Mechanisms of left ventricular early-diastolic untwisting and lengthening

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Oslo, January 2013

Anders Opdahl

List of Papers

I. Opdahl A, Helle-Valle T, Remme EW, Vartdal T, Pettersen E, Lunde K, Edvardsen T, Smiseth OA; Apical Rotation by Speckle Tracking Echocardiography: A Simplified Bedside Index of Left Ventricular Twist; *Journal of the American Society of Echocardiography. 2008;21:1121-1128*[1]

II. Opdahl A, Remme EW, Helle-Valle T, Edvardsen T, Smiseth OA; Myocardial Relaxation, Restoring Forces, and Early-Diastolic Load Are Independent Determinants of Left Ventricular Untwisting Rate; *Circulation. 2012;126:1441-1451*[2]

III. Opdahl A, Remme EW, Helle-Valle T, Lyseggen E, Vartdal T, Pettersen E, Edvardsen T, Smiseth OA; Determinants of Left Ventricular Early-Diastolic Lengthening Velocity: Independent Contributions from Left Ventricular Relaxation, Restoring Forces and Lengthening Load; *Circulation. 2009;119:2578-2586*[3]

Selected Abbreviations

A	-	Peak atrial induced transmitral flow rate/velocity
a’	-	Peak atrial induced mitral annulus velocity
dP/dt	-	Time derivative of LVP
E	-	Peak early-diastolic transmitral flow rate/velocity
e’	-	Peak early-diastolic mitral annulus velocity
ε_{CL}	-	LV circumferential-longitudinal shear strain
FRP_{Est}	-	Estimated fully relaxed LV transmural pressure
IVR/IVRT	-	Isovolumic relaxation/isovolumic relaxation time
L_0	-	Unstressed- or resting length of a myocardial segment (“slack length”)
LA	-	Left atrium/atrial
LAP	-	Left atrial pressure
LAD	-	Left anterior descending coronary artery
LVP	-	Left ventricular (LV) pressure
LVP_{MVO}	-	LV intracavitary (Paper II) or transmural (Paper III) pressure at the time of mitral valve opening
MRI	-	Magnetic resonance imaging
MVO	-	Mitral valve opening
STE	-	Speckle tracking echocardiography
τ	-	Tau; time constant of LV isovolumic pressure decline
TDI	-	Tissue Doppler imaging
$Twist_A$	-	Absolute LV twist measured with respect to a fixed LV reference configuration
$Twist_C$	-	Conventional LV twist
UTR	-	Peak early-diastolic LV untwisting rate
UTR_{IVR}	-	Peak early-diastolic LV untwisting rate during isovolumic relaxation

Introduction

Congestive heart failure is a clinical syndrome that can result from any structural or functional cardiac disorder that leads to failure of the heart to deliver oxygen at a rate commensurate with the requirements of the metabolising tissues. The condition is typically associated by dyspnoea, fatigue, and fluid retention, in addition to cardiac dilatation and reduced left ventricular (LV) systolic function. Over the last two decades this concept has been challenged by studies which show that up to 50 % of patients with congestive heart failure have normal or preserved LV ejection fraction (EF).[4-8] It appears that in most of these patients the heart failure is due to abnormal diastolic function.[9] When abnormal diastolic function can be identified, the condition has been described as diastolic heart failure.[10] However, there are studies which suggest that patients with heart failure with preserved LV ejection fraction (HF-PEF) may actually have reduced systolic function.[11] It can also be argued that the differentiation into systolic and diastolic heart failure is somewhat artificial since patients with systolic heart failure also have diastolic dysfunction.

HF-PEF is relatively uncommon in younger patients, but increases in importance in the elderly and the frequency is higher in females than in males and is associated with a history of hypertension.[12] The proportion of heart failure patients who have preserved EF seems to be increasing.[8] Diastolic heart failure or HF-PEF is not a specific disease and may have several aetiologies, including arterial hypertension, coronary artery disease, restrictive cardiomyopathy, cardiac amyloidosis, and hypertrophic cardiomyopathy. Each of the conditions are associated with variable degrees of structural LV remodelling and stiffening[13] and/or slowing of LV relaxation.

Diastolic dysfunction is in principle caused by at least one of the following components; prolonged LV relaxation and increased LV passive myocardial stiffness. Left sided heart catheterization provides the measurements regarded as the reference method for the assessment of diastolic function. It allows for quantification of LV relaxation rate as the time constant (τ) for isovolumic LV pressure (LVP) decline,[14] and LV end-diastolic (ED) pressure. By combining LV pressure with volume measurements at different preload levels, LV stiffness can be quantified. However, being an invasive approach, this method is rarely used. Echocardiography is widely used for assessment of diastolic function and a number of echocardiographic indices have been introduced.[15-18] Early-diastolic mitral annulus velocity (e'),[19, 20] and early-diastolic LV untwisting rate (UTR)[21] are indices based on myocardial velocities or deformation that have recently been introduced as markers of diastolic function which may be relatively load independent.

In this thesis we investigate the hemodynamic- and mechanical determinants of early-diastolic lengthening and untwisting, as well as their relationship to the overall mechanical systolic- and diastolic LV deformation.

The papers that have been published as part of this thesis are listed in the References.[1-3]

LV Twisting Deformation

As seen from the LV apex the apex rotates counter clockwise during systole and the base rotates clockwise, reflecting the normal twisting motion of the LV about its long

axis.[22, 23] The LV twisting motion is a consequence of myocardial fibre orientation, which changes from an approximately longitudinal, but slightly oblique orientation in the subendocardium (~80° relative to circumferential direction) to a circumferential orientation in the mid-wall (~0°) and to an oblique orientation in the subepicardium (~-60°).[24, 25] Thus, the subendocardial and subepicardial fibres represent 2 oppositely directed spirals. Because of larger radii, the torque of subepicardial fibres dominates and accounts for the normal counter clockwise systolic rotation of the LV apex.[26]

Because the magnitude of LV twist is determined by contractile force, it has been suggested that measurement of LV twist could be implemented as a clinical index of contractility and potentially become a sensitive marker of myocardial dysfunction. This concept is supported by experimental work and a few clinical studies using either magnetic resonance imaging (MRI) or invasive methods to measure LV twist.[27-29] Unfortunately, none of these methods can be implemented in clinical routine. To explore the clinical potential of measuring LV twist, there is a need for simpler and faster methods that can be applied bedside.

Speckle tracking echocardiography (STE) has recently been introduced as a bedside method for measuring LV twist.[23, 30] Rotation is measured in LV short-axis images as angular displacement of myocardial speckles, and LV twist is calculated as the difference in rotation between image planes through the LV base and apex. Although the initial testing has been promising, measurement of LV twist by STE is confounded by suboptimal speckle tracking at the LV base.[23, 31, 32] The distal apical short-axis plane provides better acoustic conditions, and out-of-plane motion is minimal because of the limited longitudinal motion of the apex. Accordingly, speckle tracking at a distal apical short-axis plane is superior to what is achieved at the LV base.[23] In addition, the magnitude of rotation at the apex is greater than at the base, and this reduces the signal-to-noise ratio. In the present thesis the relationship between apical rotation and twist was investigated in an experimental- as well as clinical study.

Rate of LV Early-Diastolic Untwisting

Assessment of LV untwisting rate by STE[33] or tagged MRI[21] has been introduced as a promising index in the evaluation of diastolic function. This is partly due to demonstration of a close association between peak untwisting rate (UTR) and LV relaxation measured as the time constant (τ)[14] for LV isovolumic pressure decay.[21, 33, 34] In addition, UTR has been associated with LV restoring forces measured indirectly as LV end-systolic volume (ESV),[29, 33, 35] and as systolic twist.[33, 34, 36, 37] It is not clear, however, whether LV relaxation and restoring forces are independent determinants of UTR. Furthermore, a recent study[38] showed increased UTR during volume loading, which tends to increase τ (slower relaxation) and ESV (decreased restoring forces), as well. Increased UTR despite slowed relaxation and reduced restoring forces suggests that UTR is not solely determined by these 2 factors. An alternative explanation may be that elevated LV diastolic pressure during volume loading has direct effects on UTR. Some previous reports indicate that systolic twisting is preload-dependent,[28, 29, 38] whereas others have reported only a minor preload dependency or none at all.[27, 39] We hypothesized that diastolic pressure has a direct effect on UTR similar to the effect of early-diastolic load on LV lengthening rate.[3, 40]

Because twist is conventionally calculated as the difference between apical and basal rotation relative to ED configuration within the same heart beat ($Twist_C$), the possibility that diastolic pressure modifies the degree of twist at end-diastole is not taken into account.[41] In principle, this is a significant limitation since a change in $Twist_C$ may be due to a change in ED twist with no change in systolic twist configuration.

To determine the relationship between systolic twist configuration and UTR, it is essential to measure twist in absolute terms ($Twist_A$). In Paper II this was achieved by measuring twist with respect to a fixed reference configuration,[29] and circumferential-longitudinal shear angle (ε_{CL}) was used as a measure of restoring forces. As peak UTR occurs early in diastole we used LVP at mitral valve opening (LVP_{MVO}) as a measure of diastolic load in the assessment of a possible direct effect on UTR. In the present thesis the potential mechanisms that regulate peak UTR was studied in a dog model during different levels of contractility, different loading conditions, and during acute myocardial ischaemia.

Early-Diastolic LV Lengthening Velocity

Assessment of left ventricular (LV) lengthening velocities by tissue Doppler imaging (TDI) plays an important role in the evaluation of patients with suspected diastolic dysfunction. Lengthening velocities are recorded at the mitral annulus from apical views, and there are 2 main velocity waves that represent early-diastolic (e') and atrial-induced (a') myocardial lengthening. Most of the clinical focus has been on e', which has demonstrated a close correlation with global LV relaxation rate,[19, 42-44] quantified invasively as the time constant of LV isovolumic pressure fall (τ). Accordingly, reduced e' has been proposed as a sign of diastolic dysfunction. Furthermore, the ratio of peak early transmitral flow velocity (E) over e' correlates with LV end-diastolic pressure, and an elevated E/e' ratio has been introduced as a clinical marker of elevated LV diastolic pressure.[19] In patients with heart failure and normal ejection fraction, an elevated E/e' ratio is consistent with diastolic heart failure.[10] Therefore, e' could become a key measure in the echocardiographic evaluation of LV function, however, insight into the mechanisms that regulate the magnitude of e' is limited.

The observed correlation between e' and τ does not imply that e' is determined exclusively by LV relaxation, and both loading conditions and diastolic recoil have been suggested as determinants of myocardial lengthening rate.[10, 43, 45, 46] Previous studies of isolated cardiac muscle preparations[47, 48] and of in situ dog hearts[49] have shown that myocardial lengthening rate is determined by end-systolic length and loading conditions. Furthermore, load applied during myocardial relaxation (late load) increased lengthening rate.[48, 49] In the present thesis the potential mechanisms that regulate e' were studied in a dog model during different levels of contractility, different loading conditions, and acute myocardial ischaemia.

Aims of the Thesis

General Aim:

The general objective was to determine the mechanisms of LV early-diastolic lengthening and untwisting and to investigate how LV twist can be measured clinically.

Specific Aims:

1) To determine whether LV apical rotation could serve as a clinical surrogate for LV twist (Paper I).
2) To determine whether LV relaxation, restoring forces and early-diastolic load are independent determinants of untwisting rate (Paper II)
3) To determine if diastolic pressure modifies the degree of LV twist at end-diastole (Paper II).
4) To determine whether LV relaxation, restoring forces and early-diastolic load are independent determinants of early-diastolic lengthening rate (Paper III).

Material

Experimental Studies

Paper I:

Nine mongrel dogs of either sex with an average body weight 26.2±1.9 kg were studied.

Paper II:

Ten mongrel dogs of either sex and with a body weight 26.4±1.8 kg were studied.

Paper III:

Twelve mongrel dogs of either sex and with a body weight of 27.6±4.0 kg were studied. Two animals were excluded owing to complications that occurred during surgery. In addition, 1 dog was excluded during dobutamine and ischaemia because of persistent arrhythmia.

Clinical Study

Paper I:

The study population included 18 healthy individuals (56% male, age 31±6 years) and 27 patients (78% male, age 57±9 years) with previous (6-8 months) myocardial infarctions in the perfusion territory of the left anterior descending coronary artery (LAD) (n=18), right coronary artery (n=6), and left circumflex coronary artery (n=3).

Methods

Experimental Studies- Animal Model

The experimental part of this thesis (Papers I, II and III) was performed in a well-established dog model for studies of LV function. The main reason for using anesthetized mongrel dogs is their tolerance to the extensive instrumentation that was needed for these studies.

After a median sternotomy, the pericardium was split from apex to base, and loosely re-sutured after the instrumentation. Inflatable vascular occluders were positioned around both caval veins. Ischaemia was achieved by constricting a snare applied around the left anterior descending coronary artery (LAD) immediately distal to the first diagonal branch.

Recordings were done with the dogs in the supine position and with the ventilator off, thus preventing influence from the respirator. All hemodynamic and sonomicrometric data were digitized at 200 Hz. After termination of each experiment, the dogs were euthanized with an intracardial injection of pentobarbital.

The National Animal Experimentation Board approved all studies. The laboratory animals were supplied by Centre for Comparative Medicine, Rikshospitalet, Oslo, Norway.

Pressure Measurements

Micromanometer-tipped catheters (MPC-500, Millar Instruments Inc., Houston, TX, USA) were positioned in the LV through a carotid artery, and in the left atrium (LA) through the left atrial appendage (Figure 1). Pericardial pressure was measured with a flat fluid-containing balloon (Paper III).[50]

To serve as an absolute pressure reference for the micromanometers, a fluid filled catheter was placed in the LA. All pressure transducers were calibrated against a mercury manometer, and the pressures were zero-referenced against the fluid-filled LA catheter during LA and LV pressure equilibrium (long diastasis after induced LV extra systole). Pressures were processed via preamplifiers (Gould Instruments Systems Inc., Cleveland, OH, USA) and digitized.

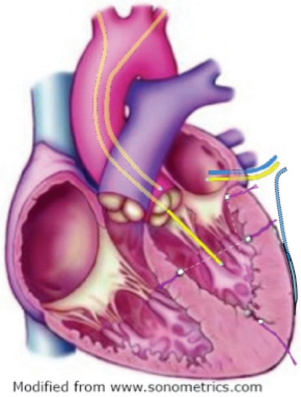

Figure 1. *Schematic illustration of the experimental set-up (Paper III) showing pressure catheters and ultrasonic crystals (white circles). Shown are aortic, left ventricular (LV) and left atrial (LA) micromanometers (yellow), fluid filled LA catheter (blue) and fluid containing pericardial balloon at the LV lateral epicardium (light blue).*

Definition of Cardiac Phases

The onset of the QRS complex in ECG was used to identify ED (Figure 2). ES was defined as aortic valve closure, identified as the time of peak negative first derivative of LVP (dP/dt_{min}).[51] The isovolumetric relaxation time (IVR) was defined as the time from ES to onset of filling. Onset of filling was defined as the time of mitral valve opening (MVO), identified as first diastolic LA-LV pressure crossover.

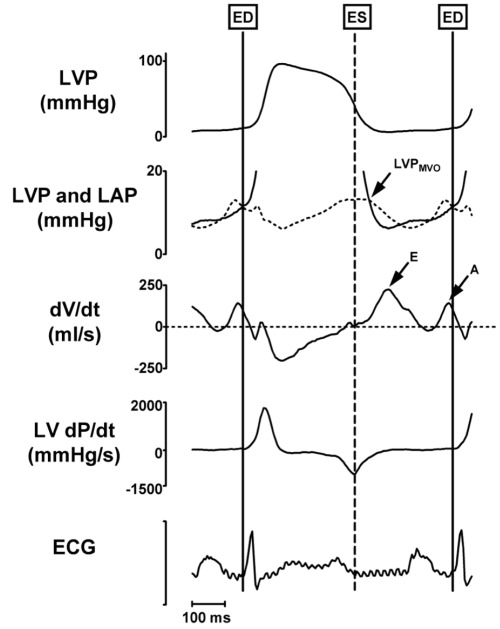

Figure 2. *Representative traces showing variables used for determining the cardiac phases. From top left ventricular pressure (LVP), left atrial pressure (LAP), time derivative of LV volume (dV/dt), time derivative of LVP (dP/dt) and ECG. ED- end-diastole; ES- end-systole; LVP_{MVO}- intracavitary (Paper II) or transmural (Paper III) LVP at onset of filling (mitral valve opening identified as first diastolic LA-LV pressure crossover); E- early-diastolic transmitral flow rate; A- atrial induced transmitral flow rate.*

Sonomicrometry and Crystal Setup

Sonomicrometry was used as a reference method for evaluation of LV dimension in the experimental studies.[52] The principal measure provided by sonomicrometry is distance between two crystals (Figure 3) at a high temporal resolution. When several crystals are implanted in the myocardium the distances between all crystals can be measured directly.

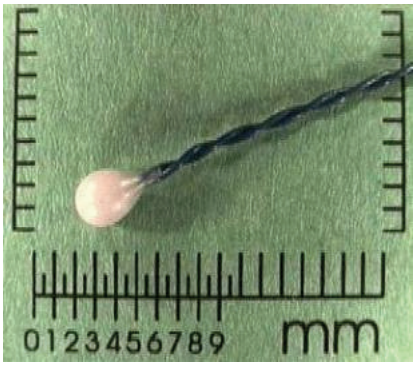

Figure 3. *Ultrasonic crystal (courtesy of Sonometrics Corp., London, Ontario, Canada). Sonomicrometry is the measurement of distances between transmitting and receiving ultrasonic crystals (piezoelectric ceramic crystals) by using the speed of sound principle. Having transmitter as well as receiver capabilities, the 2 mm diameter crystal transmits ultrasound bursts (travelling at 1540 m/s in soft tissue), which are being received by another crystal. The elapsed time from transmission to reception is a direct and linear representation of the spatial distance between the crystals. Wires leading from the crystals are connected to a digital sonomicrometer (Sonometrics Corp.), which is able to transmit from a single crystal to multiple receiving crystals, and thus rendering multiple distanced per ultrasound burst. Transit time is measured in steps of 15 nanoseconds, resulting in a spatial resolution of 0.024 mm.*

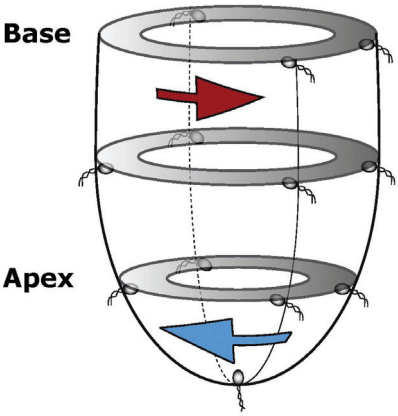

Figure 4. *Schematic illustration of the ultrasonic crystal positions in Papers I and II, and direction of basal (red) and apical (blue) systolic rotation.*

In Papers I and II we implanted 1 crystal at the apex, 4 crystals at the apical level, and 3 and 4 crystals at the basal- and equatorial levels, respectively (Figure 4). In order to limit myocardial damage and to achieve reproducible and parallel planes, the crystals were carefully implanted subepicardially (outer layer of the LV wall, Papers I and II).

In paper III LV apex-to-base long-axis diameter and anteroposterior and septum–to–lateral wall short-axis diameters were measured by sonomicrometry. The ultrasonic crystals that were used in this study were implanted in the inner layer of the LV wall. The basal crystal used to measure long-axis diameter was positioned beneath the bifurcation of the left main coronary artery.

Measurements and Calculations

The velocity of change in diameter was calculated by temporal differentiation of the diameter vs. time. The LV twisting deformation (Papers I and II) was calculated in a more complex approach utilizing information from the 3D grid of the LV, which was constructed from 12 crystals covering the entire LV myocardium.[23]

LV Volume

LV volume was calculated as a modified general ellipsoid by combining the long- and short-axis diameters.[53]

Transmural LV Pressure

Transmural LV pressure (Paper III) was calculated by subtracting pericardial pressure from the intracavitary LV pressure.

Operative LV Stiffness:

Operative LV chamber stiffness was calculated as the slope of the end-diastolic LV intracavitary pressure-volume relationship (Paper II), or the end-diastolic LV transmural pressure-long-axis diameter relationship (Paper III) during transient caval constrictions.

Early-Diastolic Load

In isolated muscle preparations[48] and in situ dog hearts,[49] forces applied to the myocardium during relaxation and filling was defined as “late load”, and an increase in late load resulted in faster myocardial lengthening. In a clinical context, it may be more intuitive to use the term “lengthening load”[54] or early-diastolic load rather than “late load”, because the former refers directly to the physiology of filling. Early-diastolic load may modulate filling by acting as an external expanding force on the LV. As a measure of LV early-diastolic load (lengthening load), we used intracavitary (Paper II) or transmural (Paper III) LV pressure at the time of mitral valve opening (LVP_{MVO}). The rationale for using LVP_{MVO} as a measure of early-diastolic load is that it reflects the external distending load during early filling and is a function of the force that pushes blood into the ventricle at the onset of filling.

Measurement of Restoring Forces– Myocardial Shortening Below Resting Length and Estimated Fully Relaxed Pressure

Another mechanism that may contribute to early-diastolic filling is the restoring forces that have been generated during previous systole. This is analogous to a

spring that has been compressed below its slack length and lengthens when the compression is released. In Paper III restoring forces were quantified by 2 different approaches (Figure 5). First, because restoring forces are generated when myocardium contracts to dimensions less than unstressed or resting length (L_0), we used the extent of long-axis shortening below L_0 as an indirect measure of restoring forces. Second, we derived an estimated value for fully relaxed LV transmural pressure (FRP_{Est}) from LV pressure- diameter curves and used this as a pressure equivalent of restoring forces. The latter served as an estimate of what the fully relaxed pressure would have been at this systolic compression level in case of a nonfilling diastole.[55, 56]

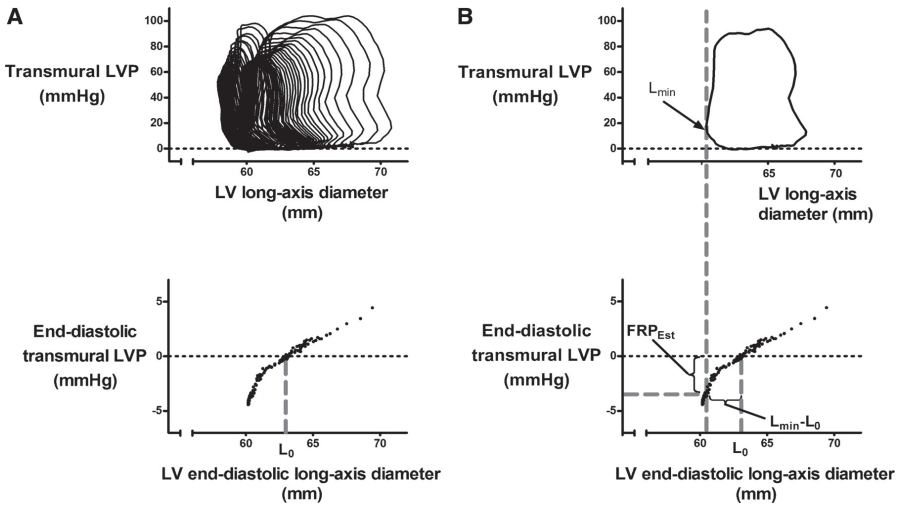

Figure 5. *Panel A: Measurement of unstressed LV diameter (Paper III). The upper left panel displays transmural LV pressure-diameter loops. To obtain a wide range of dimensions, 2 sets of caval constrictions were done, starting at baseline and after volume loading, respectively. The lower left panel displays the LV end-diastolic transmural pressure-diameter coordinates for the loops in the upper panel. Unstressed diameter (L_0) was assessed as the diameter at zero transmural pressure.*

Panel B: Assessment of LV restoring force as shortening below unstressed diameter and estimated fully relaxed pressure (Paper III). The upper right panel displays a representative LV transmural pressure-diameter loop obtained during caval constriction, and the lower panel indicates how the 2 different indices of restoring forces were measured. FRP_{Est}- Estimated fully relaxed pressure; LVP- LV pressure.

Early-Diastolic Mitral Annulus Velocity by Sonomicrometry

Assuming a fixed apex in the LV longitudinal direction, the time-derivate of LV long axis diameter reflects mitral annulus velocity (Figure 6). The first early-diastolic elongation velocity peak was defined as e' and given a positive value.

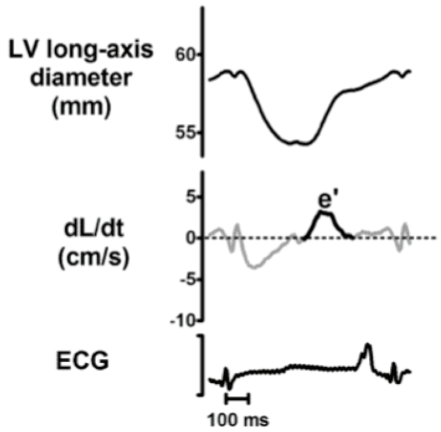

Figure 6. *Representative recording demonstrating LV long axis diameter, long axis shortening/lengthening rate (dL/dt), and ECG. Shortening/lengthening rate was calculated as the time derivative of LV long axis diameter. Peak early-diastolic lengthening rate (e') was defined as first positive wave during diastole.*

LV Rotation and Twist by Sonomicrometry

With signals obtained from the 3D grid of crystals, the coordinates of each crystal were automatically determined in space as a function of time (200 Hz). Parallel apical, equatorial, and basal LV planes were constructed by interpolation of the corresponding crystal coordinates, and the in-plane positions were approximated by cubic Hermite interpolation (Matlab 7, Mathworks, Natick, MA, USA). The centre of rotation for each LV plane was determined as the centre of a best-fit circle through the interpolated coordinates. For each plane, the angular movements of the interpolated coordinates were averaged, and LV twist was estimated as the difference in angular movement between apical and basal planes at isochronal points. Apical and basal rotation were calculated by measuring the difference in angular motion between the equatorial level, where rotation is known to be minimal,[41, 57-59] and the apical and basal levels, respectively.

Twist as conventionally measured was defined using ED configuration within the same heartbeat as reference. Thus, LV twist (Paper I) and $Twist_C$ (Paper II) were calculated as the net difference in apical and basal rotation angle (Figure 7), and rotation was set to zero at ED. As seen from the apex, normal counter-clockwise rotation was defined as positive rotation. Systolic twist was given a positive (Paper I) or negative number (Paper II).

Whereas twist as conventionally measured ($Twist_C$) was calculated using ED configuration within the same heartbeat as reference, absolute LV twist ($Twist_A$) was calculated with respect to ED twist configuration at baseline. This allowed us to investigate how changes in preload modified the degree of untwisting (Figure 8) by measuring ED twist configuration during preload alterations relative to ED twist configuration at baseline.[29, 60]

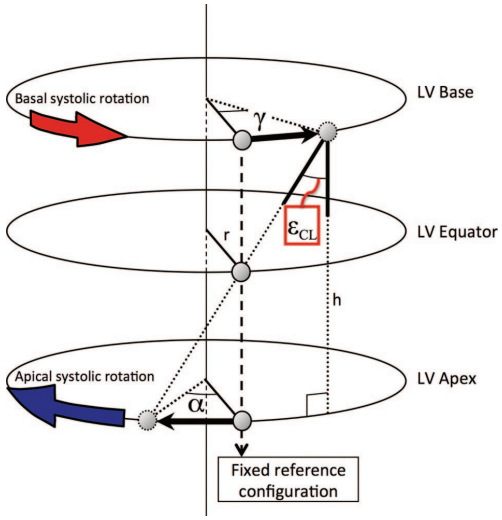

Figure 7*. Cylindrical left ventricular (LV) model illustrating direction and magnitude of systolic basal (red arrow, γ), and apical (blue arrow, α) rotation. Absolute LV twist (Twist$_A$= γ-α) was calculated relative to end-diastolic twist configuration at baseline (fixed reference configuration). Absolute LV circumferential-longitudinal shear strain (ε_{CL}) was calculated as:*

$$\varepsilon_{CL}(t) = tan^{-1}\left[\frac{2 \cdot r(t) \cdot sin\left(\frac{\theta_A(t)}{2}\right)}{h(t)}\right],$$

where t=time, r= LV radius (assuming a cylindrical shape), θ_A = Twist$_A$, and h= LV length measured as distance between apical and basal short-axis planes at each time point during the cardiac cycle.

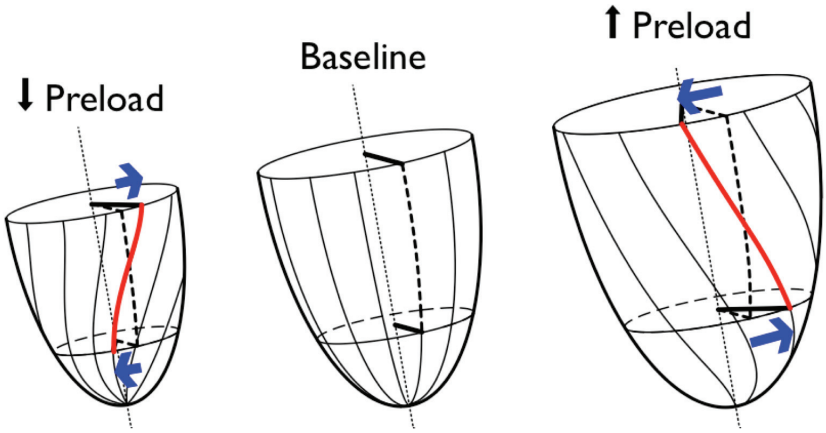

Figure 8. *Passively mediated changes in left ventricular (LV) end-diastolic twist configuration: sketch of LV end-diastolic twist configurations as passive response to preload variations. The center sketch represents the untwisted configuration at baseline. Longitudinal solid black lines are drawn through corresponding points in short-axis planes, and blue arrows indicate direction of change in rotation. In comparison with baseline, reduced preload is associated with a more twisted end-diastolic LV configuration, whereas increased preload is associated with a more untwisted configuration.*

LV Untwisting Rate by Sonomicrometry

Twisting rate was calculated as the time-derivate of LV twist. Peak untwisting rate (UTR) was defined as the early-diastolic peak in the twisting rate trace (Figure 9). Maximal untwisting rate occurring within the IVRT was denoted UTR_{IVR}.

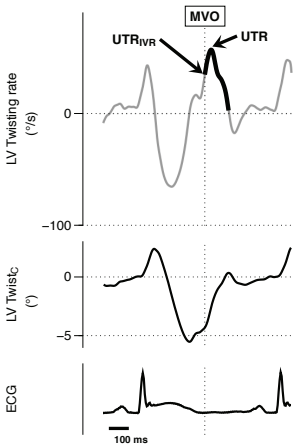

Figure 9. *Representative recording demonstrating LV twisting rate, conventional twist, and ECG. Twisting rate was calculated as the time derivative of LV twist. Peak early-diastolic untwisting rate (UTR) was defined as first positive wave during diastole, and maximal isovolumic untwisting rate (UTR_{IVR}) as the maximum untwisting rate between end-systole and mitral valve opening. $Twist_C$– conventional LV twist.*

LV Circumferential-Longitudinal Shear Strain (ε_{CL}) as a Measure of Restoring Forces

The degree of LV twist does not directly indicate the degree of myocardial deformation, and it is the degree of deformation that is related to restoring forces. Furthermore, neither conventional twist nor torsion (twist normalised by LVED length) takes into account the time varying longitudinal or radial diameter change (r) during the cardiac cycle. Therefore, we calculated circumferential longitudinal shear strain angle (ε_{CL}) as an index of restoring forces. Refining previous measurements of LV ε_{CL},[61] we used instantaneous distance between the apical and basal planes (Figure 7), as well as twisting with respect to a fixed twist position as reference ($Twist_A$).

The first step in the assessment of ε_{CL} was to define the ED LV twist configuration at baseline. Serving as a reference for all subsequent ε_{CL} calculations for the same animal, we assumed that this LV configuration had a fixed offset relative to the resting/unstressed configuration (V_0) with zero transmural LVP.[62] This approach allowed the ε_{CL} calculation to be independent of preload-mediated changes in LV ED twist configuration.[29] As shown in the equation (Figure 7), increased absolute LV twist, increased LV radius, and reduced LV length would all increase myocardial circumferential-longitudinal shear strain as indicated by a more negative ε_{CL}.

Echocardiographic Recordings

All exams were performed with a Vivid 7 ultrasound scanner (GE Vingmed Ultrasound, Horten, Norway), using a phased-array transducer. Tissue Doppler (Paper III) and grey scale recordings (Papers I and II) were obtained in apical- as well as in short axis views. We used ultrasound-gel as standoff to make sure the echocardiographic probe did not mechanically influence LV function. Recordings for subsequent speckle tracking analyses were obtained by transducer frequencies of 1.7-2.0 MHz. Sampling rates (70-110 frames/second) were adjusted for optimal speckle quality.

Early-Diastolic Mitral Annulus Velocity by Tissue Doppler Imaging

Tissue Doppler imaging (TDI) utilizes the fact that moving red blood cells travel at high speed and reflect low amplitude Doppler signals, whereas moving myocardial tissue travels at low speed and reflects very high amplitude Doppler signals. The tissue velocities are obtained by bypassing the high-pass filter and lower the gain amplification. TDI may be used to quantify myocardial velocities in multiple myocardial segments, and from different acquisition views.

The velocities obtained represent the component of motion of a given segment in a direction parallel to the ultrasonic beam. Importantly, this motion is not only caused by myocardial contraction and relaxation, but also by translation and rotation of the cardiac structures. The technical principles and limitations are similar to those encountered by standard Doppler flow systems.

In apical projections of the LV a typical tissue Doppler velocity curve obtained from the mitral annulus consists of three distinct waves (Figure 10), of which the first negative diastolic wave is the early-diastolic mitral annulus velocity.

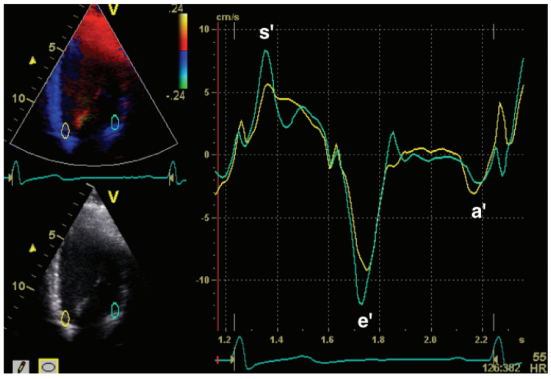

Figure 10. *Shown are color-coded (upper left) and grey-scale (lower left) images of left ventricle (LV) in 4-chamber projection and typical tissue Doppler velocity traces (right panel) of septal (yellow) and lateral (blue) mitral annulus velocity. Systolic positive velocity (s'), and two distinct diastolic negative peaks; early- (e') and atrial induced (a') velocities.*

LV Rotation, Twisting and Untwisting Rate by Speckle Tracking Imaging

Speckle Tracking Echocardiography (STE) is a relatively new approach for assessment of myocardial deformation that was used in Papers I and II. STE utilizes the phenomenon in which natural acoustic markers in grey scale ultrasound images form

interference patterns (speckles) within myocardial tissue. Dedicated software identifies the speckle patterns, and myocardial deformation is automatically being tracked on a frame-by-frame basis. The principal measurement is 2D displacement vs. time for the region of interest. Subsequently, regional deformation measures, such as strain and strain rate, can be calculated from each LV segment in circumferential, longitudinal or radial direction. Furthermore, in short axis projections average angular motion can be quantified as average rotation.[23]

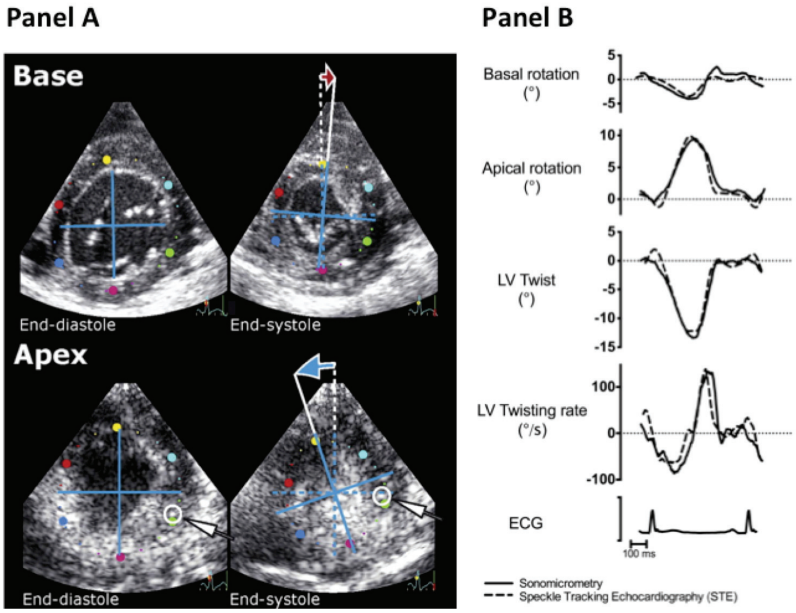

Figure 11. *Panel A: Illustrations of LV rotation by speckle tracking echocardiography (STE). Representative ultrasound recordings from basal and apical short-axis. Blue and red arrows indicate directions of systolic rotation at apex and base, respectively. White circles and arrows indicate the locations of a representative speckle pattern. When viewed from apex, there is counter clockwise rotation of the apex and clockwise rotation of the base. The centre of the area of interest is indicated by the coloured dots, and change of the position of the blue crosses indicates average rotation.*

Panel B: Representative traces of LV rotation, twist and twisting rate by STE (dashed lines) and sonomicrometry (solid lines). LV twist was calculated off-line by subtraction of apical rotation from basal rotation for each time frame during the cardiac cycle. Systolic twisting was presented by a positive number in Paper I and by a negative number in Paper II. Twisting rate was subsequently calculated as the time derivative of LV twist (Paper II).

In a short-axis projection, the endocardial border was traced manually on a frame with well-defined endocardium. An area covering the myocardium of interest was automatically delineated and adjusted manually to include at least two thirds of myocardial thickness excluding pericardium. Inclusion of rotation traces for analysis was based on automated tracking score and visual tracking evaluation. In case of inadequate tracking, the tracing of the endocardial border was manually adjusted or

drawn in another frame until better tracking was achieved. The software automatically divided the LV short-axis into 6 equiangular segments and calculated the rotation trace for each segment. The average rotation for each plane was used for subsequent calculations. LV twist was calculated off-line by subtracting the apical rotation from basal rotation. Systolic twisting was presented by a positive value in Paper I and by a negative value in Paper II.

Experimental Protocol and Interventions

After a 30-minute stabilization period, baseline recordings were performed. To avoid interference between sonomicrometry and ultrasound, we first recorded pressures, ECG, and echocardiographic data, and then pressures, ECG, and sonomicrometric data. Recordings were obtained with the ventilator off. Hemodynamic variables were allowed to return to baseline values before the start of each intervention.

Preload reduction was achieved by transient caval constrictions and preload elevation by rapid infusion of isotonic saline. Increased contractility was induced by Dobutamine infusion. Ischaemia was achieved by LAD occlusion. Dominant collaterals supporting the LAD territory were also ligated.

Clinical Study

Healthy individuals and patients with previous myocardial infarction were studied by echocardiography, and MRI tagging was used as reference method (Paper I). The study protocol was approved by the National Committee for Medical Research Ethics of Norway. All participants gave written, informed consent.

Echocardiographic Recordings

In Paper I, recordings were obtained by 2-dimensional grey-scale echocardiography (same scanner, probe, and optimization approach for speckle quality as were used in the experimental study). All echocardiographic analyses were done without knowledge of the results from the MRI exams.

In addition to apical and basal short-axis recordings, apical 4- and 2-chamber echocardiographic recordings were obtained in a supine left lateral position within 5 to 10 minutes before or after MRI examinations during breath-holds. Short-axis recordings at the basal level were obtained from a standard parasternal transducer position, and apical level recordings were obtained from a more distal anterior or anterolateral position[23] at the most distal level that did not show luminal closure during systole. LV ejection fraction (EF) was assessed by the modified Simpson's method. Speckle tracking analyses were performed in the same manner as in the experimental part of Paper I. In the clinical study the speckle quality improved progressively from the epicardium to the endocardium in the short-axis recordings that were analysed. Therefore, we limited the clinical study to assessment of rotation of the mid- and subendocardial layers.

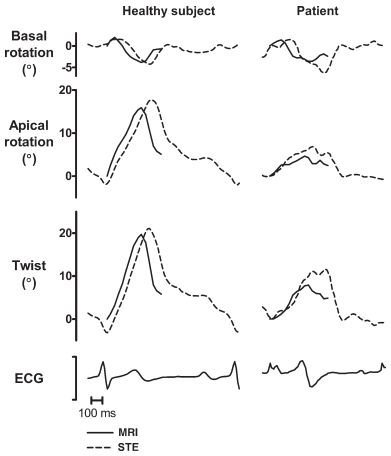

Figure 12. *Representative recordings of LV rotation and twist by speckle tracking echocardiography (STE) and tagged magnetic resonance imaging (MRI). Traces are shown for basal and apical rotation and LV twist by MRI (solid line) and STE (dotted line) from a healthy subject (left) and a patient with LAD-related prior myocardial infarction and reduced LV ejection fraction (right). Rotation by MRI is not feasible for the entire heart cycle because of tag fading in diastole. ECG, Electrocardiogram.*

Magnetic Resonance Tagging

Images were obtained with a 1.5 T scanner (Magnetom Vision Plus, Siemens, Erlangen, Germany). The LV basal image plane was defined just apical to the fibrous mitral ring, and the apical image plane was defined just basal to the level with luminal closure at end-systole. Striped tags were prescribed separately in 2 orthogonal orientations (45 and 135°, Figure 13) with spatial modulation of magnetization in a grid pattern with an 8-mm distance between tags and a time resolution of 35 ms. Images were acquired during 12- to 18-second breath holds and triggered by ECG. Consistent with STE measurements, rotation by MRI tagging was calculated as the average of measurements obtained in the mid- and subendocardial layers (Figure 12). Recordings were analysed by Harmonic Phase Imaging (HARP version 1.1, Diagnosoft Inc., Palo Alto, CA).[63]

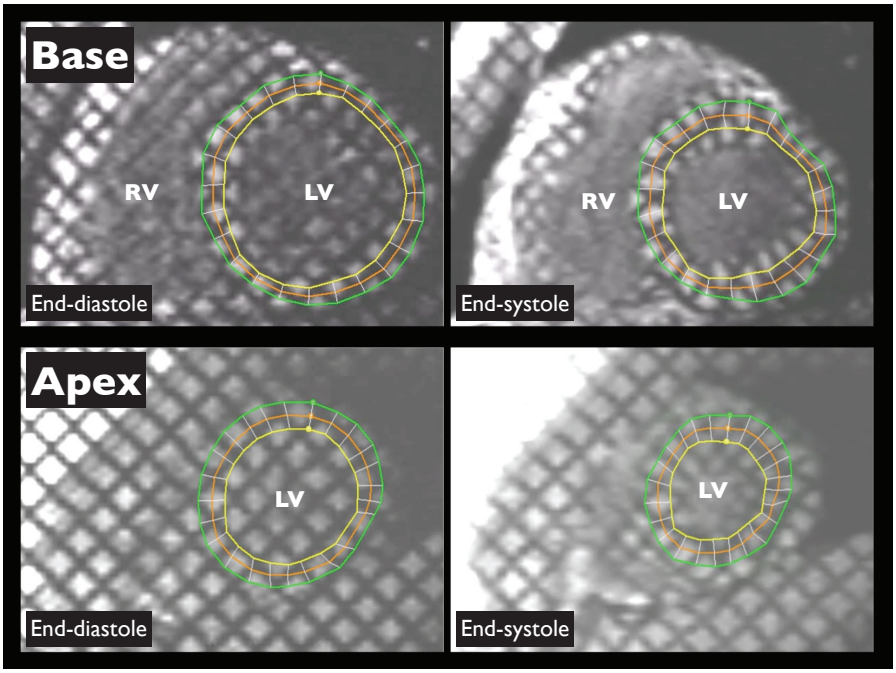

Figure 13. *Representative recordings of tagged cardiac Magnetic Resonance Imaging (MRI), illustrating basal (upper panels) and apical (lower panels) short-axis projections at end-diastolic (left) and end-systolic frames (right). Outer (green) and inner (yellow) circles indicate region of interest for the HARP analysis. Orthogonal tagging lines (dark grid) are applied at end-diastole, and follow myocardium during systolic displacement. RV- right ventricle; LV- left ventricle.*

Reproducibility of Speckle Tracking Measurements

In Paper I measurements by STE of peak rotation by 2 independent observers showed a mean difference between the 2 analyses of 0.1±2.4 degrees, with an intraclass correlation of 0.97. The intraclass correlation for intraobserver variability in 2 different recordings was 0.68 and 0.95 for basal and apical rotation, respectively.

Statistics

Values are expressed as mean±SD, unless otherwise stated. Statistical differences were considered significant at $P<0.05$. Variables from independent measurements were compared by a least squares linear regression and the Bland-Altman method[64] (Paper I).

To assess interobserver variability of peak rotation by STE (Paper I), experimental and clinical recordings were randomly selected and analysed by using intraclass correlation coefficient (α value) and the Bland-Altman method. To assess intraobserver variability of peak rotation by STE (Paper I), clinical echocardiographic recordings were randomly selected and analysed using the intraclass correlation coefficient.

Independence and homogeneity of variance are fundamental assumptions at the basis of many standard statistical techniques, such as t-test, ANOVA and multiple linear regression. Therefore, these methods are not optimal for the repeated

measurements in our experimental model. In order to handle the dependencies in repeated measurements within the same subject, as well as heterogeneous variability (Paper I, II and III), we analysed the variables by a mixed model procedure[65] with structured covariance matrix (SPSS 14, 16 and 18, SPSS Inc., Chicago, Illinois). Quadratic terms were considered and only included if significant. Covariance structures that were appropriate to the experimental protocol were considered, and chosen according to the lower information criteria (Akaike). Goodness of fit and normal distribution was assessed by residuals inspection.

Summary of Results

Paper I: Apical Rotation by Speckle Tracking Echocardiography: A Simplified Bedside Index of Left Ventricular Twist

OBJECTIVE: Magnitude of left ventricular (LV) twist has been proposed as a sensitive marker of LV function, but clinical implementation has not been feasible because of the complexity and limitations of present methodologies. The study objective was to determine whether LV apical rotation by speckle tracking echocardiography (STE) might serve as a clinically feasible index of LV twist.

METHODS AND RESULTS: The relationship between apical rotation and LV twist was investigated in anesthetized dogs (n=9) and a clinical study that included healthy controls (n=18) and patients (n=27) with previous myocardial infarction. Rotation by STE was compared with twist measured by magnetic resonance imaging and sonomicrometry in humans and dogs, respectively. In dogs, apical rotation by STE correlated well with LV twist over a wide range of loading conditions and inotropic states, and during myocardial ischaemia ($R=0.94$, $P<0.01$). Similarly, in humans there was a strong correlation between apical rotation and twist ($R=0.88$, $P<0.01$) but only a weak correlation between basal rotation and twist ($R=0.53$, $P<0.01$). Apical rotation accounted for 72±14 and 73±15% of the twisting deformation by magnetic resonance imaging in controls and patients, respectively. In dogs, apical rotation and twist decreased during myocardial ischaemia ($P<0.05$). In patients, LV twist and apical rotation were reduced ($P<0.05$) only when LV ejection fraction was less than 50%.

CONCLUSION: Apical rotation represents the dominant contribution to LV twist, and apical rotation by STE reflects LV twist over a wide range of hemodynamic conditions. These findings suggest that apical rotation by STE may serve as a simple and feasible clinical index of LV twist.

Paper II: Myocardial Relaxation, Restoring Forces, and Early-Diastolic Load are Independent Determinants of Left Ventricular Untwisting Rate

OBJECTIVE: Peak left ventricular (LV) untwisting rate (UTR) has been introduced as a clinical marker of diastolic function. This study investigates if early-diastolic load and restoring forces are determinants of UTR in addition to the rate of LV relaxation.

METHODS AND RESULTS: In 10 anesthetized dogs we measured UTR by sonomicrometry and speckle tracking echocardiography at varying LV preloads, increased contractility and myocardial ischaemia. UTR was calculated as the time derivative of LV twist. Because preload modified end-diastolic twist, LV systolic twist was calculated in absolute terms with reference to the end-diastolic twist configuration at baseline. Relaxation rate was measured as the time constant (τ) of LV isovolumic pressure decay. Early-diastolic load was measured as LV pressure at the time of mitral valve opening. Circumferential-longitudinal shear strain was used

as an index of restoring forces. In a multivariable mixed model analysis a strong association was observed between UTR and LV pressure at mitral valve opening (parameter estimate [β]=6.9; *P*<0.0001), indicating an independent effect of early-diastolic load. Furthermore, the associations between UTR and circumferential-longitudinal shear strain (β=-11.3; *P*<0.0001) and τ (β=-1.6, *P*<0.003), were consistent with independent contributions from restoring forces and rate of relaxation. Maximal UTR before mitral valve opening, however, was determined only by relaxation rate and restoring forces.

CONCLUSIONS: The present study indicates that early-diastolic load, restoring forces, and relaxation rate are independent determinants of peak UTR. However, only relaxation rate and restoring forces contributed to UTR during isovolumic relaxation.

Paper III: Determinants of Left Ventricular Early-Diastolic Lengthening Velocity: Independent Contributions from Left Ventricular Relaxation, Restoring Forces, and Lengthening Load

BACKGROUND: Left ventricular (LV) peak early-diastolic mitral annulus velocity (e') by tissue Doppler imaging has been introduced as a clinical marker of diastolic function. This study investigates whether lengthening load (early-diastolic load) and restoring forces are determinants of e' in addition to rate of LV relaxation.

METHODS AND RESULTS: In 10 anesthetized dogs, we measured e' by sonomicrometry and tissue Doppler imaging during baseline, volume loading, caval constriction, dobutamine infusion, and occlusion of the left anterior descending coronary artery. Relaxation was measured as the time constant (τ) of LV pressure decay. Lengthening load was measured as LV transmural pressure at mitral valve opening (LVP_{MVO}). Restoring forces were quantified by 2 different indices: (1) As the difference between minimum and unstressed LV diameter (L_{min}-L_0) and (2) as the estimated fully relaxed LV transmural pressure (FRP_{Est}) at minimum diameter. In the overall analysis, a strong association was observed between e' and LVP_{MVO} (β=0.49; P<0.001), which indicates an independent effect of lengthening load, as well as between e' and L_{min}-L_0 (β=-0.38; P<0.002) and between e' and FRP_{Est} (β=-0.31; P<0.002), consistent with an independent contribution of restoring forces. A direct effect of rate of relaxation on e' was observed in a separate analysis of baseline, dobutamine, and ischaemia when postextrasystolic beats were included (β=-0.06, P<0.01).

CONCLUSIONS: The present study indicates that in the nonfailing ventricle, in addition to LV relaxation, restoring forces and lengthening load are important determinants of early-diastolic lengthening velocity.

Discussion

This thesis demonstrates that apical rotation by STE reflects LV twist over a wide range of hemodynamic conditions in dogs, and in healthy controls as well as in patients with prior myocardial infarction. These findings suggest that apical rotation by STE may serve as a simple and feasible clinical index of LV twist. The clinical implications of this index should be investigated in future studies.

A load independent marker of LV relaxation would most likely be important in the assessment of diastolic function. Although, we reproduced the association between isovolumic untwisting rate and relaxation rate, peak untwisting rate was determined by restoring forces as well as diastolic load, in addition to relaxation rate. Furthermore, a load dependency of end-diastolic twist configuration was also found. Therefore, systolic function as well as diastolic loading conditions may modify untwisting rate.

Early-diastolic lengthening velocity is another index of diastolic function, which has been proposed as a marker of LV relaxation rate. In this thesis we confirmed the relationship between e' and LV relaxation rate. However, we also demonstrated that e' is dependent of restoring forces that have been generated during systole, as well as early-diastolic load. Therefore, changes in both systolic and diastolic function may cause changes in LV lengthening rate. Further studies are needed in order to determine the clinical implications of these factors in the assessment of diastolic function.

Twisting and Apical Rotation

In paper I we investigate whether apical rotation may be used as a simplified index of LV twisting. In the experimental study during a wide range of hemodynamic conditions as well as in the clinical part of this study, a close relationship between apical rotation and LV twist was found. Taken together, these results indicate that apical rotation by STE may be used as a simplified clinical index for assessing changes in LV twist.

Previous studies have confirmed the ability of STE to measure LV twist. However, there are methodological challenges that limit the clinical implementation of twist assessment.[23, 66] The suboptimal image quality of basal LV short-axis recordings is a challenge[23, 31, 32] because of acoustic problems related to the depth and the wide sector angle needed to visualize the entire LV base, and out-of-plane motion due to systolic descend toward the apex.

Both the experimental and clinical parts of this study showed that the basal rotation was of minor magnitude compared with apical rotation consistent with previous studies.[22, 61, 67, 68] There was a dominance of apical relative to basal rotation especially for the human data. In contrast with the strong correlation between apical rotation and twist, there was only a weak correlation between basal rotation and twist. This reflects that differences in basal rotation between individuals were small. In addition, inherent technical limitations of imaging the LV base may have contributed to the weak relationship. Therefore, because the apical short-axis view provides optimal imaging quality and apical rotation accounts for most of the

twisting deformation, we suggest that apical rotation may serve as a practical clinical index of LV twist. Our findings have been supported by subsequent studies in dogs,[69] and in patients with myocardial infarction and heart failure.[70, 71]

Left Ventricular Apical Rotation and Twist During Changes in Contractility, Preload and Ischaemia

We observed marked increments in twist after preload elevation in dogs, indicating that LV twist is highly load-dependent. As predicted, inotropic stimulation by dobutamine also caused marked increments in LV twist. Apical rotation reflected changes in LV twist with either intervention.

In Paper I, apical contribution to LV twist decreased during acute ischaemia in dogs, whereas basal rotation remained unchanged, reflecting that LAD occlusion impaired LV function predominantly in the apical part of the ventricle. This implies that ischaemia caused a relative increase in basal contribution to twist.

In the clinical part of Paper I a strong correlation between apical rotation and LVEF, and between LV twist and EF was demonstrated, consistent with previous studies,[72, 73] and reflecting that reduction in LV systolic function is accompanied by reduction of twist. Patients with EF≥50% had values of rotation and twist similar to the healthy subjects, whereas patients with an EF<50% apical rotation and LV twist were reduced. Therefore, Paper I is consistent with previous studies that have described reduced twist in patients with myocardial infarction.[57, 72, 73] The findings suggest that twist and apical rotation are closely related to global LV function in healthy individuals and in patients with chronic myocardial infarctions affecting apical or basal segments.

Early-Diastolic Untwisting

In Paper II we demonstrate that *early-diastolic load* is an independent determinant of peak untwisting rate (UTR) in addition to LV *restoring forces* and LV *relaxation*. The study also demonstrates that end-diastolic twist, measured in absolute terms, is preload-dependent. Changes in peak untwisting rate during volume loading was attributed to increments in LV early-diastolic load and could not be explained by changes in myocardial relaxation or restoring forces. Increase in peak untwisting rate during dobutamine infusion was attributed to both stronger restoring forces and more rapid relaxation, and reduced untwisting rate during ischaemia, to both loss of restoring forces and slowing of relaxation.

Relationship Between LV Restoring Forces, Relaxation Rate and Untwisting Rate

In general, restoring forces are generated when myocardial tissue is being deformed, that tend to restore myocardium to its resting shape. Systolic deformation of the myocardium generates potential energy analogous to compression or twisting of a spring. This potential energy (i.e., restoring force) has been associated with rapid, early-diastolic untwisting. When myocardial fibres contract below unstressed length, the generated restoring forces will cause the fibres to recoil back to their resting length when active fibre force decays. Therefore, we predicted that absolute LV circumferential-longitudinal share strain (ε_{CL}), a measure of the left ventricle's

wringing deformation with respect to a fixed reference configuration, would be a determinant of peak UTR. This was confirmed by the demonstration of a strong association between peak UTR and absolute ε_{CL}. This is consistent with the studies by Wang et al[33] which demonstrated a relationship between LV end-systolic volume and peak UTR. However, restoring forces are not related to end-systolic volume as such, but to the extent of deformation relative to the configuration at resting LV volume at zero transmural pressure (V_0).

In Paper II we assumed that end-diastolic twist configuration at baseline had a fixed offset from resting configuration at V_0 and utilized this approach to calculate absolute twist ($Twist_A$). Subsequently, $Twist_A$ was used to quantify absolute ε_{CL} as an index of restoring forces. A strong association between UTR and absolute ε_{CL} was observed. Therefore, when the ventricle contracted and changed twist configuration leading to a more negative absolute ε_{CL}, changes in shear strain were associated with peak UTR, supporting the hypothesis that restoring forces contribute to peak UTR. Increased peak UTR during dobutamine infusion was accompanied by a decrease in time constant (τ) of LV isovolumic pressure decay, which indicates faster myocardial relaxation. Also, absolute ε_{CL} was more negative, indicating stronger restoring forces. This suggests that dobutamine may have increased peak UTR both by a direct effect on myocardial relaxation and by an effect on restoring forces.

The mixed model analysis demonstrated that both peak UTR_{IVR} as well as peak UTR was significantly and independently associated with τ as well as absolute ε_{CL}. This is in keeping with previous studies, which demonstrated that LV relaxation rate is a determinant of early-diastolic untwisting rate.[21, 29, 33-35]

Relationship Between LV Early-Diastolic Load and Untwisting Rate

Restoring forces and relaxation reflect intrinsic myocardial properties, which govern untwisting before and during filling, thus affecting the ventricle's ability to fill itself. In contrast, early-diastolic load modulates untwisting during filling by acting as an external expanding force on the LV. In Paper II we used intracavitary LV pressure at the time of mitral valve opening (LVP_{MVO}) as a measure of *early-diastolic load*. This is analogous to the forces applied to the myocardium during relaxation and filling as described in isolated muscle preparations[48] and canines.[3, 54] During a wide range of hemodynamic conditions, a close association between peak UTR and LVP_{MVO} was observed and neither τ nor ε_{CL} could explain the relationship. The increase in peak UTR during volume loading could only be attributed to an increase in LVP_{MVO}. These findings support the hypothesis that changes in LVP_{MVO} have direct effects on peak UTR by acting similarly to *late load,* as described in isolated muscle preparations.[48]

Previous reports have demonstrated that untwisting occurs during IVR and early filling and that peak UTR precedes peak early-diastolic lengthening velocity (e') and filling velocity (E).[30, 33, 60, 74] Some studies also report that peak UTR occurs after MVO.[30, 33, 34, 75, 76] According to previous studies, peak UTR is relatively insensitive to changes in LV end-diastolic pressure or preload.[21, 33] However, in apparent contrast to the earlier findings, Paper II demonstrates that peak UTR indeed depends on early-diastolic load in addition to relaxation rate and restoring forces, whereas peak UTR_{IVR} depends on relaxation rate and restoring forces only.

In previous reports, variation in peak UTR has been attributed to changes in ES LV twist as conventionally measured[33, 34, 36, 37, 57] and has, therefore, been interpreted as

changes in restoring forces. However, as demonstrated in Paper II, although increased preload increases conventional twist, absolute twist and hence restoring forces, are not increased. Therefore, increased peak UTR during volume loading was not caused by restoring forces, but rather by early-diastolic load which in turn is associated with preload. Some publications have suggested that peak UTR is preload-dependent.[38] Since *preload* acts as a regulator of LV function at end-diastole, a time when early-diastolic untwisting has already occurred, *preload* as such cannot have a direct effect on peak UTR.

Early-Diastolic Lengthening

In Paper III we demonstrate that LV restoring forces and LV lengthening load are independent determinants of e', in addition to myocardial relaxation. Changes in e' during loading interventions were attributed entirely to changes in LV lengthening load and could not be explained by changes in myocardial relaxation or restoring forces. Increments in e' during dobutamine were attributed mainly to stronger restoring forces and during ischaemia to both loss of restoring forces and slowing of relaxation. Our findings have been supported by a subsequent mathematical modelling study.[40]

Relationship Between LV Restoring Forces, Contractility and Early-Diastolic Lengthening Rate

When myocardial fibres contract below their unstressed length, restoring forces are generated that will recoil the fibres back to their resting length when active fibre force decays. However, restoring forces are not related to end-systolic length as such, but rather to extent of shortening below unstressed or resting muscle length ("slack length"). Therefore, we measured minimal LV length (L_{min}) relative to slack length (L_0) and used L_{min}- L_0 as an index of restoring forces. Measurements taken during baseline conditions, dobutamine infusion, and myocardial ischaemia demonstrated a strong association between e' and L_{min}-L_0.

Nagueh et al[20] related e' to LV minimal pressure in a dog model and demonstrated a significant inverse relationship between the 2 variables, suggesting that diastolic suction contributes to e'. Although their assumption is highly relevant, no quantification of restoring forces was performed. Minimum LV pressure during ongoing filling will tend to underestimate restoring forces, because the ventricle has already expanded before the time of minimum pressure. Furthermore, acceleration of blood and myocardial mass and viscous forces account for additional pressure components.[77] Therefore, LV pressure during rapid filling is always higher than predicted by the pressure-volume curve defined during static conditions. Direct measurement of restoring forces or their pressure equivalent is not feasible in the working heart unless additional interventions or complex methods are used.[55, 56] In Paper III, we used an estimate of pressure in a fully relaxed ventricle as an equivalent of restoring forces. When the ventricle contracted to dimensions less than L_0, estimated fully relaxed pressure became increasingly negative and was associated with an increased e', which supports the hypothesis that restoring forces contribute to e'.

In Paper III, increased e' during dobutamine was accompanied by a decrease in τ, which indicates faster myocardial relaxation. A decrease in LV minimum length to values less than L_0 was found, which indicates stronger restoring forces. This suggests that dobutamine increased e' both by a direct effect on myocardial relaxation and by an effect on restoring forces. This is in keeping with previous studies[49] which suggested that increased e' by dobutamine is attributed mainly to restoring forces, reflecting increased contractility which causes smaller end-systolic myocardial dimension.

During myocardial ischaemia, we observed a reduction in e', and LV minimum length increased and approached L_0, which indicates that loss of restoring forces contributed to the reduction in e'. Furthermore, a marked increase in τ was seen, which indicates slowing of LV relaxation. Because LVP_{MVO} was essentially similar during baseline and ischaemia, reduced e' during ischaemia could not be attributed to reduced lengthening load. Most likely, reduction of e' during ischaemia is accounted for in part by slowing of LV relaxation and in part by loss of restoring forces.

Relationship Between LV Lengthening Load and Early-Diastolic Lengthening Rate

In isolated muscle preparations, late load is defined as forces applied to the myocardium during relaxation and filling, and an increase in late load results in faster myocardial lengthening.[48] In a clinical context, it may be more intuitive to use the term "lengthening load"[54] or early-diastolic load rather than "late load", because the former refers directly to the physiology of filling. In Paper III, we used transmural LV pressure at the time of mitral valve opening (LVP_{MVO}) as a measure of lengthening load. During a wide range of hemodynamic conditions, a close association between e' and transmural LVP_{MVO} was observed, and neither τ nor measures of restoring forces could explain the relationship. The increase in e' during volume loading could only be attributed to an increase in LVP_{MVO}, because restoring forces measured as shortening below resting length and estimated fully relaxed pressure did not increase, and τ increased slightly and had no significant association with e'. These findings support the hypothesis that changes in LVP_{MVO} have direct effects on e' by acting similar to late load, as described in isolated muscle preparations.[48]

Limitations and Comments to Methodology

In this thesis we used a heavily instrumented dog model. However, it allowed our hypotheses to be tested under well-controlled measurement conditions. The animal model used in the thesis differs from a clinical setting in particular due to the open-chest condition, the extensive instrumentation, and the use of general anaesthesia. However, the responses to variation in preload, contractility, as well as responses to ischaemia are probably comparable. Therefore, we believe the model is valid for studying basic principles of cardiac mechanics.

In Paper I there was a tendency towards underestimation of LV apical rotation and twist by sonomicrometry relative to STE, and in Paper II sonomicrometry slightly underestimated UTR compared to STE. This may be attributed to the subepicardial location of the crystals. In contrast, STE measurements were obtained more mid- and subendocardially, with higher rotation.[78] In Paper III sonomicrometry slightly underestimated e' compared with TDI, and this may have been attributable to the location of the basal crystal, slightly apical to the mitral ring. The strong association between sonomicrometry and the echocardiographic variables indicates that sonomicrometry provided results that were comparable to echocardiography. The strength of sonomicrometry is that the variable in question (e.g. apical rotation, UTR or e') can be measured simultaneously with all other variables, and this allows more extensive exploration of the underlying physiology.

In Paper II we used ED twist position at baseline as the reference for calculation of absolute twist and ε_{CL} for all interventions. This approach is not equivalent to assessing LV twist configuration at V_0. We assumed, however, that a constant offset exists between the twisting state used for reference and the resting twisting state at V_0. This assumption enabled comparison of dynamic changes in ε_{CL} between and within heartbeats from various hemodynamic conditions. In Paper III we measured pericardial pressure that enabled calculation of transmural LV pressure. Thus, resting myocardial length (L_0) can be identified as myocardial length at zero transmural pressure, and restoring forces was calculated as myocardial shortening below L_0. As deformation relative to the resting state reflects restoring forces, but does not measure force directly, we also calculated estimated fully relaxed pressure as a pressure equivalent of restoring forces.

In all papers in this thesis studies were performed in an open-chest model with the pericardium loosely re-sutured. As previously shown,[50] this does not mean that pericardial constraint is absent, and pericardial constraint can be measured accurately with the flat liquid-containing balloon transducer as used in Paper III.

Conclusions

In the present thesis we demonstrate that LV apical rotation is the dominant contributor to LV twist. Furthermore, systolic deformation generates restoring forces, which, together with diastolic load are important determinants of early-diastolic lengthening and untwisting, in addition to LV relaxation rate.

1) In a combined experimental and clinical study (Paper I) we demonstrate that apical rotation represents the dominant contribution to LV twist, and apical rotation by STE reflects twist over a wide range of hemodynamic conditions. These findings suggest that apical rotation by STE may serve as a simple and feasible clinical index of LV twist.
2) In an experimental study (Paper II) we demonstrate that restoring forces and relaxation rate are independent determinants of peak UTR during the isovolumic relaxation phase. When peak UTR occurs after mitral valve opening, however, early-diastolic load is an additional determinant of UTR.
3) In an experimental study (Paper II) we demonstrate that LV end-diastolic pressure modifies the degree of end-diastolic twist. Therefore, loading conditions needs to be accounted for in the assessment of absolute LV twist configuration throughout the cardiac cycle.
4) In an experimental study (Paper III) we demonstrate that in the nonfailing ventricle, in addition to LV relaxation, restoring forces and lengthening load are important determinants of early-diastolic lengthening velocity (Paper III).

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