

LV-GLS. Conversely, PLR15 led to increased atrial loading, indicated by increase of LA area, LASr, and LVOT-VTI. (Table 2).

Table 2 Short summary of echocardiographic results from Paper 1, pre- and during manoeuvres.

CPAP n=23	Pre CPAP	CPAP	p value
LAS _{ct} (%)	11.6 (10.1 to 13.1)	12.8 (11.0 to 14.6)	0.157
LAS _r (%)	40.7 (38.3 to 43.1)	35.4 (33.1 to 37.6)	< 0.001
LA Area (cm ²)	16.0 (14.2 to 17.7)	14.1 (12.5 to 15.7)	0.026
LV GLS (%)	-19.5 (-20.4 to -18.6)	-18.2 (-19.2 to -17.1)	< 0.001
LVOT VTI (cm)	23.8 (21.9 to 25.8)	18.4 (16.4 to 20.4)	< 0.001
E (m/s)	0.85 (0.80 to 0.91)	0.70 (0.66 to 0.74)	< 0.001
PLR15 n=27			
Pre PLR15	PLR15	p value	
LAS _{ct} (%)	10.1 (9.0 to 11.2)	10.8 (9.4 to 12.3)	0.283
LAS _r (%)	37.4 (35.8 to 39.1)	41.5 (39.4 to 43.6)	0.001
LA Area (cm ²)	14.9 (13.4 to 16.4)	16.4 (15.2 to 17.6)	0.007
LV GLS %	-17.1 (-17.8 to -16.4)	-17.8 (-18.8 to -16.8)	0.129
LVOT VTI (cm)	21.1 (19.7 to 22.5)	22.6 (21.4 to 23.7)	<0.001
E (m)	0.79 (0.74 to 0.84)	0.79 (0.74 to 0.84)	0.907

Results presented as mean values with 95% confidence intervals. CPAP, continuous positive airway pressure; E, early trans mitral maximum flow velocity; LA area, left atrial area; LAS_{ct}, left atrial contraction strain; LV GLS, left ventricular global longitudinal strain; LVOT VTI, left ventricular outflow tract velocity time integral; PLR15, passive leg raise.

Paper 2. The same cohort of 38 healthy participants completed a Valsalva manoeuvre. 22 complete paired datasets (pre- and during Valsalva) were available for analysis. The Valsalva manoeuvre caused the expected changes in blood pressure and heart rate. The manoeuvre did not induce any significant change in LAS_{ct}. (Table 3) Figure 2 shows a representative example of LAS measurement.

Figure 2

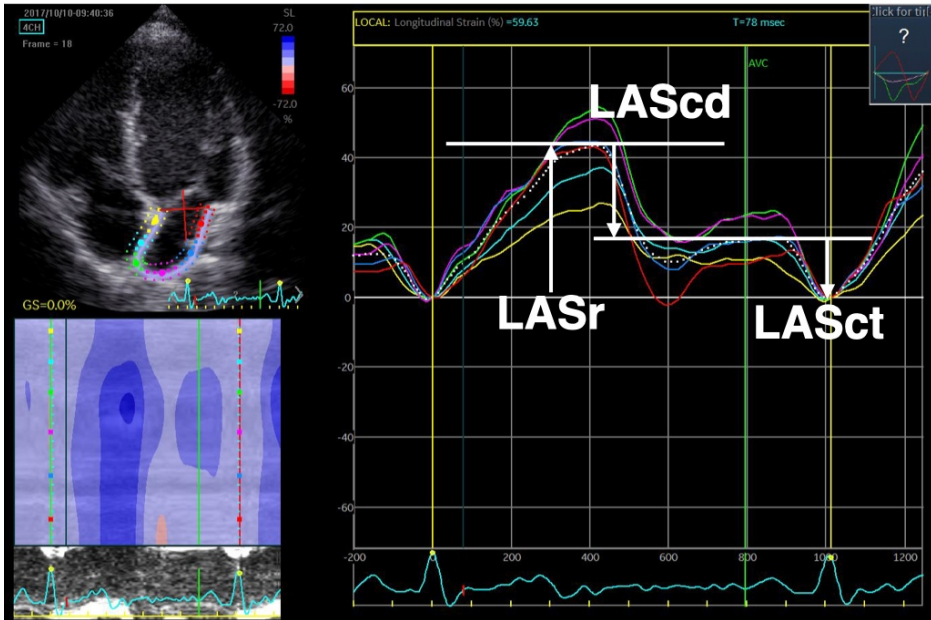


Figure 2. Left atrial strain measurement during a Valsalva manoeuvre. Left atrial conduction strain (LAScd), left atrial contraction strain (LASct) and left atrial reservoir strain (LASr) measurement are shown. The coloured curves in the diagram represent the regional strain of the left atrium, with each curve matching the corresponding region's colour. The dotted line in the diagram is the mean (global) strain curve. Note that in the left atrium there appear to be anatomical gaps in the blue field, and most likely also at the border between the green and pink fields, due to the entrance of the pulmonary veins. However, their respective (regional) strain curve patterns are similar to the other strain curves (red, yellow and blue). The ECG-curve can serve as a guide for identifying the different atrial phases. The atrial phases are clearly distinguishable in the strain curves, in these healthy individuals. This facilitates the identification of LASr and LASct phase respectively. LAScd is simply the difference between the latter two.

Table 3 Short summary of echocardiographic results from Paper 2, pre- and during manoeuvre.

N=22	Pre Valsalva	During Valsalva	P value
LAS _{ct} (%)	10.5 (2.8)	10.6 (4.6)	0.86
LAS _r (%)	44.9 (6.5)	35.4 (7.7)	<0.001
LA Area (cm ²)	17.9 (3.5)	11.5 (2.9)	<0.001
LV GLS (%)	-19.5 (2.1)	-17.5 (2.9)	<0.001
LVOT VTI (cm)	23.0 (5.9)	16.7 (4.1)	<0.001
E (m/s)	0.78 (0.04)	0.57 (0.07)	<0.001

Results presented as mean values with standard deviation. E, early trans mitral maximum flow velocity; LA area, left atrial area; LAS_{ct}, left atrial contraction strain; LV GLS, left ventricular global longitudinal strain; LVOT VTI, left ventricular outflow tract velocity time integral.

Paper 3. 41 patients with hereditary transthyretin amyloidosis and cardiac involvement (ATTR-CA) and 28 presumed healthy comparison cases over 50 years of age underwent echocardiographic assessment, including a PLR45 manoeuvre. 23 ATTR-CA patients (mean age 67 years, mean BMI 23.9 kg/m²) and 16 healthy controls (mean age 62 years, mean BMI 23.4 kg/m²) were retrospectively included in the final analysis. At rest, the ATTR-CA group exhibited reduced LV ejection fraction (45.9% vs. 51.6%) and markedly lower LA and LV strain compared to healthy controls. PLR45 resulted in an increased LVOT VTI in the control group, and both groups showed increases in trans mitral E-wave, e' velocity consistent with preload augmentation. (Figure 3) No significant change in LAS_{ct} was observed during PLR45 in either group. (Table 4 and Figure 4)

Figure 3

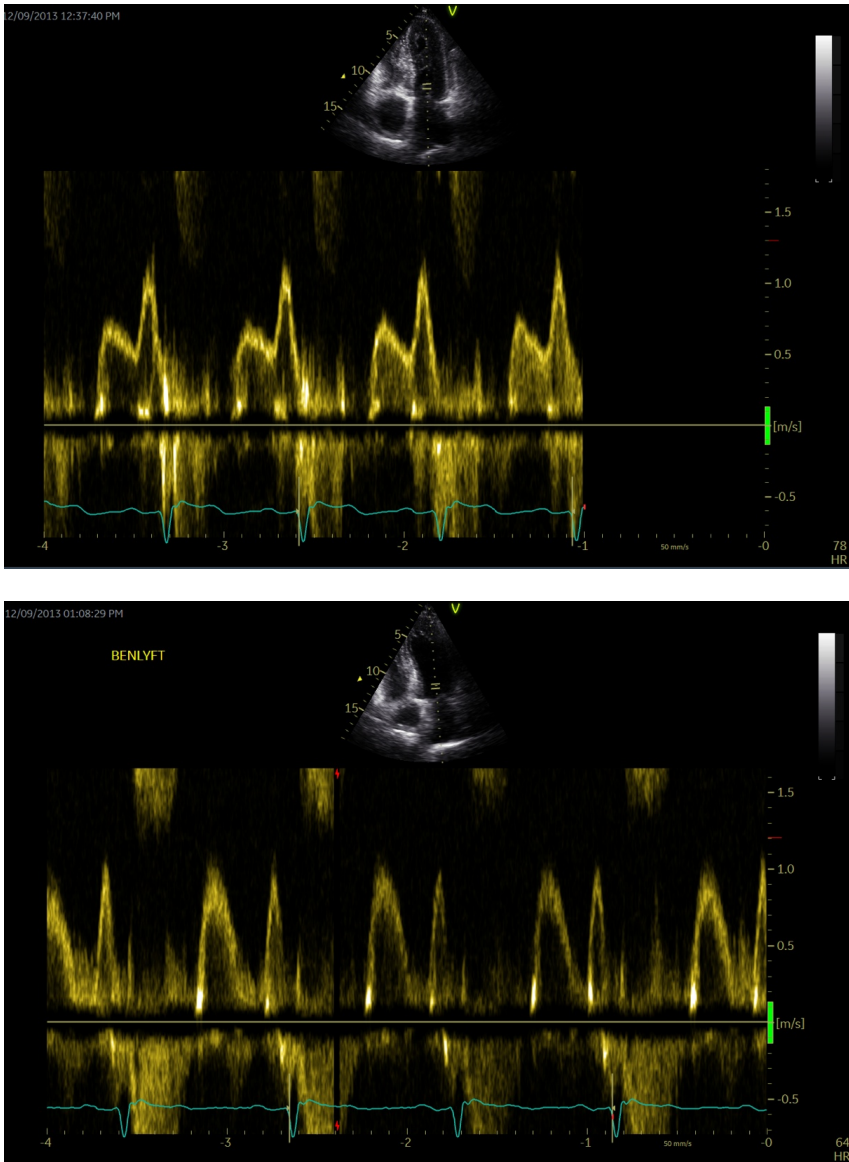
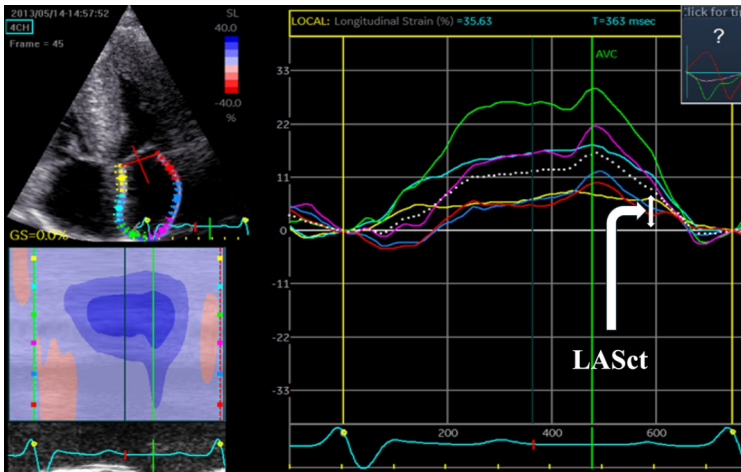


Figure 3. A representative example of mitral flow assessed by pulsed Doppler, before and during a PLR45 in a cardiac amyloidosis study participant. The top panel shows the diastolic flow over the mitral valve assessed by pulsed Doppler in a study participant with cardiac amyloidosis lying supine. The bottom panel shows an increase of the early diastolic flow (E-wave) in the same person during a passive leg lift.

Figure 4 Panel A.



Panel B.

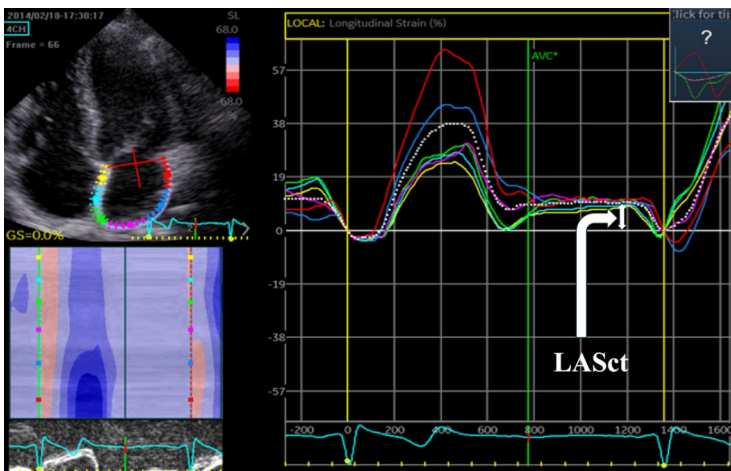


Figure 4. Examples of left atrial contraction strain measurement from study participants in Paper 3. Left atrial contraction strain measurement is demonstrated here, taken at the onset of atrial contraction (late p-wave). In cardiac amyloidosis, the left atrial strain is reduced in the whole heart cycle. Note the difference in appearance between cardiac amyloidosis (panel A) and healthy participants (panel B) regarding the plateau phase prior to atrial contraction. In cardiac illness, such as cardiac amyloidosis, the pathological strain curve increases the reliance on ECG-curve or strain rate curve analysis (Figure 5) for distinguishing the onset of atrial contraction phase. LASct, left atrial contraction strain. (Figure derived from Paper 3.)

Table 4 Short summary of echocardiographic results from Paper 3, pre- and during manoeuvre

	Healthy controls (n=16) Baseline	Healthy controls PLR45	P-value	ATTR-CA (n=24) Baseline	ATTR-CA PLR45	P-value	P-value* between baseline -groups
IVSd (mm)	10 (1)			18.5 (5)			<0.001
LVDD (mm)	46.6 (44.8 to 48.3)			44.0 (41.9 to 46.1)			0.10
LASct (%)	17.4 (15.0 to 19.9)	16.4 (14.9 to 18.0)	0.42	9.0 (7.1 to 10.9)	8.6 (6.5 to 10.6)	0.53	<0.001
LASr (%)	37.5 (32.8 to 42.2)	40.0 (36.4 to 43.6)	0.18	19.3 (15.9 to 22.6)	19.1 (15.3 to 22.8)	0.87	<0.001
LAVI (ml/m ²)	20.8 (11.1)**	23.1 (9.6)**	0.82	31.5 (14.6)	30.7 (10.1)	0.20	0.01
LV-GLS (%)	-19.2 (-20.3 to -18.0)	-19.1 (-20.4 to -17.7)	0.90	-13.5 (-14.9 to - 12.1)	-13.4 (-15.1 to - 11.8)	0.91	<0.001
LVOT- VTI (cm)	21.7 (19.9 to 23.5)	23.9 (21.9 to 25.9)	<0.001	21.0 (19.5 to 22.5)	21.1 (19.2 to 23.0)	0.66	0.55
E (cm/s)	72.9 (64.0 to 81.8)	84.3 (74.7 to 94.0)	<0.001	70.3 (61.8 to 78.8)	84.3 (77.5 to 91.1)	<0.0 01	0.68

Table 4. Results are presented as mean values with 95% confidence intervals or median values with interquartile range, as appropriate. P-values for the paired measurements are presented from a paired T-test or a Wilcoxon signed rank test, as appropriate. E, early trans mitral maximum flow velocity; IVSd, intraventricular septum diastole; LAVI, left atrial volume index; LASct, left atrial contraction strain; LASr, left atrial reservoir strain; LV GLS, left ventricular global longitudinal strain; LVOT VTI, left ventricular outflow tract velocity time integral; LVDD, left ventricular end-diastolic diameter. *) P-value presented from an independent T-test or a Mann-Whitney U-test as appropriate, between the baselines of the healthy group and the ATTR-CA group. **) n=14, only for LVAI due to missing body surface area for two healthy participants.

Paper 4. The study group was the same as in Paper 1, where 26 participants were analysed for the PLR15 and 13 for the CPAP. The slope representing the relationship between LAS and LV GLS, throughout the cardiac cycle (Figure 1 in Paper 4), the left atrioventricular strain regression slope (AVS-slope, Figure 2 in Paper 4), did not change significantly from baseline to PLR15, -2.18 (-2.03 to -2.33) to -2.26 (-2.09 to -2.43), $p = 0.40$. Likewise, during the atrial contraction phase, the AVS-slope remained stable, -3.34 (-2.70 to -3.98) to -3.37 (-3.04 to -3.71), $p = 0.92$. The AVS-slope for the entire cardiac cycle decreased significantly during CPAP, from -2.32 (-2.14 to -2.50) to -1.97 (-1.76 to -2.18), $p = 0.003$, reflecting the global reduction in cardiac loading. However, during the specific atrial contraction phase, no significant change in the AVS-slope was detected, -3.08 (-2.53 to -3.64) to -3.49 (-2.77 to -4.20), $p = 0.28$. (Table 5)

Table 5 Short summary of left atrioventricular strain regression slope results from Paper 4, pre- and during manoeuvre.

CPAP n=13	Pre CPAP	CPAP	P value
AVS-slope cardiac cycle (LAS)	-2.32 (-2.14 to -2.50)	-1.97 (-1.76 to -2.18)	0.003
AVS-slope late diastole (LASct)	-3.08 (-2.53 to -3.64)	-3.49 (-2.77 to -4.20)	0.28
PLR15 n=26			
Pre PLR15	PLR15	P value	
AVS-slope cardiac cycle (LAS)	-2.18 (-2.03 to -2.33)	-2.26 (-2.09 to -2.43)	0.40
AVS-slope late diastole (LASct)	-3.34 (-2.70 to -3.98)	-3.37 (-3.04 to -3.71)	0.92

Results presented as mean values with 95% confidence intervals. AVS-slope, left atrioventricular strain regression slope; LAS, left atrial strain; LASct, left atrial contraction phase.

Across the four studies included in this thesis, acute alterations in preload did not result in measurable changes in LASct. This finding was consistent across different preload-modifying interventions, including PLR15, PLR45, CPAP, the VM, and was observed in both healthy individuals and patients with CA. Furthermore, the relationship between LA and LV strain during the atrial contraction phase was not affected by changes in preload.

In healthy participants, preload augmentation and reduction induced the expected load-dependent changes in conventional echocardiographic parameters, including LA size, LASr, and indices of LV filling and stroke volume. The CPAP and VM induced a larger deviation from baseline for LVOT VTI, E, e, LV EDV compared to PLR15 and PLR45. Despite these physiological responses, LASct remained unchanged across all experimental conditions. Sympathetic activation during the VM likewise had no measurable effect on LASct.

In patients with ATTR-CA, LA and LV strain parameters were markedly reduced compared with healthy controls at rest. Preload augmentation resulted in an increased LVOT VTI only in the control group. Both groups showed increases in trans mitral E-wave, e velocity consistent with preload augmentation. LASct remained unchanged in both CA and controls.

Analysis of atrioventricular deformation interdependence demonstrated that the larger preload alteration induced during CPAP, influenced the global relationship between LA and LV strain, but the PLR15 did not. The specific interdependence during atrial contraction remained stable during both interventions.

DISCUSSION

In healthy participants, all preload interventions resulted in the expected changes in conventional preload markers, such as LA area, LASr, trans mitral flow velocities, and LV stroke volume. These findings confirm that the experimental models successfully induced measurable changes in cardiac loading.

In Paper 1-3 LASct remained unaffected by acute preload alterations, regardless of whether preload increased or decreased, and across both healthy individuals and patients with cardiac illness (amyloidosis). Although CPAP seem to induce a larger preload alteration compared with PLR15 and PLR45, neither caused a change i LASct. In Paper 4 the atrioventricular mechanical relationship during the atrial contraction phase appears to be preserved under varying loading conditions.

The principal finding of these explorative studies suggest that LASct is largely independent of acute preload changes.

The properly performed Valsalva manoeuvre is recognized to simultaneously reduce preload and increase sympathetic nervous system activity. The absence of a consistent change in LASct during phase 2 of the manoeuvre may reflect a net neutral effect, whereby preload reduction and sympathetic stimulation exert opposing influences on atrial contractility.

In the study cohort with ATTR-CA, LASct was markedly reduced at rest compared with healthy controls, reflecting impaired intrinsic atrial myocardial function. Preload augmentation by PLR45 did not result in a significant change in LASct in either group. In the amyloidosis cohort, preload augmentation increased indices of filling pressure without a corresponding increase in stroke volume, consistent with restrictive ventricular physiology. The stability of LASct despite altered loading conditions may reflect a fixed limitation in atrial contractile reserve due to amyloid infiltration and increased atrial afterload.

Paper 4 extended these observations by examining the interdependence between LA and LV strain under varying preload conditions. While global atrioventricular strain relationships across the cardiac cycle were affected by larger preload reductions during CPAP, the specific relationship during atrial contraction remained unchanged across all interventions. This suggests that although atrial deformation during reservoir and conduit phases is preload sensitive, atrial

systolic deformation during late diastole is comparatively preserved and coupled to ventricular mechanics in a preload-independent manner.

These findings support the concept that LASct may reflect intrinsic atrial contractile function rather than passive deformation secondary to acute preload changes. This distinguishes LASct from reservoir and conduit strain, which demonstrated consistent load dependency across all papers.

There are reports of LASct in cohorts during controlled load interventions. LASct with presumed preload reductions has been assessed in clinical settings. In patients being anesthetized for cardiac surgery, LASct was unaffected by positive pressure ventilation and general anaesthesia[128], though the preload changes were occurring in the midst of a complex circulatory intervention. LASct has been described in renal failure patients, before and after haemodialysis and was apparently unaffected by dialysis and preload reduction [129]. In one report, an increase in LASct was observed in response to leg lift in a hypertensive group but not in a comparison group with diastolic dysfunction and preserved ejection fraction [55] Different interventions may lead to different degrees of preload change and it seem that large preload alterations may affect LASct. Dermlim et al studied left atrial strain during acute volume loading by crystalloids (150 ml/kg/hr for 90 min) in dogs causing an increased atrial preload where LASct initially were unaffected but after 75ml/kg did increase.[58] In critically ill patients a large volume (500ml) infused in a short time-period (15 minutes) caused an increase in LASct in a fluid responder group, but not in the non-responders. [130] Genovese et al. studied left atrium preload-dependence by the use a preload decreasing model, a tilt test (designed for testing autonomic nervous system response). Their manoeuvre caused a large preload reduction by stepwise raising a tilting board from supine to an almost upright position. This decreased atrial preload substantially and LASct. However there was also activation of the autonomic nerve system demonstrated by an increase in heart rate at the upright position. [59]

Cardiac strain, including left atrial strain is shown to have a large variability in health [131]. When performing post hoc comparisons [132, 133] for the grouped results of LASct from our Papers 1-3, it is noted that the variability of LAct is relatively higher than that of LASr when comparing with their means respectively. This is in accordance with other studies [134]. It is possible that LASct exhibits a similar preload-dependence as LASr, but the higher variability in these measurements may limit the ability to detect such effects. When planning these studies, care was taken to choose a clinical meaningful effect to try to detect.[135] However, less is known how to interpret changes in LASct, and in these explorative studies a change of 20% from baseline were assumed to be of importance to detect.

MODEL VALIDITY

The preload intervention models used across the included studies were chosen to induce controlled and reproducible changes in cardiac loading while minimizing confounding effects from abrupt autonomic activation (except in Paper 2). Successful preload modulation was confirmed by consistent changes in LA size, LASr, trans mitral flow parameters, and LVOT VTI. Baseline strain values were in agreement with previously reported reference ranges for healthy individuals, supporting the physiological validity of the models. The observed preload changes induced by the PLR15 and PLR 45 were modest, suggesting that a more provocative alteration in preload increase could be relevant for further investigation. Such a modification may more closely mimic the clinical scenario of cardiovascular insufficiency during acute illness. The relatively modest PLR15 adjustment in this thesis represents an initial step in exploring this phenomenon.

The use of paired within-subject comparisons enhanced the study power to detect intervention-related changes, despite relatively small sample sizes. However, the preload alterations achieved were intentionally modest to avoid pronounced sympathetic activation (except the VM), which may have limited the magnitude of atrial loading changes and the ability to detect very small preload-dependent effects on LASct.

In this thesis, if the data was normally distributed a parametric test was used. If the data was not normally distributed, a non-parametric test was used. It is debated whether parametric or non-parametric tests should be used in small datasets where a strong central tendency is expected for the assessed data, but normality is not confirmed. [136, 137]

STRENGTHS AND LIMITATIONS

A strength of this thesis is the consistent application of multiple preload-modifying interventions across independent cohorts, allowing convergence of evidence from complementary physiological and clinical perspectives. The inclusion of both healthy individuals of different ages, and patients with cardiac amyloidosis strengthens the generalizability of the findings across different age periods, normal and pathological conditions. Standardized echocardiographic acquisition and analysis protocols were applied, and strain measurements were obtained from paired cardiac cycles, increasing internal validity. An intra- and interdependent test between operators were performed and showed good agreement.

Several limitations should be acknowledged. Sample sizes were modest, and the studies were not powered to detect small changes in LASct. The participants of Papers 1 and 2 were young and presumed healthy, limiting extrapolation to older populations and other disease states. In the amyloidosis study, LAS analysis was performed retrospectively, and imaging was not optimized specifically for atrial strain. Technical challenges inherent to atrial strain imaging, including thin atrial walls, image foreshortening, and respiratory motion, may have contributed to measurement variability. Strain analyses were limited to apical four-chamber views and to a single observer (except in Paper 3), precluding assessment of interobserver variability. The technical aspects of atrial strain assessment present challenges concerning adequate image quality, where this could vary within the same sequence. The assessor doing the strain measurements was not blinded to the interventions, since assessment of the whole sequence was necessary to get the optimal heart cycles. The performed manoeuvres to alter preload were different, hence no direct comparison between results in the thesis can be made. The measuring of LASct can be challenging, as it may be difficult to precisely determine the exact moment for the onset of atrial contraction. P-wave from the ECG can be used as well as the shape of the strain curve, however in cardiac illness the shape of the strain curve it is not always clear regarding the start of the atrial contraction phase. Assessment of the LASR curves from a single cardiac cycle can be utilized to determine the onset of the left atrial (LA) contraction phase. (Figure 5)

Figure 5

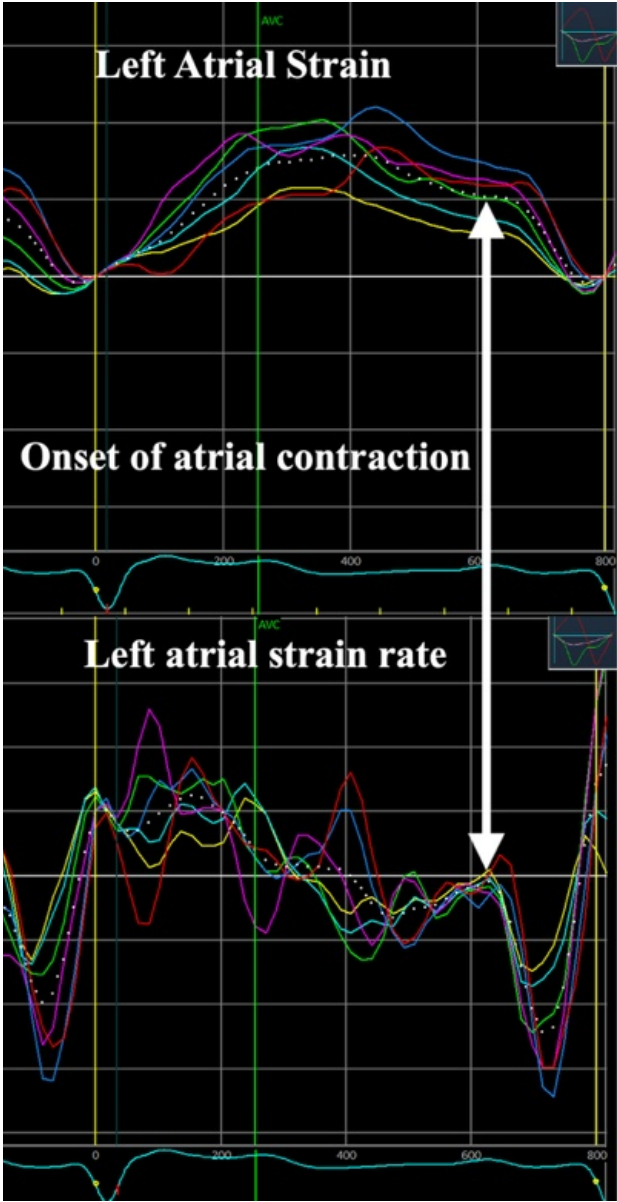


Figure 5. Left atrial strain and strain rate from a study participant with cardiac amyloidosis. The downward-pointing arrow indicates the initiation of left atrial contraction in the strain rate curve. Consequently, the onset is marked identically in the left atrial strain curve, as indicated by the upward-pointing arrow.

APPLICABILITY IN CLINICAL PRACTICE

In clinical practice, the considerable variability in LAct measurements makes it challenging to interpret a single measurement with confidence. Repeated measurements over time, particularly during shifts in pathophysiological conditions within the same patient, may provide more valuable insights when assessing changes from baseline.

CONCLUSION

Across four papers in this thesis, LASct demonstrated stability in response to acute preload alterations in both healthy individuals of different age and patients with cardiac amyloidosis. These findings support the concept that LASct is largely preload independent within clinically relevant loading ranges and may therefore serve as a robust marker of intrinsic left atrial contractile function. LASct may have a potential as a marker of myocardial function, both in healthy individuals and in critical illness. Intensive care settings, particularly those involving fluid resuscitation, provide a natural context for further cohort studies aimed at assessing how these strain measures correlate with clinical conditions before, during, and after treatment.

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