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

Running title: Opdahl et al.; Determinants of LV untwisting rate

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Abstract:

**Background** - 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 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 ischemia. 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 ($\tau$) of LV isovolumic pressure (LVP) decay. Early-diastolic load was measured as LVP at the time of mitral valve opening (LVP$_{MVO}$). Circumferential-longitudinal shear strain ($e_{CL}$) was used as an index of restoring forces. In a multivariable mixed model analysis a strong association was observed between UTR and LVP$_{MVO}$ (Parameter estimate $[\beta] = 6.9; P<0.0001$), indicating an independent effect of early-diastolic load. Furthermore, the associations between UTR and $e_{CL}$ ($\beta = -11.3; P<0.0001$) and $\tau$ ($\beta = -1.6, P<0.003$), were consistent with independent contributions from restoring forces and rate of relaxation. Maximal UTR prior to 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.

**Key words:** animal model; diastolic dysfunction; left ventricular torsion; relaxation; ventricular mechanics
Introduction

Left ventricular (LV) peak untwisting rate (UTR) by speckle tracking echocardiography (STE)\(^1\) or tagged magnetic resonance imaging (MRI)\(^2\) have been proposed as markers of diastolic function due to their association with the time constant (\(\tau\)) of LV isovolumic pressure decay.\(^1,3\) In addition, UTR has been associated with LV restoring forces measured indirectly as LV end-systolic volume (ESV),\(^1,4,5\) and as systolic twist.\(^1,3,6,7\) It is not clear, however, whether LV relaxation and restoring forces are independent determinants of UTR. Furthermore, a recent study\(^8\) showed increased UTR during volume loading, which tends to increase \(\tau\) (slower relaxation) as well as ESV (decreased restoring forces). Increased UTR despite slowed relaxation and reduced restoring forces suggests that UTR is not solely determined by these two 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,\(^5,8,9\) whereas others have reported only a minor preload dependency or none at all.\(^10,11\) We hypothesized that diastolic pressure has a direct effect on UTR similar to the effect of early-diastolic load on LV lengthening rate.\(^12,13\)

The aim of the present study was to determine whether LV relaxation and restoring forces are independent determinants of UTR and if LV diastolic pressure has direct effects on UTR. Because twist is conventionally calculated as the difference between apical and basal rotation relative to end-diastolic configuration within the same heart beat (\(\text{Twist}_C\)), the possibility that diastolic pressure modifies the degree of twist at end-diastole is not taken into account.\(^14\) In principle, this is a significant limitation since a change in \(\text{Twist}_C\) may be due to a change in end-diastolic twist with no change in peak 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 the present study this was achieved by measuring twist with respect to a fixed reference configuration.$^5$ Furthermore, twist per se does not directly quantify the myocardial deformation which is responsible for generation of restoring forces. The same twist amplitude in a long ventricle causes less myocardial deformation (and, thus, less restoring forces) than in a short ventricle, and an LV with a large short-axis radius will be more deformed compared to one with a shorter radius for the same degree of twist. Thus, to better quantify the relationship between twist and restoring forces we also express twisting deformation in terms of circumferential-longitudinal shear strain ($\varepsilon_{CL}$), which takes into account LV length and short-axis radius. The $\tau$ of LV isovolumic pressure decay was used as a measure of rate of LV relaxation, and $\varepsilon_{CL}$ was used as a measure of restoring forces. Because peak UTR occurs early in diastole we used LVP at mitral valve opening rather than end-diastolic pressure as a measure of diastolic load. The study was done in a dog model during different levels of contractility, different loading conditions, and during acute myocardial ischemia.

**Methods**

Ten mongrel dogs of either sex and body weight of 26±2 kg were anesthetized, ventilated and surgically prepared with pressure catheters, electrocardiogram and sonomicrometric crystals as previously described.$^{15}$ They were euthanatized at the end of the experiments by a lethal dose of pentobarbital. The National Animal Experimentation Board approved the study. The laboratory animals were supplied by the Center for Comparative Medicine, Oslo University Hospital.

The time constant of LV isovolumetric relaxation ($\tau$) was used as a marker of rate of LV relaxation.$^{16}$ Time of first diastolic LA-LV pressure crossover served as a marker of time of MVO. LV intracavitary pressure at MVO (LVP$_{MVO}$) was used as a measure of LV early-diastolic
load. ED and ES were defined as onset of the R-wave in the ECG and as time of minimum LV dP/dt, respectively.

For dimension and twist measurements, 1 sonomicrometric crystal was implanted at the tip of the apex and 11 crystals along the LV circumference at basal (n=3), equatorial (n=4), and apical (n=4) short-axis levels as previously described. To minimize myocardial damage and to achieve reproducible and parallel planes, the crystals at each level were placed subepicardially at distances ≈ 20, 40, and 60 mm from the LV apex. With signals obtained from the 3D grid of crystals, the coordinates of each crystal were automatically determined in space as a function of time at a sampling rate of 200 Hz.

Calculation of LV volume and stiffness

LV volume (LVV) was calculated as a modified general ellipsoid. Operative LV stiffness was calculated as the slope of the LV EDV versus EDP relationship during transient caval constrictions.

Calculation of LV twist

LV TwistC was calculated as basal rotation minus apical rotation (Figure 1) using ED configuration within the same heartbeat as reference. As seen from the apex, counter-clockwise rotation was defined as positive rotation. By these conventions an increasingly negative twist value indicates that the ventricle is increasingly twisted. LV TwistA was calculated with respect to ED twist configuration at baseline. This allowed us to investigate how changes in preload changed the degree of untwisting (Figure 2) by measuring ED twist configuration during preload alterations relative to ED twist configuration at baseline. Peak UTR was defined as the maximal time derivative of LV twist after ES. The maximum UTR value during isovolumic relaxation (IVR) was also extracted and termed UTR_{IVR}.
Calculation of LV circumferential-longitudinal shear strain ($\varepsilon_{CL}$) as an index of restoring forces

In general, restoring forces is a function of degree of myocardial deformation relative to a resting state. As twist does not take into account the dynamic longitudinal or radial diameter change ($r$), we calculated absolute circumferential-longitudinal shear strain angle ($\varepsilon_{CL}$) as an index of restoring forces. Refining previous measurements of LV $\varepsilon_{CL}$, we used instantaneous distance between the apical and basal planes. Calculation of $\varepsilon_{CL}$ is shown in Figure 1. Furthermore, to take into account preload effect at end-diastole, we used twisting with respect to a fixed twist position as reference (absolute twist, $\text{T}_w$).

The first step in the assessment of absolute $\varepsilon_{CL}$ was to define the ED LV twist configuration at baseline. Serving as a reference for all subsequent calculations of absolute $\varepsilon_{CL}$ 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. This approach allowed the absolute $\varepsilon_{CL}$ calculation to be independent of preload-mediated changes in LV ED twist configuration.

From the equation in legend to Figure 1 it can be derived that increased counterclockwise apical rotation, increased LV radius, and reduced LV length would all increase absolute myocardial circumferential-longitudinal shear strain as indicated by a more negative absolute $\varepsilon_{CL}$. Conventional $\varepsilon_{CL}$ was calculated in a similar fashion, but conventional twist was used, rather than absolute twist. We extracted peak twist and peak $\varepsilon_{CL}$ as the minimal value at end-systole or after, including contribution from post-systolic twist to restoring forces.

Echocardiography

To validate the principles that were studied with a method which is used clinically we also
assessed LV twist by STE from 2D gray scale short-axis recordings as previously described.\textsuperscript{15} The echocardiographic analyses were performed without knowledge of the results from the reference method. Using crystals as anatomic landmarks, short-axis echocardiograms were recorded in the same planes as were used for rotation by sonomicrometry.\textsuperscript{15}

**Experimental Protocol**

To determine how UTR responded to changes in loading, preload was reduced by transient caval constrictions and was elevated by rapid intravenous infusion of body tempered isotonic saline.

To determine how UTR responded to increased inotropy and acute ischemia, respectively, dobutamine was given intravenously (5.0 \(\mu g/kg/min\)) and the left anterior descending coronary artery (LAD) was occluded for 15 minutes by a snare placed immediately distal to the first diagonal branch.

**Statistics**

Variables were analyzed by a mixed model procedure\textsuperscript{22} with structured covariance matrix (SPSS 18, SPSS Inc., Chicago, Illinois) to handle the dependencies in repeated measurements within the same subject. Quadratic terms were considered and only included if significant. Prior to assessment of the regression model analysis, the covariance structure with lowest information criteria (Akaike) was chosen from structures considered appropriate to the experimental protocol. Goodness of fit and normal distribution were assessed by residuals inspection. First, the 3 proposed determinants of UTR (\(\tau\), \(LVP_{MVO}\), and \(e_{CL}\)) were assessed in a multivariable mixed model analysis. Second, in a separate analysis, \(Twist_C\), ESV, and LV stiffness were added to the mixed model as potential determinants of UTR and kept only if significant. Parameter estimates (\(\beta\)) with 95% confidence intervals (CI) were reported. Values are reported as fitted means and estimated differences with 95% CI if not stated otherwise. Statistical differences were considered...
significant at $P<0.05$ ($P<0.01$ for multiple comparisons). The authors had full access to the data and take responsibility for their integrity. All authors have read and agree to the manuscript as written.

**Results**

**Figure 3** displays representative recordings from the different interventions. **Tables 1 and 2** present mean data. There were increments in peak UTR with volume loading and dobutamine infusion and a trend towards reduction with caval constriction and LAD-occlusion.

Values of UTR using STE were slightly larger than by sonomicrometry, but UTR by the 2 methods showed close association ($\beta=0.85$ [0.72, 0.98]; $P<0.0001$). Due to interference between sonomicrometry and ultrasound signals, UTR using STE could not be measured simultaneously with LV dimensions. In order to calculate LV twist using STE, merging of data from 2 different heartbeats is necessary. Therefore, in the subsequent presentation we used UTR by sonomicrometry since this allowed analysis of all variables from the same heartbeat.

**Relationships between LV end-diastolic pressure and twisting state**

When using conventional twist, ED twist is by definition zero. As illustrated in **Figure 4** elevation of EDP during volume loading and reduction during caval constriction were accompanied by a positive and a negative ED Twist$_A$ value, respectively. I.e. relative to baseline the ED configuration was more untwisted during volume loading and less untwisted during caval constriction. During dobutamine infusion, EDP and ED Twist$_A$ remained unchanged, whereas the elevated EDP during ischemia was accompanied by a more positive (more untwisted) ED Twist$_A$. As indicated by **Figure 5**, a 1 mmHg increment in EDP was associated by a $0.47^\circ$ increase in ED Twist$_A$ ($\beta=0.47$ [0.38, 0.55]; $P<0.0001$), suggesting that ED Twist$_A$ is preload-
dependent. To simplify, we may say that the LV gets more untwisted with a higher end-diastolic pressure (Tables 1 and 2). Due to the preload dependent ED twist configuration, systolic TwistC was overestimated relative to systolic TwistA during volume loading and underestimated during caval constriction (Figure 6).

**Changes in untwisting rate and hemodynamic variables during dobutamine infusion and ischemia**

Dobutamine infusion caused a more rapid relaxation as indicated by a reduction in \( \tau \) (\( \Delta \tau = -9 \text{ms} \ [ -11, -7] \); \( P < 0.01 \)) compared to baseline (Table 1). Furthermore, it was accompanied by an increase in restoring forces as indicated by a more negative peak absolute \( \varepsilon_{\text{CL}} \) (\( \Delta \varepsilon_{\text{CL}} = -2.9^\circ \ [-3.8, -2.0] \); \( P < 0.01 \)). Figure 7 (first and second panels) shows that a decrease in \( \tau \) and a more negative absolute \( \varepsilon_{\text{CL}} \) were accompanied by a significant increase in peak UTR. In contrast, changes during ischemia, including prolonged relaxation as indicated by an increase in \( \tau \) (\( \Delta \tau = 14 \text{ms} \ [8, 19] \); \( P < 0.01 \)) and a decrease in restoring forces as indicated by a less negative peak absolute \( \varepsilon_{\text{CL}} \) (\( \Delta \varepsilon_{\text{CL}} = 1.6^\circ \ [0.4, 2.8] \); \( P < 0.01 \)), were associated with a trend towards a reduction in UTR (\( \Delta \text{UTR} = -10^\circ/\text{s} \ [-28, 7] \); \( P = 0.2 \)) (Table 1). There was no change in early-diastolic load during dobutamine infusion, and only a slight increase during ischemia. These results indicate that during changes in contractility changes in UTR are mediated predominantly by changes in relaxation rate and restoring forces.

**Changes in untwisting rate and hemodynamic variables during caval constriction and volume loading**

During volume loading, early-diastolic load was elevated as indicated by an increase in LVP\(_{\text{MVO}}\) (\( \Delta \text{LVP}_{\text{MVO}} = 6.0 \text{mmHg} \ [6.7, 8.4] \); \( P < 0.01 \)) and was accompanied by an average increase in UTR (\( \Delta \text{UTR} = 27^\circ/\text{s} \ [7, 47] \); \( P < 0.01 \)) compared to baseline (Table 1). The increase in UTR occurred
Despite a trend of slowing of relaxation ($\Delta \tau=8\text{ms}$ [2, 15]; $P<0.05$) and reduced restoring forces (peak absolute $\varepsilon_{\text{CL}}$ became somewhat less negative: $\Delta \varepsilon_{\text{CL}}=1.0^\circ$ [0.0, 1.9]; $P<0.05$). Changes during caval constriction, including reduced early-diastolic load as indicated by a $\text{LVP}_{\text{MVO}}$ decline ($\Delta \text{LVP}_{\text{MVO}}=-6.5\text{mmHg}$ [-7.3, -5.8]; $P<0.01$) and increased restoring forces as indicated by a more negative peak absolute $\varepsilon_{\text{CL}}$ ($\Delta \varepsilon_{\text{CL}}=-1.2^\circ$ [-2.2, -0.3]; $P<0.01$), were associated with a reduction in UTR ($\Delta \text{UTR}=-16^\circ/\text{s}$ [-25, -7]; $P<0.01$). Caval constriction did not induce changes in relaxation rate.

Changes in UTR with volume loading and caval constriction could not be attributed to changes in $\tau$ or to changes in absolute $\varepsilon_{\text{CL}}$ and were, therefore, not related to relaxation or restoring forces. As indicated in Figure 7 (third panel), changes in early-diastolic load were closely associated with changes in UTR. This indicates that during interventions which primarily involve load variation, changes in UTR are mediated predominantly by variation in early-diastolic load.

While post-systolic shortening in the longitudinal or circumferential direction could be observed in the ischemic region in all animals, there were only 3 animals that exhibited post-systolic twist, resulting in a more negative $\varepsilon_{\text{CL}}$ ($\Delta \varepsilon_{\text{CL}}=-1.3\pm1.2^\circ$ [mean$\pm$SD]). During the non-ischemic interventions there were only a few incidences where post-systolic twist was observed (10%) and the magnitude of post-systolic twist was small in these cases ($\Delta \varepsilon_{\text{CL}}=-0.5\pm0.2^\circ$).

**Relationships between untwisting rate and hemodynamic variables**

The magnitude of the individual effect of the 3 proposed determinants of peak UTR (Figure 7) was evaluated using a mixed model including all interventions. A strong association was observed between peak UTR and $\text{LVP}_{\text{MVO}}$ ($\beta=6.9$ [5.4, 8.4]; $P<0.0001$), indicating an independent effect of early-diastolic load. Furthermore, the association between peak UTR and

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absolute ε_{CL} (β=-11.3 [-15.9, -6.7]; P<0.0001) was consistent with an independent contribution from restoring forces. A significant association was also observed between peak UTR and τ (β=-1.6 [-2.6, -0.6]; P<0.003), confirming previous reports of τ as an independent determinant of UTR. In a separate analysis, peak Twist_c, ESV, and operative LV stiffness were added to the model, but no statistically significant independent effects were observed. The different β-values in the multivariable mixed model indicate the magnitude of change of UTR for a 1-unit change of each determinant as shown simplified in the equation:

\[
UTR = 6.9 \text{s}^{-1} \text{mmHg}^{-1} \bullet LVP_{MVO} - 11.3 \text{s}^{-1} \bullet ε_{CL} - 1.6 \text{s}^{-1} \bullet τ + β_0
\]

On average peak UTR occurred after MVO (Table 1). Of the 50 cases (10 animals x 5 interventions in each), peak UTR occurred prior to MVO in 14 cases (7 during caval constriction), whereas the remaining occurred during filling.

Maximum value of the time derivative of LV twist measured during IVR (UTR_{IVR}) for all 50 cases is shown in Figure 8 and was associated with absolute ε_{CL} (β=-3.9 [-7.1, -0.8]; P<0.017), and with τ (β=-0.9 [-1.6, -0.3]; P<0.009). However, there was no significant association between UTR_{IVR} and early-diastolic load (β=0.8 [-0.3, -1.9]; P=0.13), indicating that UTR_{IVR} is determined by restoring forces and relaxation rate, but not by early-diastolic load.

Discussion

The present study demonstrates that early-diastolic load is an independent determinant of peak UTR in addition to LV restoring forces and LV relaxation. The contribution from each determinant, however, differed markedly between the hemodynamic interventions that were studied. The present study also demonstrates that end-diastolic twist, measured in absolute terms, is preload-dependent. With increased preload the LV became more untwisted in end-diastole.
Furthermore, increasing peak UTR 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 UTR during dobutamine infusion was attributed to both stronger restoring forces and more rapid relaxation and, reduced UTR during ischemia, to both loss of restoring forces and slowing of relaxation.

**Relationship between LV restoring forces and UTR**

In general, when myocardial tissue is being deformed, restoring forces are generated that tend to restore myocardium to its resting shape. Systolic deformation of the extracellular matrix and myocyte components, such as titin, generates potential energy analogous to compression of a spring. This potential energy (i.e., restoring force) has been associated with rapid, early-diastolic untwisting. When myocardial fibers contract below unstressed length, the generated restoring forces will cause the fibers to recoil back to their resting length when active fiber force decays. Therefore, we predicted that absolute εCL, a measure of the left ventricle’s wringing deformation with respect to a fixed reference configuration, would be a determinant of UTR. This was confirmed by the demonstration of a strong association between UTR and absolute εCL. This is consistent with the studies by Wang et al1 which demonstrated a relationship between LV ESV and peak untwisting rate. However, because heart size may vary between subjects, a similar ESV may reflect highly different magnitudes of restoring forces in different subjects. Thus, restoring forces should ideally be related to the extent of deformation relative to the configuration at resting LV volume at zero transmural pressure (V0).

In the present study we did not measure pericardial pressure. Therefore, we could not calculate LV transmural pressure and V0. We assumed, however, that end-diastolic twist configuration at baseline had a fixed offset from resting configuration at V0 and utilized this
approach to calculate TwistA. Subsequently, TwistA, LV length and radius were used to quantify peak absolute $\varepsilon_{CL}$, which was used as an index of restoring forces. As indicated in Figure 7, a strong association between UTR and peak absolute $\varepsilon_{CL}$ was observed. Therefore, when the ventricle contracted and became more twisted as reflected by a more negative absolute $\varepsilon_{CL}$, changes in shear strain were associated with peak UTR, supporting the hypothesis that restoring forces contribute to peak UTR.

If preload-mediated variations in end-diastolic twist position are ignored by using conventional twist, this may lead to misinterpretation of the relationship between restoring forces and UTR. Therefore, with increased preload, which is associated with a more untwisted end-diastolic state, conventional twist may overestimate restoring forces. In contrast, conventional twist during reduced preload may underestimate restoring forces.

**Relationship between relaxation rate and UTR**

In the present study, increased UTR during dobutamine infusion was accompanied by a decrease in $\tau$, which indicates faster myocardial relaxation. Also, $\varepsilon_{CL}$ was more negative, indicating stronger restoring forces. This suggests that dobutamine increased UTR both by a direct effect on myocardial relaxation and by an effect on restoring forces.

During myocardial ischemia we observed a trend towards reduction in UTR, and absolute $\varepsilon_{CL}$ approached zero, indicating that reduction of restoring forces contributed to the reduction in UTR. Furthermore, there was a marked increase in $\tau$, indicating slowing of LV relaxation. Since $LVP_{MVO}$ was slightly increased, reduced UTR during ischemia could not be attributed to reduced early-diastolic load. The mixed model analysis demonstrated that both UTR$_{IVR}$ as well as peak UTR were significantly and independently associated with $\tau$ as well as absolute $\varepsilon_{CL}$. This is in keeping with previous studies which demonstrated that LV relaxation rate is a determinant of
early-diastolic untwisting rate.\textsuperscript{1-5}

**Relationship between LV early-diastolic load and UTR**

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 the present study we used LVP\textsubscript{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,\textsuperscript{24} in canines,\textsuperscript{12} and in a clinical context.\textsuperscript{25} During a wide range of hemodynamic conditions, a close association between UTR and LVP\textsubscript{MVO} was observed and neither τ nor ε\textsubscript{CL} could explain the relationship. The increase in UTR during volume loading could only be attributed to an increase in LVP\textsubscript{MVO}, as neither the magnitude of restoring forces nor rate of relaxation increased. These findings support the hypothesis that changes in LVP\textsubscript{MVO} have direct effects on UTR by acting similarly to *late load*, as described in isolated muscle preparations.\textsuperscript{24}

The statistical associations between UTR and its proposed determinants do not prove their causality of UTR. However, in a recent mathematical simulation study\textsuperscript{13} we derived the physical relationship between the same determinants and LV lengthening velocity, which also confirmed similar findings in a previous experimental study.\textsuperscript{12} We believe the principles that restoring forces are released by relaxation and that load acts as an external force that deforms the LV, applies to both rate of lengthening and untwisting, and hence that these variables are more than just associated with, but also determine peak UTR.

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
Some studies also report that peak UTR occurs after MVO.\textsuperscript{1,3,27-29} According to previous studies, UTR is relatively insensitive to changes in LV EDP or preload.\textsuperscript{1,2} However, in apparent contrast to the earlier findings, the present study demonstrates that peak UTR is indeed dependent on early-diastolic load in addition to relaxation rate and restoring forces, whereas UTR\textsubscript{IVR} is dependent on relaxation rate and restoring forces only.

One reason for the previous findings of apparent load independency of UTR might be that, in contrast to the present study, Dong et al\textsuperscript{2} calculated UTR or recoil rate as the slope of the linear regression through the first 64 ms after peak torsion on the torsion versus time plot, and did not include data points after IVR. Given that peak UTR may occur after MVO, they may have underestimated peak UTR during increased early-diastolic load. Their findings, however, are consistent with ours for UTR\textsubscript{IVR}, as we found a significant correlation with $\tau$, whereas no significant relationship was found with early-diastolic load, as expected.

Previous studies have shown a strong association between UTR and conventional ES twist,\textsuperscript{1,3,6,7} which was also seen in our data (Figure 7, right panel). Conventional twist is the difference between diastolic and systolic twist configuration, which are associated with diastolic load and restoring forces, respectively. It is therefore reasonable that conventional twist correlates well with UTR as it includes the effect of 2 of the independent determinants of UTR. Increased conventional twist has been interpreted as a sign of larger restoring forces that could explain the associated increase in UTR. However, as demonstrated in the present study, although increased preload was associated with larger systolic conventional twist amplitude, systolic peak absolute twist was not more negative and hence restoring forces were not increased. Therefore, increased 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
UTR is preload-dependent. 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 UTR.

**Clinical implications**

Measurement of peak UTR by STE has the potential to be used clinically in the evaluation of diastolic function. The present experimental study confirmed the relationship between peak UTR and rate of LV relaxation as previously described. Our study also confirmed a relationship between peak UTR and restoring forces. Previous studies have described a weak or insignificant relationship between peak UTR and diastolic load. The present study demonstrated that early-diastolic load is an important and independent determinant of peak UTR. Therefore, when peak UTR is used clinically in the evaluation of LV diastolic function, all three determinants should be taken into account, i.e. relaxation, restoring forces, and diastolic load. Importantly, as demonstrated in the present study maximal UTR measured during isovolumic relaxation was determined by LV relaxation rate as well as restoring forces, but was not significantly associated to early-diastolic load. Therefore, measurement of maximal isovolumic UTR has the potential of becoming a preload independent marker of LV relaxation and magnitude of restoring forces. Because restoring forces are generated by systolic contraction, the dependency of UTR on restoring forces implies that changes in UTR may be due to changes in systolic function. Therefore, UTR should not be considered a pure measure of diastolic function.

**Limitations and comments to methodology**

Sonomicrometry slightly underestimated UTR compared to STE, and this may be attributed to the subepicardial location of the crystals. In contrast, STE measurements were obtained in the mid- and subendocardial part of the LV wall, which has higher rotation. The association
between UTR by the 2 methods, however, indicates that STE provided results that are comparable to measurements by sonomicrometry. The strength of sonomicrometry is that UTR can be measured simultaneously with all other variables, allowing for more extensive exploration of the underlying physiology.

The lower conventional twist magnitudes seen in the present study relative to previously reported values may, in addition to the subepicardial crystal location, be accounted for by the open chest animal model, anesthesia and extensive instrumentation. The LV long axis level of the ultrasonic crystals that were used for apical rotation measurement was also somewhat more basal than the short-axis level normally used when assessing rotation by STE. Since apical rotation, which is the dominating contributor to LV twist, increases progressively towards the apex, our slightly more basal measurement level may also explain the lower twist values in the present study.

LAD-occlusion, which causes regional LV dysfunction, as well as the heavy instrumentation, may have introduced more post-systolic shortening than in a normal heart with potential effects on timing of untwisting. However, our findings regarding timing of both onset of untwisting relative to end-systole and peak UTR relative to MVO, seem to be in the range of previously published results.

In the current study we used ED twist position at baseline as the reference for calculation of absolute twist and absolute $e_{CL}$ for all interventions. This approach is not equivalent to assessing LV twist configuration with reference to $V_0$, and will strictly speaking not provide an accurate measure of restoring forces. However, we assumed a constant offset between the twisting state at our reference configuration and the twisting configuration at $V_0$, an assumption that enabled comparison of changes in $e_{CL}$ between and within heartbeats from various
hemodynamic conditions.

**Conclusions**

The present study supports the hypothesis that *early-diastolic load* as well as *restoring forces* are independent determinants of peak early-diastolic untwisting rate in addition to *rate of LV relaxation*. For untwisting rate during isovolumic relaxation, however, only restoring forces and LV relaxation rate are independent determinants. Furthermore, end-diastolic twisting state is preload-dependent and, therefore, loading conditions need to be accounted for when calculating twist by the conventional methodology. Thus, conventional twist which is calculated as change from the end-diastolic twist for a given heart beat, has limited ability to serve as a marker for restoring forces.

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**Conflict of Interest Disclosures:** None.

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Table 1. Hemodynamic and twist responses to changes in loading conditions, contractility, and ischemia

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<tr>
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<th>Baseline</th>
<th>Cavai constriction</th>
<th>Volume loading</th>
<th>Dobutamine infusion</th>
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<td>106(96,116)</td>
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<tr>
<td>LV dP/dt max, mmHg/s</td>
<td>1433(1295,1572)</td>
<td>942(794,1090)*</td>
<td>1538(1342,1734)</td>
<td>2714(2117,3311)</td>
<td>1229(986,1473)</td>
</tr>
<tr>
<td>LV EDP, mmHg</td>
<td>7.8(7.1,8.6)</td>
<td>1.5(0.6,2.4)*</td>
<td>15.0(12.5,17.6)*</td>
<td>9.3(8.2,10.4)†</td>
<td>13.0(10.4,15.1)*</td>
</tr>
<tr>
<td>LV ED volume, ml</td>
<td>60(54,65)</td>
<td>47(39,55)*</td>
<td>74(64,85)*</td>
<td>65(57,74)†</td>
<td>78(67,90)*</td>
</tr>
<tr>
<td>LV ES volume, ml</td>
<td>43(37,49)</td>
<td>38(31,42)*</td>
<td>48(41,56)*</td>
<td>39(32,45)*</td>
<td>64(52,77)*</td>
</tr>
<tr>
<td>LV t, ms</td>
<td>38(36,40)</td>
<td>37(35,39)</td>
<td>46(39,53)†</td>
<td>29(27,31)*</td>
<td>52(46,57)*</td>
</tr>
<tr>
<td>LVP MVO, mmHg</td>
<td>10.2(9.6,10.8)</td>
<td>3.7(2.9,4.4)*</td>
<td>16.2(13.7,18.7)*</td>
<td>11.2(9.7,12.7)</td>
<td>12.1(10.4,13.9)†</td>
</tr>
<tr>
<td>Operating stiffness, mmHg/ml</td>
<td>0.4(0.3,0.5)</td>
<td>0.4(0.4,0.4)</td>
<td>0.4(0.3,0.5)</td>
<td>0.5(0.2,0.8)</td>
<td></td>
</tr>
<tr>
<td>Time to onset of untwisting, ms</td>
<td>273(246,301)</td>
<td>250(232,269)†</td>
<td>290(271,310)†</td>
<td>268(244,292)</td>
<td>268(247,289)</td>
</tr>
<tr>
<td>Time to ES, ms</td>
<td>306(281,331)</td>
<td>250(278,317)</td>
<td>322(306,339)</td>
<td>285(260,320)</td>
<td>306(292,320)</td>
</tr>
<tr>
<td>Time to UTR TIVR by Sonomicrometry, ms</td>
<td>345(310,381)</td>
<td>321(295,346)</td>
<td>350(327,373)</td>
<td>329(298,359)</td>
<td>326(303,349)</td>
</tr>
<tr>
<td>Time to MVO, ms</td>
<td>369(340,398)</td>
<td>371(346,395)</td>
<td>379(350,409)</td>
<td>344(313,375)</td>
<td>384(363,405)</td>
</tr>
<tr>
<td>Time to peak UTR by Sonomicrometry, ms</td>
<td>390(34/.432)</td>
<td>347(309,385)†</td>
<td>402(364,441)</td>
<td>358(324,391)</td>
<td>386(347,425)</td>
</tr>
<tr>
<td>Peak UTR, °/s</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sonomicrometry</td>
<td>63(56,70)</td>
<td>47(39,54)*</td>
<td>90(70,110)*</td>
<td>140(105,174)*</td>
<td>52(35,70)</td>
</tr>
<tr>
<td>STE</td>
<td>64(52,76)</td>
<td>43(32,54)</td>
<td>39(31,47)</td>
<td>81(66,95)†</td>
<td>34(17,51)</td>
</tr>
<tr>
<td>UTR TIVR by Sonomicrometry, °/s</td>
<td>42(34,50)</td>
<td>43(32,54)</td>
<td>102(80,123)*</td>
<td>149(114,184)*</td>
<td>52(36,69)</td>
</tr>
<tr>
<td>Peak conventional twist, °</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-4.1(-3.5,-3.9)</td>
<td>-2.8(-4.2,-1.4)*</td>
<td>-6.3(-5.2,-5.1)†</td>
<td>-10.4(-12.4,-8.4)*</td>
<td>-4.1(-5.6,-2.5)</td>
</tr>
<tr>
<td>Peak absolute twist, °</td>
<td>-4.7(-5.6,-3.8)</td>
<td>-6.8(-7.9,-5.8)*</td>
<td>-3.0(-4.5,-1.6)†</td>
<td>-10.0(-11.8,-8.2)*</td>
<td>-2.0(-3.9,0.0)*</td>
</tr>
<tr>
<td>Peak conventional e CL, °</td>
<td>-2.7(-3.2,2.2)</td>
<td>-1.5(-2.4,-0.5)†</td>
<td>-3.6(-4.3,-2.8)†</td>
<td>-5.8(-6.7,-4.8)*</td>
<td>-2.4(-3,3,-1.4)</td>
</tr>
<tr>
<td>Peak absolute e CL, °</td>
<td>-2.7(-3.2,-2.2)</td>
<td>-3.8(-4.6,-3.0)†</td>
<td>-1.6(-2.4,-0.8)†</td>
<td>-5.5(-6.3,-4.7)*</td>
<td>-1.2(-2.3,0.0)*</td>
</tr>
</tbody>
</table>

Values are fitted means with 95% confidence intervals, times are milliseconds (ms) after onset of systole.

*P<0.01 and †P<0.05 vs. baseline (mixed models)

LAD= left anterior descending coronary artery; LV= left ventricle/ventricular; LVP= LV pressure; ED= end-diastolic; ES= end-systolic; UTR= LV peak early-diastolic untwisting rate; UTR TIVR= maximal untwisting rate during isovolumic relaxation; STE= speckle tracking echocardiography; t= time constant of LV isovolumic pressure decay; IVR= isovolumic relaxation; dP/dt max= maximal time derivate of LVP; e CL= circumferential-longitudinal shear strain; MVO= mitral valve opening.
Table 2. End-diastolic (ED) twist responses to changes in loading conditions, contractility, and ischemia

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Caval constriction</th>
<th>Volume loading</th>
<th>Dobutamine infusion</th>
<th>LAD-occlusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>ED conventional twist, º</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ED absolute twist, º</td>
<td>0 (reference)</td>
<td>-4.0(-5.0,-3.0)*</td>
<td>3.3(2.1,4.5)*</td>
<td>0.4(-0.6,1.4)</td>
<td>2.1(0.6,3.7)*</td>
</tr>
<tr>
<td>ED conventional ε_{CL}, º</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ED absolute ε_{CL}, º</td>
<td>0 (reference)</td>
<td>-2.3(-3.0,-1.6)*</td>
<td>1.9(1.2,2.7)*</td>
<td>0.3(-0.3,0.9)</td>
<td>1.2(0.3,2.1)*</td>
</tr>
</tbody>
</table>

Values and abbreviations as in Table 1.
Figure Legends:

Figure 1. Cylindrical left ventricular (LV) model illustrating direction and magnitude of systolic basal (red arrow, γ), and apical (blue arrow, α) rotation. Absolute LV twist (TwistA = γ-α) was calculated relative to end-diastolic twist configuration at baseline (fixed reference configuration).

Absolute LV circumferential-longitudinal shear strain ($\varepsilon_{CL}$) 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), $\theta_A$ = TwistA, and h= LV length measured as distance between apical and basal short-axis planes at each time point during the cardiac cycle.

Figure 2. 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 middle panel represents the untwisted configuration at baseline. Longitudinal solid black lines are drawn through corresponding points in short-axis planes and arrows indicate direction of change in rotation.

Compared to baseline, reduced preload is associated with a more twisted end-diastolic LV configuration, whereas increased preload is associated with a more untwisted configuration.

Figure 3. Traces from a representative experiment during the different interventions. The upper panels show twisting rate where the untwisting rate (UTR) following onset of early-diastolic
filling is emphasized by a thick line. MVO: mitral valve opening; LV: left ventricle/ventricular; LVP: LV pressure; LAP: left atrial pressure; ε_{CL}: circumferential-longitudinal shear strain; Twist_{C}: conventional twist; Twist_{A}: absolute twist.

Decreased UTR was observed during caval constriction and ischemia, whereas increased UTR was observed during volume loading and even more during dobutamine infusion. Due to a more twisted LV state at end-diastole (ED) during caval constriction, diastolic return of absolute ε_{CL} did not reach zero but remained negative until next systole. During volume loading the opposite occurred; because of a more untwisted LV state at ED, diastolic return of absolute ε_{CL} proceeded above zero and remained positive until the next systole. These preload-mediated changes in LV passive twist configuration were not shown in Twist_{C} due to definition of ED magnitude as zero.

**Figure 4.** Absolute left ventricular (LV) twist during different interventions. Data points and error bars are average values (all animals) and standard deviations, respectively. Time is shown in percentage of systolic duration: end-diastole (ED): 0% and end-systole (ES): 100%. Absolute twist was calculated using a fixed LV reference configuration (ED at baseline) for all interventions. Absolute twist values are shown at: ED, at the time of peak twist, ES, and at the time of mitral valve opening. Due to a more twisted LV state during caval constriction, diastolic absolute twist remained negative until next systole. During volume loading, however, diastolic untwisting led to a positive LV absolute twist configuration at onset of systole.

**Figure 5.** Left ventricular (LV) end-diastolic (ED) absolute twisting state (Twist{A}) versus LV pressure (EDP) at various hemodynamic states. Twist_{A} was calculated using a fixed LV
reference configuration (ED at baseline) for all interventions. An elevated EDP was associated by an increased TwistA (i.e. a more untwisted LV). The close association between LV EDP and ED TwistA, indicates that LV twist configuration at onset of systole is preload dependent. Least-squares linear regression line is displayed for visual clarification.

**Figure 6.** Left ventricular (LV) peak absolute twist (TwistA) versus peak conventional twist (TwistC) at various hemodynamic states. As seen relative to the identity line, TwistC overestimated systolic LV twist configuration during volume loading and underestimated it during caval constriction.

**Figure 7.** Relationships between peak untwisting rate (UTR) and rate of relaxation (τ, first panels from left), restoring forces (absolute εCL, second panels), early-diastolic load (LVP_{MVO}, third panels), and conventional εCL (right panels), respectively. The upper panels display all interventions. The lower panels show interventions with primarily variations in contractility (first, second and forth panel from the left: baseline, dobutamine infusion, and ischemia), and primarily variations in preload (third panel from left: baseline, caval constriction, and volume loading).

There was a close association between UTR and τ (bottom left), and absolute εCL (bottom, second from left) for variations in contractility, consistent with effects from relaxation rate and restoring forces, respectively. Changes in UTR during variation in preload, however, could not be attributed to relaxation rate or restoring forces. The close association between UTR and LVP_{MVO} indicates that these changes could only be accounted for by variation in early-diastolic load and that early-diastolic load is a determinant of peak untwisting rate. Conventional
twist was closely associated with UTR. As conventional twist is associated with 2 of the other
determinants, diastolic load and restoring forces, its close association with UTR may be
explained mainly as an effect of these two. Least-squares linear regression lines are displayed for
visual clarification.

**Figure 8.** Relationships between maximal untwisting rate during isovolumic relaxation (UTRIVR)
and rate of relaxation (τ, first panel), restoring forces (absolute εCL, second panel), early-diastolic
load (LVP_MVO, third panel), and conventional εCL (right panel) respectively. There was a close
association between isovolumic untwisting rate and τ, and absolute εCL for all interventions,
consistent with effects from relaxation rate and restoring forces. However, as expected, there was
no significant association between isovolumic untwisting rate and LVP_MVO, indicating that early-
diastolic load has no effect on isovolumic untwisting rate. Least-squares linear regression lines
are displayed for visual clarification.
Myocardial Relaxation, Restoring Forces, and Early-Diastolic Load are Independent Determinants of Left Ventricular Untwisting Rate
Anders Opdahl, Espen W. Remme, Thomas Helle-Valle, Thor Edvardsen and Otto A. Smiseth