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Myocardial deformation in African hypertensive patients with heart failure : an analysis using speckle tracking echocardiography

Maharaj, Nirvarthi

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MYOCARDIAL DEFORMATION IN AFRICAN HYPERTENSIVE PATIENTS WITH HEART FAILURE- AN ANALYSIS USING SPECKLE TRACKING ECHOCARDIOGRAPHY

Nirvarthi Maharaj

A thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand, in fulfilment of the requirements for the degree

of

Doctor of Philosophy

Johannesburg, 2013

DECLARATION

I, Nirvarthi Maharaj, declare that this thesis is my own work. It is being submitted for the degree of Doctor of Philosophy at the University of Witwatersrand, Johannesburg. It has not been submitted before any degree or examination at this or any other university.

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_____ day of _____, 2013.

ABSTRACT

Hypertension and heart failure are intimately related with the incidence of heart failure among hypertensive subjects between 1% and 2% per year. Structural and functional myocardial abnormalities identified in hypertensive patients contribute to the progression of myocardial dysfunction. Systolic abnormalities in hypertension begin to develop in the early stages of the disease despite normal left ventricular (LV) ejection fraction (EF) and contribute to the progressive deterioration of LV systolic performance. However, these systolic abnormalities are initially not detectable by conventional echocardiographic methods. Speckle tracking echocardiography (STE) is a sensitive quantitative technique for assessing LV function. LV twist is an important contributing factor to the systolic function of the LV in health and disease and may be a better index of systolic function than ejection fraction (EF) in hypertensive patients (HTP).

The remodelling process of the left ventricle in hypertension entails a complex interplay between myocyte hypertrophy and dysfunction, with qualitative changes in the extracellular matrix contributing to progressive dysfunction. Adverse LV remodeling in HTP is associated with an imbalance in collagen degradation and may contribute to the remodelling phenotype and systolic dysfunction in hypertension. Increased matrix metalloproteinase-1 (MMP1) levels contribute to development of LV dilatation and failure with higher levels of MMP1 in the myocardium of hypertensive patients with low EF than those with normal EF.

Hypertension can cause systolic dysfunction as a consequence of adverse remodelling and LV hypertrophy, but given the multitude of factors involved in LV decompensation mediated by mechanical, neurohormonal and cytokine routes, the exact mechanisms that contribute to the adverse remodelling and EF deterioration are not fully elucidated. LV twist may be a contributing factor to systolic dysfunction independent of other factors, thus, a focus on abnormalities in the cardiac mechanics of twist in the left ventricle may be helpful in understanding the pathogenesis behind the transition from compensated to decompensated heart failure. Furthermore, the changes in the extracellular matrix may account for the varying morphology, EF and LV twist in HTP.

The purpose of this thesis was to 1) determine LV twist in healthy adults of different age groups (n=127), 2) evaluate LV twist changes in African HTP with low (EF<50%) and preserved EF (EF ≥ 50%) (n=82) and 3) examine the relationship between LV twist and biomarkers of collagen degradation in HTP with preserved and low EF.

Parasternal short-axis images of three consecutive end-expiratory cardiac cycles at LV basal and apical levels were obtained. Apical rotation (AR) and basal rotation (BR) during ejection and instantaneous LV peak systolic twist (net twist, defined as maximal value of instantaneous AR minus BR) were measured. 127 normal subjects were divided into four age groups: 20-29 (n=34); 30-39 (n=33); 40-49 (n=29); and 50-65 (n=31) years. LV twist and markers of collagen turnover (serum concentrations of matrix metalloproteinase -1 (MMP1), tissue inhibitor of MMP1 (TIMP1) and ratio of MMP1:TIMP1) were measured in 82 hypertensive patients, 41 with EF < 50% (HTLEF) and 41 with EF ≥ 50% (HTNEF). Rigid body rotation (RBR) was defined as AR and BR occurring in the same direction. Serum biomarkers were log transformed before analysis.

LV twist increased with age in normal subjects. Multivariate linear regression analysis showed age as the main predictor of net LV twist (R^2=0.82, P<0.0001) in normal subjects. Net LV twist was lower in HTLEF compared with HTNEF (3.34 + 1.10 vs. 11.70 + 0.67, p < 0.0001). Of 41 HTLEF patients, 28 (68%) had normal twist pattern while 13 (32%) exhibited RBR. The subgroup with RBR showed greater LV dysfunction (EF: 27.9±5.8% vs. 35±7.5%; p=0.005) and more spherical LV geometry (p=0.0009) compared with those who had normal pattern of twist. Log TIMP1, Log MMP1 and Log MMP1:TIMP1 ratio levels were higher in HTLEF compared with HTNEF (12.32 ± 0.25 vs. 11.81 ± 0.13, p<0.0001; 9.08 ± 0.32 vs. 8.00 ± 0.18, p<0.0001; -3.25 ± 0.30 vs. -3.81 ± 0.18, p<0.0001; respectively). There was an inverse correlation between Log MMP1:TIMP1 and net LV twist after adjusting for EF (r = -0.41, p <0.0001).

This study established normative data and patterns for myocardial deformation (strain and LV twist) in a normal black-African adult population across different age groups and can be used as a

baseline for future studies. Age was the major determinant of increased LV twist in a normal black population. LV twist may be a compensatory mechanism to preserve EF and maintain normal systolic function with advancing age and in hypertension. LV twist varies with the degree of remodeling and systolic function in hypertension. RBR represents a novel assessment of more severe LV remodeling and LV systolic dysfunction in hypertensive patients. Alterations in collagen turnover not only accompanies more adverse remodelling but also contributes to LV twist differences observed between HTLEF and HTNEF patients. The inverse relation between LV twist and loss of myocardial collagen scaffolding suggests that integrity of the extracellular matrix may play an important role in preservation of LV twist. These findings highlight the value of LV twist as a sensitive global parameter of LV systolic myocardial performance. Longitudinal studies assessing LV twist may provide significant value in clinical practice as an early marker for risk stratification in hypertensive patients who may benefit from aggressive medical therapy to prevent LV remodelling and heart failure.

DEDICATION

Dedicated to God and my family without whom this dream would never have been realised.

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NOMENCLATURE

Angiotensin-converting enzyme inhibitors/angiotensin receptor blockers (ACE/ARB)

Apical rotation (AR)

Basal rotation (BR)

Basal anterior (BA)

Basal anterolateral (BAL)

Basal anteroseptal (BAS)

Basal inferior (BI)

Basal inferolateral (BIL)

Basal inferoseptal (BIS)

Blood pressure (BP)

Circumferential strain (CS)

Deceleration time (Decel time)

End-diastolic diameter (EDD)

End-diastolic volume (EDV)

End-diastolic volume index (EDVI)

Ejection fraction (EF)

E` lateral wall velocity (E`L)

E` medial wall velocity (E` M)

End-systolic volume (ESV)

End-systolic volume index (ESVI)

Extracellular matrix (ECM)

Heart failure (HF)

Hypertensive patients (HTP)

Hypertensive heart disease (HHD)

Hypertensive patients with low ejection fraction (HTLEF)

Hypertensive patients with normal ejection fraction (HTNEF)

Interventricular septum end-diastolic diameter (IVSD)

Left atrial volume index (LAVI)

Left ventricular (LV)

Left ventricular hypertrophy (LVH)

Left ventricular posterior wall diameter (LVPWD)

Left ventricular sphericity index (LVSPHI)

Longitudinal strain (LS)

Magnetic resonance imaging (MRI)

Matrix metalloproteinases (MMPs)

New York Heart Association (NYHA)

Pulse pressure (PP)

Radial strain (RS)

Rigid body rotation (RBR)

Speckle tracking echocardiography (STE)

Subendocardial apical rotation (Subendo AR)

Subepicardial apical rotation (Subepi AR)

Subendocardial basal rotation (Subendo BR)

Subepicardial basal rotation (Subepi BR)

Tissue inhibitor of matrix metalloproteinases (TIMPs)

1.0 INTRODUCTION

1.1 HYPERTENSIVE HEART DISEASE

Hypertension is the commonest cardiovascular disease in black Africans (Steyn et al., 2001). In South Africa about 1/5 to 1/4 individuals of African ancestry have hypertension with a large proportion not at target blood pressure (BP) levels (Steyn et al., 2001, Steyn et al., 1996, Alberts et al., 2005). Hypertension is the most important risk factor for ischaemic heart disease, stroke, peripheral vascular disease, chronic renal disease and heart failure (HF) (Alberts et al., 2005, Mabuza, 2008). The increased prevalence of hypertension in the black community in South Africa seems to be associated with changes in lifestyle such as urbanisation, alcohol consumption, smoking, and obesity (Alberts et al., 2005). As a consequence of the high prevalence of hypertension there is an increased risk of cardiovascular diseases (Alberts et al., 2005) in rural communities and presumably in urban communities (Seedat, 1998, Connor et al., 2006).

The identification of target organ damage such as left ventricular hypertrophy (LVH) is important for the prediction of cardiovascular risk (Williams B, 2004) with significant therapeutic and prognostic implications. LV (left ventricular) geometric patterns, regardless of categorization criteria, are reliable markers of cardiovascular risk (Cuspidi et al., 2010) with LVH associated with adverse outcomes. Furthermore, patients with concentric remodelling have been shown to have similarly poor outcomes to those with LV hypertrophy when compared to patients with normal LV geometry (Kizer et al., 2004).

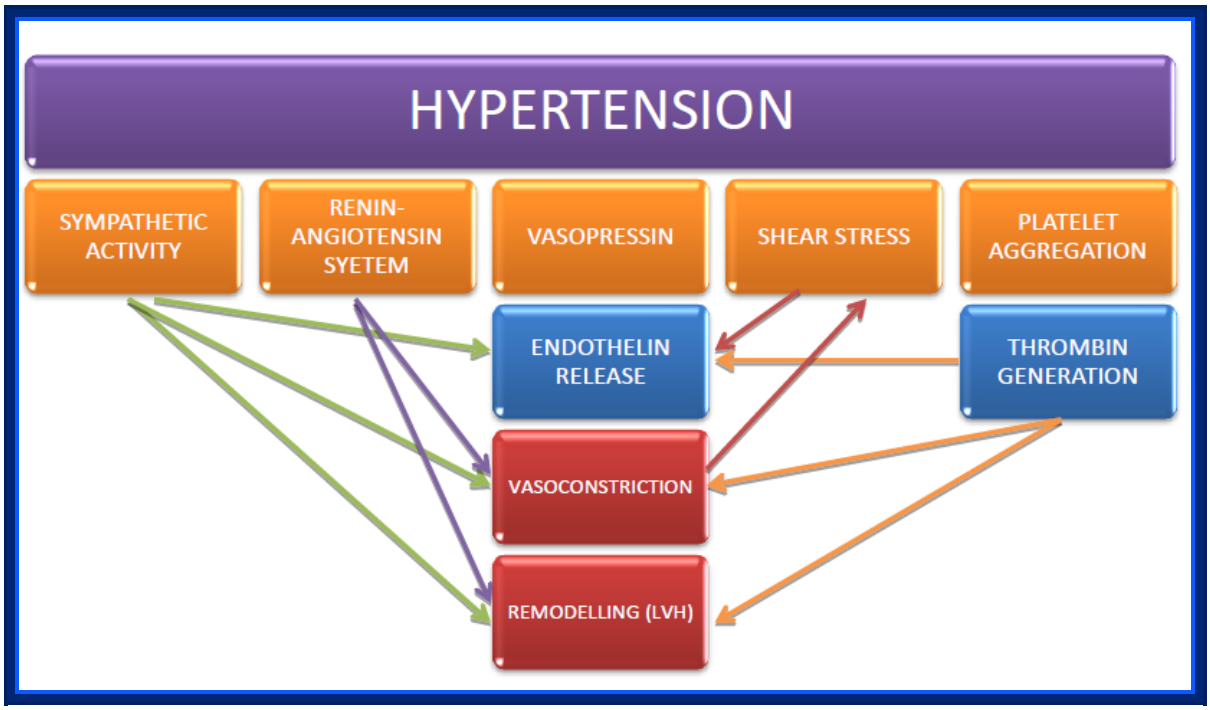


Figure 1.1.1 Factors contributing to LVH in hypertension

The response of the left ventricle to pressure overload is different in black patients, with a high prevalence and severity of concentric geometry as demonstrated in the Baragwanath Ambulatory Monitoring Study in hypertension (Sareli et al., 2001). In a large population-based cohort of treated hypertensive patients (HTP), black patients had higher mean LV mass index and relative wall thickness than Caucasian patients—differences that persisted after adjustment for conventional demographic and clinical risk factors (Kizer et al., 2004). Accordingly, the prevalence of LV hypertrophy and concentric geometry in black patients was twice that of whites (Kizer et al., 2004) independent of clinical covariates, measures of systemic haemodynamics and vascular stiffness (Kizer et al., 2004). In an African-American population, LVH as detected by echocardiography was a precursor of premature cardiovascular morbidity and mortality after adjusting for traditional risk factors (age, diabetes and smoking) including BP (Nunez et al., 2005). The jury is still out on the mechanisms (ethnic propensity or greater severity of hypertension) behind the genesis of LV remodelling in black patients (Kizer et al., 2004). Furthermore, the determinants of LV geometry [Fig. 1.1.1] and indeed the mechanism whereby altered geometry mediates increased risk is poorly defined.

Initially, LVH represents a compensatory response to pressure overload in hypertension (Drazner, 2011, Drazner, 2005). However, over time and with continuing excessive load, a variety of changes occur in the structure, function, architecture, microcirculation and metabolism of the myocardium that collectively have been termed remodelling (Frohlich, 1999, Frohlich et al., 1992, Drazner, 2011). Despite normal left ventricular ejection fraction (EF), decreases in systolic function have been identified in patients with concentric LV hypertrophy as measured by midwall shortening, implicating abnormal myocardial function proceeding to developing dilated cardiac failure.

Although concentric LVH is associated with subtle abnormalities in systolic function, it has not been shown in longitudinal studies whether such patients subsequently develop ventricular dilation in the absence of coronary artery disease. Furthermore, LVH was also associated with a decrease in left ventricular EF, independent of conventional BP in HTP (Drazner et al., 2005). Therefore, it is important to assess accurately left ventricular systolic function in HTP with or without LVH using sensitive measures other than EF.

1.1.1 HYPERTENSION INDUCED HEART FAILURE

Hypertension is the leading cause of HF in black patients indicating an advanced and often irreversible degree of organ damage (Drazner, 2011, Drazner, 2005). Adverse remodelling and decreased myocardial contractile function contribute to hypertension induced heart failure. Based on published data, black patients have HF at an earlier age and more advanced left ventricular dysfunction with poorer functional class at the time of diagnosis. The precursor to decompensated HF is thought to primarily be LVH and not coronary artery disease (Yancy, 2003).

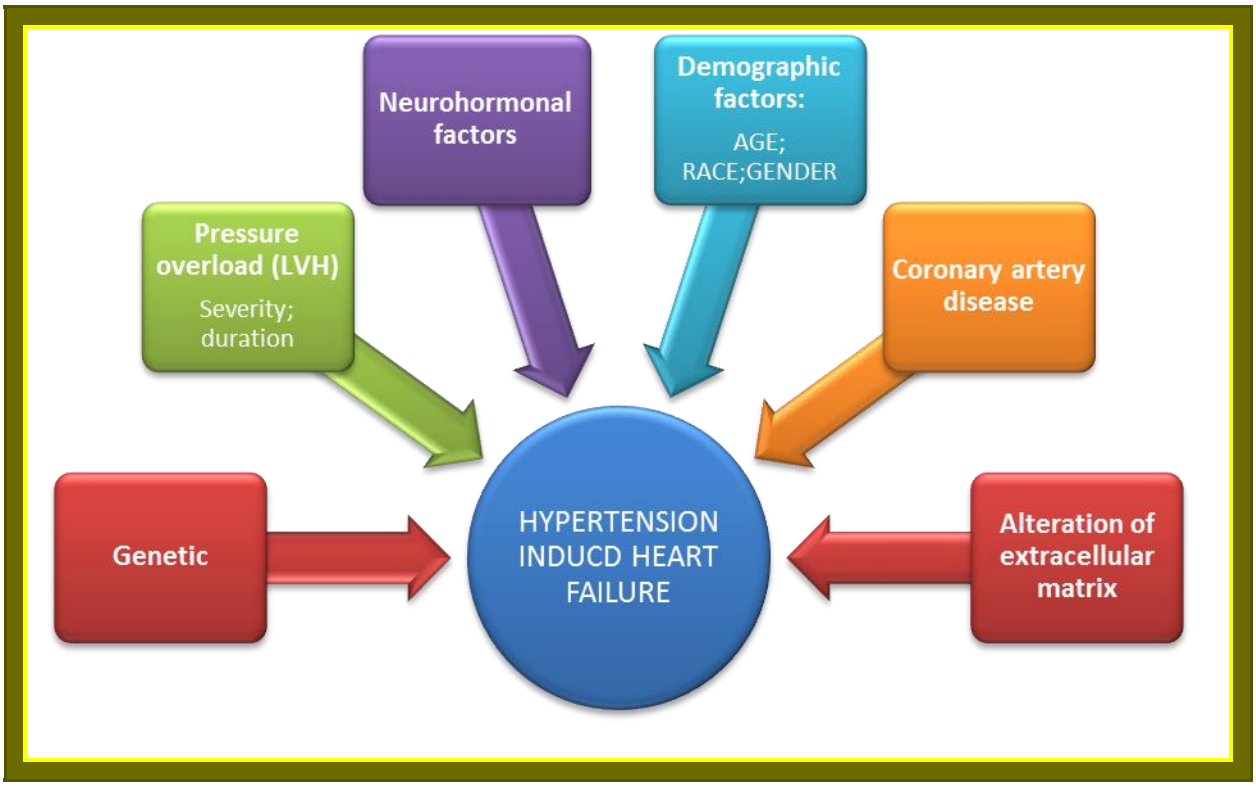


Figure 1.1.2 Factors contributing to hypertension induced heart failure

The connection between hypertension and HF appears to represent a continuum of cardiovascular disease, initiated as hypertension reflecting primarily vascular endothelial dysfunction (or vascular remodeling), which then progresses to the clinical scenario of HF reflecting ventricular dysfunction (or ventricular remodeling) (Yancy, 2003). Thus, a predisposition toward ventricular remodeling and LVH may likewise contribute to a higher incidence of HF, which, when present, is a more malignant disease (Yancy, 2003). Although eccentric hypertrophy is a common precursor to reduced EF, concentric LV hypertrophy may also be important in the deterioration to LV systolic dysfunctionand may allow refinement of phenotypic characterization of HTP (HTP) identifying risk factors for LV response to hypertension (Drazner, 2005) and likely progression to HF. However, there are no longitudinal studies documenting the transition from normal EF to low EF in the absence of coronary artery disease in HPT with LVH.

Traditionally, global systolic function is assessed by calculating EF using the Modified Simpsons biplane method (Lang et al., 2005). LV EF is the most common clinically used echocardiographic parameter to quantify the severity of LV systolic dysfunction in patients with HF. However, EF describes LV systolic chamber function and does not always reflect global LV myocardial contractile performance or provide information about individual myocardial deformation components, which may have prognostic utility in hypertensive heart disease (HHD). Whereas wall stress remains difficult to measure, myocardial strain and LV twist may be measured using speckle tracking echocardiography (STE) to detect subclinical myocardial dysfunction in HHD (Kang et al., 2008, Narayanan et al., 2009, Mizuguchi et al., 2010b, Mizuguchi et al., 2009, Mizuguchi et al., 2008, Mizuguchi et al., 2010a) as an adjunct to conventional echocardiographic parameters.

1.2 SPECKLE TRACKING ECHOCARDIOGRAPHY

1.2.1 STRAIN

Strain imaging has emerged as a useful technique to estimate myocardial function and contractility (Hoffmann et al., 1996, D'hooge et al., 2000). Two-dimensional (2D) STE enables objective assessment of left ventricular function through the analysis of myocardial strain based on similar principles that are used in tagged magnetic resonance imaging (MRI). The measurement of strain by STE has been validated against sonomicrometry and MRI (Gilman et al., 2004). In a sub study, comparing STE with tagged MRI (the current gold standard for deformation analysis), strain measured by STE correlated well with that obtained by MRI (Goffinet et al., 2009).

2D strain utilizes natural acoustic markers for frame-to-frame tracking of myocardial motion by tracking speckles in an ultrasonic B mode image over numerous frames using specialized software (Marwick et al., 2009, Marwick, 2010, Marwick, 2006, Perk et al., 2007, Nesbitt et al., 2009). The speckles are the result of constructive and destructive interference of ultrasound, back-scattered from structures smaller than a wavelength of ultrasound (Mor-Avi et al., 2011). This gives each

small area a rather unique speckle pattern that remains relatively constant from one frame to the next (Mor-Avi et al., 2011). Therefore, a suitable pattern-matching algorithm can identify the frame-to-frame displacement of a speckle pattern, allowing myocardial motion to be followed in two dimensions (Mor-Avi et al., 2011).

The appearance of these acoustic markers is considered to be relatively stable between subsequent image frames, so that a change in their position is assumed to follow tissue motion. Tracking is based on searching for the new location of the marker in the subsequent frame. Two dimensional strain and strain rate are then calculated from the displacement and rate of displacement of each marker, respectively. Since tracking is based on gray-scale B-mode images, it is in principle, angle-independent and affords good temporal and spatial resolution. Strain is defined as a dimensionless index which is the ratio of final to initial length of a myocardial fibre (Nesbitt et al., 2009) [Fig.1.2.1.1]. Consequently, positive strain occurs if the segment length exceeds its original length, and negative strain exists if the segment length is shorter than its original length. Strain occurs in radial (narrowing or compression and widening or expansion), longitudinal (shortening and lengthening), and circumferential (tangential) directions [Fig. 1.2.1.2].

$$\text{Strain} = \frac{\text{Change in length (e)}}{\text{Original length (Lo)}}$$



Figure 1.2.1.1 Strain formula

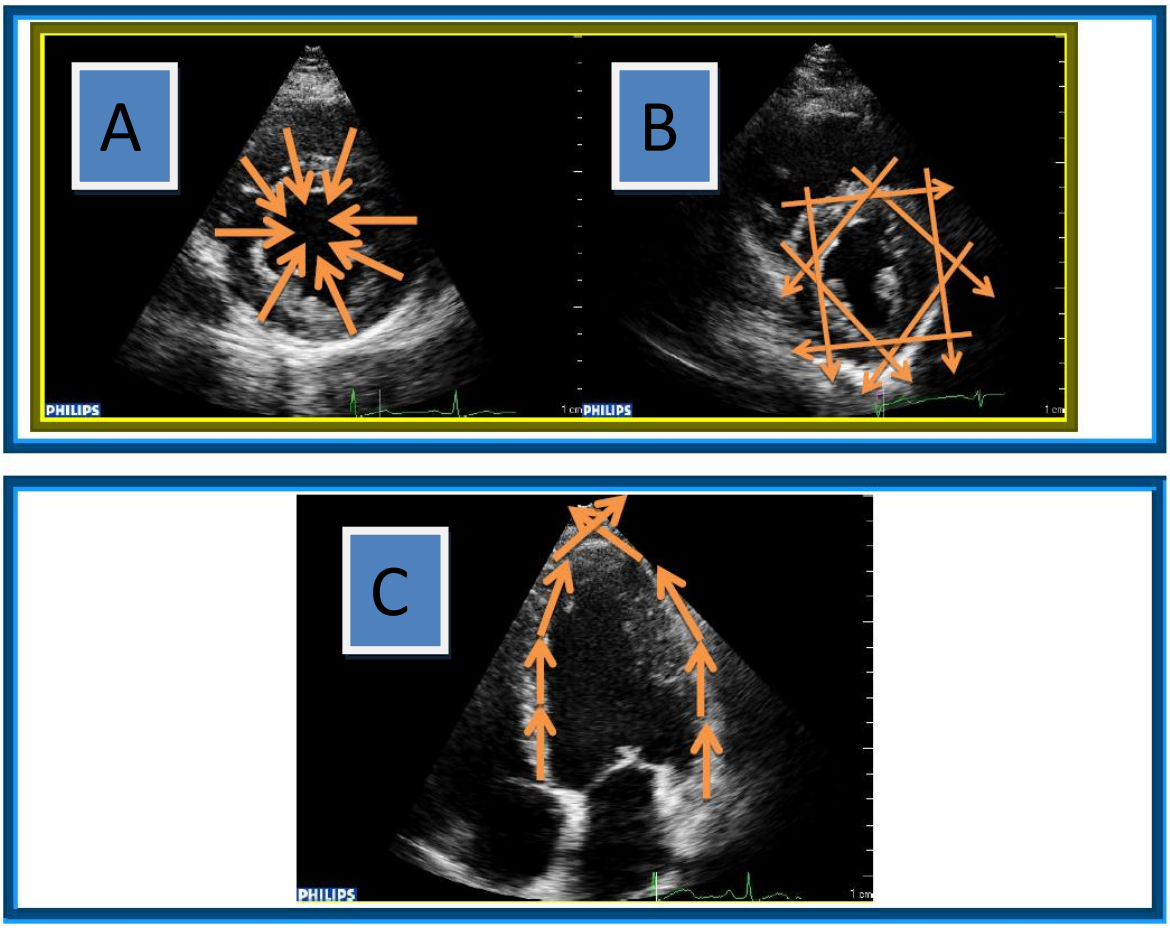


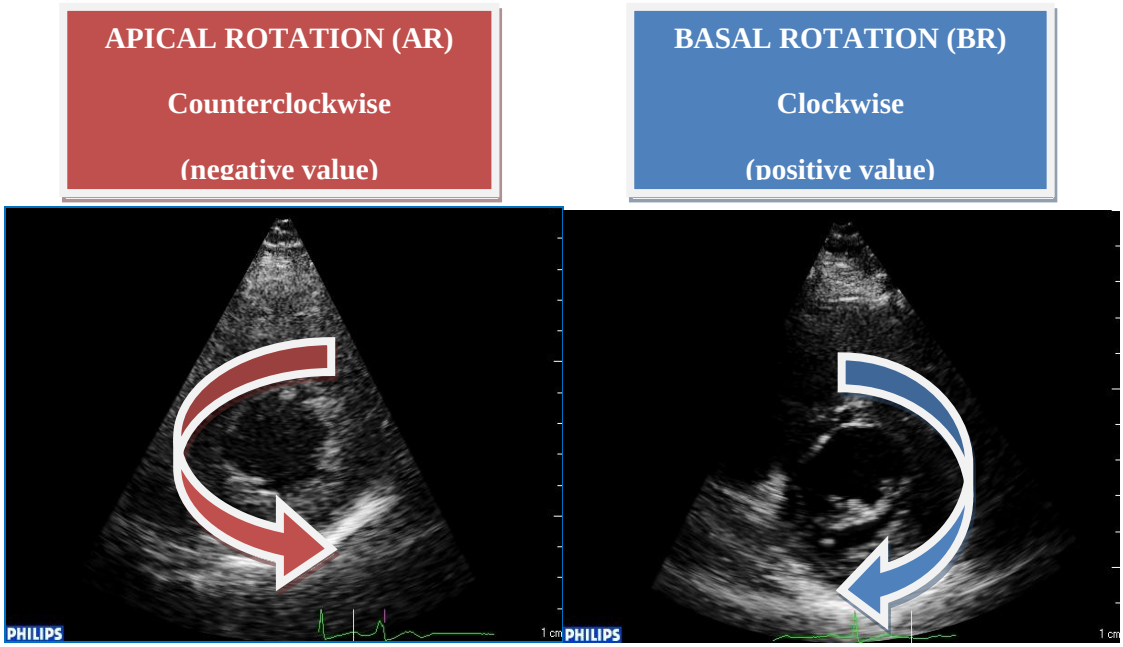
Figure 1.2.1.2 A - Radial strain; B- Circumferential strain; C- Longitudinal strain

The advent of 2D STE has allowed a comprehensive and quantitative assessment of left ventricular function (Mor-Avi et al., 2011). Over the past 5 years, numerous studies have demonstrated that impaired myocardial strain is an indicator of global and regional dysfunction in various cardiac conditions, although different speckle tracking software have been used (Mor-Avi et al., 2011). Data regarding accuracy, validity, and clinical application of STE are rapidly accumulating with low intra- and interobserver variability (Korinek et al., 2005, Amundsen et al., 2006, Buckberg et al., 2004). The clinical utility of these measurements has been demonstrated in a spectrum of cardiovascular pathophysiologies (Mor-Avi et al., 2011). The accuracy and rapid analysis of 2D STE converts this technique into a suitable method for objective assessment of myocardial function

in clinical practice. As such, speckle tracking could potentially be used as a bedside tool to improve the assessment of the global and regional functional status of the myocardium.

1.2.2 LEFT VENTRICULAR TWIST

LV twist is defined as the wringing motion whereby the LV apex rotates with respect to the LV base about the LV long axis (Sengupta et al., 2008b). Viewed from the LV apex, systolic apical rotation (AR) is counterclockwise and basal rotation (BR) is clockwise (Sengupta et al., 2008b) [Fig. 1.2.2.1]. The main factors determining the degree of twist is the angle of motion and the amount of shortening of myocardial fibre which closely relates to the transmural fibre strain gradient between epicardial and endocardial myocardial fibres (Goffinet et al., 2009, Sengupta et al., 2008b, Sengupta et al., 2008a).



TWIST = AR-BR (systole)

Figure 1.2.2.1 Twist calculation

LV myocardial fibre architecture is important for LV function (Sengupta et al., 2008b, Sengupta et al., 2008a). The left ventricle has a myocardial architecture, which is a transmural continuum

between two helical fibre arrangements, in which a subendocardial fibre wraps in a right-handed helix and a subepicardial fibre wraps in a left-handed helix (Sengupta et al., 2008b) [Fig. 1.2.2.2]. From the endocardium to the epicardium, the helix angle changes continuously (Sengupta et al., 2008b) [Fig. 1.2.2.2]. LV twist is a result of equilibrium between torques caused by the oblique myofibres in the subepicardium and subendocardium (Alharthi et al., 2009) [Fig. 1.2.2.2].

When both layers contract simultaneously, a larger radius of rotation for the outer epicardial layer results in epicardial fibres having a mechanical advantage in dominating the overall direction of rotation (Sengupta et al., 2008b) [Fig. 1.2.2.2]. Cardiac motion begins as a result of muscle fibre contraction in the base to narrow and elongate the ventricular chamber (McDonald, 1970, Ormiston et al., 1981). LV twist is greatest toward the apex to achieve maximal reduction in chamber volume (Bogaert and Rademakers, 2001) and may be explained by the fact that the right- and left-handed helical segments converge toward the apex to form the vortex of the double helical loop (Bogaert and Rademakers, 2001).

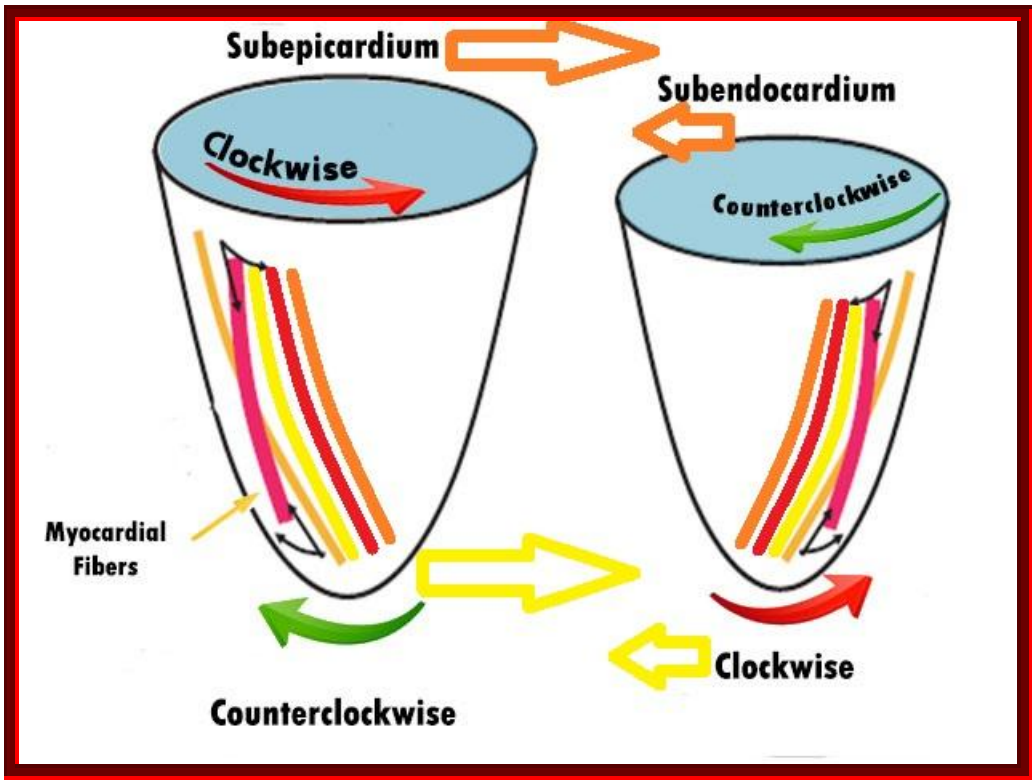


Figure 1.2.2.2 Rotation of myocardial fibres at subendocardium and subepicardium (apical and basal levels)

LV twist may also be important to equalize transmural differences in sarcomere shortening, end-systolic fibre stress, and contractile work during the ejection phase (Sengupta et al., 2007). The transmural spread of electrical activation results in sequential subendocardial-to-subepicardial fibre shortening (Sengupta et al., 2008a). The myocardium shortens along the entire transmural course, resulting in longitudinal and circumferential shortening of the left ventricle (Sengupta et al., 2008a). Twist helps bring a uniform distribution of LV fibre stress and fibre shortening across the wall (Alharthi et al., 2009).

To provide a framework for interpreting LV twist, Taber and colleagues (Taber et al., 1996) proposed a model of helical layer architecture on a one-layer cylinder composed of obliquely aligned muscle fibres embedded in an isotropic matrix. It has been demonstrated in a mathematical model that normal twist causes sarcomere shortening of 0.20 mm in the epicardium and 0.48 mm in the endocardium (Sengupta et al., 2007). Elimination of the twist, however, decreases epicardial shortening (0.10 mm) and increases endocardial shortening (0.55 mm) (Alharthi et al., 2009). Thus, disappearance of twist would increase endocardial stress and strain and increase oxygen demand, thereby reducing the efficiency of LV systolic function (Alharthi et al., 2009).

Assessment of LV rotation and twist was initially obtained by MRI (Goffinet et al., 2009). STE has been validated as a reliable method to quantify LV twist. The potential clinical applications of LV twist indices are currently expanding. The magnitude of LV twist is determined by contractile force. Therefore, measurements of LV twist may be effective as a clinical index of local and global cardiac contractility, and could be used to estimate disease-related changes of myocardial function (Sengupta et al., 2008a, Sengupta et al., 2008b).

1.2.3 LV STRAIN IN HYPERTENSION

In general, LV longitudinal strain (LS), which is predominantly governed by the subendocardial region, is the most vulnerable component of LV myocardial deformation in hypertension and therefore most sensitive to the presence of myocardial disease (Cho et al., 2006). The midmyocardial and epicardial function may remain relatively unaffected initially, hence circumferential strain (CS), radial strain, and LV twist may remain normal or show exaggerated compensation for preserving LV systolic performance (Cho et al., 2006). Two studies in the Japanese population using 2D STE have reported a decrease in some of the contractility indices (LS) in HTP with LVH as compared to normal controls (Amundsen et al., 2006, Mizuguchi et al., 2010b).

Furthermore, peak systolic strain was significantly reduced in patients with concentric hypertrophy and concentric remodelling when compared with patients with normal LV geometry (Hare et al., 2008). These differences remained significant when corrected for age, systolic BP, wall stress, and baseline EF (Hare et al., 2008) indicating that there are abnormalities in baseline myocardial deformation that may be identified in patients with altered LV geometry. However, these findings were not predictive of the evolution of LV geometry (Hare et al., 2008).

Plaksej showed in his study that LVH predominantly influences longitudinal and CS, but has a lesser impact on radial strain (RS) (Plaksej R, 2009). However, in an Asian population, all LV strain parameters were impaired in patients with hypertension and concentric hypertrophy (Mizuguchi et al., 2010b). The definition of values for myocardial deformation in HTP are dependent on the vendors used and have only been validated in western populations (Minoshima et al., 2009, Narayanan et al., 2009, Quinones et al., 2002, Becker et al., 2006). Moreover, limited data exists on strain parameters in hypertensive patients with heart failure. In HTP, the reduction in myocardial function with evidence of LV hypertrophy and remodelling support further investigation of myocardial deformation and their role in reflecting the mechanisms linking altered

LV geometry with progression to decompensated LV systolic function (Hare et al., 2008, Drazner, 2011).

1.2.4 LV TWIST IN HEART FAILURE

The dynamic interaction between subendocardial and subepicardial fibre helices in the left ventricle leads to a twisting deformation which plays an important role in optimizing LV EF (Sengupta et al., 2008b). It has been shown experimentally as well as clinically that LV twist closely parallels changes in LV global ejection performance. In previous work, it has been shown that LV twist is of fundamental importance to systolic LV function, with LV twist showing a positive correlation with EF (Notomi et al., 2005). An acute transmural insult or progression of disease results in concomitant midmyocardial and subepicardial dysfunction, leading to a reduction in LV twist mechanics and a reduction in LV EF (Cho et al., 2006). 2D STE showed a significant correlation between peak systolic twist and EF (Popescu et al., 2009) consistent with magnetic resonance tagging methods (Kanzaki et al., 2006) where an impairment in peak LV systolic twist is proportional to the degree of global LV dysfunction. Recently, in a population of advanced HF patients a significant positive correlation between LV twist and EF ($r = 0.53$, $p<0.001$) was noted (Bertini et al., 2010).

Recent studies have demonstrated the influence of cardiac shape on LV function, showing that LV myocardial fibre architecture is vital for an efficient LV performance. The geometry of the normal LV is ellipsoid with its long axis directed from apex to base (Streeter and Ramon, 1983, Streeter Jr et al., 1969). The size and shape of the LV changes, with an enlarged ventricle developing a spherical configuration (Popescu et al., 2009) resulting in significant changes in LV rotation. As demonstrated in a mathematical model (Taber et al., 1996), LV dilation and thinning, present in dilated cardiomyopathy, equalizes the radii of the subepicardial and subendocardial layers; as a result, the mechanical advantage of the subepicardial myofibres (the major determinants of LV twist under physiological conditions) is reduced (Popescu et al., 2009).

Consequently, LV twist decreases with increasing cavity volume. Furthermore, the intrinsic processes causing a dilated cardiomyopathy result in myofibre disarray and alterations in myofibre angle (Popescu et al., 2009). These phenomena eventually lead to the loss of the physiological spiral architecture of the LV and to the impairment of LV twist (Popescu et al., 2009). Last but not least, slowed transmural fibre activation, related to fibrosis and remodelling of gap junctions, may delay the activation of the epicardial myofibres, determining an initial clockwise twist (because of the unopposed rotation of the endocardial myofibres) thereby impairing the peak LV twist (Adamu et al., 2009).

As compared with ischemic heart disease patients, HF patients have a more pronounced impairment of LV rotational mechanics, irrespective of HF aetiology as a result of reduced LV basal and AR (Mor-Avi et al., 2011). In particular, the typical counterclockwise rotation of the LV apex may be completely abolished, or even reversed in a clockwise rigid body rotation [apical and BR in the same direction] (RBR). Additionally, in patients with dilated cardiomyopathy, LV AR was significantly related to LV diameters, volumes, and sphericity index (Popescu et al., 2009). RBR was observed in a high percentage of patients with dilated cardiomyopathy (52%) with more severe LV remodelling seen in these patients compared with those with normally directed AR (Popescu et al., 2009). However, studies reporting the effects of myocardial ischaemia on LV twist described a reduction in LV AR but not clockwise RBR (Garot et al., 2002, Takeuchi et al., 2007b, Bansal et al., 2008, Tibayan et al., 2004, Nagel et al., 2000, Nucifora et al., 2010).

RBR in patients with dilated cardiomyopathy and severely dilated ventricles could be explained by increased LV sphericity leading to widening of the apex (Popescu et al., 2009). In patients with severe LV remodelling in term of size, shape and severe systolic dysfunction, LV twist is depressed, due to the loss of the oblique architecture of the ascending and descending apical loop fibres (Popescu et al., 2009) which become more transverse and closely resemble the horizontal fibre orientation of the basal loop (Popescu et al., 2009). Clockwise RBR, assessed by tagging

MRI, was also found in 12/21 patients with dilated cardiomyopathy (Setser et al., 2007) and in 11/37 patients with idiopathic dilated cardiomyopathy using 2D STE (Meluzin et al., 2009). These studies are in agreement with Van Dalen et al (van Dalen et al., 2010) who have demonstrated that an optimal myofibre helix angle exists and is related to a certain value of LV sphericity index. A change in cardiac shape may indeed lead to a change in the arrangement of myocardial fibres and thereby a change in LV twist (van Dalen et al., 2010).

Hypertension is intimately related to left ventricular dysfunction and congestive HF, with the incidence of HF reported between 1% and 2% per year in HTP after excluding prior ischemic heart disease as a risk factor for HF (Lloyd-Jones et al., 2002, Drazner, 2011). Therefore, early detection of LV systolic dysfunction in HTP is a crucial issue. LV twist is a fundamental component of LV wall motion in assessing ventricular function. LV twist may provide incremental information on LV systolic performance apart from EF. Various clinical conditions such as hypertrophy may affect LV twist and consequently LV function by altering the distribution of LV myocardial wall stress (Ahmed et al., 2012). LV twist could be considered as a compensatory mechanism to counterbalance contraction abnormalities in order to maintain LV output in HHD (Cho et al., 2006).

Furthermore, little work has been done to examine the contribution of regional tissue deformation to myocardial dysfunction and subsequent HF in patients with hypertension. Whereas the association between hypertension and the development of HF is well recognized, the exact mechanism by which this process occurs has been difficult to elucidate (Drazner, 2005). The assumption that pure diastolic dysfunction is responsible for HF with preserved systolic function has been challenged over recent years (Sanderson, 2003, Burkhoff et al., 2003) with emerging evidence suggesting that many of these patients actually have detectable abnormalities in myocardial systolic function, if sensitive enough measures are used (Yip et al., 2002, Poulsen et al., 2003, Yu et al., 2002). Evaluation of LV twist using speckle tracking is a sensitive technique used to assess cardiac performance and may be a better index of systolic function than EF in HTP. In an

African population, we postulate that myocardial strain and LV twist abnormalities (contractility indices) may be a contributing factor to systolic dysfunction in HTP with HF (normal EF and low EF).

1.3 BIOMARKERS IN HYPERTENSIVE HEART DISEASE AND MYOCARDIAL DEFORMATION

HHD has been considered as one of the most common aetiological conditions predisposing to HF. Although, hypertension increases the risk for developing HF (Levy et al., 1996), LVH itself is an independent risk factor for HF (Levy et al., 1996, Kannel et al., 1987). Furthermore, in spite of progressive advances in antihypertensive strategies over the years, the incidence of HF in HTP with LVH remains high and comparable with that of major cardiovascular events, such as stroke (Tocci et al., 2008). LV dilation and LVH are markers of LV remodelling, a key precursor of HF (Aurigemma et al., 2001). HF is a progressive disorder initiated after a primary insult with loss of functioning myocytes, or loss of myocardial contractility with pump dysfunction (Mann et al., 2012) [Fig 1.3.1]. Patients with LV dysfunction may be asymptomatic as a result of compensatory mechanisms (remodelling) activated to maintain physiological LV function with preserved functional capacity of the patient (Mann et al., 2012). However, compensatory mechanisms (remodelling) result in adverse changes in myocardium (secondary damage) with a decline in LV function [Fig. 1.3.1].

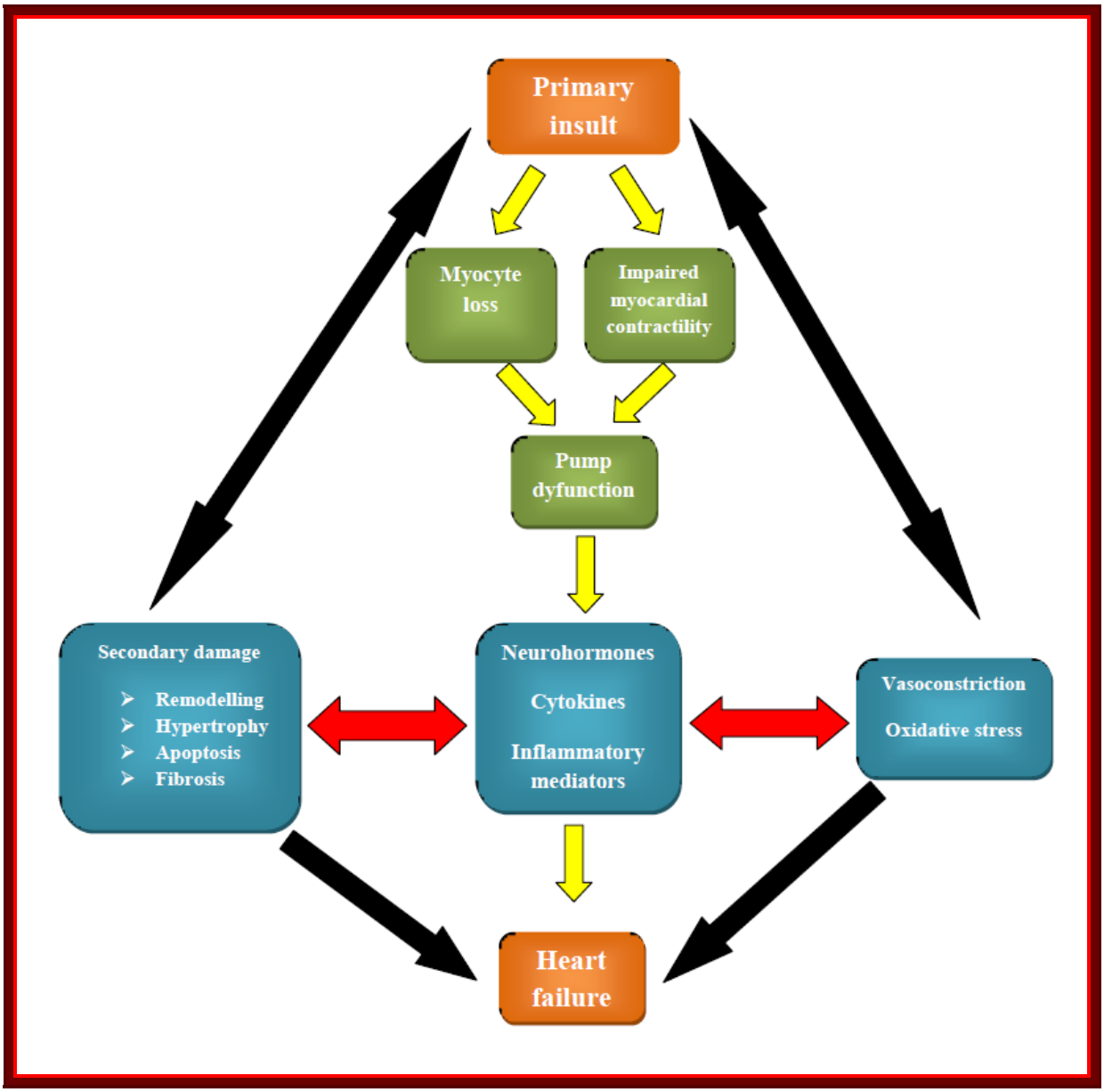


Figure 1.3.1 Pathophysiology of heart failure in hypertension

The process of LV remodelling has an important effect on the biology of the cardiac myocyte, on changes in the myocyte and nonmyocyte components of the myocardium, and on the geometry and architecture of the LV (Mann et al., 2012) [Fig. 1.3.2]. Alterations of the structure and composition of cardiomyocyte and noncardiomyocyte compartments of the myocardium appear to play a central role in the pathogenesis of HF (Mann et al., 2012) [Fig. 1.3.2]. Among these alterations, changes in the quantity and quality of the extracellular matrix (ECM), including the collagen network, have been characterized that induce remodelling of the myocardium and ultimately deteriorate left

ventricular function and facilitate the development of HF. It is not the quantity but rather the quality (structural remodeling) of myocardium that contributes to compromised function. Myocardial remodelling results from modifications that are not necessarily adaptive to the initial insult, but pathologic and potentially self-perpetuating in a progressive vicious cycle (Mann et al., 2012).

Alterations that occur in failing myocardium involve cardiac myocytes and composition of the extracellular matrix (Mann et al., 2012). Failing human cardiac myocytes undergo changes that lead to a progressive loss of contractile function including cell hypertrophy; abnormal myocardial energetics secondary to mitochondrial abnormalities and altered substrate metabolism; progressive loss of myofilaments in cardiac myocytes; alterations in cytoskeletal proteins with disarray of the cytoskeleton and alterations in excitation-contraction coupling (Mann et al., 2012) [Fig. 1.3.2]. The cardiac myocyte component of the myocardium undergoes progressive myocyte loss, through necrotic, apoptotic, or autophagic cell death pathways, which contribute to progressive cardiac dysfunction and LV remodelling (Mann et al., 2012) [Fig. 1.3.2] .

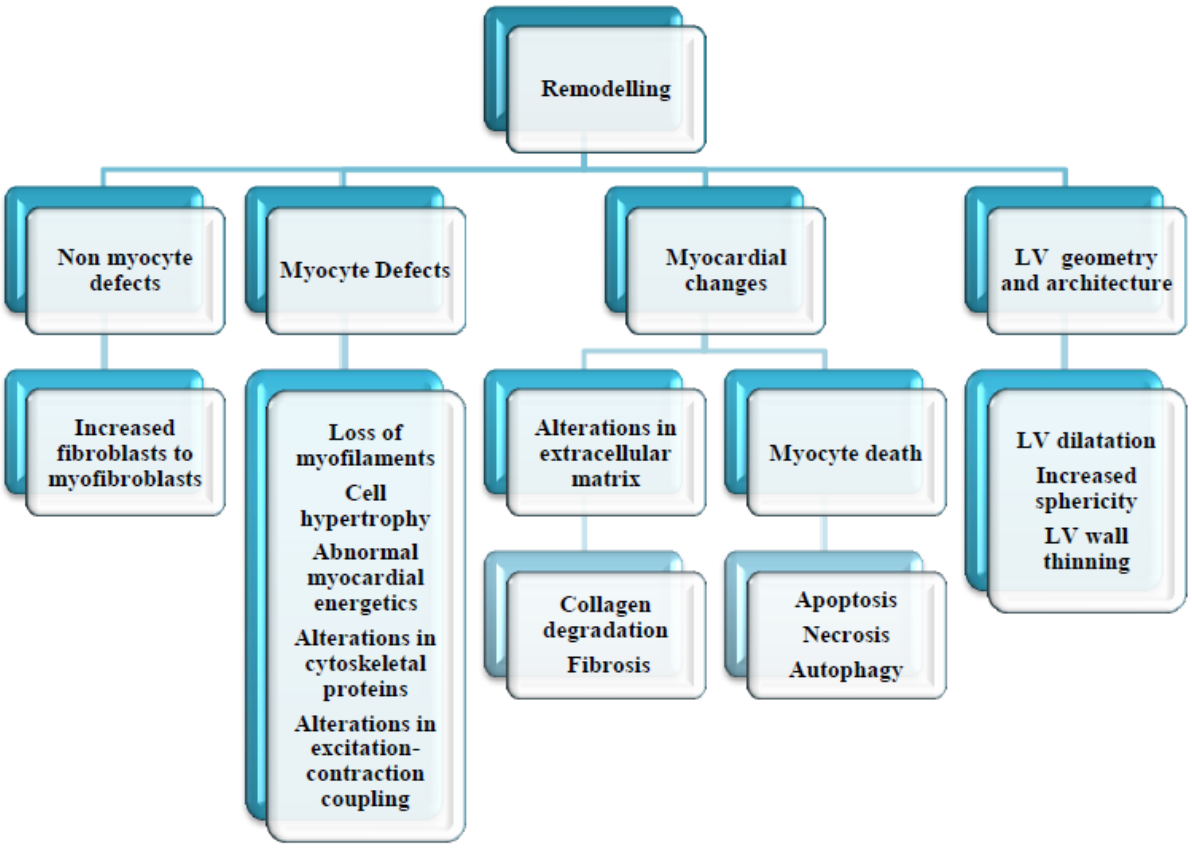


Figure 1.3.2 Cardiac Remodelling

Changes within the ECM constitute the second important myocardial adaptation that occurs during cardiac remodelling (Mann et al., 2012). The ECM forms a continuum between different cell types within the myocardium and provides a structural supporting network to maintain myocardial geometry during the cardiac cycle (Li et al., 1998, Thomas et al., 1998, Timms et al., 2002, Laviades et al., 1998) . Disruption or discontinuities within the fibrillar ECM network will result in a loss of normal structural support and continuity, resulting in myocyte fascicles being subjected to abnormal stress-and strain patterns during the cardiac cycle, which, in turn, will result in changes in myocardial geometry and function (Li et al., 1998, Thomas et al., 1998, Timms et al., 2002, Laviades et al., 1998) . Although the fibrillar collagen matrix was initially considered to form a relatively static complex, it is now recognized that these structural proteins can undergo rapid turnover and is highly responsive to biological and mechanical stimuli (Li et al., 1998, Thomas et

al., 1998, Timms et al., 2002, Laviades et al., 1998). During cardiac remodelling, important changes in the ECM include changes in fibrillar collagen synthesis and degradation, changes in the degree of collagen cross-linking, as well as loss of collagen struts that connect the individual cardiac myocytes (Li et al., 1998, Thomas et al., 1998, Timms et al., 2002, Laviades et al., 1998).

In pressure overload hypertrophy as seen in hypertension, the increase in systolic wall leads to the addition of sarcomeres in parallel, an increase in myocyte cross-sectional area and increased LV wall thickening without chamber enlargement (Mann et al., 2012). Pressure overload is characterized by a period of compensation in which left ventricular concentric hypertrophy normalizes systolic wall stress and contractile function is preserved. The transition to declining LV performance is characterized by the onset of chamber dilatation with the failure of further concentric hypertrophic growth to normalize load, and progressive contractile dysfunction (Drazner, 2011). A number of observations suggest that the transition to failure relates mainly to cardiomyocyte loss due to both apoptosis and necrosis, changes in the composition of motor unit and cytoskeleton of cardiomyocytes and alterations in the metabolism of the extracellular matrix (Díez et al., 2005). Changes in the biology of the failing myocyte, as well as in the failing myocardium, are largely responsible for the progressive LV dilation and LV dysfunction that occur during cardiac remodelling with increasing sphericity and LV dilatation (Mann et al., 2012) [Fig. 1.3.2].

HHD is characterized by complex changes in myocardial structure (e.g. enhanced cardiomyocyte growth, excessive cardiomyocyte apoptosis, accumulation of interstitial and perivascular collagen fibres, disruption of endomysial and perimysial collagen network) that induce the remodelling of the myocardium, and ultimately, impair left ventricular function and facilitate the development of HF (Díez et al., 2005). Hypertensive myocardial remodelling is the consequence of a number of pathological processes mediated by mechanical, neurohormonal, and cytokine routes, occurring in the cardiomyocyte and the non-cardiomyocyte compartments of the myocardium (Swynghedauw, 1999). LV remodelling is associated with derangements in the dynamic balance between the

accumulation and breakdown of cardiac ECM (Li et al., 1998, Thomas et al., 1998, Timms et al., 2002, Laviades et al., 1998). Alterations to the properties of the cardiac ECM, which consists of predominantly fibrillar collagen (Caulfield and Borg, 1979), are central to this remodelling process (Graham and Trafford, 2007, Jalil et al., 1989) as the cardiac ECM serves to maintain correct myocyte geometry and cardiac extension properties, and provides tensile strength to the tissue.

The scaffolding of cardiomyocytes is provided by a network of fibrillar collagen subdivided into three components (Li et al., 1998, Thomas et al., 1998, Timms et al., 2002, Laviades et al., 1998). The epimysium is located on the endocardial and epicardial surfaces of the myocardium, where it provides support for endothelial and mesothelial cells (Li et al., 1998, Thomas et al., 1998, Timms et al., 2002, Laviades et al., 1998). The perimysium surrounds muscle fibres, and perimysial strands connect groups of muscle fibres together sustaining the tension between different muscle bundles(Li et al., 1998, Thomas et al., 1998, Timms et al., 2002, Laviades et al., 1998). The endomysium arises from the perimysium and surrounds individual muscle fibres (Li et al., 1998, Thomas et al., 1998, Timms et al., 2002, Laviades et al., 1998). Struts of endomysium tether muscle fibres together and function as the sites for connections to cardiomyocyte cytoskeletal proteins (Li et al., 1998, Thomas et al., 1998, Timms et al., 2002, Laviades et al., 1998). The remodeling of collagen occurs mainly in the perimysial and endomysial components and leads to the thickening of the collagen fibrils and fibres, an increase in the density of the endomysial weaves around the cardiomyocytes, and an increase in newly formed collagen fibres (Li et al., 1998, Thomas et al., 1998, Timms et al., 2002, Laviades et al., 1998).

Matrix metalloproteinases (MMPs) are a family of proteolytic enzymes synthesized and secreted by fibroblasts, endothelial, and inflammatory cells (Nagase et al., 2006) that are involved in the protein degradation in the ECM and play an important role in remodelling (López et al., 2006). Tissue inhibitors of metalloproteinases (TIMPs) are low-molecular-weight molecules that bind to MMPs forming MMP–TIMP complexes that exhibit an inhibitory control on MMPs (Nagase et al., 2006). In fact, TIMPs bind noncovalently to MMPs in a 1: 1 stoichiometry, preventing access to the

substrate. In physiological conditions, collagen turnover is precisely regulated to maintain a finely balanced equilibrium between synthesis and degradation.

The interaction between the enzyme matrix metalloproteinase1 (MMP1) that initiates the degradation of collagen fibres within the heart, and its inhibitor tissue inhibitor of metalloproteinases1 (TIMP1) is of critical relevance in the maintenance of the integrity of the collagen network. In pathologic conditions, such as hypertension, the excessive inhibition, as anticipated by a relative excess of TIMPs over MMPs, will reduce collagen degradation and facilitate the deposition of fibrotic tissue [Fig 1.3.3], whereas the insufficient inhibition, as anticipated by a relative abundance of MMPs over TIMPs, will increase collagen degradation and promote the disruption and loss of the physiological collagen scaffold [Fig. 1.3.3] (Briones et al., 2010, Spinale, 2007).

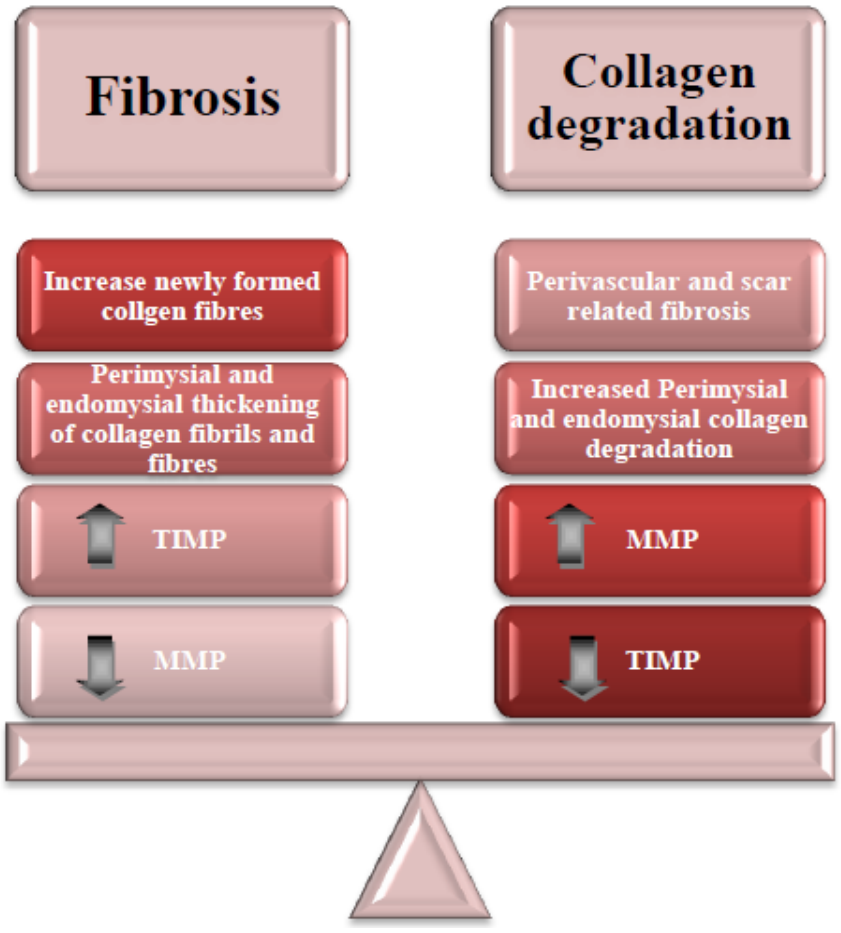


Figure 1.3.3 <u>Abnormalities in collagen turnover in hypertension</u>

The pathology of the end-stage failing human heart is characterized by myocyte loss, myocardial collagen accumulation and collagen fibril disruption, remodelling of the ECM, and disorganization of the cardiac myofibrils (Weber et al., 1993, Alla et al., 2006, Varo et al., 1999). Congestive HF has been associated with maladaptive ECM remodelling resulting from an imbalance in the synthesis and degradation of ECM components (Weber et al., 1993, Laviades et al., 1998). It has been proposed that this maladaptive remodelling contributes to the diminished systolic performance as well as the decreased compliance of the failing human heart (Brilla CG, 1994). The mechanisms responsible for the alterations in the ECM in the failing human heart remain undefined. Indeed, increased MMP activity has been observed in the failing human heart (Tyagi et al., 1996). An increase in the expression or activity of MMPs results in increased proteolytic activity in the extracellular spaces, leading to increased extracellular remodelling. Alternatively, decreased expression of TIMPs can elicit a similar tissue response. Increased activity of MMPs or decreased expression of TIMPs can result in enhanced proteolytic activity, fibrillar collagen degradation, progressive myocyte loss, and ventricular dilatation and sphericalization.

The identification of biomarkers of potential usefulness for the clinical handling of cardiac diseases evolving to HF has been a prolific field recently with the investigation of novel circulating biochemical markers of myocardial remodelling in HHD accelerating at a remarkable pace. Biomarkers either determined in the circulation as biochemical markers or detected in the heart by imaging technologies, can be defined as alterations in the constituents of tissues or body fluids that can be applicable in at least five clinical areas: screening, diagnosis, prognostication, prediction of disease recurrence, and therapeutic monitoring. In order for a circulating molecule to be considered a biochemical marker of myocardial remodelling it must fulfil several criteria.

First of all, there must be a relationship between its expression in the myocardium and its blood concentration (González et al., 2009); secondly, there has to be a positive gradient from its concentration in coronary sinus blood towards its concentration in peripheral vein blood, proving its predominant cardiac origin (González et al., 2009); thirdly, there must be an association between

its concentration in blood with the cardiac structural and/or functional parameters reflecting the hallmarks of the myocardial changes under study (González et al., 2009); finally, its levels should vary in parallel with the changes in the above parameters induced by pharmacological treatment. On the other hand, the method of determination must be easy (i.e. ELISA), reproducible and low cost, and the biochemical marker must have a good sensitivity and specificity to detect the pathology under study.

During the initial phases of HHD, the predominant process is increased synthesis of cardiomyocyte and ECM proteins (López et al., 2006). Much less is known about the subsequent phases of HHD, especially the transition from the compensated state to clinically apparent heart failure. In HHD, an increase in interstitial collagen (accumulated perimysium) is associated with diastolic heart failure, whereas degradation of endomysial and perimysial components of the collagen scaffolding is accompanied by ventricular dilatation and systolic heart failure (López et al., 2006). However, in impaired systolic function, the collagen content of the heart is increased and is mainly scar-related collagen deposition and perivascular fibrosis (López et al., 2006). Recently, Diez and colleagues revealed that enhanced MMP-mediated collagen degradation contributed to the LV dilation and decline in ejection fraction in HHD with systolic dysfunction (López et al., 2006).

The relationship between excessive myocardial MMP1 activity, increased mysial collagen degradation, and progression to systolic HF has been shown experimentally through the use of transgenic models and humans (Baicu et al.,2003, Kim et al., 2000). The volume of myocardial tissue occupied by perimysial and endomysial collagen was lower in HTP with low EF than in HTP with preserved EF (López et al., 2006). These findings suggest that excessive MMP1 dependent degradation of mysial collagen may be related to the compromise of systolic function in HTP. Loss of inhibitory control of TIMP on MMP correlates with progression of left ventricular remodelling via increased MMP activity, ECM proteolysis, and myocardial remodelling changes (López et al., 2006). These data suggest that the transition from compensated LVH to heart failure is associated with degradation of ECM.

In patients with systolic heart failure, perivascular fibrosis and scarring occupied a greater portion of the myocardium, while the number of interstitial collagen fibres was reduced (López et al., 2006). Perivascular and scar-related fibrosis secondary to excessive collagen synthesis and exaggerated perimysial and endomysial collagen degradation may contribute critically to deterioration of systolic function in HTP (López et al., 2006). Depending on the temporal sequence of the disease process and on the localization, either diffuse or focal of the injury, this pattern may coexist at variable degrees within the same myocardium. MMP activation can contribute to the fibrotic process by participating in a vicious circle in which ECM degradation promotes ECM protein synthesis and fibrosis. This pathway is particularly detrimental because the nature and organization of the newly synthesized ECM differs from that of the native ECM.

The expression of MMPs is regulated at the transcriptional level by a variety of inflammatory cytokines, hormones and growth factors. It is likely that up-regulation of MMP1 in low EF HTP is a dynamic process and is determined by the co-occurrence of a number of intracellular and extracellular signals (López et al., 2006). For instance, a number of humoral factors (i.e., inflammatory mediators, neurohormones, and bioactive peptides) may contribute to MMP1 up-regulation in HTP with low EF (Siwik and Colucci, 2004). Although MMPs are proteolytic enzymes that degrade ECM proteins, they have other functions beyond this activity, affecting different regulatory pathways in the remodeling process.

Moreover, increased MMP expression and activation are associated with inflammation, oxidative stress, and the activation of the renin–angiotensin–aldosterone system (Brassard et al., 2005, Deschamps et al., 2005). In addition, experimental evidence has been provided showing that following chronic neurohormonal activation, increased synthesis and release of MMPs into the local ECM of the cardiomyocyte occurs (Coker et al., 1999), which in turn could contribute to endomysial and perimysial collagen degradation and disruption. MMPs have significant effects on important biologically active proteins and peptides, such as transforming growth factor-b, and other profibrotic proteins and pathways (Wang et al., 2010). Indeed, the disruption of the ECM in

hypertensive cardiac remodeling may involve both profibrotic and proteolytic pathways, providing a possible explanation for the variability of results regarding MMP modulation. In addition, mechanical signals linked to LV wall stress may result in the induction of myocardial MMP1 (Spinale, 2002). It has been demonstrated that acute MMP activation and disruption of the fibrillar collagen network in the pressure overload hypertrophied myocardium causes a decrease in systolic performance without changing cardiomyocyte contractility (Baicu et al., 2003).

A higher concentration of MMP1 has been found in coronary sinus blood compared with antecubital vein blood in patients with hypertensive but not in normotensive subjects (López et al., 2006). Moreover, a highly significant direct correlation has been found between MMP1 detected in coronary blood and peripheral blood in HTP (López et al., 2006). Of interest, in patients with hypertension and HF, MMP1 concentration measured in peripheral blood increases in parallel with the increase in myocardial MMP1 expression and is higher in patients with loss of myocardial collagen scaffold (López et al., 2006). Thus, in the setting of steady-state production by extracardiac sources, an excess of circulating MMP1 in patients with HHD can be considered of cardiac origin.

The peripheral MMP1:TIMP1 ratio may be useful as an index of the MMP1/TIMP1 balance within the myocardium (López et al., 2006). Furthermore, the ratio MMP: TIMP is higher in HTP with low EF than in patients with preserved EF (López et al., 2006). The clinical relevance of the MMP1:TIMP1 ratio determined in peripheral vein blood is further supported by its associations with parameters assessing LV systolic dysfunction (depressed EF) and remodelling (increased LVEDD) (López et al., 2006).

Therefore, the determination of MMP1 and TIMP1 in serum might be a useful biochemical marker of alterations in the perimysial and endomysial collagen network involved in systolic deterioration and geometric dilatation of the left ventricular chamber in HTP with HF (López et al., 2006) adding further diagnostic and prognostic information for the assessment of these patients. It is possible that

changes in the ECM reflected by the MMP1:TIMP1 ratio, account for the varying morphology, EF and LV twist in HTP who present with HF. Thus far, no study has assessed the relationship between LV twist and markers of collagen turnover in HTP with HF. In this context, the assessment of myocardial deformation of the left ventricle with the markers of collagen turnover may help to understand the progression towards hypertensive HF and may help clarify its clinical impact on the prognosis of HHD.

Several published studies describe LV twist forces in normal adults (Villari et al., 1997, Park et al., 2008, Takeuchi et al., 2007a, Wang et al., 2007, Rothfeld et al., 1998, Helle-Valle et al., 2005, Notomi et al., 2005). There appears to be an increase in left ventricular twist with advancing age (Notomi et al., 2005, Sun et al., 2012a, Reckefuss N, 2011, Helle-Valle et al., 2005, Rothfeld et al., 1998, Park et al., 2008, Takeuchi et al., 2007a, Wang et al., 2007). Whether this relates solely to the aging process or is associated with concomitant physiological or comorbid conditions that occur with aging is unknown. Normal deformation values vary among publications and depend on the vendor used, suggesting that reference values have to be obtained for each method and software (Carasso et al., 2012, Cameli et al., 2011, Sun et al., 2004, Marwick et al., 2009, Manovel et al., 2010, Mor-Avi et al., 2011). Furthermore, the interpretation of functional data across a spectrum of ages requires consideration of the ventricular loading conditions and vascular impedance. In subjects of African descent, no data exist on the analysis of these parameters in normal individuals. Therefore, it is pertinent to establish normative reference values in an African population.

1.4 IMPLICATIONS

Assessment of myocardial mechanics may be tailored according to the clinical goals. The detection of altered longitudinal mechanics alone may suffice if the overall goal of analysis is to detect the presence of early myocardial disease. Further characterization of radial strain, CS, and twist mechanics may provide assessment of the transmural disease burden and pathophysiologic insight into the mechanism of LV dysfunction (Cho et al., 2006). The screening for hypertension and

provision of appropriate anti-hypertensive therapy remain important public health measures both in South Africa and elsewhere. Although, LVH has been shown to be an important predictor of cardiac morbidity and mortality in patients with hypertension, identification of more sensitive pre-clinical markers of LV dysfunction remains an important treatment goal in the arena of hypertension. Additional sensitive markers of LV remodelling may not only serve to further elucidate the mechanism of progression from hypertension to HF, but may also allow for more intensive and targeted therapy in at risk patients to curb the epidemic of HF.

2. OBJECTIVES

1. To establish normative values for myocardial deformation (systolic longitudinal, circumferential and radial) and twist mechanics in healthy subjects.
2. To determine the twist mechanics and patterns of rotation in HTP with systolic LV dysfunction without coronary artery disease.
3. To determine the relationship of biomarkers of collagen turnover (MMP1, TIMP1, MMP1: TIMP1 ratio) with myocardial deformation and twist mechanics in HTP with HF with normal EF and low EF without coronary artery disease.

3. PUBLICATION CHAPTERS

3.1 Left Ventricular Twist in a Normal African Adult Population

Nirvarthi Maharaj, MD, Ferande Peters, MD, Bijoy K. Khandheria, MD, FACP, FESC, FAHA, FACC, FASE, Elena Libhaber, PhD, Mohammed R. Essop, MD, FACC, FRCP, *Johannesburg, South Africa; Milwaukee, Wisconsin*

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3.1.1 ABSTRACT

Background: Speckle tracking has emerged as a quantitative technique for assessing left ventricular (LV) function. However, no normative data for LV twist using speckle tracking echocardiography (STE) are available in the black population. This study assessed myocardial mechanics by determining LV twist parameters in different age groups using STE, and evaluated the effect of aging on LV twist in this population.

Methods: The study population consisted of 127 healthy volunteers divided into four age groups: 20-29 (n=34); 30-39 (n=33); 40-49 (n=29); and 50-65 (n=31) years. Parasternal short-axis images of three consecutive end-expiratory cardiac cycles at LV basal, papillary muscle and apical levels and apical four-chamber images were obtained. Apical rotation (AR) and basal rotation (BR) LV peak systolic rotation during ejection and instantaneous LV peak systolic twist (net twist, defined as maximal value of instantaneous AR minus BR) were measured.

Results: Mean strain values were -17.28 ± 3.30% for longitudinal, -17.40 ± 3.29% for circumferential and 57.49 ± 3.32% for radial strain. Mean rotational values were: AR (5.56° ± 1.98), BR (-3.31° ± 0.92) and net twist (8.87° ± 2.21). AR, BR and net twist increased with age, whereas longitudinal, circumferential and RS decreased with age. Multivariate linear regression analysis showed age as the main predictor of net twist (R^2=0.82, P<0.0001).

Conclusions: These data establish values for strain and twist in a normal black adult population. Net twist increases with age whereas strain parameters decrease. Age is the strongest independent predictor of LV twist.

3.1.2 INTRODUCTION

Left ventricular (LV) twist is defined as the wringing motion whereby the LV apex rotates in an opposite direction with respect to the LV base about the LV long axis (Takeuchi et al., 2009). LV twist has a vital role in ensuring that blood is ejected in an efficient manner during systole. Twist mechanics of the left ventricle obtained using STE offer new insights into the assessment of LV performance. Speckle tracking is a novel echocardiographic technique for the evaluation of LV twist that has been validated against MRI tagging (Goffinet et al., 2009) and has contributed to an explosion of research utilizing this technique in various cardiac abnormalities. Several pathological states have been identified in which abnormality of LV twist is a significant contributing factor to LV systolic dysfunction.

In healthy humans, there is a continuum of expression of cardiac structural and functional alterations that occur with aging (Lakatta, 2003c, Lakatta, 2003a, Lakatta, 2003b). These age-associated cardiac changes may have relevance to the increased incidence of LV hypertrophy, chronic HF and atrial fibrillation seen with increasing age (Lakatta, 2003c, Lakatta, 2003a, Lakatta, 2003b). Several published studies describe LV twist forces in normal adults (Villari et al., 1997, Park et al., 2008, Takeuchi et al., 2007a, Wang et al., 2007, Rothfeld et al., 1998, Helle-Valle et al., 2005, Notomi et al., 2005). There appears to be an increase in left ventricular twist with advancing age (Notomi et al., 2005, Sun et al., 2012a, Reckefuss N, 2011, Helle-Valle et al., 2005, Rothfeld et al., 1998, Park et al., 2008, Takeuchi et al., 2007a, Wang et al., 2007). Whether this relates solely to the aging process or is associated with concomitant physiological or comorbid conditions that occur with aging is unknown. The aim of this study was to establish normal values for left ventricular twist in black Africans and evaluate the effect of aging on left ventricular twist in this population.

3.1.3 METHODS

This was a single centre, observational, cross-sectional, prospective study. The study population comprised randomly chosen volunteers recruited from Chris Hani Baragwanath Hospital staff and visitors and Soweto community members from January 2011 to May 2012. All subjects underwent clinical evaluation, which included history and physical examination. Only subjects who were asymptomatic and normotensive, had no evidence of cardiovascular or systemic diseases on history and physical examination, and had normal 12-lead electrocardiogram underwent echocardiography. All subjects who had entirely normal echocardiograms and adequate image quality for speckle-tracking analysis were enrolled in the study after informed consent was obtained. A total of 150 subjects were screened for inclusion. Twenty-three subjects were excluded as a result of inadequate image quality, which did not allow for complete segmental assessment of LV rotation at both the basal and apical left ventricle. The remaining 127 subjects made up the study group. The subjects were classified into four age groups: 20-29 (n=34), 30-39 (n=33), 40-49 (n=29) and 50-65 (n=31) years. This study was approved by the University of Witwatersrand Ethics Committee (clearance number: M110204) (see appendix a) and Institutional Review Board.

BP measurements

A Dinamap automated BP monitor (1846 SX; CritikonInc, Tampa, Fla) was used to measure BP readings. BP was recorded in the sitting position. Patients sat quietly for 10 min prior to readings being taken. The patient's arm was supported at heart level during BP measurements. The pneumatic bag must be 20% wider than the diameter of the upper arm and fully deflated between measurements. BP readings were taken every 3 minutes for 30 minutes. The mean of these 10 readings (SBP, DBP, MAP, and HR) were calculated and recorded.

Echocardiography

Comprehensive transthoracic echocardiography was performed using a commercially available system (iE33 xMATRIX, Philips Healthcare, Andover, MA) equipped with an S5-1 transducer (frequency: 1.7 MHz transmitted, 3.4 MHz received) according to a standardized protocol. Images were acquired with the patient in the left lateral decubitus position during quiet respiration. All data were transferred to an Xcelera workstation (Philips Healthcare) and analyzed offline. All echocardiographic measurements were averaged from three heartbeats. Measurements relating to chamber size and function were performed in accordance with the American Society of Echocardiography (ASE) chamber quantification guidelines (Lang et al., 2005) and the ASE 2010 guidelines on right heart assessment (Rudski et al., 2010). Severity of mitral and tricuspid regurgitation were analyzed in accordance with the ASE guidelines on native valvular regurgitation (Zoghbi et al., 2003). EF was calculated from LV volumes by the modified biplane Simpson rule in accordance with the guidelines (Lang et al., 2005).

From the mitral-inflow pattern, peak early (E) and late (A) filling velocities, E/A ratio, and E-velocity deceleration time were measured. Tissue Doppler was performed at end-expiration in the pulsed-wave Doppler mode at the level of the inferoseptal side of the mitral annulus from an apical four-chamber view. To acquire the highest wall tissue velocities, the angle between the Doppler beam and the longitudinal motion of the investigated structure was adjusted to a minimal level. The spectral pulsed-wave Doppler velocity range was adjusted to obtain an appropriate scale. The time intervals between the peak of the R wave on the electrocardiogram and the aortic valve opening and closure, and between the R wave and the mitral valve opening and closure were measured using pulsed Doppler acquired from the LV outflow and inflow, respectively.

Left ventricular mass was calculated using the formula: LV mass = $0.8 \times [1.04[(\text{LVIDd} + \text{PWTd} + \text{SWTd})^3 - (\text{LVIDd})^3]] + 0.6$ g, where LVIDd, PWTd and SWTd are left ventricular internal diameter at end diastole, posterior wall thickness at end diastole and septal wall thickness at end

diastole, respectively (Lang et al., 2005, Ganau et al., 1992). Relative wall thickness was calculated using the formula: (2 × PWTd)/LVIDd (Lang et al., 2005, Ganau et al., 1992). The formula for body surface area: BSA =[(0.0001x weight in kg)* 0.425] x[height in cm)* 0.725] (Lang et al., 2005).

Speckle-Tracking Analysis

Two-dimensional images were obtained at a frame rate of 60 to 80 frames/s. Parasternal short-axis images at the LV basal level showing the tips of the mitral valve leaflets were obtained with cross sections as circular as possible. To obtain a short-axis image at the true LV apical level, the transducer was positioned one or two intercostal spaces more caudally and acquired in a manner described by (van Dalen et al., 2008c). LV LS analysis was performed using an apical four-chamber view. Foreshortened images were avoided by looking for the largest long-axis dimensions. From short-axis views, radial strain, CS and rotational data were averaged across six wall segments (anteroseptal, anterior, lateral, posterior, inferior and septal) at the basal and apical levels. Global CS was also measured from parasternal short-axis views at papillary muscle level. The width of the region of interest was adjusted to include the entire myocardium. Analysis of the datasets was performed using QLAB Advanced Quantification Software Version 8.0 (Philips, Best, Netherlands). In all instances the mean longitudinal, circumferential and RS for each subject was calculated by the sum of the strain measured in each anatomic region divided by the number of segments analyzed in that particular view.

To assess LV rotation, six tracking points were placed on an end-diastolic frame (Van Dalen et al., 2009) in each parasternal short-axis image automatically encompassing endocardium and epicardium. Tracking points were separated about 60 degrees from each other and placed at 1 o'clock (30°, anteroseptal insertion into the LV via the right ventricle), 3 o'clock (90°), 5 o'clock (150°), 7 o'clock (210°), 9 o'clock (270°, inferoseptal insertion into the LV via the right ventricle) and 11 o'clock (330°) to fill the total LV circumference (Van Dalen et al., 2009). In all instances in

which tracking points were manually manipulated to optimize speckle tracking, no tracking points were allowed to be moved more than 30 degrees. Data were exported to a spreadsheet program (Excel, Microsoft Corp., Redmond, WA). Counterclockwise rotation as viewed from the apex was expressed as a positive value; clockwise rotation was expressed as a negative value. End systole was defined as the point of aortic valve closure.

Analysis was performed to evaluate the peak basal and apical bulk rotations during the ejection phase and the net instantaneous twist of the LV, which was calculated as peak apical rotation (AR) minus basal rotation (BR) at a time isochronous with the peak AR. Analysis was performed to note the patterns of AR and BR during isovolumic contraction and during the ejection phase characterized by clockwise BR and counterclockwise AR in systole. The curves generated by the QLAB software include automated analysis of subendocardial and subepicardial regions by speckle tracking at the base and apex. In all subjects, the direction, timing and peak values of subendocardial and subepicardial rotations at both the apex and base were documented. The untwisting rate was defined as the mean diastolic untwisting velocity from instantaneous peak systolic twist to mitral valve opening and calculated as follows: (twist at mitral valve opening − peak systolic twist)/(time interval from peak systolic twist to mitral valve opening).

Statistical Analysis

Database management and statistical analyses were performed with SAS software (Version 9.2, SAS Institute Inc., Cary, NC). Data are presented as mean ± standard deviation. Continuous variables between age categories were compared using ANOVA, or Kruskal-Wallis test when the distribution was non-normal. Two-by-two comparisons were then performed applying Bonferroni correction with a p-value <0.0083 denoting significance. Univariate and multivariate linear regression analysis were used to identify possible independent determinants of net twist. Six separate models to predict net twist were used in a multiple linear regression analysis. The interobserver variability was assessed for twist measurements in 20 randomly selected patients and

calculated as the standard deviation of the differences between measurements of two independent observers who were unaware of the other patient data; these calculations were expressed as a percentage of the average value. The intraobserver variability was calculated as the standard deviation of the differences between a first and second determination of a single observer, and expressed as a percentage of the average value. P-values <0.05 were considered statistically significant.

3.1.4 RESULTS

Patient Selection

A total of 150 normal subjects were screened for inclusion in the study. Inadequate visualization of the endocardial border precluded correct wall motion tracking in 23 individuals, who were eventually excluded from the analysis. The remaining 127 subjects showed adequate tracking for apical and basal short-axis views and were consecutively included.

Baseline and Echocardiographic Characteristics

The baseline general characteristics of the study sample are represented in Table 3.1.1. A significant increase in systolic BP (p=0.03), indexed LV mass (p=0.002) and relative wall thickness (p=0.008) was observed with increasing age. The indices of diastolic function showed that the peak E velocity decreased (p=0.06), while the A velocity increased (p=0.01), resulting in a progressive decrement in E/A ratio (p<0.0001) (Table 3.1.1). Peak E` medial wall velocity and peak E` lateral wall velocity decreased significantly with age (p=0.03 and p<0.0001, respectively) (Table 3.1.1).

TABLE 3.1.1 BASELINE CLINICAL AND ECHOCARDIOGRAPHIC CHARACTERISTICS

Variable	Total	20-29	30-39	40-49	50-65	*P*
No.	127	34	33	29	31	
Age (years)	39.7 ± 12.1	25.0 ± 2.8	34.9 ± 2.2	45.0 ± 2.7	55.8 ± 4.9	<0.0001
Sex (F:M ratio)	64:63	18:16	15:18	15:14	16:15	0.45
BMI (kg/m^2)	24.9 ± 2.4	24.2 ± 2.6	24.2 ± 3.0	25.5 ± 2.0	25.7 ± 1.3	0.06
BSA (m^2)	1.7 ± 0.2	1.8 ± 0.2	1.7 ± 0.2	1.8 ± 0.1	1.7 ± 0.1	0.10
SBP (mmHg)	124 ± 5	123 ± 5	123 ± 6	125 ± 5	126 ± 4	0.03
DBP (mmHg)	77 ± 9	74 ± 10	76 ± 9	80 ± 6	78 ± 8	0.05
PP (mmHg)	47 ± 8	49 ± 9	47 ± 8	45 ± 6	47 ± 8	0.30
Heart rate (bpm)	73 ± 10	70 ± 10	75 ± 9	77 ± 9	71 ± 11	0.01
EDD (mm)	45 ± 4	45 ± 4	45 ± 4	45 ± 3	46 ± 3	0.89
IVSD (mm)	10 ± 2	9 ± 2	10 ± 2	10 ± 1	10 ± 2	0.007
LVPWD (mm)	9 ± 1	8 ± 1	8 ± 1	9 ± 1	9 ± 1	0.009
EDV (ml)	72.8 ± 9.7	73.4 ± 6.7	72.0 ± 9.7	73.9 ± 10.2	72.1 ± 11.8	0.75
EDVI (ratio)	42.2 ± 6.1	42.0 ± 5.5	42.2 ± 5.9	41.9 ± 6.0	42.7 ± 7.4	0.77
ESV (ml)	31.9 ± 8.2	32.5 ± 8.6	32.8 ± 7.9	32.8 ± 8.1	29.6 ± 8.4	0.30
ESVI (ratio)	18.5 ± 4.7	18.4 ± 4.4	19.3 ± 5.1	18.6 ± 4.6	17.5 ± 4.8	0.55
EF (%)	62.4 ± 7.0	61.8 ± 7.1	60.4 ± 6.9	62.3 ± 6.1	65.2 ± 7.4	0.06
RWT (ratio)	0.38 ± 0.05	0.36 ± 0.05	0.37 ± 0.06	0.39 ± 0.04	0.40 ± 0.04	0.008
LVMI (g/m^2)	81.4 ± 18.0	72.5 ± 19.7	80.8 ± 17.2	83.9 ± 15.0	89.4 ± 15.5	0.002
LAVI (ml/m^2)	22.6 ± 3.2	22.6 ± 2.4	22.2 ± 4.7	22.4 ± 2.5	23.4 ± 2.5	0.34
E (cm/s)	86.9 ± 14.5	88.8 ± 14.7	91.4 ± 17.2	84.4 ± 14.0	82.4 ± 9.8	0.06
A (cm/s)	60.0 ± 11.0	54.6 ± 10.9	62.3 ± 11.1	60.5 ± 10.0	62.9 ± 10.3	0.01
Decel time (ms)	171± 15	173 ± 13	167 ± 16	172 ± 14	171 ± 16	0.36
E/A ratio	1.47 ± 0.22	1.66 ± 0.26	1.48 ± 0.19	1.40 ± 0.12	1.32 ± 0.12	<0.0001
E` M (cm/s)	11.6 ± 1.2	12.0 ± 1.3	11.8 ± 1.2	11.6 ± 1.2	11.1 ± 0.7	0.03
E` L (cm/s)	14.5 ± 2.1	15.6 ± 2.3	15.5 ± 1.8	13.8 ± 1.3	13.0 ± 1.3	<0.0001
E/E` ratio	6.66 ± 0.92	6.47 ± 0.98	6.70 ± 1.06	6.63 ± 0.78	6.86 ± 0.79	0.40

Data reported as mean ± standard deviation.
BMI, body mass index; *BSA*, body surface area; *DBP*, systolic blood pressure; *Decel time*, Deceleration time; *EDD*, end-diastolic diameter; *EDV*, end-diastolic volume; *EDVI*, end-diastolic volume index; *EF*, ejection fraction; *E` L*, peak E` lateral wall velocity; *E` M*, peak E` medial wall velocity; *ESV*, end-systolic volume; *ESVI*, end-systolic volume index; *F:M*, female to male; *IVSD*, interventricular septum end-diastolic diameter; *LAVI*, left atrial volume index; *LVMI*, left ventricular mass index; *LVPWD*, left ventricular posterior wall diameter; *PP*, pulse pressure; *RWT*, relative wall thickness; *SBP*, systolic blood pressure.

Left Ventricular Strain and Rotation Analysis

Mean strain values for all subjects were -17.28 ± 3.30% for LS, -17.40 ± 3.29% for CS and 57.49 ± 3.32% for RS (Table 3.1.2). All subjects had normal pattern of rotation with AR moving in a counterclockwise direction and BR recorded in a clockwise direction during systole. Mean rotational values for the study sample were 5.56 ± 1.98° for AR, -3.31 ± 0.92° for BR and 8.87 ±

2.21° for net LV twist (Table 3.1.3). Mean subendocardial and subepicardial rotational values for both AR (5.74 ± 2.04° vs. 5.44 ± 1.96°, respectively) and BR (-3.33 ± 0.96° vs. -3.10 ± 1.14°, respectively) were evaluated in all subjects (Table 3.1.3). Both subendocardial and subepicardial rotation values for the apex (counterclockwise, positive values) and base (clockwise, negative values) were recorded in the same direction (Figure 3.1.1) in all subjects. The mean untwisting rate was 52.19 ± 5.56 degrees/sec (Table 3.1.3).

TABLE 3.1.2 STRAIN PARAMETERS WITH AGE

Variable	Total	20-29	30-39	40-49	50-65	*P*
No.	127	34	33	29	31	
LS (%)	-17.28 ± 3.30	-21.76 ± 0.84	-17.71 ± 0.76	-15.66 ± 0.76	-12.90 ± 1.17	<0.0001
CS (%)	-17.40 ± 3.29	-21.56 ± 0.96	-18.41 ± 0.29	-15.69 ± 0.74	-12.88 ± 1.17	<0.0001
RS (%)	57.49 ± 3.32	61.71 ± 0.91	58.51 ± 0.52	55.71 ± 0.72	52.80 ± 1.28	<0.0001

Data reported as mean ± standard deviation.
CS, circumferential strain*; LS*, Longitudinal Strain*; RS*, Radial Strain.

TABLE 3.1.3 ROTATIONAL PARAMETERS WITH AGE

Variable	Total	20-29	30-39	40-49	50-65	*P*
No.	127	34	33	29	31	
AR (deg)	5.56 ± 1.98	4.11 ± 0.74	4.41 ± 0.88	5.73 ± 1.21	8.22 ± 1.56	<0.0001
Subendo AR (deg)	5.74 ± 2.04	4.30 ± 0.81	4.53 ± 0.94	5.92 ± 1.32	8.45 ± 1.62	<0.0001
Subepi AR (deg)	5.44 ± 1.96	4.00 ± 0.68	4.32 ± 0.81	5.58 ± 1.19	8.09 ± 1.62	<0.0001
BR (deg)	-3.31 ± 0.92	-2.64 ± 0.64	-3.37 ± 0.87	-3.52 ± 0.73	-3.77 ± 1.03	<0.0001
Subendo BR (deg)	-3.39 ± 0.94	-2.77 ± 0.67	-3.43 ± 0.94	-3.59 ± 0.70	-3.85 ± 1.07	<0.0001
Subepi BR (deg)	-3.16 ± 1.12	-2.34 ± 1.26	-3.29 ± 0.88	-3.41 ± 0.82	-3.68 ± 0.98	<0.0001
Time to peak rotation (ms)	357 ± 41	342 ± 38	353 ± 44	361 ± 28	372 ± 46	0.02
Net twist (deg)	8.87 ± 2.21	6.75 ± 0.81	7.79 ± 0.74	9.25 ± 0.90	12.00 ± 1.38	<0.0001
Untwist rate (deg/sec)	52.19 ± 5.56	55.40 ± 2.82	49.14 ± 6.38	50.22 ± 5.50	53.75 ± 4.61	<0.0001

Data reported as mean ± standard deviation.
AR, apical rotation; *BR*, basal rotation; *Subendo AR*, subendocardial apical rotation; *Subepi AR*, subepicardial apical rotation; *Subendo BR*, subendocardial basal rotation; *Subepi BR*, subepicardial basal rotation.

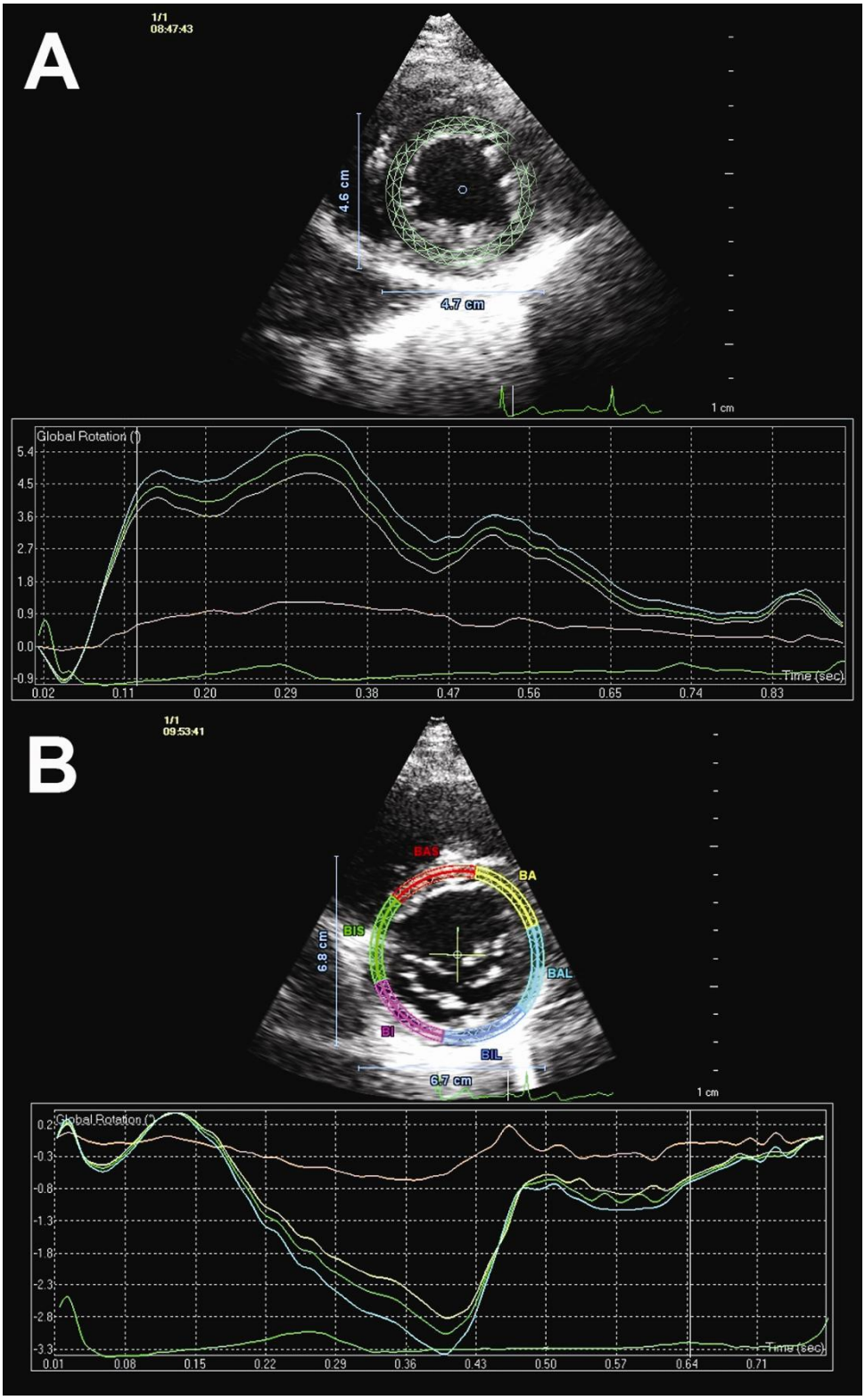


Figure 3.1.1 Subendocardial and subepicardial rotations at apex (Panel A) and base (Panel B). *BA* = basal anterior; *BAL* = basal anterolateral; *BAS* = basal anteroseptal; *BI* = basal inferior; *BIL* = basal inferolateral; *BIS* = basal inferoseptal.

Age-Related Changes in Strain and Left Ventricular Twist

There was a significant decrease in longitudinal ($p<0.0001$), circumferential ($p<0.0001$) and RS ($p<0.0001$) with advancing age (Table 3.1.2). Net twist increased with advancing age ($p<0.0001$) and was accompanied by an increment in both AR ($p<0.0001$) and BR ($p<0.0001$) (Table 3.1.3, Figure 3.1.2). Differences in AR were progressively more pronounced after age 30 (4.41 ± 0.88° [30-39]; 5.73 ± 1.21° [40-49]; 8.22 ± 1.56° [50-65]; $p<0.0001$) (Table 3.1.3, Figure 3.1.2).There were no significant differences in BR among age groups ≥40 ($p>0.05$) (Figure 3.1.2). Net twist changed substantially in those ≥40 years old (9.25 ± 0.90° [40-49]; 12.00 ± 1.38° [50-65]) (Figure 3.1.2). Subendocardial and subepicardial rotational values at the apex and base increased with age ($p<0.0001$) (Table 3.1.3). There was a significant increase in time to peak rotation during aging ($p=0.02$) (Table 3.1.3).

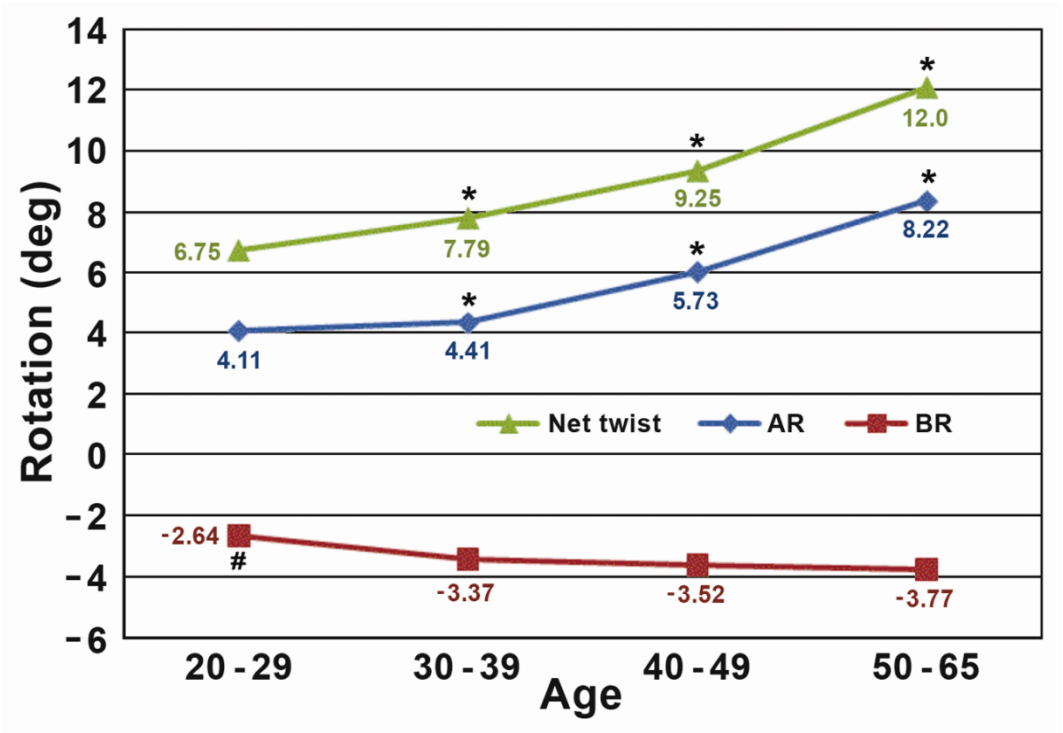


Figure 3.1.2 Age-related values in peak rotation and twist parameters. *p<0.0001 when compared to the age 20-29 group. #p=0.0005 when compared to the age 30-39 group. *AR* = apical rotation; *BR* = basal rotation.

Factors Determining Left Ventricular Twist

Univariate linear regression analysis showed that age, EF, left ventricular mass index (LVMI), E/A ratio, and longitudinal, circumferential and RS were independently associated with net twist ($p<0.05$ to $p<0.0001$) (Table 3.1.4). Table 3.1.5 summarizes the results of a multivariate linear regression analysis identifying predictors of net twist in different models. Insertion of EF, systolic BP, LVMI, end-systolic volume index, body surface area, pulse pressure, E/A ratio, peak E` lateral wall velocity, untwist rate, and longitudinal, radial and CS as potential covariates in six different models of multivariate analyses did not change the final models generated, which demonstrated age and male sex as the main independent predictors of net twist (Table 3.1.5).

TABLE 3.1.4 UNIVARIATE LINEAR REGRESSION ANALYSIS FOR NET TWIST

Variable	β coefficients ± standard error	R^2	*P*
Age	0.164 ± ± 0.007	0.80	<0.0001
Sex (F:M ratio)	0.476 ± ± 0.392	0.01	0.23
Body surface area (m^2)	-2.654 ± ± 1.340	0.03	0.05
Systolic blood pressure (mmHg)	0.066 ± 0.039	0.02	0.09
Pulse pressure (mmHg)	-0.019 ± 0.025	0.005	0.44
Heart rate (bpm)	0.003 ± 0.020	0.0002	0.87
LVMI (g/m^2)	0.037 ± 0.010	0.09	0.0006
Ejection fraction (%)	0.063 ± 0.028	0.04	0.02
ESVI (ratio)	-0.067 ± 0.042	0.02	0.11
E/A (ratio)	-4.765 ± 0.776	0.23	<0.0001
Peak E` lateral wall velocity (cm/s)	-0.004 ± 0.016	0.0005	0.80
Untwist rate (deg/sec)	0.001 ± 0.036	0.0001	0.97
Longitudinal strain (%)	0.577 ± 0.031	0.74	<0.0001
Circumferential strain (%)	0.590 ± 0.029	0.77	<0.0001
Radial strain (%)	-0.524 ± 0.033	0.67	<0.0001

ESVI, end-systolic volume index; *LVMI*, left ventricular mass index.

TABLE 3.1.5 MULTIVARIATE LINEAR REGRESSION ANALYSIS FOR NET TWIST

Variable	β coefficients ± standard error	*P*
Model 1		
Age (years)	0.167 ± 0.008	<0.0001
Sex (F:M ratio)	0.382 ± 0.176	0.03
Systolic blood pressure (mmHg)	-0.015 ± 0.018	0.41
Pulse pressure (mmHg)	-0.004 ± 0.005	0.49
Model 2		
Age (years)	0.167 ± 0.008	<0.0001
Sex (F:M ratio)	0.382 ± 0.176	0.03
Pulse pressure (mmHg)	0.004 ± 0.011	0.73
LVMI (g/m^2)	-0.003 ± 0.005	0.57
Model 3		
Age (years)	0.159 ± 0.007	<0.0001
Sex (F:M ratio)	0.491 ± 0.177	0.006
Body surface area (m^2)	-1.230 ± 0.617	0.05
ESVI (ratio)	-0.031 ± 0.021	0.14
Ejection fraction (%)	0.011 ± 0.014	0.45
Model 4		
Age (years)	0.162 ± 0.007	<0.0001
Sex (F:M ratio)	0.412 ± 0.175	0.02
ESVI (ratio)	-0.027 ± 0.011	0.19
Ejection fraction (%)	0.009 ± 0.014	0.53
Model 5		
Age (years)	0.165 ± 0.009	<0.0001
Sex (F:M ratio)	0.360 ± 0.180	0.04
Untwist rate (deg/sec)	0.017 ± 0.016	0.30
Peak E` lateral wall velocity (cm/s)	0.068 ± 0.492	0.89
E/A (ratio)	-0.005 ± 0.007	0.50
Model 6		
Age (years)	0.133 ± 0.034	0.0002
Sex (F:M ratio)	0.376 ± 0.177	0.04
Longitudinal strain (%)	-0.002 ± 0.108	0.99
Circumferential strain (%)	0.127 ± 0.127	0.32
Radial strain (%)	0.009 ± 0.072	0.90

Multiple correlation coefficients are R^2=0.81, p<0.0001 for Models 1, 2, 4, 5, and 6; and R^2=0.82, p<0.0001 for Model 3.

Reproducibility of results

Intraobserver and interobserver variability for LV rotation parameters (AR, BR net twist, LS, CS, and radial strain) in our center varies from 2 ± 3% to 10 ± 9%, and from 4 ± 4% to 12 ± 8%, respectively.

3.1.5 DISCUSSION

This study describes normative values for LV twist and its determinants in a black adult population. The pattern of rotation during systole in our study was that of the apex rotating counterclockwise while the base rotated in a clockwise direction. Regional subepicardial and subendocardial rotation at the apex and base were both counterclockwise and clockwise, respectively, in keeping with the overall global pattern of rotation observed at these anatomical sites. There was a trend of increasing AR, BR and net twist with aging, whereas longitudinal, radial and CS decreased with aging. Age and male gender were the main independent predictors of LV twist following multivariate regression analysis.

Twist mechanics of the LV occur as a result of torsional deformation about the longitudinal axis and are initiated by the interaction between the systolic clockwise rotation of the base and the counterclockwise rotation of the apex (Sengupta et al., 2008b). Twist is the net difference between AR and BR when the LV is viewed from the apex (Sengupta et al., 2008b, van Dalen et al., 2008b). Speckle tracking is a novel method for obtaining these parameters and can be performed in the clinical setting. In subjects of African descent, no data exist, to our knowledge, on the analysis of these parameters in normal individuals. Our findings reveal that the values of BR, AR and net twist using the QLAB software do not differ from findings in other populations using this vendor's technology (van Dalen et al., 2008b). Additionally, the values obtained for AR and BR in this study are less than those reported in normal populations using different vendor technologies (van Dalen et al., 2008b, Zhang et al., 2007, Zhang et al., 2010, Takeuchi et al., 2006).

To our knowledge, this study is the first to evaluate the rotation of the subepicardial and subendocardial regions at the base and apex using speckle tracking in a normal black population. Our findings reveal that, at the base and apex, the subendocardial and subepicardial rotations occur in the same direction as each other, peak simultaneously but differ in magnitude. These findings are in keeping with those of other studies (Bertini et al., 2010, Carasso et al., 2012, Nottin et al., 2008)

in which speckle tracking was performed to evaluate the subendocardial and subepicardial regions. In these studies, the direction of rotation of the subendocardium and subepicardium at basal and apical levels are the same as the direction of the global rotation at apical and basal levels (Nottin et al., 2008, Bertini et al., 2010, Carasso et al., 2012). These patterns of rotation are noted irrespective of the speckle-tracking vendor technology used (Carasso et al., 2012, Bertini et al., 2010, Nottin et al., 2008).

It is postulated that subendocardial fibres in the apex and base move in the same direction, whereas the subepicardial fibres in these regions move in an opposite direction and that the interaction between these fibres in the helical architecture contributes to LV twist (van Dalen et al., 2008b). Net rotation is thought to be predominantly counterclockwise at the apex due to the longer arm of rotation of the subepicardial fibres. This postulate cannot be assessed in this study since the technique of speckle tracking only tracks the movement of speckles in these anatomical regions. This is not synonymous with an assessment of myocardial fibre movement in these regions. We postulate that perhaps current speckle-tracking technology is unable to sufficiently assess fibre movement due to its limitations with regard to both spatial and temporal resolution. A further limitation is that the technology utilized in this study is only a two-dimensional assessment of speckle movement in these regions, which may be more accurately assessed by three-dimensional speckle tracking. Thus, the assessment of myocardial fibre arrangement in these regions and the actual rotatory forces exerted by these fibres in humans cannot be detected by current speckle-tracking technology.

Aging affects many aspects of cardiovascular function (Lakatta, 2003c, Lakatta, 2003a, Lakatta, 2003b, Lakatta and Sollott, 2002, Oxenham et al., 2003). Advancing age is associated with structural and functional changes in the myocardium, e.g. myocardial fibrosis and impaired coronary perfusion (Oxenham et al., 2003, Lakatta, 2003c, Lakatta, 2003a, Lakatta, 2003b). Increased LV wall thickness, alterations in the diastolic filling pattern, impaired LV ejection and heart rate reserve capacity are the most dramatic changes in cardiac function that occur with aging

in healthy persons (Lakatta, 2003c, Lakatta, 2003a, Lakatta, 2003b). Although these age-associated changes do not always reflect clinical heart disease per se, they may compromise cardiac reserve and alter the threshold for disease manifestation.

To our knowledge this is the first study to evaluate all aspects of twist and strain with aging using STE in a black population. Our findings reveal that net twist increased with age due to an increase in both BR and AR. However, there appears to be a temporal dispersion in the increment of apical and BR. We observed that AR increased substantially after age 40 whereas no significant increment occurred in BR after age 40. The result is that the major increment in net twist observed occurred after age 40. In accordance with our study, BR also was less influenced by age in other published data (van Dalen et al., 2008b, Zhang et al., 2007, Takeuchi et al., 2006, Zhang et al., 2010). Van Dalen et al. reported a mean value of peak LV twist as 7.7 ± 3.58° in a white population (van Dalen et al., 2008b), with peak LV twist significantly greater in subjects aged ≥60 years (10.8 ± 4.98°) compared with those aged ≤40 years (6.7 ± 2.98°) and those aged 41 to 59 years (8.0 ± 3.08°) (van Dalen et al., 2008b). Takeuchi et al. examined the effect of aging on twist in 113 Asian volunteers; peak LV twist increased significantly in those aged ≥60 years with similar values as published on whites (Takeuchi et al., 2006). Furthermore, (Zhang et al., 2010) showed a greater increment in AR from subjects aged 45 to 55 years (7.94 ± 1.20°) to those aged 55 to 65 years (9.65 ± 1.56°), and a lesser increase in BR [-6.50 ± 2.49° (45-55 years) vs. -7.07 ± 1.25° (55-65 years)].

The characteristics of LV twist undergo substantial changes during the aging process. The increase in LV twist can be partially explained by lessened opposition to AR, which results from a gradual decrease in subendocardial function with aging (Zhang et al., 2010, van Dalen et al., 2008b, Takeuchi et al., 2006, Zhang et al., 2007). Impairment of subendocardial contractile function may be on the basis of ischemia or fibrosis and results in diminished clockwise systolic BR, which leads to less opposition of counterclockwise systolic AR, causing net twist to increase (Zhang et al., 2007, Zhang et al., 2010, van Dalen et al., 2008b, Takeuchi et al., 2006).

An analysis of other aspects of myocardial mechanics revealed a concurrent reduction in longitudinal, circumferential and RS with increasing age. Whereas some researchers described reduced strain with increasing age (tissue Doppler imaging (Dalen et al., 2010, Kuznetsova et al., 2008) and MRI (Fonseca et al., 2003)) or increasing strain with aging (STE) (Reckefuss N, 2011), others found no correlation between strain and age (tissue Doppler imaging) (Sun et al., 2004) in other populations. Moreover, some studies using speckle tracking found some strain parameters (circumferential and radial strain) increased (Sun et al., 2012a, Reckefuss N, 2011) while LS decreased (Sun et al., 2012a). Another possible rationale for the increase in twist with aging could be as a compensatory mechanism for decline in other strain parameters (Carasso et al., 2012). This may explain our observation that the time to peak twist increases with increasing age, which may occur as a result of greater compensatory rotation to counteract the concomitant declining function in other strain parameters.

LV twist is an effective method to preserve LV systolic function, as the helical fibre architecture of the heart doubles the LV EF (Bertini et al., 2010, Nottin et al., 2008). We postulate that increased net twist with advancing age may demonstrate a possible contribution of net twist to preserving EF and maintaining normal systolic function of the left ventricle as found in other studies (Nottin et al., 2008, Bertini et al., 2010). AR is the major contributing factor of LV twist, with an increase in AR resulting in an increase in twist. This implies that apical function is perhaps the most important determinant for LV twist, and one would expect that pathological states that impair apical function to a greater degree than other anatomic sites may result in greater LV dysfunction. However, age was the main independent predictor of net LV twist in our study. Furthermore, despite an increase in relative wall thickness, LVMI, systolic BP and changes in diastolic indices with advancing age in our study, conventional echocardiographic and clinical variables were not able to predict net LV twist. Cameli et al. showed an independent correlation of indexed end-systolic LV volume, LV relaxation, heart rate, gender and age with LV twist mechanics in normal individuals (Cameli et al., 2011). However, echocardiographic and clinical variables were not independent predictors of LV twist mechanics (Cameli et al., 2011). Moreover, strain parameters showed an independent

correlation (longitudinal, circumferential and radial strain) with net LV twist in our study but were unable to predict net LV twist.

Limitations

The technique of speckle tracking used in this study only provides a two-dimensional analysis, and the consistency of these findings using other two-dimensional vendor technology is uncertain. MRI tagging or sonomicrometry were not employed as the gold standard to compare speckle-tracking findings. Normal deformation values vary among publications and depend on the vendor used, suggesting that reference values have to be obtained for each method and software (Carasso et al., 2012, Cameli et al., 2011, Sun et al., 2004, Marwick et al., 2009, Manovel et al., 2010, Mor-Avi et al., 2011). The interpretation of functional data across a spectrum of ages requires consideration of the ventricular loading conditions and vascular impedance. Further research is required to assess the fibre structure and loading conditions that change with aging between different populations, as the response of the left ventricle in black patients in pathological states does differ (Marwick, 2010, Leitman et al., 2004, Kizer et al., 2004, Nunez et al., 2005). Further research is required to determine the effect of aging on strain and rotation using three-dimensional speckle tracking, preferably in an international multicenter cohort.

3.1.6 CONCLUSION

The data reported in this study establish the normal values and patterns for left ventricular strain and rotational mechanics in a normal black-African adult population and can be used as a baseline for future studies. Aging is associated with increased net LV twist whilst decreasing strain parameters are associated with advancing age. Age is the major determinant of increased LV twist in this population.

3.2 Time to Twist: Marker of Systolic Dysfunction in Africans With Hypertension

Nirvarthi Maharaj[1], MD, Bijoy K. Khandheria[2]*, MD, Ferande Peters[1], MD, Elena Libhaber[1], PHD, Mohammed R. Essop[1], MD,*Johannesburg, South Africa; Milwaukee, Wisconsin*

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3.2.1 ABSTRACT

Aims: Speckle tracking echocardiography (STE) has emerged as a quantitative technique to assess left ventricular (LV) function via twist mechanics. Hypertension-associated changes in LV twist have not been previously evaluated in African patients with LV systolic dysfunction. This study evaluates LV twist dynamics in HTP with low EF.

Methods and Results: Forty-one HTP with EF <45% and normal coronary angiography (HTLEF Group) were compared with 41 control subjects using STE. Peak apical rotation (AR) and isochronous BR (BR) were measured, as was net twist (maximal value of simultaneous AR – BR). Rigid body rotation (RBR) was defined as AR and BR occurring in the same direction. Of 41 HTLEF patients, 28 (68%) had normal twist pattern while 13 (32%) exhibited RBR; 11 of those 13 exhibited clockwise rotation. AR (1.89°±0.99 vs. 7.15°±2.26; p<0.0001), BR (1.44°±0.78 vs. –3.75°±1.61; p<0.0001) and net twist (3.3°±1.17 vs. 10.9°±2.7; p<0.0001) were lower in HTLEF patients. The subgroup with clockwise RBR showed greater LV dysfunction (EF: 27.9±5.8% vs. 35±7.5%; p=0.005) and more spherical LV geometry in both diastole and systole (p=0.0009 and p=0.0002, respectively) compared with those who had normal pattern of twist. In the HTLEF subgroup with normal twist pattern, AR (r=0.57; p=0.002) and net twist (r=0.57; p=0.002) correlated with EF.

Conclusions: Left ventricular twist in patients with hypertension and EF less than 45% is diminished. AR and net twist are good correlates for EF in HTP with EF <45%.

3.2.2 INTRODUCTION

Hypertension is a well-documented precedent to left ventricular (LV) dysfunction and congestive HF. In the Framingham Heart Study, lifetime risk for congestive HF was 20% for both men and women (Lloyd-Jones et al., 2002). Even after exclusion of patients with antecedent myocardial infarction, risk for HF remains significant (Drazner, 2011). Although the contribution of hypertension to HF is known, the mechanism is multifactorial and unclear (Drazner, 2011). It is generally postulated that the development of systolic dysfunction in HTP is either due to a myocardial infarction or as a consequence of concomitant ischemic heart disease (Drazner, 2011). Hypertension may cause systolic dysfunction as a consequence of adverse remodelling, but this has not been clearly documented (Drazner, 2011). LV twist may be a contributing factor to this dysfunction independent of ischemic heart disease, thus, a focus on abnormalities in the cardiac mechanics of twist in the left ventricle may be helpful in understanding the pathogenesis behind the transition from compensated to decompensated HF.

LV twist occurs when the LV apex moves in a counterclockwise direction during systole while the base moves in a clockwise direction. LV twist can now be accurately assessed using STE, which has been validated against MRI (Helle-Valle et al., 2005, Notomi et al., 2005). Evaluation of LV twist has proven a sensitive technique to assess cardiac performance (Taber et al., 1996, Greenbaum et al., 1981, Bell et al., 2000). We hypothesized that LV rotational parameters and net LV twist are diminished in hypertensive black patients with low EF in the absence of ischemic heart disease. In a population with no concomitant coronary disease, we compared LV twist and its components in HTP who presented with HF and low EF to healthy, normal subjects.

3.2.3 METHODS

This single centre, cross-sectional study was approved by the University of Witwatersrand Ethics Committee (clearance number: M110204) (see appendix a) and the Institutional Review Board.

Subjects were consecutively recruited from the Chris Hani Baragwanath Hospital Cardiac Clinic from January 2011 to October 2011. Inclusion criteria were: documented prior diagnosis of hypertension (measurements on three separate occasions where the systolic BP was ≥140 mmHg, or the diastolic BP was ≥90 mmHg taken over a period of two months (Seedat and Rayner, 2012)) at the Hypertension Clinic, documented HF (past or on presentation as evidenced by symptoms [New York Heart Association functional class], clinical examination and/or admissions for HF), sinus rhythm, EF <45% on echocardiography, and normal coronary angiography.

Exclusion criteria were: previous myocardial infarction, anaemia (haemoglobin <12 g/dL in females, <13 g/dL in males), excess alcohol intake (maximum alcohol intake no more than 40 grams per day in males and 20 grams per day in females with two alcohol-free days per week), renal dysfunction (glomerular filtration rate <60 mL/min/1.73 m^2), documented diagnosis of diabetes and/or glycated haemoglobin >7%, organic valvular disease, dilated cardiomyopathy of any aetiology, cardiac infiltrative diseases, post-viral myocarditis, any systemic illness (e.g. human immunodeficiency virus [HIV]), thyroid disease, and any primary organ dysfunction or failure (e.g. chronic renal disease). Subjects who fulfilled the criteria and provided voluntary informed consent were enrolled and underwent a detailed clinical and echocardiographic evaluation at baseline. A total of 50 patients were screened for inclusion in the study. Nine patients were excluded as a result of inadequate image quality that did not allow for complete segmental assessment of LV rotation at both the basal and apical left ventricle. The remaining 41 HTP with low ejection fraction (HTLEF) made up the study group.

The control group was recruited from the staff at Chris Hani Baragwanath Hospital, patient escorts and community members from Soweto. All members of the control group were unrelated to the patients in the HTLEF group. Individuals younger than 50 years were matched with a tolerance of 5 years in terms of age, while individuals older than 50 years were allowed a tolerance of up to 10 years. All members of the control group were asymptomatic, normotensive, had no evidence of cardiovascular or systemic diseases and had a normal 12-lead electrocardiogram prior to

undergoing echocardiography for the study. The control group was age- and sex-matched with the HTLEF cohort. Echocardiographic evaluation of the control group is part of an ongoing prospective study to provide normal reference ranges for echocardiographic measurements in subjects of African ancestry.

BP measurements

Please refer to 3.1.3.

Echocardiography

Please refer to 3.1.3. Concentric hypertrophy was defined as RWT >0.42 and left ventricular mass index (LVMI) (g/m^2) >95 in females and >115 in males, while eccentric hypertrophy was defined as RWT <0.42 and LVMI >95 in females and >115 in males (Lang et al., 2005, Ganau et al., 1992). LV sphericity index was calculated by dividing the LV maximal long-axis internal dimension by the maximal short-axis internal dimension at end diastole and end systole (van Dalen et al., 2010) using apical four-chamber view.

Speckle-tracking analysis

Two-dimensional images were obtained at a rate of 50 to 80 frames per second. Parasternal short-axis images at the LV basal level showing the tips of the mitral valve leaflets were obtained with the cross-section as circular as possible. To obtain a short-axis image at the true LV apical level, the transducer was positioned one or two intercostal spaces more caudal and acquired in a manner described by van Dalen et al (van Dalen et al., 2008c). Analysis of datasets was performed using QLAB advanced quantification Version 8.0 software (Philips Healthcare, Best, Netherlands) by a physician experienced in speckle tracking. The assessment of LV twist using QLAB software had previously been validated against MRI tagging (Goffinet et al., 2009).

To assess LV rotation, six tracking points were placed on the myocardium (Van Dalen et al., 2009), avoiding the pericardium on an end-diastolic frame in each parasternal short-axis image as determined automatically by the nature of the software algorithm. At the base, tracking points were separated about 60 degrees from one another to fit the total LV circumference as described by Van Dalen et al (Van Dalen et al., 2009). Repositioning of tracking points was allowed provided the position was moved no more than 30 degrees. At the apex, tracking points were placed per the software algorithm from the endocardium to epicardium. After positioning the tracking points, the program tracked these points. The operator ensured that cardiac cycles with heart rate variability <10% were selected for the measurements of LV rotation parameters. Manual correction for region of interest repositioning was required in 6 (14.6%) control subjects and 10 (20%) HTP screened for inclusion in the study. Time needed for analysis was, on average, 2-3 minutes per patient.

Counterclockwise rotation, as viewed from the apex, was expressed as a positive value; clockwise rotation was expressed as a negative value (Van Dalen et al., 2009). End systole was defined as the point of aortic valve closure. Analysis was performed to evaluate the peak apical rotation (AR) during the ejection phase, the basal rotation (BR) at a time isochronous with that of peak AR during the ejection phase and the net instantaneous twist of the left ventricle (net twist), which was calculated as peak AR minus the BR at a time isochronous with peak AR.

In addition to quantifying AR and BR, we analyzed the direction of rotation in systole. We focused on the pattern of rotation during the ejection phase of systole and identified rotation as either normal or having evidence of RBR, which was either counterclockwise or clockwise at both the basal and apical levels. Normal patterns evaluated were characterized by clockwise systolic BR and counterclockwise systolic AR (van Dalen et al., 2011). The untwisting rate was defined as the mean diastolic untwisting velocity from instantaneous peak systolic twist to mitral valve opening and calculated thusly: (twist at mitral valve opening − peak systolic twist)/(time interval from peak systolic twist to mitral valve opening).

Statistical analysis

Database management and statistical analyses were performed with SAS Version 9.2 software (SAS Institute Inc., Cary, NC). Data were presented as mean ± standard deviation. Continuous variables were compared using independent t-test or Wilcoxon-Mann-Whitney test when the distribution was non-normal. Spearman correlation coefficients for the relationships between LV rotation parameters against conventional echocardiographic parameters were determined. The interobserver variability was assessed for twist measurements in 20 randomly selected patients, calculated as the standard deviation of the differences between the measurements of two independent observers who were unaware of the other patient data, and expressed as a percentage of the average value. The intraobserver variability was calculated as the standard deviation of the differences between a first and second determination of a single observer and expressed as a percentage of the average value. Intraobserver and interobserver variability for LV rotation parameters in our centre varies from 2% ± 3% to 10% ± 9%, and from 4% ± 4% to 12% ± 8%, respectively. P-values less than 0.05 were considered statistically significant.

3.2.4 RESULTS

Patient selection

A total of 50 patients with HTLEF were screened for inclusion in the study. Inadequate visualization of the endocardial border precluded correct wall-motion tracking in 9 patients, who were then excluded from the analysis. The remaining 41 patients showed adequate tracking for apical and basal short-axis views and were consecutively included (Table 3.2.1). The control group was comprised of 41 individuals. All patients from the HTLEF group were on optimal doses of beta blocker (carvedilol) and the angiotensin-converting enzyme inhibitors, spironolactone and furosemide.

Baseline and echocardiographic characteristics

Baseline characteristics of the study population are represented in Table 3.2.1. Patients with HTLEF differed significantly from the patients in the control group with respect to age, body mass index and baseline BP. Adjustments were made in the data analysis to accommodate for these differences. As expected, patients with HTLEF differed significantly from the control group with regard to all echocardiographic measurements (Table 3.2.1). Two major geometric patterns were noted in subjects with HTLEF: concentric hypertrophy in 22 patients (54%) and eccentric hypertrophy in 19 patients (46%).

TABLE 3.2.1 BASELINE CLINICAL AND ECHOCARDIOGRAPHIC CHARACTERISTICS

	Control group	**HTLEF group**
No. patients	41	41
Age, years	50.1 ± 9.3	55.0 ± 9.1[#]
Male gender, no. (%)	19 (46.3)	19 (46.3)
Body mass index, kg/m^2	24.7 ± 1.8	29.5 ± 5.0*
Body surface area, m^2	1.72 ± 0.14	1.86 ± 0.17*
NYHA functional capacity, no. (%)		
Class I	41 (100)	12 (29)*
Class II		14 (34)*
Class III		15 (37)*
Duration of hypertension, years	---	12.2 ± 6.5
Duration of heart failure, years	---	3.2 ± 1.6
Medication (ACE/ARB, diuretics, beta blocker, spironolactone)	---	41
Systolic blood pressure, mmHg	120 ± 7	156 ± 8*
Diastolic blood pressure, mmHg	71 ± 6	89 ± 10*
Heart rate, bpm	73 ± 10	81 ± 10*
EDD, mm	43 ± 5	58 ± 5[†]
IVSD, mm	10 ± 1	12 ± 2[†]
IVPWD, mm	8 ± 1	12 ± 2[†]
End-diastolic volume, ml	80.6 ± 13.8	133.2 ± 41.2[†]
End-diastolic volume index, ml/m^2	46.8 ± 7.7	72.3 ± 23.6[†]
End-systolic volume, ml	32.6 ± 9.8	91.1 ± 38.7[†]
End-systolic volume index, ml/m^2	18.9 ± 5.6	49.4 ± 21.4[†]
Ejection fraction, %	69.3 ± 9.5	33.0 ± 7.6[†]
Relative wall thickness ratio	0.38 ± 0.03	0.41 ± 0.06[#]
Left ventricular mass index, g/m^2	73.8 ± 13.9	161.4 ± 32.8[†]
Left atrial volume index, ml/m^2	23.0 ± 2.2	31.0 ± 16.3[#]
Functional mitral regurgitation	---	1.83 ± 0.8
End-diastolic LVSPHI ratio	1.88 ± 0.30	1.36 ± 0.12[†]
End-systolic LVSPHI ratio	2.13 ± 0.54	1.39 ± 0.10[†]
Peak E velocity (cm/s)	80.2+7.8	65.1+8.2[†]
Peak A velocity (cm/s)	49.5 ± 8.4	85.3 ± 7.6[†]
Deceleration time (ms)	170 ± 31	245 ± 44[†]
E/A ratio	1.70 ± 0.34	0.77 ± 0.11[†]

[#]p<0.05.
*p<0.0005.
[†]p<0.0001.
A = late (atrial) ventricular filling velocity; ACE/ARB = angiotensin-converting enzyme inhibitors/angiotensin receptor blockers; E = early ventricular fillingvelocity; EDD = end-diastolic diameter; HTLEF = patient study group with hypertension and low ejection fraction (<45%); IVPWD = interventricular posterior wall at end-diastolic dimension;
IVSD = interventricular septal thickness at end-diastolic dimension; LVSPHI = left ventricular sphericity index; NYHA = New York Heart Association.

Left ventricular rotation analysis

All subjects in the control group had normal pattern of rotation (Fig. 3.2.1). Three distinct rotational patterns were identified in the HTLEF group. Of the 41 patients with HTLEF, 28 patients (68%) had normal pattern of twist (Fig. 3.2.2, Table 3.2.2) while 13 (32%) exhibited RBR. Eleven of the 13 patients had evidence of clockwise RBR (Fig. 3.2.3 and Table 3.2.2) and two had counterclockwise RBR. AR (1.89° ± 0.99 vs. 7.15° ± 2.26; $p<0.0001$), BR (1.44° ± 0.78 vs. -3.75° ± 1.61; $p<0.0001$) and net twist (3.3° ± 1.2 vs. 10.9° ± 2.7; $p<0.0001$) were lower in HTLEF patients compared to controls (Table 3.2.3). The untwisting rate was reduced in HTLEF patients compared to controls ($p= 0.03$) (Table 3.2.3).

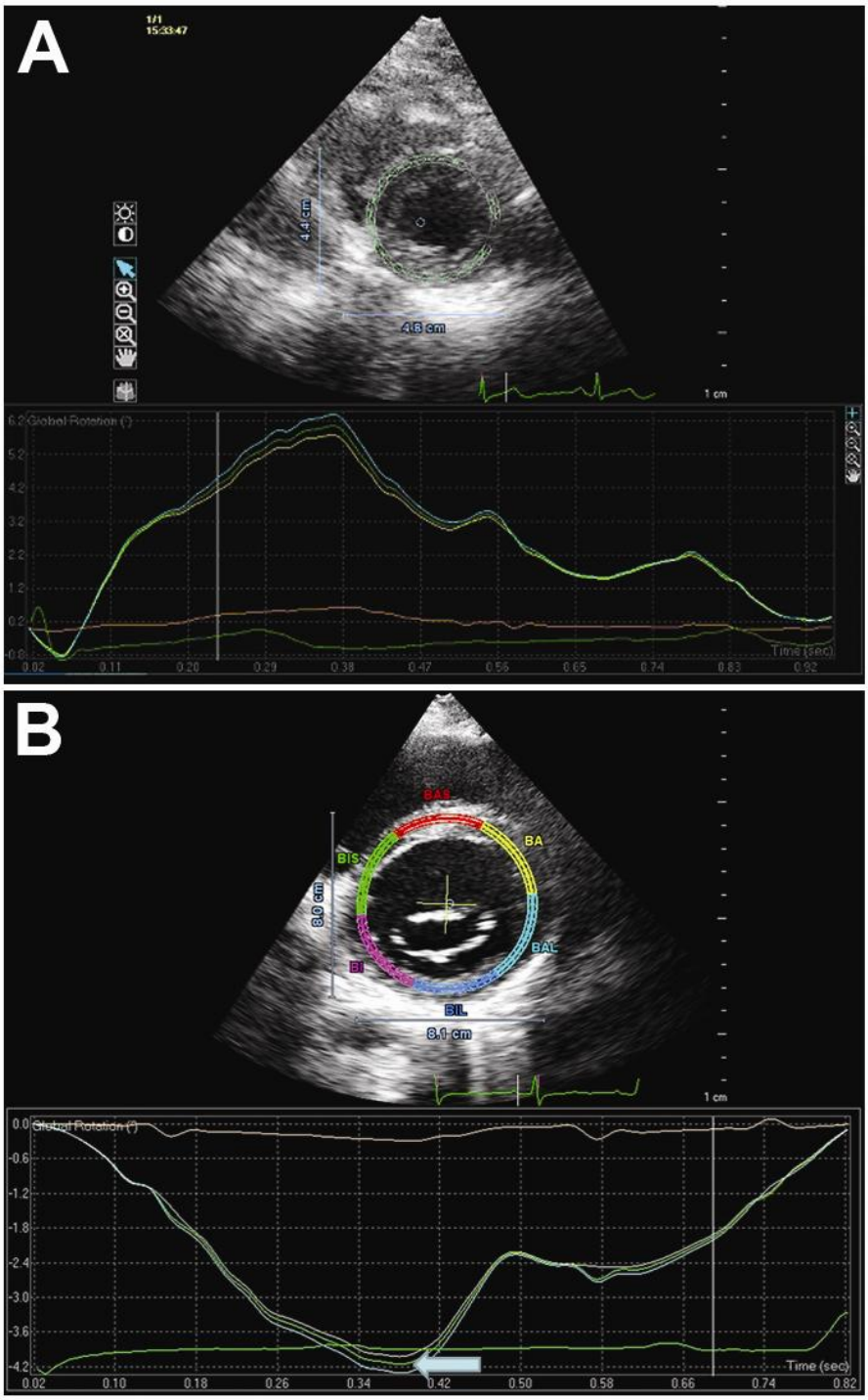


Figure 3.2.1 (A**) Normal apical rotation in control patients. Tracking points at the left ventricular apical level. Left ventricular rotation–time curve (x-axis: time in ms; y-axis: left ventricular apical rotation in degrees). Electrocardiogram is displayed at the bottom. (**B**) Normal basal rotation in control patients. Tracking points at the left ventricular basal level. Left ventricular rotation–time curve (x-axis: time in ms; y-axis: left ventricular basal rotation in degrees). Electrocardiogram is displayed at the bottom (arrow indicates peak global basal rotation at time of aortic valve closure).**

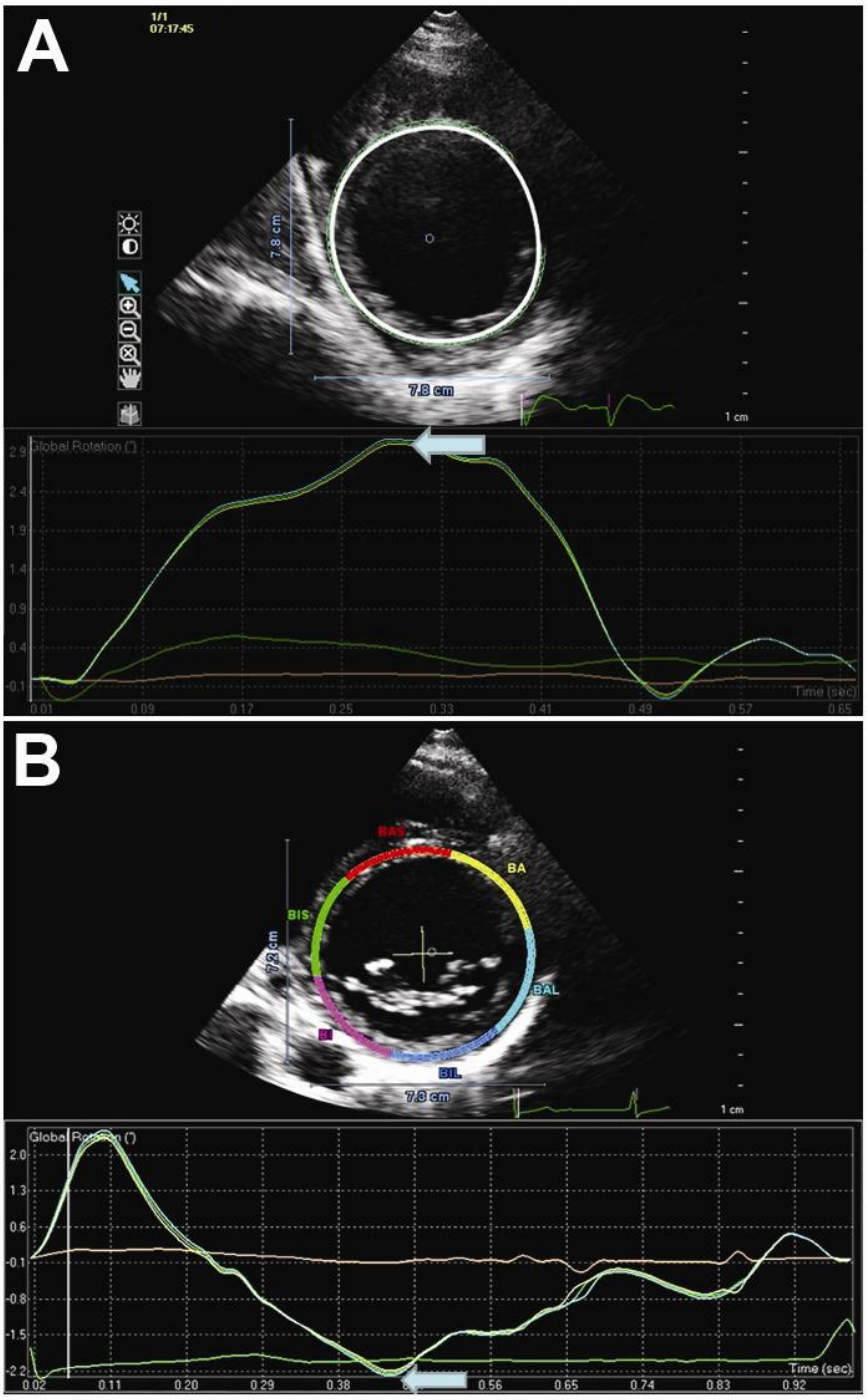


Figure 3.2.2 (A**) Apical rotation with normal pattern in hypertensive patients with ejection fraction <45%. Tracking points at the left ventricular apical level. Left ventricular rotation–time curve (x-axis: time in ms; y-axis: left ventricular apical rotation in degrees). Electrocardiogram is displayed at the bottom (arrow indicates peak global apical rotation at time of aortic valve closure). (**B**) Basal rotation with normal pattern in hypertensive patients with ejection fraction <45%. Tracking points at the left ventricular basal level. Left ventricular rotation–time curve (x-axis: time in ms; y-axis: left ventricular basal rotation in degrees). Electrocardiogram is displayed at the bottom (arrow indicates peak global basal rotation at time of aortic valve closure).**

TABLE 3.2.2 COMPARISON BETWEEN HTLEF PATIENTS WITH DIFFERENT PATTERNS OF TWIST

	Normal pattern (n=28)	**Clockwise RBR (n=11)**	***P*-value**
Age, years	55.0 ± 9.7	56.0 ± 8.2	0.61
Male gender, no. (%)	16 (57.1)	2 (18.1)	0.07
Body mass index, kg/m^2	28.8 ± 5.2	31.4 ± 4.6	0.17
Body surface area, m^2	1.86 ± 0.17	1.88 ± 0.18	0.99
Systolic blood pressure, mmHg	156 ± 8	154 ± 9	0.49
Diastolic blood pressure, mmHg	89 ± 10	93 ± 11	0.35
Heart rate, bpm	78 ± 10	87 ± 8	0.02
NYHA functional capacity, no. (%)			
Class I	12 (43)	0 (0)	
Class II	8 (28.5)	4 (36)	0.017
Class III	8 (28.5)	7 (64)	
Duration of hypertension, years	11.3 ± 6.9	13.5 ± 4.2	0.06
Duration of heart failure, years	3.1 ± 1.6	3.2 ± 1.1	0.60
End-diastolic diameter, mm	58 ± 6	59 ± 3	0.15
IVSD, mm	12 ± 2	13 ± 2	0.01
IVPWD, mm	12 ± 2	12 ± 1	0.86
End-diastolic volume, ml	122.8 ± 36.1	159.1 ± 45.7	0.02
End-diastolic volume index, ml/m^2	66.8 ± 22.1	85.2 ± 23.3	0.03
End-systolic volume, ml	79.2 ± 29.5	119.6 ± 45.9	0.008
End-systolic volume index, ml/m^2	43.0 ± 17.2	64.2 ± 23.8	0.009
Ejection fraction, %	35.0 ± 7.5	27.9 ± 5.8	0.005
Relative wall thickness ratio	0.41 ± 0.07	0.40 ± 0.04	0.31
Left ventricular mass index, g/m^2	152.4 ± 32.6	176.1 ± 30.1	0.08
Left atrial volume index, ml/m^2	33.2 ± 15.0	43.9 ± 12.2	0.04
End-diastolic LVSPHI ratio	1.40 ± 0.09	1.26 ± 0.11	0.0009
End-systolic LVSPHI ratio	1.43 ± 0.08	1.29 ±0.07	0.0002

HTLEF = patient study group with hypertension and low ejection fraction (<45%); IVPWD = interventricular posterior wall at end-diastolic dimension; IVSD = interventricular septal thickness at end-diastolic dimension; LVSPHI = left ventricular sphericity index; NYHA = New York Heart Association; RBR = rigid body rotation.

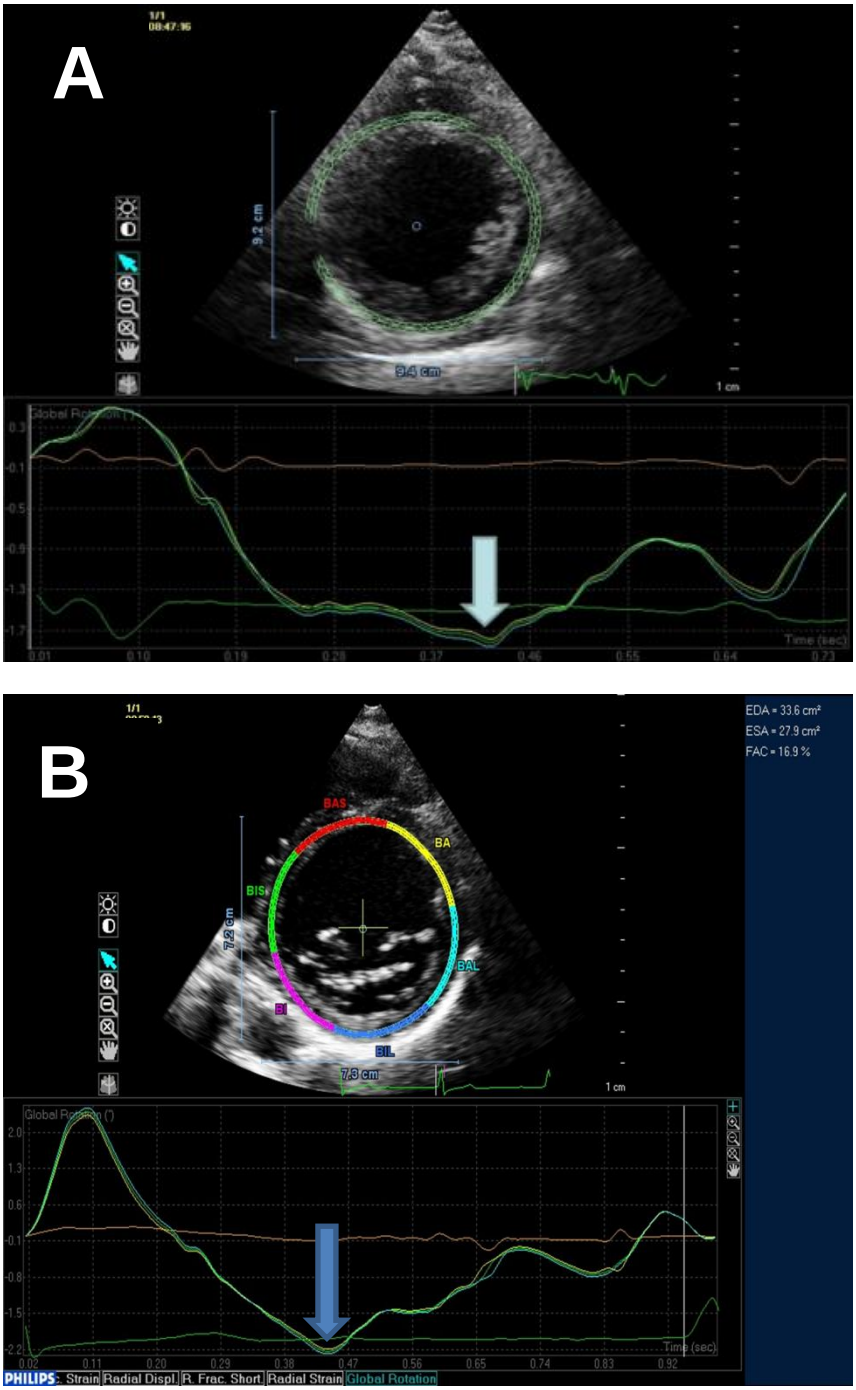


Figure 3.2.3 (A**) Apical clockwise rotation in hypertensive patients with ejection fraction <45%. Tracking points at the left ventricular apical level. Left ventricular rotation–time curve (x-axis: time in ms; y-axis: left ventricular apical rotation in degrees). Electrocardiogram is displayed at the bottom (arrow indicates peak global apical rotation at time of aortic valve closure). (**B**) Basal rotation with normal pattern in hypertensive patients with ejection fraction <45%. Tracking points at the left ventricular basal level. Left ventricular rotation–time curve (x-axis: time in ms; y-axis: left ventricular basal rotation in degrees). Electrocardiogram is displayed at the bottom (arrow indicates peak global basal rotation at time of aortic valve closure).**

TABLE 3.2.3 COMPARISON OF LEFT VENTRICULAR TWIST BETWEEN CONTROL AND HTLEF PATIENTS WITH NORMAL PATTERN OF TWIST

	Control group (n=41)	**HTLEF group (n=28)**	***P*-value**
Apical rotation, degrees	7.15 ± 2.26	1.89 ± 0.99	<0.0001
Basal rotation, degrees	-3.75 ± 1.61	-1.44 ± 0.78	<0.0001
Time to peak apical rotation, ms	348 ± 120	332 ± 70	0.52
Net twist, degrees	10.90 ± 2.70	3.30 ± 1.17	<0.0001
Untwisting rate, degrees/sec	-45 ± 17	-30 ± 15	0.03

HTLEF = patient study group with hypertension and low ejection fraction (<45%).

Comparison of HTLEF patients with different patterns of twist

Patients with HTLEF exhibited three different patterns of twist (normal, clockwise RBR and counterclockwise RBR). Since only two patients were observed to have counterclockwise RBR, they were not included in the analysis. There were no significant differences between the normal pattern and the clockwise RBR subgroups in terms of age, sex, body surface area, baseline BP and duration of hypertension (Table 3.2.2). NYHA class was statistically significant (p=0.017). No patients in the HTLEF subgroup who had clockwise RBR were NYHA class I, 64% were NYHA class III and 36% were NYHA class II. In contrast, 43% of patients in the HTLEF subgroup who had normal pattern of twist were NYHA class I, and 28% were NYHA class II or III (Table 3.2.2). Patients with clockwise RBR had significantly larger LV volumes (p=0.02 and p=0.008 for end-diastolic volume and end-systolic volume, respectively), lower EF (p=0.005), and a more spherical LV geometry in both diastole and systole (p=0.0009 and p=0.0002, respectively) (Table 3.2.2).

Correlation analysis

Significant positive correlations were noted between net twist and EF (r=0.83, p<0.0001) and AR and EF (r=0.80, p<0.0001) in in both groups (control and HTLEF with normal pattern of twist). A negative correlation was seen between BR and EF in both groups (control and HTLEF with normal pattern of twist) (r=-0.59, p<0.0001). Subanalysis of the control group showed no significant correlation between LV rotation parameters and EF (AR: r=-0.02, p=0.88; BR: r=-0.19, p=0.23, net twist: r=0.08, p=0.60). However, there was a significant correlation between AR and EF (r=0.57, p=0.002) (Fig. 3.2.4, Panel A) and between net twist and EF (r=0.57, p=0.002) (Fig. 3.2.4, Panel C)

in the subset of the HTLEF group with normal pattern of rotation; BR showed no correlation to EF (r=-0.14; p=0.49) (Fig. 3.2.4, Panel B). There was no association between RWT and LV rotational parameters and net twist (net twist: r=0.12, p=0.58; AR: r=0.32, p=0.13; BR: r=0.24, p=0.25) in both groups (control and HTLEF with normal pattern of twist). There was no association between LVMI and net twist (r=-0.069, p=0.66 in controls; r=-0.015, p=0.94 in HTLEF with normal pattern of twist). Furthermore, there was no relationship between LVMI and untwist rate (r=-0.037, p=0.82 in controls; r=-0.31, p=0.11 in HTLEF with normal pattern of twist).

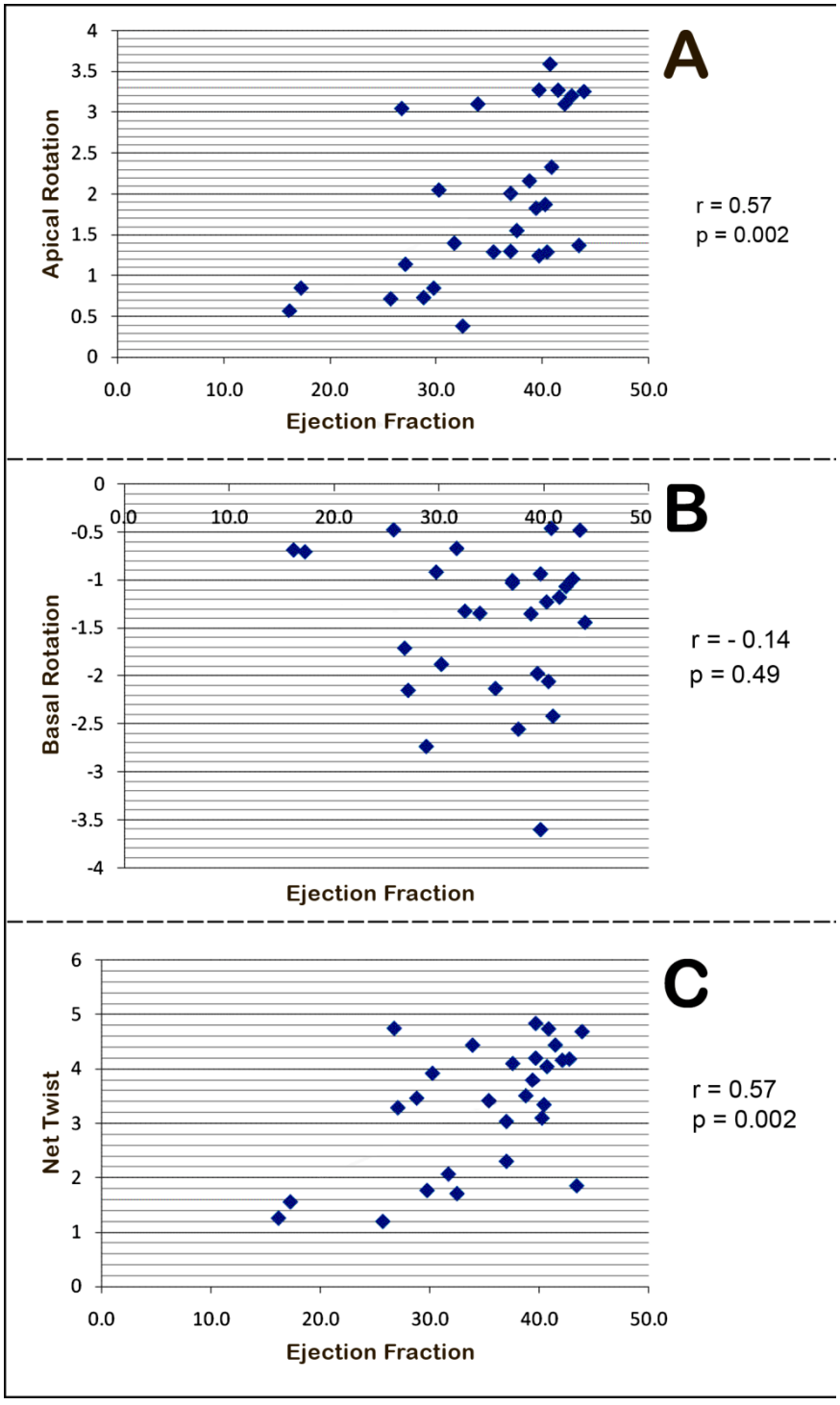


Figure 3.2.4 Correlation between rotational parameters and ejection fraction in subgroup of hypertensive patients with ejection fraction <45% and normal pattern of twist (n=28). Shown are correlations between (A) apical rotation and ejection fraction; (B) basal rotation and ejection fraction; and (C) net twist and ejection fraction.

3.2.5 DISCUSSION

In this study of patients with hypertension and low EF, we found that (a) net instantaneous twist and its determinants are diminished; (b) patients exhibiting RBR have more advanced LV dysfunction and adverse remodelling; and (c) AR and net twist correlate well with EF.

This is the first study to our knowledge that has evaluated twist mechanics in HTP with low EF. The clinical profile of this cohort with HTLEF is unique in that all subjects had no angiographic evidence of epicardial coronary artery disease (CAD) and no evidence of previous myocardial infarction on electrocardiography, echocardiography or left ventricular angiography. This challenges the long-held postulate that progression to severe systolic dysfunction in patients with hypertension is associated with ischemia secondary to CAD or previous myocardial infarction. In addition, the degree of remodeling and LV dysfunction in this cohort of patients is not influenced by other comorbidities that may cause LV dysfunction such as diabetes, atrial fibrillation and volume-overloaded conditions like renal failure and anemia. Thus, hypertension is more likely to be the sole initiator of the remodeling process. The second important finding of this study is the fact that both concentric and eccentric hypertrophy were present in patients with HTLEF.

Twist is determined by the rotation of the apex with respect to the base along its long axis and is produced by contraction of the heart's obliquely spiraling myocardial fibre layers. Due to their longer radii of rotation, subepicardial fibres are thought to be the predominant cause of the net counterclockwise rotation at the apex and the net clockwise rotation at the base (Sengupta et al., 2008a). We noted that diminished net twist occurred in all HTLEF patients, caused by both a reduction in AR and BR. However, only AR correlated positively with degree of LV dysfunction, suggesting degree of apical dysfunction is more important in LV dysfunction than the degree of basal dysfunction. The absence of ischemia or infarction in our study population is important, as previous studies have shown impaired AR and LV twist deformation in patients with myocardial infarction (Garot et al., 2002, Takeuchi et al., 2007b, Bansal et al., 2008b, Tibayan et al., 2004,

Nucifora et al., 2010). The reduction in both AR and BR may imply that both areas are affected by a more global abnormality that affects both subendocardial and subepicardial fibres. One such process may be microvascular ischemia, either from microcirculatory dysfunction or due to oxygen supply/demand mismatch. All are well documented as occurring in HTP in the absence of epicardial CAD.

In this study we have documented RBR in patients with HTLEF for the first time. The predominant pattern was clockwise, implying that the apex was moving in a completely opposite direction to normal. In the patients with clockwise RBR, left ventricles were significantly more dilated, spherical and dysfunctional. We postulate that ventricular remodeling and abnormal fibre orientation (Beyar and Sideman, 1986) may contribute to change in the normal twist pattern. In severely dilated ventricles, increased LV sphericity may lead to widening of the apex resulting in significant changes in LV AR values (Hutchins et al., 1978, Popescu et al., 2009). Consequently, widening of the apex brings about the loss of the oblique architecture of the apical loop fibres, which become more transverse and resemble the horizontal fibre orientation of the basal loop (Beyar and Sideman, 1986). Thus the apex moves in a reverse direction, similar to the base, resulting in clockwise RBR. However, this postulate does not explain the mechanism of counterclockwise rotation observed in two patients.

RBR and reversed systolic AR have been documented in patients with isolated LV noncompaction and idiopathic dilated cardiomyopathy (Hutchins et al., 1978, Popescu et al., 2009, van Dalen et al., 2008a, Liu and Li, 2010). Some of these reports suggest patients with abnormal twist patterns have more severe LV remodeling than patients with normal patterns of twist (Liu and Li, 2010, Hutchins et al., 1978, Popescu et al., 2009, van Dalen et al., 2008a). We propose that the presence of RBR, especially in a clockwise direction, represents a novel assessment of more severe LV remodeling in HTP with LV systolic dysfunction in the absence of ischemia. The loss of LV twist may result in further decline in LV function and initiate a continuous, vicious cycle of remodeling. This raises

the question whether RBR represents a more advanced state of remodeling or if it is the precipitant to the development of more advanced remodeling.

Study limitations

Our study has a number of limitations. This study cohort represents a select population, rare in Western populations where comorbidities do exist. Moreover, as this study was cross-sectional, further follow-up and prospective research is required to determine the progression of the disease and long-term outcome in patients with decreased LV twist and RBR associated with hypertension and LV systolic dysfunction. The technique of speckle tracking utilized in this study only provides a two-dimensional analysis; reproducibility of these findings using other technology is uncertain. In addition, the gold standards of MRI tagging or sonomicrometry were not employed to confirm speckle-tracking findings in our study. Rotational values are lower using this specific vendor technology, and reproducibility of these findings using other vendors needs to be assessed. Further prospective studies with larger sample sizes are needed to confirm the clinical implications of our findings. Finally, a three-dimensional speckle-tracking analysis may offer new insight on patients with HTLEF and provide a good comparison with the reported findings using two-dimensional STE.

3.2.6 CONCLUSION

This study provides important insight into the left ventricular twist characteristics of HTP with systolic dysfunction and African ancestry. We found patients with hypertension and low EF in the absence of coronary artery disease had reduced net twist as a result of diminished apical and basal rotation. RBR can occur in association with hypertension and low EF and may result in adverse remodelling and a greater degree of left ventricular dysfunction.

3.3 Relationship between Left Ventricular Twist and Circulating Biomarkers of Collagen Turnover in Hypertensive Patients with Heart Failure

Nirvarthi Maharaj, MD, Bijoy K. Khandheria, MD, Elena Libhaber, PhD, Samantha Govender, BSc, Raquel Duarte, PhD, Ferande Peters, MD,

Mohammed R. Essop, MD,*Johannesburg, South Africa; Milwaukee, Wisconsin*

Submitted for publication; currently under review.

3.3.1 ABSTRACT

Left ventricular twist may be a compensatory mechanism to preserve EF. In HTP, twist varies depending on the left ventricle's degree of remodelling and systolic function; it is increased in those with preserved EF and diminished in those with low EF. The ratio of collagen-degradation biomarkers in HTP is higher in those with low EF than those with preserved EF and may contribute to remodelling and systolic dysfunction. This study evaluated the relationship between these biomarkers and left ventricular twist in 82 HTP – 41 with EF <50% (HTLEF group) and 41 with EF ≥50% (HTNEF group) with heart failure. Net left ventricular twist was measured using speckle-tracking echocardiography. Markers of collagen turnover, including serum concentrations of matrix metalloproteinase1 (MMP1), tissue inhibitor of MMP1 (TIMP1) and ratio of MMP1:TIMP1, were measured. Log TIMP1, Log MMP1 and Log MMP1:TIMP1 ratio levels were higher in the HTLEF group versus the HTNEF group (12.32 ± 0.25 vs. 11.81 ± 0.13, p<0.0001; 9.08 ± 0.32 vs. 8.00 ± 0.18, p<0.0001; -3.25 ± 0.30 vs. -3.81 ± 0.18, p<0.0001; respectively). Net left ventricular twist was lower in the HTLEF group versus the HTNEF group (3.34 ± 1.10 vs. 11.70 ± 0.67, p<0.0001). An inverse correlation existed between Log MMP1:TIMP1 and net left ventricular twist after adjusting for EF (r=-0.41, p<0.0001). This inverse correlation between twist and loss of myocardial collagen scaffolding suggests the integrity of the ECM plays an important role in preserving myocardial deformation in hypertensive patients with heart failure.

3.3.2 INTRODUCTION

Left ventricular (LV) twist is defined as the wringing motion of the heart during systole whereby the apex rotates in a counterclockwise direction with respect to the base rotating in a clockwise direction (Helle-Valle et al., 2005, Notomi et al., 2005, Taber et al., 1996, Sengupta et al., 2008b, Mor-Avi et al., 2011). LV twist is an important contributing factor to the systolic function of the left ventricle in health and disease (Helle-Valle et al., 2005, Notomi et al., 2005, Taber et al., 1996, Sengupta et al., 2008b, Mor-Avi et al., 2011). Evaluation of LV twist using speckle tracking is a sensitive technique used to assess cardiac performance (Helle-Valle et al., 2005, Notomi et al., 2005, Taber et al., 1996, Sengupta et al., 2008b, Mor-Avi et al., 2011) and can be a better index of systolic function than EF in HTP. In HTP with preserved EF, LV twist is increased while LS is diminished, suggesting that LV twist may be a compensatory mechanism to preserve EF (Ahmed et al., 2012). LV twist is diminished in HTP with low EF who initially present with HF (Maharaj et al., 2012a) and has been found to be more diminished in HTP with eccentric LV hypertrophy compared to concentric hypertrophy (Mizuguchi et al., 2010b). This suggests LV twist varies with the degree of remodelling and systolic function caused by hypertension.

The remodelling process of the left ventricle in hypertension entails a complex interplay between myocyte hypertrophy and dysfunction, with qualitative changes in the ECM contributing to progressive dysfunction (Laviades et al., 1998, Li et al., 1998, Timms et al., 2002, Thomas et al., 1998). Adverse LV remodelling and hypertrophy in HTP is associated with derangements in the dynamic balance between the accumulation and breakdown of collagen in the cardiac ECM (Laviades et al., 1998, Li et al., 1998, Timms et al., 2002, Thomas et al., 1998). Furthermore, increased matrix metalloproteinase1 (MMP1) levels – reflecting collagen degradation – may contribute to the development of LV dilatation and failure in HTP matrix (Laviades et al., 1998, Li et al., 1998, Timms et al., 2002, Thomas et al., 1998, López et al., 2006). (López et al., 2006) found that a greater excess of MMP1 to the tissue inhibitor of matrix metalloproteinase1 (TIMP1) occurred in the myocardium of HTP with low EF than those with normal EF. Moreover, Lopez et

al. found that the circulating MMP:TIMP ratio was associated with greater LV dilatation and systolic dysfunction (López et al., 2006). This landmark study implied the varying morphology and function in HHD was related to the equilibrium of MMP1 and TIMP1 in maintaining collagen homeostasis (López et al., 2006). Hypertension can cause systolic dysfunction as a consequence of adverse remodelling and LV hypertrophy, but given the multitude of factors involved in LV decompensation mediated by mechanical, neurohormonal and cytokine routes, the exact mechanisms that contribute to the adverse remodelling and EF deterioration are not fully elucidated (Drazner, 2011, Frohlich, 1999).

We postulate that changes in the ECM as reflected by MMP1:TIMP1 ratio account for the varying morphology, EF and LV twist in HTP who present with HF. The aim of this study was to evaluate LV twist mechanics and their relationship with biomarkers of collagen degradation in HTP.

3.3.3 METHODS

This cross-sectional study was approved by the University of Witwatersrand Ethics Committee and the Institutional Review Board. Subjects were consecutively recruited from the Chris Hani Baragwanath Hospital Cardiac Clinic from January 2011 to June 2012. Inclusion criteria were: documented prior diagnosis of hypertension (measurements on three separate occasions where systolic BP was ≥140 mmHg or diastolic BP was ≥90 mmHg taken over a period of two months (Seedat and Rayner, 2012) at the Hypertension Clinic), documented HF using Framingham Study criteria (Lloyd-Jones et al., 2002), sinus rhythm, and normal coronary angiography. Exclusion criteria were: previous myocardial infarction, previous arrhythmia, anaemia (haemoglobin <12 g/dL in females, <13 g/dL in males), excess alcohol intake (maximum alcohol intake no more than 40 grams per day in males and 20 grams per day in females with two alcohol-free days per week), renal dysfunction (glomerular filtration rate <60 mL/min/1.73 m^2), documented diagnosis of diabetes and/or glycated haemoglobin >7%, organic valvular disease, dilated cardiomyopathy of any etiology, cardiac infiltrative diseases, post-viral myocarditis, any systemic illness (e.g. human

immunodeficiency virus [HIV]), thyroid disease, any primary organ dysfunction or failure (e.g. chronic renal disease), and patients with evidence of RBR on speckle tracking.

A total of 107 subjects who fulfilled the criteria and provided voluntary informed consent were enrolled and underwent a detailed clinical and echocardiographic evaluation at baseline. After echocardiography, 25 patients were excluded as a result of inadequate image quality that did not allow for complete segmental assessment of LV rotation at both the basal and apical left ventricle. The remaining 82 HTP, 41 of whom had EF <50% (HTLEF group) and 41 of whom had EF ≥50% (HTNEF group), made up the study group and were given adequate doses of HF therapy as per individual patient's requirements.

The control group (n=41) was recruited from staff at Chris Hani Baragwanath Hospital, patient escorts and community members from Soweto. All controls were unrelated to patients in the study groups and were asymptomatic, normotensive, had no evidence of cardiovascular or systemic disease, and had a normal 12-lead electrocardiogram prior to undergoing echocardiography for the study. The control group was age- and sex-matched with the HTLEF and HTNEF cohorts. Individuals younger than 50 years were matched with a tolerance of 5 years in terms of age, whereas individuals older than 50 years were allowed a tolerance of up to 10 years.

BP measurements

Please refer to 3.1.3.

Echocardiography

Please refer to 3.1.3. Concentric hypertrophy was defined as RWT >0.42 and left ventricular mass index (LVMI) (g/m^2) >95 in females and >115 in males, while eccentric hypertrophy was defined as RWT <0.42 and LVMI >95 in females and >115 in males (Lang et al., 2005, Ganau et al., 1992). LV sphericity index was calculated by dividing the LV maximal long-axis internal dimension by

the maximal short-axis internal dimension at end diastole and end systole (van Dalen et al., 2010) using apical four-chamber view.

Speckle-tracking analysis

Please refer to Methods in chapter 3.2.3.

Biomarkers

All blood samples were taken at the time of echocardiographic examination. Samples were collected in a serum separator tube, which allowed samples to clot for 30 minutes before centrifuging for 15 minutes at 1000 × gravity. Serum was removed immediately and samples stored at less than or equal to -20°C. Total serum levels of TIMP1 and MMP1 were determined using the Fluorokine® MAP multiplex kits (R&D Systems, Minneapolis, MN) designed for use with the Luminex® 100 dual-laser, flow-based analyzers (Luminex Corp., Austin, TX), which detect antibodies to human TIMP1 and MMP1. The inter- and intra-assay coefficients of variation were 12.7% and 5.9% for MMP1 and 10.1% and 6.5% for TIMP1, respectively. Lower detection limits were 4.40 pg/ml of MMP1 and 1.54 pg/ml of TIMP1. Because the actual activity of MMP1 depends on the balance between active enzyme and inhibitor (i.e. TIMP1), the serum MMP1:TIMP1 ratio was considered an index of MMP1 activity.

Statistical Analysis

Database management and analyses were performed with SAS software (Version 9.2, SAS Institute Inc., Cary, NC). Data was presented as mean ± standard deviation. Continuous variables between groups were compared using ANOVA, or Kruskal-Wallis test when the distribution was non-normal. Two-by-two comparisons were then performed applying Bonferroni correction with $p<0.0083$ denoting significance. Biomarker levels (TIMP1, MMP1 and MMP1:TIMP1 ratio) were log-transformed before analysis when distribution was non-normal. Pearson correlation coefficients

were determined for the relationships between LV myocardial deformation parameters and biomarker levels. Interobserver variability was assessed for twist and strain measurements in 20 randomly selected patients and calculated as the standard deviation of the differences between measurements of two independent observers who were unaware of the other patient data, then expressed as a percentage of the average value. Intraobserver variability was calculated as the standard deviation of the differences between a first and second determination of a single observer, and expressed as a percentage of the average value. Intra- and interobserver variability for LV rotation parameters in our centre varies from 2 ± 3% to 10 ± 9%, and from 4 ± 4% to 12 ± 8%, respectively (Maharaj et al., 2012b).

3.3.4 RESULTS

Baseline and Echocardiographic Characteristics

There were no statistically significant differences in age, sex or body mass index between the HTLEF and HTNEF groups ($p>0.05$) (Table 3.3.1). Mean duration of hypertension was similar between the two groups (12.2 ± 6.5 years in HTLEF and 15.5 ± 8.4 years in HTNEF, $p>0.05$) (Table 3.3.1), but mean duration of HF was higher in the HTLEF group (3.2 ± 1.6 years) than the HTNEF group (1.8 ± 1.0 years), $p<0.005$. All patients in the HTLEF group had LV hypertrophy compared with 85% in the HTNEF group. The HTLEF group had significantly higher LV mass index and end-diastolic volume index and lower sphericity index ($p<0.01$) (Table 3.3.2).

TABLE 3.3.1. CLINICAL CHARACTERISTICS COMPARISON BETWEEN CONTROLS AND HYPERTENSIVE PATIENTS

	Control Group	HTNEF Group	HTLEF Group
No. patients	41	41	41
Age, years	50.1±9.3^	55.5±8.4	55.0±9.1*
Gender, F:M	22:19	22:19	22:19
Body mass index, kg/m^2	24.7±1.8^	30.2±4.9	28.9±4.2$^{\#}$
Body surface area, m^2	1.72±0.14^	1.86±0.15	1.85±0.16$^{\#}$
NYHA functional capacity, no. (%)			
Class I	41 (100%)	26 (63%)$^{\#\#}$	12 (29%)$^{\#}$
Class II		15 (37%)	14 (34%)
Class III			15 (37%)
Duration of hypertension, years	---	15.5±8.4	12.2±6.5
Duration of heart failure, years	---	1.8±1.0$^{\#\#}$	3.2±1.6
Systolic blood pressure, mmHg	128±10^	141±14$^{\#\#}$	156±8$^{\#}$
Diastolic blood pressure, mmHg	79±8	84±11	89±10$^{\#}$
Heart rate, bpm	73±10	75±11$^{\#\#}$	81±10$^{\#}$
Medication, no. (%)			
Furosemide	0	41 (100%)	41 (100%)
ACE-I or ARB	0	41 (100%)	41 (100%)
Beta blockers	0	28 (68%)	41 (100%)
Amlodopine	0	41 (100%)	41 (100%)
Spironolactone	0	0	41 (100%)

Values are mean ± standard deviation. $^{\#}$p<0.001 for comparison among all groups; *p=0.03 for age among all groups; $^{\#\#}$p<0.005 for comparison between HTNEF and HTLEF. ^p<0.001 for comparison between controls and HTNEF. ACE-I indicates angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; F:M, female-to-male ratio of number of patients; HTLEF, patient study group with hypertension and low ejection fraction (<50%); HTNEF, patient study group with hypertension and normal ejection fraction (≥50%); NYHA, New York Heart Association.

TABLE 3.3.2. ECHOCARDIOGRAPHIC DATA COMPARISON BETWEEN CONTROLS AND HYPERTENSIVE PATIENTS

	Control Group	HTNEF Group	HTLEF Group
No. patients	41	41	41
End-diastolic diameter, mm	43±5	45±1$^{\#\#}$	58±5$^{\#}$
IVSD, mm	10±1^	13±2$^{\#\#}$	12±2$^{\#}$
IVPWD, mm	8±1^	10±1	11±1$^{\#}$
End-diastolic volume index, ml/m^2	46.8±7.7	45.5±13.8$^{\#\#}$	71.7±21.6$^{\#}$
End-systolic volume index, ml/m^2	18.9±5.6	18.4±7.3$^{\#\#}$	47.1±17.0$^{\#}$
Ejection fraction, %	69.3±9.5^	60.9±7.3$^{\#\#}$	33.4±7.1$^{\#}$
Left ventricular mass index, g/m^2	73.8±13.9^	106.3±21.3$^{\#\#}$	152.0±25.3$^{\#}$
Left atrial volume index, ml/m^2	23.0±2.2^	26.8±3.8$^{\#\#}$	31.0±16.3$^{\#}$
LVSPHI (end diastole), ratio	1.9±0.3^	1.7±0.1$^{\#\#}$	1.4±0.1$^{\#}$
LVSPHI (end systole), ratio	2.1±0.5^	1.8±0.2$^{\#\#}$	1.4±0.1$^{\#}$
E/E` ratio	5.6±1.8^	11.6±4.6$^{\#\#}$	18.7±12.1$^{\#}$

Values are mean ± standard deviation. $^{\#}$p<0.001 for comparison among all groups; $^{\#\#}$p<0.01 for comparison between HTNEF and HTLEF. ^p < 0.001 for comparison between controls and HTNEF.

HTLEF indicates patients with hypertension and ejection fraction <50% on echocardiography; HTNEF, patients with hypertension and ejection fraction ≥50% on echocardiography; IVPWD, interventricular posterior wall at end-diastolic dimension; IVSD, interventricular septal thickness at end-diastolic dimension; LVSPHI, left ventricular sphericity index.

Myocardial Deformation and Biomarkers Analysis

Net LV twist was significantly higher in the HTNEF group (11.70° ± 0.67) compared to controls (10.9° ± 2.7) and the HTLEF group (3.34° ± 1.10) [p<0.001] (Table 3.3.3). AR was higher in the HTNEF group than in controls (p<0.001), while BR was similar (p=0.36) (Table 3.3.3). AR and BR values were significantly higher in HTNEF compared with HTLEF (p<0.0001) (Table 3.3.3). Longitudinal, circumferential and RSwere diminished in the HTNEF and HTLEF groups compared with controls, with a greater decrement in the HTLEF group (p<0.0001) (Table 3.3.3). Log TIMP1, Log MMP1 and Log MMP1:TIMP1 ratio were increased in HTLEF compared with HTNEF (12.32 ± 0.25 vs. 11.81 ± 0.13; 9.08 ± 0.32 vs. 8.00 ± 0.18; and -3.25 ± 0.30 vs. -3.81 ± 0.18, p<0.0001; respectively) (Table 3.3.3).

TABLE 3.3.3 MYOCARDIAL DEFORMATION AND BIOMARKERS COMPARISON BETWEEN CONTROLS AND HYPERTENSIVE PATIENTS

	Control Group	HTNEF Group	HTLEF Group
No. patients	41	41	41
Apical rotation, degrees	6.8±1.5^	7.7±0.4$^{\#\#}$	1.7±1.0$^{\#}$
Basal rotation, degrees	-3.8±1.6	-4.0±0.6$^{\#\#}$	-1.6±0.9$^{\#}$
Time to peak apical rotation, ms	348±20	356±14$^{\#\#}$	326±6*
Net twist, degrees	10.6±2.3^	11.7±0.7$^{\#\#}$	3.3±1.1$^{\#}$
Untwisting rate, degrees/s	-50.6±4.3^	-39.1±3.4$^{\#\#}$	-26.8±6.4$^{\#}$
Longitudinal strain, %	-14.6±2.2^	-11.4±0.2$^{\#\#}$	-8.7±1.2$^{\#}$
Circumferential strain,%	-14.6±2.3^	-11.5±0.3$^{\#\#}$	-9.0±1.4$^{\#}$
Radial strain, %	54.6±2.3^	42.2±2.4$^{\#\#}$	29.0±1.3$^{\#}$
TIMP1, pg/ml	86240.2±12405.2^	135969.3±20767.1$^{\#\#}$	232612.2±66117.2$^{\#}$
Log TIMP1	11.4±0.1^	11.8±0.1$^{\#\#}$	12.3±0.3$^{\#}$
MMP1, pg/ml	894.8±213.1^	3036.8±553.7$^{\#\#}$	9203.1±2981.0$^{\#}$
Log MMP1	6.8±0.2^	8.0±0.2$^{\#\#}$	9.1±0.3$^{\#}$
MMP1:TIMP1 ratio	0.011±0.003^	0.023±0.004$^{\#\#}$	0.041±0.014$^{\#}$
Log MMP1:TIMP1 ratio	-4.6±0.3^	-3.8±0.2$^{\#\#}$	-3.3±0.3$^{\#}$

Values are mean ± standard deviation. $^{\#}$p<0.0001 for comparison among all groups; *p<0.01 for comparison among all groups; $^{\#\#}$p<0.005 for comparison between HTNEF and HTLEF. ^p<0.001 for comparison between controls and HTNEF.

HTLEF indicates patients with hypertension and ejection fraction <50% on echocardiography; HTNEF, patients with hypertension and ejection fraction ≥50% on echocardiography; MMP1, matrix metalloproteinase-1; TIMP1, tissue inhibitor of matrix metalloproteinase-1.

Relationship between Biomarkers and LV Twist

As longitudinal and CS and BR decreased (increasing to a positive value), Log MMP1:TIMP1 ratio increased in the HTNEF and HTLEF groups (Table 3.3.4). Log MMP1:TIMP1 ratio inversely correlated with net LV twist and EF (r=-0.74, p<0.0001; r=-0.67, p<0.0001, respectively) in HTP (Table 3.3.4). However, after adjusting for EF, Log MMP1:TIMP1 ratio explained 16.8% of the net LV twist variation (p<0.0001) (Table 3.3.4).

Table 3.3.4 Correlation of Log MMP1:TIMP1 With Myocardial Deformation Parameters in Hypertensive Patients

	Unadjusted	Adjusted for ejection fraction
Ejection fraction, %	r=-0.67	---
	p<0.0001	
Twist, degrees	r=-0.74	**r=-0.41**
	p<0.0001	**p<0.0001**
Apical rotation, degrees	r=-0.74	r=-0.40
	p<0.0001	p=0.0002
Basal rotation, degrees	r=0.64	r=0.30
	p<0.0001	p=0.006
Longitudinal strain, %	r=0.53	r=0.08
	p<0.0001	p=0.50

MMP1:TIMP1 indicates ratio of matrix metalloproteinase-1 to tissue inhibitor of matrix metalloproteinase-1.

3.3.5 DISCUSSION

This study's main findings were: 1) LV twist is increased in HTNEF patients, whereas all other strain parameters are diminished compared with controls; 2) In HTLEF patients, LV twist is decreased while all strain parameters are diminished to a greater extent than that observed in HTNEF patients; 3) Circulating biomarkers of collagen turnover are increased to a greater degree in HTLEF patients than in HTNEF patients; and 4) There is a modest inverse correlation between LV twist and Log MMP1:TIMP1 ratio even when adjusted for EF.

LV twist originates from the dynamic interaction between oppositely wound subepicardial and subendocardial myocardial fibre helices and has an important role in LV ejection (Sengupta et al., 2008b). The contraction of the subepicardial fibres rotates the apex in a counterclockwise direction and the base in a clockwise direction. The contraction of the subendocardial region rotates the apex and base in exactly the opposite directions (Sengupta et al., 2008b). When both layers contract simultaneously, a larger radius of rotation for subepicardial fibres results in a mechanical advantage determining the overall direction of rotation at apex (counterclockwise) and base (clockwise) (Sengupta et al., 2008b).

This study confirms that LV twist varies with the degree of remodelling and dysfunction in HHD, with similar abnormalities reported in prior studies of HTP with either normal or low EF (Maharaj et al., 2012a, Mizuguchi et al., 2010b, Ahmed et al., 2012). Studies (Maharaj et al., 2012a, Mizuguchi et al., 2010b, Ahmed et al., 2012, van Dalen et al., 2010, Garot et al., 2002, Takeuchi et al., 2007b) show a positive correlation between LV twist and EF, reflecting that reduction in LV systolic function is accompanied by reduction of twist. In HTP with normal EF, LS decreases while an increment in LV twist ensures preservation of normal EF (Maharaj et al., 2012a, Mizuguchi et al., 2010b, Ahmed et al., 2012). HTNEF patients have less adverse remodelling; consequently their subendocardial and subepicardial fibres are less affected. Thus, while there is a decline in all strain modalities compared with controls, LV twist increases to preserve EF in these patients(Maharaj et

al., 2012a, Mizuguchi et al., 2010b, Ahmed et al., 2012), especially those with preserved apical function. However, LV twist is decreased in HTP with low EF, in keeping with the overall decrease in other strain parameters. The major mechanism for this appears to relate to apical function (Maharaj et al., 2012a, Mizuguchi et al., 2010b, Ahmed et al., 2012). A prior study depicted that AR correlated positively with the degree of LV dysfunction, suggesting degree of apical dysfunction is more important than the degree of basal dysfunction (Maharaj et al., 2012a, Mizuguchi et al., 2010b, Ahmed et al., 2012). The absence of ischemia or infarction in our study population is important, since these confounding factors may decrease apical function and, consequently, LV twist (Takeuchi et al., 2007b, Bansal et al., 2008b, Tibayan et al., 2004, Nucifora et al., 2010). It cannot be ascertained whether the difference in LV twist observed in normal versus low EF represents a cause or consequence of a decline in EF in hypertensive-associated HF.

The second finding of this study is that circulating biomarkers of collagen are elevated to a greater degree – with decrements in myocardial deformation – in HTP with low EF compared to those with normal EF. These findings are similar to those documented by (López et al., 2006) who demonstrated an excess of MMP1 relative to TIMP1 in the myocardium of HTP with low EF compared with those with preserved EF in 39 patients with no coronary artery disease (16 with low EF, 23 with preserved EF). Their study documented that HTP with low EF had a lower volume of myocardial tissue occupied by endomysial and perimysial collagen but increased perivascular and scar-related collagen deposits compared with HTP with normal EF (López et al., 2006). They also found that the serum MMP1:TIMP1 ratio was inversely correlated with EF, whereas the degree of LV dilatation was positively correlated (López et al., 2006). Thus, the phenotype of LV dilatation associated with reduced EF may be a consequence of this remodelling that occurs in the ECM due to excessive MMP1 activity relative to TIMP1.

Our study shows an inverse relationship between MMP1:TIMP1 ratio and LV twist even when adjusting for EF, implying that the decline in LV twist may be related to the remodelling process of the ECM. HTP with normal EF have lower MMP1:TIMP1 ratios, suggesting less extracellular

remodelling that does not affect all regions of the myocardium to the same extent. Prior studies have suggested the major pathophysiological abnormality in HTNEF patients is that of subendocardial ischemia (Fujii et al., 1992), which occurs in the absence of coronary disease and initially affects subendocardial fibres (Kang et al., 2008). Subendocardial ischemia results from diminished coronary flow autoregulation in the subendocardium compared to the subepicardium with increased transmural pressure loss within the microvasculature contributing to subendocardial underperfusion and vulnerability to ischemic injury (Fujii et al., 1992).

In HTP with normal EF, the degree and location of collagen degradation may relate to the myocardial deformation indices, i.e. interstitial and perivascular fibrosis and collagen degradation is likely to initially affect the subendocardial fibres in the presence of subendocardial ischemia (Kang et al., 2008), with decrement in LS due to their prominent subendocardial location (Kang et al., 2008), and thereafter the development of circular fibre dysfunction in the midwall (decreases in radial and CS) (Kang et al., 2008). If the greatest area of involvement is the subendocardium and perhaps some degree of involvement in the midmyocardium, then LS will be most affected with varying decrements of circumferential and RS noted. Subendocardial fibre dysfunction will cause less opposition to the subepicardial fibres, thus the predominant rotatory effects of the subepicardial fibres dominate during systole and result in enhanced BR, AR and net LV twist. In our study, this postulate would explain the strain, AR and net LV twist observed in HTNEF. A nonsignificant trend of increased BR also was noted.

Higher MMP1:TIMP1 ratio was detected in HTP with low EF compared with HTNEF patients. This finding is similar to (López et al., 2006) and suggests this process is characterized by more extensive extracellular remodelling. We propose that perhaps this adverse remodelling is a more diffuse process that affects all regions of the myocardium to a similar degree, such that the relatively preserved subepicardial fibre function seen in HTNEF is abolished, resulting in impairment of all myocardial strain and LV twist. As collagen degradation increases, there is global dysfunction of both the subendocardial and subepicardial fibres, with diffuse collagen degradation

in subepicardial and subendocardial fibres resulting in greater impairment in AR and LV twist. This accompanies greater decrements in strain parameters in HTP with low EF.

The decline in LV twist may relate both to factors that cause a global reduction in systolic function and specific remodelling factors that decrease LV twist. The loss or disruption of this collagen network may compromise systolic function by three mechanisms. Firstly, the loss of mysial collagen results in discontinuity of the ECM, compromising the alignment and support of the matrix (López et al., 2006). A second factor relates to the possible loss of synchronized sarcomere contraction (López et al., 2006). The final mechanism occurs as a consequence of increased MMP1 activity where there is myocardial slippage and, consequently, a decreased number of muscle layers in the left ventricle (López et al., 2006). Collagen loss promotes myocyte slippage, thereby altering LV structure and function with ventricular dilatation, increasing LV sphericity and greater systolic dysfunction if the process is exaggerated (Laviades et al., 1998, Li et al., 1998, Timms et al., 2002, Thomas et al., 1998, López et al., 2006). The loss of the oblique architecture of loop fibres and abnormal muscle fibre orientation (Popescu et al., 2009) responsible for the wringing motion results in a decline in LV twist (van Dalen et al., 2010).

Limitations

This study cohort represents a select population, rare in Western populations where comorbidities do exist. Speckle-tracking echocardiography software used only provides a two-dimensional analysis, and the consistency of these findings using other two-dimensional vendor technology is uncertain. Tagged MRI or sonomicrometry were not employed as the gold standard to compare speckle-tracking findings. We did not histologically investigate the correlation between echocardiographic parameters and the collagen volume fraction in myocardium or assessment of tissue MMP activity (i.e. zymography). Moreover, as this study was cross-sectional with a small sample size, further follow-up and prospective research is required to determine how the changes in LV twist, extracellular collagen degradation and remodelling affect progression of HHD.

3.3.6 CONCLUSION

HTP with low EF have more collagen degradation and impaired twist compared to HTP with normal EF. Log MMP1:TIMP1 ratio inversely correlates with net LV twist even when adjusted for EF. These preliminary findings may indicate that increased loss of myocardial collagen scaffolding not only accompanies more adverse remodelling but also contributes to the differences in LV twist mechanics observed between HTLEF and HTNEF patients. Alterations in collagen turnover can affect the oblique spiral myocardial fibre architecture, resulting in abnormalities in LV twist mechanics.

4.0 DISCUSSION

4.1 SUMMARY OF KEY FINDINGS

Firstly, this study established normative data and patterns for myocardial deformation (strain and LV twist) in a normal black-African adult population across different age groups and can be used as a baseline for future studies. Aging was associated with increased net LV twist whilst decreasing strain parameters were associated with advancing age. Age was the major determinant of increased LV twist in this population. Additionally, despite an increase in relative wall thickness, left ventricular mass index, systolic BP and changes in diastolic indices with advancing age in our study, conventional echocardiographic and clinical variables were not able to predict net LV twist. Moreover, strain parameters showed an independent correlation (longitudinal, circumferential and radial strain) with net LV twist but were unable to predict net LV twist.

Secondly, diminished net twist occurred in all HTLEF patients, caused by both a reduction in AR and BR. However, only AR correlated positively with the degree of LV dysfunction, suggesting that the degree of apical dysfunction is more important in LV dysfunction than the degree of basal dysfunction. AR and net twist correlate well with EF whilst end diastolic diameter showed no correlation with LV twist. RBR can occur in association with hypertension and low EF and may result in adverse remodelling and a greater degree of left ventricular dysfunction.

Finally, all strain parameters are diminished in HTNEF and HTLEF while LV twist is increased in HTNEF compared to HTLEF patients. LV twist varies with the degree of remodelling and dysfunction in HHD. This study revealed an inverse relationship between MMP1:TIMP1 ratio and LV twist even when adjusting for EF, implying that the decline in LV twist may be related to the remodelling process of the ECM. HTP with low EF have more collagen degradation and impaired twist compared to HTP with normal EF. These preliminary findings may indicate that increased loss of myocardial collagen scaffolding not only accompanies more adverse remodelling but also

contributes to the differences in LV twist mechanics observed between HTLEF and HTNEF patients. Alterations in collagen turnover can affect the oblique spiral myocardial fibre architecture, resulting in abnormalities in LV twist mechanics.

4.2 MYOCARDIAL DEFORMATION IN NORMAL ADULTS

STE provides assessment of cardiac chamber function through the largely angle independent quantification of LV myocardial deformation (Bohs and Trahey, 1991). The routine use of 2D strain and LV twist in clinical practice requires the definition of a normal range, but the majority of studies have defined their own normal ranges using a relatively small number of subjects who are of a limited age range. Our study provides the age specific normal range of 2D strain and LV twist parameters which serve as reference for the parameters measured to investigate changes that may occur in disease states in an African population.

Our findings reveal that the values of BR, AR and net twist using the QLAB software do not differ from findings in other populations using this vendor's technology (van Dalen et al., 2008b). Additionally, the values obtained for AR and BR in this study are less than those reported in normal populations using different vendor technologies (van Dalen et al., 2008b, Zhang et al., 2007, Zhang et al., 2010, Takeuchi et al., 2006). The mean strain values in our study were -17.28 ± 3.30% for longitudinal, -17.40 ± 3.29% for circumferential and 57.49 ± 3.32% for radial strain. Marwick et al reported an average LV peak systolic LS of –18.6 in 242 healthy volunteers using a General Electric (GE) system (Marwick et al., 2009). Another recent report described an average global LS of –20.6±2.6% and an average RS of 48.2±13.8% in 144 healthy subjects using a GE system (Reckefuss N, 2011). The average values of longitudinal and CS were also significantly lower in Caucasians than in Asians. This difference is thought to be related to body surface area, which is significantly higher in Caucasians than in Asians.

Few studies have addressed the inter-vendor variability of 2D strain measurements (Bansal et al., 2008a, Biaggi et al., 2011, Koopman et al., 2010, Manovel et al., 2010). A recent study performed 2D speckle tracking analysis on 28 healthy adult subjects using ultrasound systems and software from GE and Toshiba, and found no significant vendor differences in the global longitudinal, circumferential or RS measurements (Manovel et al., 2010). They also reported that global LS had a more narrow limit of agreement (–2.25 to 3.65) compared to radial and CS and concluded that global LS is a more robust parameter for evaluating myocardial function when a different cardiac ultrasound system is used for assessment (Manovel et al., 2010).

Although the fundamental concept of 2D STE may be the same, 2D STE image tracking ability on a frame-by-frame basis in 3 vendors was different, resulting in a maximum difference of 18% in the feasibility between vendors (Takigiku et al., 2012). Image quality could affect the tracking feasibility observed in all of the systems from the different vendors (inferior in the Philips system compared to the GE or Toshiba) (Takigiku et al., 2012). Therefore, it must be acknowledged that the contribution of image quality to tracking feasibility differs with vendors and as a result the normal range of 2D strain differs (Takigiku et al., 2012). Systems from different vendors are not interchangeable when acquiring and analyzing 2D strain data to assess myocardial deformation from the same subjects (Takigiku et al., 2012).

Physiological variables such as preload, afterload, and contractility alter the extent of LV twist (Dong et al., 1999, Hansen et al., 1991, MacGowan et al., 1996). Twist is greater with higher preload. For example, higher LV end-diastolic volumes, with LV end-systolic volume held constant, produce higher LV twist (Sengupta et al., 2008b). Similarly, afterload affects twist, that is, twist decreases at higher end-systolic volumes when end-diastolic volumes are held constant (Sengupta et al., 2008b). The effect of preload on twist is about two-thirds as great as that of afterload. Like changes in loading conditions, increasing contractility increases LV twist; for example, positive inotropic interventions such as dobutamine infusion and paired pacing increase LV twist (Buchalter et al., 1994, Moon et al., 1994, Hansen et al., 1991), whereas negative

inotropic interventions markedly reduce twist (Buchalter et al., 1994). In the intact circulation, changes in contractility are often accompanied by changes in loading conditions, thus, altering LV twist mechanics (Sengupta et al., 2008b).

4.3 EFFECT OF AGING ON MYOCARDIAL DEFORMATION

The increasing number and proportion of aged individuals in the population warrants knowledge of normal physiological changes of myocardial deformation with advancing age. Aging affects many aspects of cardiovascular function (Lakatta, 2003c, Lakatta, 2003a, Lakatta, 2003b, Lakatta and Sollott, 2002, Oxenham et al., 2003). Advancing age is associated with structural and functional changes in the myocardium, e.g. myocardial fibrosis and impaired coronary perfusion (Oxenham et al., 2003, Lakatta, 2003c, Lakatta, 2003a, Lakatta, 2003b). Increased LV wall thickness, alterations in the diastolic filling pattern, impaired LV ejection and heart rate reserve capacity are the most dramatic changes in cardiac function that occur with aging in healthy persons (Lakatta, 2003c, Lakatta, 2003a, Lakatta, 2003b). Although these age-associated changes do not always reflect clinical heart disease per se, they may compromise cardiac reserve and alter the threshold for disease manifestation.

Age-related changes is via changes in sarcomere protein isoforms and titin development which alters myocardial contractility (Lakatta et al., 1975, Lakatta, 2003a). In addition, BP increases with aging, but the proportions of LV shape and ventricular wall thickness and size remained constant, which causes increased interventricular wall strain and drives the changes in LV myocardial deformation (Lakatta, 2003c, Lakatta, 2003a).

The characteristics of LV twist undergo substantial changes during the aging process. Our findings observed that AR increased substantially after age 40 whereas no significant increment occurred in BR after age 40. In accordance with our study, BR also was less influenced by age in other published data (van Dalen et al., 2008b, Zhang et al., 2007, Takeuchi et al., 2006, Zhang et al.,

2010). Van Dalen et al. reported a mean value of peak LV twist as 7.7 ± 3.58° in a white population (van Dalen et al., 2008b), with peak LV twist significantly greater in subjects aged ≥60 years (10.8 ± 4.98°) compared with those aged ≤40 years (6.7 ± 2.98°) and those aged 41 to 59 years (8.0 ± 3.08°) (van Dalen et al., 2008b). Takeuchi et al. examined the effect of aging on twist in 113 Asian volunteers; peak LV twist increased significantly in those aged ≥60 years with similar values as published on whites (Takeuchi et al., 2006). Furthermore, (Zhang et al., 2010) showed a greater increment in AR from subjects aged 45 to 55 years (7.94 ± 1.20°) to those aged 55 to 65 years (9.65 ± 1.56°), and a lesser increase in BR [-6.50 ± 2.49° (45-55 years) vs. -7.07 ± 1.25° (55-65 years)].

The increase in LV twist can be partially explained by reduced opposition to AR, which results from a gradual decrease in subendocardial function with aging (Zhang et al., 2010, van Dalen et al., 2008b, Takeuchi et al., 2006, Zhang et al., 2007). In a tagged MRI study, aging was associated with a decrease of contractile function in the sub-endocardium relative to that in the sub-epicardium without changes in EF (Lumens et al., 2006). Impairment of subendocardial contractile function may be on the basis of ischemia or fibrosis and results in diminished clockwise systolic BR, which leads to less opposition of counterclockwise systolic AR, causing net twist to increase (Zhang et al., 2007, Zhang et al., 2010, van Dalen et al., 2008b, Takeuchi et al., 2006). Subendocardial dysfunction develops with aging due to subendocardial fibrosis and fibre rearrangement reducing negative torque in the subendocardium, causing augmentation of positive myocardial twist. The net effect is increased LV twist and the preservation of EF in the elderly and reducing myocardial oxygen demand (Tibayan et al., 2004, Taber et al., 1996).

An analysis of other aspects of myocardial mechanics revealed a concurrent reduction in longitudinal, circumferential and RS with increasing age. Whereas some researchers described reduced strain with increasing age (tissue Doppler imaging (Dalen et al., 2010, Kuznetsova et al., 2008) and MRI (Fonseca et al., 2003)) or increasing strain with aging (STE) (Reckefuss N, 2011), others found no correlation between strain and age (tissue Doppler imaging) (Sun et al., 2004) in other populations. Moreover, some studies using speckle tracking found some strain parameters

(circumferential and radial strain) increased (Sun et al., 2012a, Reckefuss N, 2011) while LS decreased (Sun et al., 2012a).

LV systolic twist gradually increases from infancy to adulthood (Burns et al., 2008, Sengupta et al., 2008b, Esch and Warburton, 2009). LV systolic twist continues to increase with aging as a result of increased AR. AR is the major contributing factor of LV twist, with an increase in AR resulting in an increase in twist. This implies that apical function is perhaps the most important determinant for LV twist, and one would expect that pathological states that impair apical function to a greater degree than other anatomic sites may result in greater LV dysfunction. In this study age remained the main independent predictor of net LV twist.

4.4 MYOCARDIAL MECHANICS IN HYPERTENSIVE PATIENTS WITH PRESERVED EF

The deleterious effects of hypertension on LV function are multifactorial, comprising increased mechanical stress with subsequent LV hypertrophy, alterations in the phenotype of cardiomyocytes, enhanced apoptosis of the cardiomyocytes with interstitial and perivascular fibrosis, endothelial dysfunction, microvascular insufficiency, and upregulation of neurohormonal factors, such as the sympathetic and renin-angiotensin-aldosterone system (de Leeuw and Kroon, 1998, Fouad, 1987). Many pathophysiological mechanisms, including reductions in coronary blood flow reserve (CBFR), have been proposed to explain the progression of compensated LVH to ventricular dysfunction (Frohlich, 1999). Coronary microcirculation alterations in hypertension have already been reported in a previous study (Hamasaki et al., 2000). Endothelial dysfunction in hypertension has been shown in coronary vasculature in patients without LV systolic dysfunction (Hamasaki et al., 2000, Treasure et al., 1993). It is possible that impairment of endothelium-dependent coronary vasodilatation capacity is one of the mechanisms responsible for inadequate myocardial flow, and therefore contributes to ventricular maladaptation before the development of morphological alterations.

The results of large epidemiologic trials indicate that hypertension is one of the key factors associated with the increased incidence of congestive HF (Levy et al., 1996). Both structural and functional myocardial abnormalities identified in HTP contribute to the progression of myocardial dysfunction from an asymptomatic state to clinically apparent heart disease (de Leeuw and Kroon, 1998). Considerable variability in the cardiac phenotype of HHD exists, with Black patients being more prone to concentric LVH (Kizer et al., 2004). Growing evidence suggests that systolic abnormalities in HT begin to develop in the early stages of the disease when global LV EF is still normal. The same mechanisms might be implicated in the progressive deterioration of LV systolic performance during the development of clinical HF (Vinch et al., 2005, Bountioukos et al., 2006, Yuda et al., 2002, Pela et al., 2001). Accordingly, functional abnormalities causing HF symptoms may be the long-term consequence of systolic functional deficits that are initially not detectable by conventional echocardiographic methods.

The measurement of global LV function is the most common indication for echocardiography and LV dysfunction is known to be a strong prognostic marker of adverse outcome (Quiñones et al., 2000), most frequently assessed by the calculation of EF by Modified Simpsons biplane method. This Modified Simpsons and other methods are heavily dependent on image quality, image orientation, and reader experience. Whereas global systolic LV function in HTP with HF is preserved according to standard LV EF assessment, a number of previous studies identified systolic LV abnormalities in a substantial portion of asymptomatic or mildly symptomatic HTP using advanced echocardiographic and MRI techniques (Vinch et al., 2005, Bountioukos et al., 2006, Yuda et al., 2002, Pela et al., 2001, Rosen et al., 2006, Palmon et al., 1994, Aurigemma et al., 1995).

The need for formal quantitative assessment of myocardial systolic function remains a significant challenge. STE is an innovative technique in assessing LV twist mechanics quantitatively identifying global and regional dysfunction in a multitude of cardiac conditions. Two dimensional

strain and LV twist is not angle-dependent and has been shown to correlate well with EF measured both by echocardiography (Belghiti et al., 2008, Delgado et al., 2008) and MRI (Brown et al., 2009, Cho et al., 2006). LV twist occur as result of the apex of the LV moving in a counter clockwise direction while the base moves in a clockwise direction in systole. The resulting twist is thought to be pivotal in achieving a 60% reduction in LV volume despite only a 15% reduction in myocardial fibre shortening. Over and above the pattern of left ventricular geometric response to hypertension, abnormalities of cardiac mechanics based on LV twist mechanics may be important in understanding the pathogenesis of the transition from compensated to decompensated HF.

LV hypertrophy is associated with a variety of alterations including: fibrosis, impaired coronary artery vasodilatory capacity, depressed LV wall mechanics, and abnormal LV diastolic filling pattern (Weber and Brilla, 1991, Querejeta et al., 2000, Conrad et al., 1995, Brilla et al., 1991, Martinez et al., 2003). It is well known that a preclinical systolic dysfunction occurs in HTP with LV hypertrophy (Mizuguchi et al., 2010b, Narayanan et al., 2009) but, in addition, an impairment of longitudinal systolic function is seen even in patients with normal LV geometry. In this respect, STE appears more sensitive than conventional echocardiography in identifying a reduction of intrinsic myocardial contractility, evident in HTP long before LV hypertrophy becomes detectable. These early changes of myocardial contractility may be secondary to hemodynamic and/or biochemical alterations.

Our study documented a reduction in all strain parameters and increased LV twist in HTNEF. All patients with HTNEF had abnormal geometric patterns (85% LVH; 15% concentric remodelling). A reduction in LV LS with impairment in LV radial and CS in patients of NYHA III and IV functional class was found in 64 Caucasian patients with normal EF (Kosmala et al., 2008). The decline in LS with worsening HF precedes any other strain parameter and may be ascribed to the hypertension-associated damage of subendocardial myocardial fibres, which are mainly responsible for longitudinal function ((Kang et al., 2008, Kosmala et al., 2008). These fibres are more vulnerable to increased wall stress, stress-induced ischemia and microvascular dysfunction

accompanying hypertension. Furthermore, the impairment in radial and circumferential function, reflected by decreased respective strain values, appears later in the course of HHD and thereby postulated to contribute to global cardiac dysfunction (Kosmala et al., 2008). However, Palmon et al (Palmon et al., 1994) found that radial, circumferential, and LS appear to decline in parallel in hypertensive disease. Stanton et al (Stanton et al., 2009) speculated that LS < 12% could be recommended as diagnostic cut-off for LV dysfunction. There are discrepancies in myocardial strain abnormalities reported in HTP and this could be attributed to vendor variability, population diversity and variances in burden of disease.

Regionally differing patterns of changes in morphology and deformation in the basal LV segments are determined by the fibre structure of the myocardium and its interaction with local wall stress. The sub-endocardial fibres are mainly longitudinally oriented, while mid-wall fibres are circumferential (Greenbaum et al., 1981). Local wall stress is determined by curvature, with a higher level of wall stress seen with an increasing radius of curvature. While in the apex, the radii of curvature are similar in all directions, at the base, they are substantially smaller in the radial compared to the longitudinal direction. Both fibre orientation and local geometry result in a non-uniform wall stress distribution, with a decrease from endo- to epicardium and from equator to apex (Büchi et al., 1990, Hood Jr et al., 1969, Wong and Rautaharju, 1968). Due to a greater local radius of curvature, stress in basal segments will increase more than in mid and apical segments when LV pressure increases. Since hypertrophy is directly related to wall stress (Grossman et al., 1975) there must be a gradual increase in the hypertrophic response from apex to base.

In models of LV myocardial deformation, it has subsequently been shown that the LV myocardial fibre architecture is important for LV function (Arts et al., 1979, Ingels Jr, 1997). Adequate pressure generation is probably primarily produced via circumferential mid-wall fibres but also by subendocardial and subepicardial spiralling fibres that generate LV twist and shortening of the LV long axis (Ingels Jr, 1997). In previous work, it has been shown that LV twist is of fundamental importance to systolic LV function (Arts et al., 1979, Ingels Jr et al., 1989, Sengupta et al., 2008b,

Sengupta et al., 2006). LV twist may also be an important manner to equalize transmural differences in sarcomere shortening, end-systolic fibre stress, and contractile work during the ejection phase (Arts et al., 1979). The increased end-systolic wall stress, an expression of afterload, as well as the loading conditions, are likely to play a role in determining myocardial deformation dysfunction in HHD. The chronic increase of end systolic wall stress may promote subendocardial synthesis of collagen, thereby contributing to reduction of myocardial deformation.

Various factors, not only those that are directly related to the increased LV wall stress, are involved in the deterioration of myocardial deformation in the course of hypertension (Kosmala et al., 2008). It is well known that in LV hypertrophy (Kosmala et al., 2008), myocardial fibrosis related to pressure overload appears frequently in the more vulnerable subendocardial layer. Increased left ventricular mass index, one of the principle pathophysiologic determinants of LV dysfunction in hypertension, was independently associated with impaired myocardial deformation assessed by 2D strain [longitudinal, radial, and circumferential] (Kosmala et al., 2008). The same holds true for the duration of hypertension with a greater extent of systolic dysfunction accompanying a longer disease history (Kosmala et al., 2008).

It is well known that the net LV twist is the result of subepicardial contraction and subendocardial counterbalancing contraction (Ingels Jr et al., 1989). The epicardial fibres govern the direction of LV twist, mainly owing to their longer arm of movement. Therefore, it can be anticipated that the epicardial fibres may become even more dominant when the LV walls are thicker, in particular relative to LV cavity dimension, because in such walls the differences in the arms of movement will be greater. This may be due to the increased concentric hypertrophy leading to an increased lever arm for the epicardial fibres. In the normal LV, a counterclockwise-rotating torque in the subepicardium dominates a clockwise-rotating torque in the subendocardium, because the subepicardium fibres have a larger radius. An increase in relative wall thickness with hypertrophy will produce larger radius differences between endocardium and epicardium, resulting in an augmentation of peak twist.

Another mechanism is that reduced endocardial shortening may also lead to increased LV twist since the forces opposing twist are reduced. LVH impairs subendocardial perfusion with contractile dysfunction (Zhang et al., 1993, Lima et al., 1985, Johansson et al., 2008). The impairment of subendocardial myofibre shortening leads to a greater transmural shortening gradient, which is defined as epicardial minus endocardial myofibre shortening normalized to the mean value (Van der Toorn et al., 2002). Therefore, in patients with LV hypertrophy, transmural gradient of myofibre shortening may be greater resulting in an enhanced LV twist.

In addition, the possible mechanisms for the relative lack of compensatory increase in BR to AR may be related to subendocardial ischemia as a result of oxygen supply /demand mismatch, microvascular dysfunction and altered capillary/myocyte ratio in the absence of epicardial coronary artery disease. Due to the larger radii, the torque of subepicardial fibres dominates and accounts for the enhanced counterclockwise rotation of the LV apex. Previous studies have found that in LV hypertrophy, AR and LV twist are augmented (Wang et al., 2007, Takeuchi et al., 2007a). In HTP, this could be a compensatory mechanism aimed at maintaining relatively normal LV systolic function (Przewlocka-Kosmala et al., 2006).

However, we postulate that the LV dilatation after compensatory LV hypertrophy – a response to persistent pressure overload and augmented wall stress – is an important contributor to reduced myocardial deformation as a result of progressive deterioration of contractile function as the heart increases in size. Myocardial fibrosis is one of the factors responsible for myocardial function deterioration in hypertension (Komuro et al., 1991, Brilla et al., 1991). In HTP with normal EF, impaired LS and increased LV twist are directly related with serum levels of TIMP1, a marker of myocardial fibrosis (Martinez et al., 2003). This suggests that abnormal collagen turnover and myocardial fibrosis may contribute to early LV contractile dysfunction (Kang et al., 2008) with increasing collagen degradation contributing to greater systolic dysfunction. In the early stage the subendocardial longitudinal fibres are primarily affected so that unopposed epicardial torque will

increase apical twist, whereas later more widespread fibrosis or damage will affect global function and lead to reduced twist (Phan et al., 2009). It is likely that only transmural damage is associated with reduction of EF and twist, whereas subendocardial dysfunction (regional damage) results in myocardial strain impairment (Sengupta and Narula, 2008) and compensatory increase in LV twist.

It is unclear whether increased LV twist is a compensatory response to maintain the intracavitary pressure or a secondary change of abnormal fibre structure caused by subendocardial dysfunction in HTP with normal EF. Early detection of the LV dysfunction, before development of LVH, may represent a clinical finding that would justify aggressive treatment aimed at reducing cardiovascular mobility and mortality. Prevention of LV remodelling favourably impacts the untoward natural history of HF, which may be, at least partly, related to the preservation of LV twist. Therefore, assessment of LV twist may be of importance in the evaluation and guidance of therapies in HHD, especially if these therapies are to influence cardiac remodelling.

4.5 TWIST MECHANICS IN HYPERTENSIVE PATIENTS WITH LOW EF

LV twist was decreased in HTLEF, with a reduction in AR and BR. All patients in the HTLEF group had LV hypertrophy (concentric and eccentric hypertrophy). The reduction in both AR and BR may imply that both areas are affected by a more global abnormality that affects both subendocardial and subepicardial fibres. AR correlated positively with degree of LV dysfunction, suggesting degree of apical dysfunction is more important in LV dysfunction than the degree of basal dysfunction. LV twist is directly correlated with left ventricular systolic function (Pacileo et al., 2011). Furthermore, LV twist or AR alone provides a more accurate quantification of LV contractility than does LVEF (Kim et al., 2009).

Left ventricular AR represents the dominant contribution to LV twist. The mechanisms responsible for changes in twist dynamics are unclear, but might relate to LV dilatation, remodelling of cardiac myocytes and connective tissue matrix (Jin et al., 2007). The loss of LV twist is mainly due to a

decrease in counterclockwise AR. With the progression of the HHD, as a consequence of LV dilatation, the LV takes on a more spherical geometry and this advanced LV remodeling and the consequent loss of the oblique architecture of loop fibres, leads to impaired LV twist (Popescu et al., 2009). The differences in short-axis and long-axis dilatation result in changes in fibre angles that may further impair LV twist and thus cardiac function (Hutchins et al., 1978).

The progressive impairment of LV contractility and function involves not only the increase in LV dimensions and sphericity but also significant changes in the magnitude and pattern of LV rotation, especially RBR. In a recent study of patients with dilated cardiomyopathy (Popescu et al., 2009), RBR signified the expression of more advanced disease, with more severe LV remodelling, dyssynchrony, and systolic dysfunction compared to patients with normally directed AR. This study illustrates the intimate relationship between LV size, shape, and function: progressive LV dilation and increased sphericity leads to changes in myofibre orientation and LV twist mechanics with deterioration of systolic LV function.

Normally, looking at the heart directly from the anterior wall, the LV fibre helix angle varies from the subendocardium to the subepicardium (Geerts et al., 2002, Ingels Jr, 1997). Previous mathematical models revealed the essential role of LV twist in optimizing LV oxygen demand and the efficiency of LV systolic thickening by uniformly distributing myofibre stress across the myocardial wall (Sengupta et al., 2008a). The transmural oxygen demand was found to be a function of the structure of the LV fibres, and the degree of the twist (Lumens et al., 2006). A wider fibre angle distribution is associated with a larger epicardium-endocardium (epi-endo) stress difference and is thus associated with a higher metabolic gradient (Lumens et al., 2006). The twisting motion of the LV reduces the sarcomeres length gradient and the metabolic gradient. Similarly, shorter endocardial sarcomeres decrease the endocardial sarcomere lengths and, hence, decrease the metabolic gradient (Lumens et al., 2006).

LV rotation or twisting motion has an important role in LV systolic function by generating maximum intracavitary pressures with minimum fibre shortening thereby reducing myocardial oxygen demand (Sengupta et al., 2008b). The endocardial layer consumes more oxygen than the epicardial layer, indicating that the endocardial sarcomeres develop high stresses at the beginning of systole, and then decrease toward end systole due to the shortening of the sarcomeres (Lumens et al., 2006). The epicardial outer layer shortens to a lesser extent than the inner layers, and maintains the stresses throughout the systole (Lumens et al., 2006). A higher endocardial oxygen demand corresponds linearly to higher mechanical stresses in the endocardium (Lumens et al., 2006). A significant impairment of LV twist will therefore result in increased myofibre stress and oxygen demand resulting in supply/demand imbalance. This low-efficiency state would further impair myocardial contractility, possibly representing the initial step of a vicious circle of progressive LV dilatation and decline in LV systolic function.

However, factors that relate to remodelling such as neurohormonal and growth factors, cytokines, apoptosis etc. were not evaluated and may be an important determinant of remodelling and its subsequent effect on twist mechanics. Furthermore, increase in the size of the cardiomyocyte, alterations in the ECM, and abnormalities of the intramyocardial coronary vasculature may also contribute to abnormalities in twist mechanics. Moreover, other potential factors that may contribute to abnormalities of twist may relate to genetic abnormalities that may affect sarcomere function.

Current data indicates that rotational deformation in HF patients is a more accurate marker of changes in cardiac muscle performance or predictor of outcome than conventional measures such as EF or stroke volume (Burns et al., 2008, Sengupta et al., 2008b, Esch and Warburton, 2009, Sanderson, 2008). However, it is difficult to determine which occurs first: the decline in LV twist, which results in impaired systolic function and greater adverse remodelling, or adverse pathological remodelling resulting in a decrement of LV twist in HTP. We believe the association of RBR especially in a clockwise direction represents a novel assessment of more severe LV remodelling in

HTP with left ventricular systolic dysfunction in the absence of ischemia. The loss of LV twist may result in further decline in LV function and initiate a continuous, vicious cycle of remodeling. This raises the question whether RBR represents a more advanced state of remodeling or if it is the precipitant to the development of more advanced remodeling.

4.6 RELATIONSHIP BETWEEN BIOMARKERS OF COLLAGEN DEGRADATION AND LV TWIST IN HYPERTENSION INDUCED HEART FAILURE

LV dysfunction, irrespective of the cause of HF, leads to perturbed wall stress resulting in remodelling and HF progression. Although it is known that remodelling of the myocardial ECM occurs in HF, a consensus on the nature of these alterations does not exist, with some evidence favouring fibrosis at the point of end-stage HF (Conrad et al., 1995, Weber et al., 1989, Querejeta et al., 2004), and others depicting collagen depletion (Spinale et al., 1998, Spinale et al., 1996, Dell'italia et al., 1997). MMPs are the key enzymes responsible for ECM degradation whereas the TIMPs endogenously inhibit this process. The interplay and balance of MMPs and their tissue inhibitors (TIMPs) determine the maintenance of interstitial tissue homeostasis. The collagen network (collagen associated with groups of cardiomyocytes or perimysium, and collagen which surrounds and interconnects individual cardiomyocytes or endomysium) is responsible for providing the supportive scaffolding for cardiomyocytes, but also serves to integrate the individual contractions of these cells and provide a coordinated delivery of force (pressure) to the left ventricular chamber for expulsion of blood (Weber et al., 1994).

Some recent observations suggest that the ratio between MMPs and TIMPs, which is an indirect measure of MMP activity, may serve as a biomarker of LV remodeling (López et al., 2010). Circulating MMP1:TIMP1 ratio has been shown to be a biomarker of collagen degradation (López et al., 2006). Serum MMP1:TIMP1 ratio is associated with the degradation and loss of endomysial and perimysial collagen (González et al., 2009). Excess of MMP1 relative to TIMP1 is present in the myocardium of low EF HTP compared with preserved EF HTP (López et al., 2006). The up-

regulated expression of MMP1 is detected at both the cardiomyocyte and the interstitial level in HTP with low EF. Several studies have delineated the relationship between MMPs and TIMPs, and that the changes in the MMP1:TIMP1 ratio correlate with LVH and dilation (López et al., 2006, Lindsay et al., 2002, González et al., 2010, González et al., 2009, López et al., 2004, López et al., 2010, Kang et al., 2008).

This study revealed that in HTP with preserved EF, LV twist is increased while LV twist is diminished in HTP with low EF who initially present with HF. This suggests LV twist varies with the degree of remodelling and systolic function caused by hypertension. Moreover, circulating biomarkers of collagen are elevated to a greater degree – with decrements in myocardial deformation – in HTP with low EF compared to those with normal EF. Additionally, an inverse relationship between MMP1:TIMP1 ratio and LV twist even when adjusting for EF was noted; implying that the decline in LV twist may be related to the remodelling process of the ECM. HTP with normal EF have lower MMP1:TIMP1 ratios, suggesting less extracellular remodelling that does not affect all regions of the myocardium to the same extent. Prior studies have suggested the major pathophysiological abnormality in HTNEF patients is that of subendocardial ischemia (Fujii et al., 1992), which occurs in the absence of coronary disease and initially affects subendocardial fibres (Kang et al., 2008). Subendocardial ischemia results from diminished coronary flow autoregulation in the subendocardium compared to the subepicardium with increased transmural pressure loss within the microvasculature contributing to subendocardial underperfusion and vulnerability to ischemic injury (Fujii et al., 1992).

In HTP with normal EF, the degree and location of collagen degradation may relate to the myocardial deformation indices, i.e. interstitial and perivascular fibrosis and collagen degradation is likely to initially affect the subendocardial fibres in the presence of subendocardial ischemia (Kang et al., 2008), with decrement in LS due to their prominent subendocardial location (Kang et al., 2008), and thereafter the development of circular fibre dysfunction in the midwall (decreases in radial and CS) (Kang et al., 2008). If the greatest area of involvement is the subendocardium and

perhaps some degree of involvement in the midmyocardium, then LS will be most affected with varying decrements of circumferential and RS noted. Subendocardial fibre dysfunction will cause less opposition to the subepicardial fibres, thus the predominant rotatory effects of the subepicardial fibres dominate during systole and result in enhanced BR, AR and net LV twist. In our study, this postulate would explain the strain, AR and net LV twist observed in HTNEF. A nonsignificant trend of increased BR also was noted.

Higher MMP1:TIMP1 ratio was detected in HTP with low EF compared with HTNEF patients suggesting that this process is characterized by more extensive extracellular remodelling. We propose that perhaps this adverse remodelling is a more diffuse process that affects all regions of the myocardium to a similar degree, such that the relatively preserved subepicardial fibre function seen in HTNEF is abolished, resulting in impairment of all myocardial strain and LV twist. As collagen degradation increases, there is global dysfunction of both the subendocardial and subepicardial fibres, with diffuse collagen degradation in subepicardial and subendocardial fibres resulting in greater impairment in AR and LV twist. This accompanies greater decrements in strain parameters in HTP with low EF.

The decline in LV twist may relate both to factors that cause a global reduction in systolic function and specific remodelling factors that decrease LV twist. The loss or disruption of this collagen network may compromise systolic function by three mechanisms. Firstly, the loss of mysial collagen results in discontinuity of the ECM, compromising the alignment and support of the matrix (López et al., 2006). A second factor relates to the possible loss of synchronized sarcomere contraction (López et al., 2006). The final mechanism occurs as a consequence of increased MMP1 activity where there is myocardial slippage and, consequently, a decreased number of muscle layers in the left ventricle (López et al., 2006). Collagen loss promotes myocyte slippage, thereby altering LV structure and function with ventricular dilatation, increasing LV sphericity and greater systolic dysfunction if the process is exaggerated (Laviades et al., 1998, Li et al., 1998, Timms et al., 2002, Thomas et al., 1998, López et al., 2006). The loss of the oblique architecture of loop fibres and

abnormal muscle fibre orientation (Popescu et al., 2009) responsible for the wringing motion results in a decline in LV twist (van Dalen et al., 2010).

However, despite the available information on these biomarkers in HTP, a number of major issues remain that limit their clinical usefulness. In fact, based on current knowledge, it is not possible to determine prognostic, diagnostic, or predictive cut-off values for hypertensive cardiovascular damage. Although several studies show cut-off values, these values were not used in a prospective way. Moreover, although non-invasive identification of increased collagen turnover may be possible with evolving cardiac magnetic resonance and molecular techniques, they still require further investigation. Another major limitation in the field of collagen biomarkers is the lack of systematic meta-analysis of the multiple clinical studies published in order to both analyse the discrepancies in the results among different studies and identify reproducible patterns of alterations in the biomarkers.

4.7 LIMITATIONS

2D STE has the advantages of ease of application and analysis and good data reproducibility. However, the accuracy of the measurements largely depends on the image quality and the accuracy of tracking. 2D myocardial deformation analysis generally requires higher quality images than standard 2D and Doppler echocardiography. The analysis of the LS of the lateral and anterior walls, and the basal circumferential and RS of the posterior basal segments are technically more challenging than other segments, presumably due to the suboptimal image quality as a result of rib artefacts or proximity to non-myocardial structures such as the posterior mitral annulus. In particular, suboptimal image quality of basal LV short-axis recordings is a challenge 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, when the base descends toward the apex in systole (Opdahl et al., 2008). Furthermore, the imaged epicardium is sometimes too bright, causing signal saturation, which precludes discrimination of the subtleties of image contrast that allows speckle tracking to

work (Van Dalen et al., 2009). The size of a tracking point is in reality larger as the one that is displayed (Van Dalen et al., 2009). Therefore, placement of a tracking point in the epicardium can potentially result in stationary artefacts by tracking of non-moving speckles outside the heart (Van Dalen et al., 2009). Motion of the mitral valve leaflets in the area of tracking points placed on the endocardium will potentially interfere with proper speckle tracking as well (Van Dalen et al., 2009). Finally, and probably most importantly, in a significant number of patients, true AR will be underestimated to a variable extent because of inability to visualize the true LV apex (Van Dalen et al., 2009). The short axis recordings and analyses should be done meticulously (Van Dalen et al., 2009). Currently, the anatomical landmarks are to loosely defined and in particular attention should be made to record the correct cross-section at the LV apical level (Van Dalen et al., 2009).

4.8 FUTURE RESEARCH

Vendor diversity of currently available speckle tracking software requires investigation to determine whether quantitative values obtained from every system are similar. The lack of standardization of imaging planes and different speckle-tracking algorithms among vendors make it difficult to make comparisons or establish normal values for LV twist with high levels of confidence. A multicenter study in a large number of normal subjects with different ultrasound machines is required to resolve this issue. The lack of agreement between equipment and proprietary software preventing the adoption of universally accepted thresholds of abnormality. Given differences in image processing and the information used to derive a final strain value among manufacturers, follow-up echocardiography for strain analysis using a different manufacturer's system may provide misleading results. In a recent expert consensus statement from the American Society of Echocardiography and the European Society of Echocardiography, a recommendation was made that more comparisons between vendor-specific software packages must be made before STE can become routinely adopted in clinical practice (Mor-Avi et al., 2011). Most reports evaluating differences between STE vendors report data analyzed from the images directly using echocardiographic equipment and software from the same manufacturer (Manovel et

al., 2010, Biaggi et al., 2011, Sun et al., 2012b, Koopman et al., 2010). The limitation of applying these studies to clinical practice is that many echocardiography labs use equipment from multiple vendors for image acquisition and may have STE software from one or more vendors. Furthermore, many echocardiographic labs store images in a neutral imaging format, necessitating the use of STE programs that analyze vendor-neutral images for patient follow-up.

Therefore, what remains to be resolved is to what extent such differences are clinically relevant. Caution is therefore advised when trying to generalize results between current competing STE vendors. Moreover, population samples representing diseases affecting cardiac function should be studied to determine the diagnostic power and abnormal ranges of LV twist values. Clinically, until standardization is achieved, echocardiography labs should identify lab-specific normal values with each vendor analysis package and use the same software in serial studies. Most studies, however, have been limited to images acquired by a single ultrasound machine manufacturer using the same vendor-specific analytic tool. Although appropriate for early research applications, the use of a single vendor is impractical because it prevents widespread prospective or retrospective clinical or research applications in large populations across multiple imaging platforms, software packages, and institutions.

Three-dimensional STE is a new technique that provides information regarding different parameters of LV myocardial deformation (Pérez de Isla et al., 2008) with a better assessment of regional myocardial mechanics, which might be important for diagnosis, prognosis, and therapy. 3D STE is likely to allow standardization of LV planes used for assessing twist measurements. However, currently available 3D speckle tracking echocardiographic methods are based on different algorithms, which introduce substantial differences between them making them not interchangeable with each other. Therefore, it is critical that each 3D speckle-tracking echocardiographic method is validated individually before being introduced into clinical practice.

Identifying subclinical left ventricular (LV) systolic dysfunction among hypertensive subjects might be helpful in identifying patients at higher risk for HF. Given the prevalence of hypertension in the population, identifying patients at high risk for HF might permit targeted preventive strategies. STE allows comprehensive assessment of myocardial function and the spectrum of potential clinical applications is actually expansive. In particular, a growing body of evidence suggests that the assessment of LV twisting is feasible and useful in several clinical settings. The relationship between strain and twist mechanics of the LV provides insight into the transmural heterogeneity in myocardial contractile function. The presence of a subendocardial- to-subepicardial gradient in LV mechanics may provide a useful clinical measure for early recognition of a subclinical state that is likely to progress into systolic HF. So LV twisting dynamics appears useful in detecting early LV dysfunction in the setting of systemic diseases with cardiac involvement such as arterial hypertension.

The pathway from hypertension with normal or increased twist with preserved EF to that of diminished twist in HTLEF may not be unidirectional. Further prospective research is warranted in assessing whether patients with HTLEF and normal patterns of twist develop clockwise AR during progression of LV remodelling. Moreover, further research is needed to determine if more adverse clinical sequelae will occur in patients with RBR. Furthermore, the impact of drug therapy is unknown in patients with HTLEF i.e. do some patients exhibit reverse remodelling and normalisation of twist mechanics on good medical therapy. However, whether a strategy tailored to normalise LV twist provides additional clinical benefit compared with HF controlled to conventional targets is uncertain. In addition, research is required to assess whether patients with RBR have a longer duration of congestive cardiac failure or a shorter duration of adequately treated HF.

4.9 CONCLUSION

This study established normative data and patterns for myocardial deformation (strain and LV twist) in a normal black-African adult population across different age groups and can be used as a baseline for future studies. Age was the major determinant of increased LV twist in a normal black population. LV twist may be a compensatory mechanism to preserve EF and maintain normal systolic function with advancing age and in hypertension. LV twist varies with the degree of remodeling and systolic function in hypertension. RBR represents a novel assessment of more severe LV remodeling and LV systolic dysfunction in hypertensive patients. Alterations in collagen turnover not only accompanies more adverse remodelling but also contributes to LV twist differences observed between HTLEF and HTNEF patients. The inverse relation between LV twist and loss of myocardial collagen scaffolding suggests that integrity of the extracellular matrix may play an important role in preservation of LV twist. These findings highlight the value of LV twist as a sensitive global parameter of LV systolic myocardial performance. Its impairment possibly plays an important role in the development of LV remodelling. LV twist may provide significant value over clinical and echocardiographic features in predicting LV remodelling. Accordingly, longitudinal studies assessing LV twist may provide significant value in clinical practice as an early marker for risk stratification in hypertensive patients who may benefit from aggressive medical therapy to prevent LV remodelling and heart failure, and poor outcomes.

APPENDICES

APPENDIX a - Ethics Clearance

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Dr Nirvarthi Maharaj

CLEARANCE CERTIFICATE	**M110204**
PROJECT	The progression of compensated to decompensated Left ventraicular dyfunction in an African
Hypertensive	population.
INVESTIGATORS	Dr Nirvarthi Maharaj.
DEPARTMENT	Department of Internal Medicine/ Cardiology
DATE CONSIDERED	25/02/2011
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 25/03/2011 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor : Dr Elena Libhaber

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**
PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...

APPENDIX b - Left Ventricular Twist in a Normal African Adult Population

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Left ventricular twist in a normal African adult population

Nirvarthi Maharaj[1], Ferande Peters[1], Bijoy K. Khandheria[2]*, Elena Libhaber[1], and Mohammed R. Essop[1]

[1]Division of Cardiology, Chris Hani Baragwanath Hospital and University of the Witwatersrand, Johannesburg, South Africa; and [2]Aurora Cardiovascular Services, Aurora Sinai/Aurora St. Luke's Medical Centers, University of Wisconsin School of Medicine and Public Health, 2801 W. Kinnickinnic River Parkway, Suite 840, Milwaukee, WI 53215, USA

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Aims Speckle tracking has emerged as a quantitative technique for assessing left ventricular (LV) function. However, no normative data for LV twist using speckle tracking echocardiography (STE) are available in the black population. This study assessed myocardial mechanics by determining LV twist parameters in different age groups using STE, and evaluated the effect of ageing on LV twist in this population.

Methods and results The study population consisted of 127 healthy volunteers divided into four age groups: 20–29 ($n = 34$); 30–39 ($n = 33$); 40–49 ($n = 29$); and 50–65 ($n = 31$) years. Parasternal short-axis images of three consecutive end-expiratory cardiac cycles at LV basal, papillary muscle, and apical levels, and apical four-chamber images were obtained. Apical and (AR) basal (BR) LV peak systolic rotation during ejection and instantaneous LV peak systolic twist (net twist, defined as maximal value of instantaneous AR minus BR) were measured. Mean strain values were $-17.28 \pm 3.30\%$ for longitudinal, $-17.40 \pm 3.29\%$ for circumferential, and $57.49 \pm 3.32\%$ for radial strain. Mean rotational values were AR ($5.56 \pm 1.98°$), BR ($-3.31 \pm 0.92°$), and net twist ($8.87 \pm 2.21°$). AR, BR, and net twist increased with age, whereas longitudinal, circumferential, and radial strain decreased with age. Multivariate linear regression analysis showed age as the main predictor of net twist ($R^2 = 0.82$, $P < 0.0001$).

Conclusion These data establish values for strain and twist in a normal black adult population. Net twist increases with age, whereas strain parameters decrease. Age is the strongest independent predictor of LV twist.

Keywords Speckle tracking • Twist • Torsion • Strain

Introduction

Left ventricular (LV) twist is defined as the wringing motion whereby the LV apex rotates in an opposite direction with respect to the LV base about the LV long axis.[1] LV twist has a vital role in ensuring that blood is ejected in an efficient manner during systole. Twist mechanics of the left ventricle obtained using speckle tracking echocardiography offer new insights into the assessment of LV performance. Speckle tracking is a novel echocardiographic technique for the evaluation of LV twist that has been validated against magnetic resonance imaging (MRI) tagging[2] and has contributed to an explosion of research utilizing this technique in various cardiac abnormalities. Several pathological states have been identified in which the abnormality of LV twist is a significant contributing factor to LV systolic dysfunction.

In healthy humans, there is a continuum of cardiac structural and functional alterations that occur with ageing.[3–5] These age-associated cardiac changes may have relevance to the increased incidence of LV hypertrophy, chronic heart failure, and atrial fibrillation seen with increasing age.[3–5] Several published studies describe LV twist forces in normal adults, and there appears to be an increase in LV twist with advancing age.[4–11] Whether this relates solely to the ageing process or is associated with concomitant physiological or comorbid conditions that occur with ageing is unknown.

The aim of this study was to establish normal values for LV twist in black Africans and evaluate the effect of ageing on LV twist in this population.

* Corresponding author. Tel: +1 414 649 3909; Fax: +414 649 3278, Email: publishing32@aurora.org
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Methods

The study population comprised randomly chosen volunteers recruited from Chris Hani Baragwanath Hospital staff and visitors and Soweto community members from January 2011 to May 2012. All subjects underwent clinical evaluation, which included history and physical examination. Only subjects who were asymptomatic and normotensive, had no evidence of cardiovascular or systemic diseases on history and physical examination, and had normal 12-lead electrocardiogram underwent echocardiography. All subjects who had entirely normal echocardiograms and adequate image quality for speckle-tracking analysis were enrolled in the study after informed consent was obtained. A total of 150 subjects were screened for inclusion. Twenty-three subjects were excluded as a result of inadequate image quality, which did not allow for complete segmental assessment of LV rotation at both the basal and apical left ventricle. The remaining 127 subjects made up the study group and were classified into four age groups: 20–29 ($n = 34$), 30–39 ($n = 33$), 40–49 ($n = 29$), and 50–65 ($n = 31$) years. This study was approved by the University of Witwatersrand Ethics Committee (clearance number: M110204) and Institutional Review Board.

Echocardiography

Comprehensive transthoracic echocardiography was performed using a commercially available system (iE33 xMATRIX, Philips Healthcare, Andover, MA, USA) equipped with an S5–1 transducer (frequency: 1.7 MHz transmitted, 3.4 MHz received) according to a standardized protocol. All echocardiographic measurements were averaged from three heartbeats. Measurements relating to chamber size and function were performed in accordance with the American Society of Echocardiography (ASE) chamber quantification guidelines[12] and the ASE 2010 guidelines on right heart assessment.[13] Severity of mitral and tricuspid regurgitation was analysed in accordance with the ASE guidelines on native valvular regurgitation.[14] Ejection fraction was calculated from LV volumes by the modified biplane Simpson rule in accordance with the guidelines.[12] From the mitral-inflow pattern, peak early (*E*) and late (*A*) filling velocities, *E*/*A* ratio, and *E*-velocity deceleration time were measured. Pulsed-wave tissue Doppler was performed at end-expiration at the level of the inferoseptal mitral annulus from an apical four-chamber view. To acquire the highest wall tissue velocities, the angle between the Doppler beam and the longitudinal motion of the investigated structure was adjusted to a minimal level. The spectral pulsed-wave Doppler velocity range was adjusted to obtain an appropriate scale. The time intervals between the peak of the R-wave on the electrocardiogram and the aortic valve opening and closure, and between the R-wave and the mitral valve opening and closure were measured using pulsed-wave Doppler acquired from the LV outflow and inflow, respectively. LV mass was calculated using the formula: LV mass $= 0.8 \times \{1.04[(\text{LVIDd} + \text{PWTd} + \text{SWTd})^3 - (\text{LVIDd})^3]\} + 0.6$ g, where LVIDd, PWTd, and SWTd are LV internal diameter at end-diastole, posterior wall thickness at end-diastole, and septal wall thickness at end-diastole, respectively.[12,15] Relative wall thickness was calculated using the formula: $(2 \times \text{PWTd})/\text{LVIDd}$.[12,15]

Speckle-tracking analysis

Two-dimensional images were obtained at a frame rate of 60–80 frames/s. Parasternal short-axis images at the LV basal level showing the tips of the mitral valve leaflets were obtained with cross-sections as circular as possible. To obtain short-axis images at the true LV apical level, the transducer was positioned one or two intercostal spaces more caudally and acquired in a manner described by van Dalen *et al.*[16] LV longitudinal strain analysis was performed using the apical four-chamber view. Foreshortened images were avoided by looking for the largest long-axis dimensions. From short-axis views, radial strain, circumferential strain, and rotational data were averaged across six wall segments (anteroseptal, anterior, lateral, posterior, inferior, and septal) at the basal and apical levels. Global circumferential strain was also measured from parasternal short-axis views at the papillary muscle level. The width of the region of interest was adjusted to include the entire myocardium. Analysis of the data sets was performed using QLAB Advanced Quantification Software (Version 8.0, Philips, Best, The Netherlands). In all instances, mean longitudinal, circumferential, and radial strain for each subject was calculated by the sum of the strain measured in each anatomic region divided by the number of segments analysed in that particular view.

To assess LV rotation, six tracking points were placed on an end-diastolic frame[17] in each parasternal short-axis image automatically encompassing the endocardium and epicardium. Tracking points were separated ~60° from each other and placed at 1 o'clock (30°, anteroseptal insertion into the left ventricle via the right ventricle), 3 o'clock (90°), 5 o'clock (150°), 7 o'clock (210°), 9 o'clock (270°, inferoseptal insertion into the left ventricle via the right ventricle), and 11 o'clock (330°) to fill the total LV circumference.[17] In all instances in which tracking points were manually manipulated to optimize speckle tracking, no tracking points were allowed to be moved more than 30°. Counterclockwise rotation as viewed from the apex was expressed as a positive value; clockwise rotation was expressed as a negative value. End-systole was defined as the point of aortic valve closure.

Analysis was performed to evaluate the peak basal and apical bulk rotations during the ejection phase and the net instantaneous twist of the left ventricle, which was calculated as peak apical rotation (AR) minus basal rotation (BR) at a time isochronous with the peak AR. Analysis was performed to note the patterns of AR and BR during isovolumic contraction and during the ejection phase characterized by clockwise BR and counterclockwise AR in systole. The curves generated by the QLAB software include automated analysis of subendocardial and subepicardial regions by speckle tracking at the base and apex. In all subjects, the direction, timing, and peak values of subendocardial and subepicardial rotations at both the apex and base were documented. The untwisting rate was defined as the mean diastolic untwisting velocity from instantaneous peak systolic twist to mitral valve opening and calculated as follows: (twist at mitral valve opening – peak systolic twist)/time interval from peak systolic twist to mitral valve opening.

Statistical analysis

Database management and statistical analyses were performed with the SAS software (Version 9.2, SAS Institute, Inc., Cary, NC, USA). Data are presented as mean ± standard deviation. Continuous variables between age categories were compared using ANOVA, or the Kruskal–Wallis test when the distribution was non-normal. Two-by-two comparisons were then performed applying the Bonferroni correction, with a *P*-value <0.0083 denoting significance. Univariate and multivariate linear regression analyses were used to identify possible independent determinants of net twist. Three separate models to predict net twist were used in a multiple linear regression analysis. Interobserver variability was assessed for twist measurements in 20 randomly selected patients and calculated as the standard deviation of the differences between measurements of two independent observers who were unaware of the other patient data; these calculations were expressed as a percentage of the average value. Intraobserver variability was calculated as the standard deviation of the differences between a first and second determination of a single

observer, and expressed as a percentage of the average value. *P*-values <0.05 were considered statistically significant. Intraobserver and interobserver variability for LV rotation parameters (AR and BR, net twist, and longitudinal, circumferential, and radial strain) in our centre varies from 2 ± 3 to 10 ± 9%, and from 4 ± 4 to 12 ± 8%, respectively.

Results

Baseline and echocardiographic characteristics

The baseline general characteristics of the study sample are presented in *Table 1*. A significant increase in systolic blood pressure ($P = 0.03$), indexed LV mass ($P = 0.002$), and relative wall thickness ($P = 0.008$) was observed with increasing age.

LV strain and rotation analysis

Mean strain values for all subjects were −17.28 ± 3.30% for longitudinal strain, −17.40 ± 3.29% for circumferential strain, and 57.49 ± 3.32% for radial strain (*Table 2*). Mean rotational values for the study sample were 5.56 ± 1.98° for AR, −3.31 ± 0.92° for BR and 8.87 ± 2.21° for net LV twist (*Table 3*). Mean subendocardial and subepicardial rotational values for both AR (5.74 ± 2.04 vs. 5.44 ± 1.96°, respectively) and BR (−3.33 ± 0.96 vs. −3.10 ± 1.14°, respectively) were evaluated in all subjects

Table 1 Baseline clinical and echocardiographic characteristics

Variable	Total	20–29	30–39	40–49	50–65	*P*-value
Number of subjects	127	34	33	29	31	
Age (years)	39.7 ± 12.1	25.0 ± 2.8	34.9 ± 2.2	45.0 ± 2.7	55.8 ± 4.9	<0.0001
Sex (F:M ratio)	64:63	18:16	15:18	15:14	16:15	0.45
Body mass index (kg/m^2)	24.9 ± 2.4	24.2 ± 2.6	24.2 ± 3.0	25.5 ± 2.0	25.7 ± 1.3	0.06
Body surface area (m^2)	1.7 ± 0.2	1.8 ± 0.2	1.7 ± 0.2	1.8 ± 0.1	1.7 ± 0.1	0.10
Systolic blood pressure (mmHg)	124 ± 5	123 ± 5	123 ± 6	125 ± 5	126 ± 4	0.03
Diastolic blood pressure (mmHg)	77 ± 9	74 ± 10	76 ± 9	80 ± 6	78 ± 8	0.05
Pulse pressure (mmHg)	47 ± 8	49 ± 9	47 ± 8	45 ± 6	47 ± 8	0.30
Heart rate (b.p.m.)	73 ± 10	70 ± 10	75 ± 9	77 ± 9	71 ± 11	0.01
EDD (mm)	45 ± 4	45 ± 4	45 ± 4	45 ± 3	46 ± 3	0.89
IVSD (mm)	10 ± 2	9 ± 2	10 ± 2	10 ± 1	10 ± 2	0.007
LVPWD (mm)	9 ± 1	8 ± 1	8 ± 1	9 ± 1	9 ± 1	0.009
EDVI (ratio)	42.2 ± 6.1	42.0 ± 5.5	42.2 ± 5.9	41.9 ± 6.0	42.7 ± 7.4	0.77
ESVI (ratio)	18.5 ± 4.7	18.4 ± 4.4	19.3 ± 5.1	18.6 ± 4.6	17.5 ± 4.8	0.55
Ejection fraction (%)	62.4 ± 7.0	61.8 ± 7.1	60.4 ± 6.9	62.3 ± 6.1	65.2 ± 7.4	0.06
Relative wall thickness (ratio)	0.38 ± 0.05	0.36 ± 0.05	0.37 ± 0.06	0.39 ± 0.04	0.40 ± 0.04	0.008
LVMI (g/m^2)	81.4 ± 18.0	72.5 ± 19.7	80.8 ± 17.2	83.9 ± 15.0	89.4 ± 15.5	0.002
LAVI (mL/m^2)	22.6 ± 3.2	22.6 ± 2.4	22.2 ± 4.7	22.4 ± 2.5	23.4 ± 2.5	0.34
Peak *E* velocity (cm/s)	86.9 ± 14.5	88.8 ± 14.7	91.4 ± 17.2	84.4 ± 14.0	82.4 ± 9.8	0.06
E/*A* ratio	1.47 ± 0.22	1.66 ± 0.26	1.48 ± 0.19	1.40 ± 0.12	1.32 ± 0.12	<0.0001
Peak *E*′ medial wall velocity (cm/s)	11.6 ± 1.2	12.0 ± 1.3	11.8 ± 1.2	11.6 ± 1.2	11.1 ± 0.7	0.03
Peak *E*′ lateral wall velocity (cm/s)	14.5 ± 2.1	15.6 ± 2.3	15.5 ± 1.8	13.8 ± 1.3	13.0 ± 1.3	<0.0001
E/*E*′ ratio	6.66 ± 0.92	6.47 ± 0.98	6.70 ± 1.06	6.63 ± 0.78	6.86 ± 0.79	0.40

Data reported as mean ± standard deviation.
EDD, end-diastolic diameter; EDVI, end-diastolic volume index; ESVI, end-systolic volume index; F:M, female to male; IVSD, interventricular septum end-diastolic diameter; LAVI, left atrial volume index; LVMI, left ventricular mass index; LVPWD, left ventricular posterior wall diameter.

Table 2 Strain parameters with age

Variable	Total	20–29	30–39	40–49	50–65	*P*-value
Number of subjects	127	34	33	29	31	<0.0001
Longitudinal strain (%)	−17.28 ± 3.30	−21.76 ± 0.84	−17.71 ± 0.76	−15.66 ± 0.76	−12.90 ± 1.17	<0.0001
Circumferential strain (%)	−17.40 ± 3.29	−21.56 ± 0.96	−18.41 ± 0.29	−15.69 ± 0.74	−12.88 ± 1.17	<0.0001
Radial strain (%)	57.49 ± 3.32	61.71 ± 0.91	58.51 ± 0.52	55.71 ± 0.72	52.80 ± 1.28	<0.0001

Data reported as mean ± standard deviation.

Table 3 Rotational parameters with age

Variable	Total	20–29	30–39	40–49	50–65	P-value
Number of subjects	127	34	33	29	31	
AR (degrees)	5.56 ± 1.98	4.11 ± 0.74	4.41 ± 0.88	5.73 ± 1.21	8.22 ± 1.56	<0.0001
Subendocardial AR (degrees)	5.74 ± 2.04	4.30 ± 0.81	4.53 ± 0.94	5.92 ± 1.32	8.45 ± 1.62	<0.0001
Subepicardial AR (degrees)	5.44 ± 1.96	4.00 ± 0.68	4.32 ± 0.81	5.58 ± 1.19	8.09 ± 1.62	<0.0001
BR (degrees)	−3.31 ± 0.92	−2.64 ± 0.64	−3.37 ± 0.87	−3.52 ± 0.73	−3.77 ± 1.03	<0.0001
Subendocardial BR (degrees)	−3.39 ± 0.94	−2.77 ± 0.67	−3.43 ± 0.94	−3.59 ± 0.70	−3.85 ± 1.07	<0.0001
Subepicardial BR (degrees)	−3.16 ± 1.12	−2.34 ± 1.26	−3.29 ± 0.88	−3.41 ± 0.82	−3.68 ± 0.98	<0.0001
Time to peak rotation (ms)	357 ± 41	342 ± 38	353 ± 44	361 ± 28	372 ± 46	0.02
Net twist (degrees)	8.87 ± 2.21	6.75 ± 0.81	7.79 ± 0.74	9.25 ± 0.90	12.00 ± 1.38	<0.0001
Untwist rate (degree/s)	52.19 ± 5.56	55.40 ± 2.82	49.14 ± 6.38	50.22 ± 5.50	53.75 ± 4.61	<0.0001

Data reported as mean ± standard deviation.
AR, apical rotation; BR, basal rotation.

(*Table 3*). Both subendocardial and subepicardial rotation values for the apex (counterclockwise, positive values) and base (clockwise, negative values) were recorded in the same direction (*Figure 1*) in all subjects. The mean untwisting rate was 52.19 ± 5.56°/s (*Table 3*).

Age-related changes in strain and LV twist

There was a significant decrease in longitudinal, circumferential, and radial strain with advancing age (*Table 2*). Net twist increased with advancing age and was accompanied by an increment in both AR and BR (*Table 3*, *Figure 2*). Differences in AR were progressively more pronounced after age 30 [4.41 ± 0.88° (30–39); 5.73 ± 1.21° (40–49); 8.22 ± 1.56° (50–65); $P < 0.0001$] (*Figure 2*, *Table 3*). There were no significant differences in BR among age groups ≥40 ($P > 0.05$) (*Figure 2*). Net twist changed substantially in those ≥40 years old [9.25 ± 0.90° (40–49); 12.00 ± 1.38° (50–65)] (*Figure 2*). Subendocardial and subepicardial rotational values at the apex and base increased with age (*Table 3*).

Factors determining LV twist

Univariate linear regression analysis showed that age, ejection fraction, left ventricular mass index (LVMI), and longitudinal, circumferential, and radial strain were independently associated with net twist ($P < 0.05$ to $P < 0.0001$) (*Table 4*). *Table 5* summarizes results of a multivariate linear regression analysis identifying predictors of net twist. Insertion of ejection fraction, LVMI, end-systolic volume index, body surface area, pulse pressure, and longitudinal, radial, and circumferential strain as potential covariates in three different models of multivariate analyses did not change the final models generated, which demonstrated age and male sex as the main independent predictors of net twist (*Table 5*).

Discussion

Main findings

This study describes normative values for LV twist and its determinants in a black adult population. The pattern of rotation during systole in our study was that of the apex rotating counterclockwise while the base rotated clockwise. Regional subepicardial and subendocardial rotation at the apex and base were both counterclockwise and clockwise, respectively, in keeping with the overall global pattern of rotation observed at these anatomical sites. There was a trend of increasing AR, BR, and net twist with ageing, whereas longitudinal, radial, and circumferential strain decreased with ageing. Age and male gender independently predicted LV twist following multivariate regression analysis.

Twist is the net difference between AR and BR when the left ventricle is viewed from the apex.[18,19] In subjects of African descent, no data exist on the analysis of these parameters in normal individuals. Our findings reveal that the values of BR, AR, and net twist using the QLAB software do not differ from findings in other populations using this vendor's technology.[19] Additionally, the values obtained for AR and BR in this study are less than those reported in normal populations using different vendor technologies.[19–22]

Our findings, where at the base and apex, subendocardial and subepicardial rotations occur in the same direction as each other, and peak simultaneously but differ in magnitude, are in keeping with those of other studies.[23–25] These rotational patterns are noted irrespective of the speckle-tracking vendor technology used.[23–25]

It is postulated that subendocardial fibres in the apex and base move in the same direction, whereas the subepicardial fibres in these regions move in the opposite direction and that the interaction between these fibres in the helical architecture contributes to LV twist.[19] Net rotation is thought to be predominantly counterclockwise at the apex due to the longer arm of rotation of subepicardial fibres. This postulate cannot be assessed in this study since speckle tracking only tracks the movement of speckles in these anatomical regions, which is not synonymous with assessing myocardial fibre movement in these regions. We postulate that perhaps current speckle-tracking technology is unable to sufficiently assess fibre movement due to its limitations with regard to both spatial and temporal resolution.

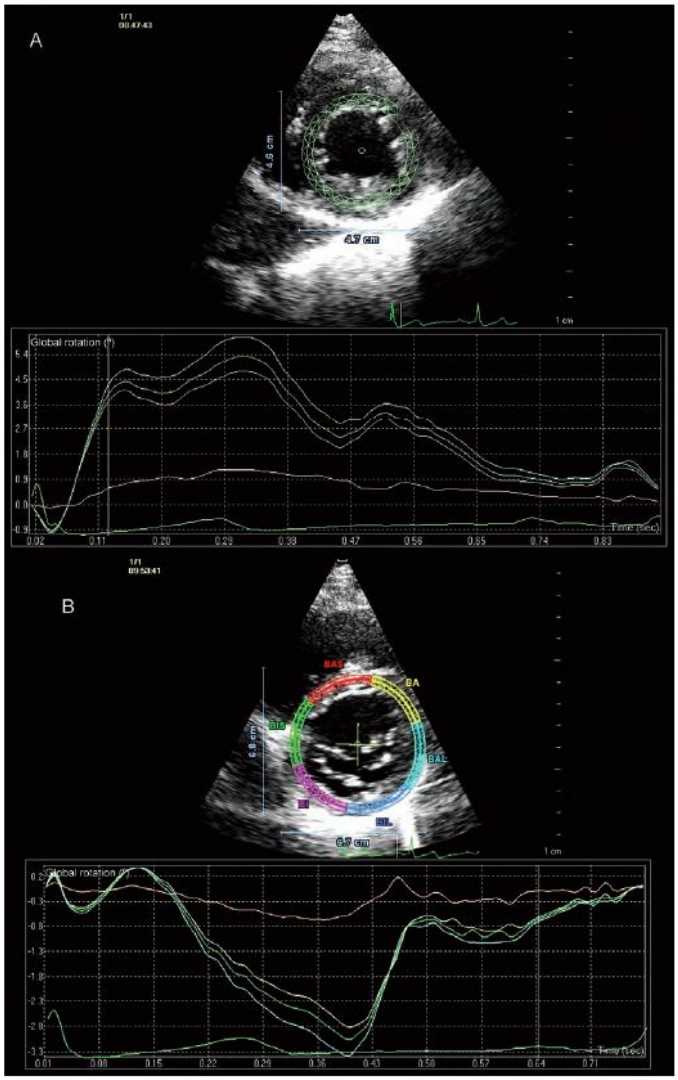


Figure 1 Subendocardial and subepicardial rotations at base (*A*) and apex (*B*). BA, basal anterior; BAL, basal anterolateral; BAS, basal anteroseptal; BI, basal inferior; BIL, basal inferolateral; BIS, basal inferoseptal.

Ageing affects many aspects of cardiovascular function.[3–5,26,27] Advancing age is associated with structural and functional changes in the myocardium, e.g. myocardial fibrosis and impaired coronary perfusion.[3–5,27] Increased LV wall thickness, alterations in the diastolic filling pattern, impaired LV ejection, and heart rate reserve capacity are the most dramatic changes in cardiac function that occur with ageing in healthy persons.[3–5] Although these age-associated changes do not always reflect clinical heart

disease per se, they may compromise cardiac reserve and alter the threshold for disease manifestation.

Our findings reveal that net twist increased with age due to an increase in both basal and AR. However, there appears to be a temporal dispersion in the increment of AR and BR. We observed that AR increased substantially after age 40, whereas no significant increment occurred in BR after age 40, resulting in a major increment in net twist after age 40. BR also was less influenced by age in other published data.[19–22] Van Dalen et al.[19] reported a mean value of peak LV twist as 7.7 ± 3.58° in a white population, with peak LV twist significantly greater in subjects aged ≥60 years (10.8 ± 4.98°) compared with those aged ≤40 years (6.7 ± 2.98°) and those aged 41–59 years (8.0 ± 3.08°).[19] Takeuchi et al.[21] examined the effect of ageing on twist in 113 Asian volunteers; peak LV twist increased significantly in those aged ≥60 years with similar values as published on whites. Furthermore, Zhang et al.[22] showed a greater increment in AR from subjects aged 45–55 years (7.94 ± 1.20°) to those aged 55–65 years (9.65 ± 1.56°), and a lesser increase in BR [−6.50 ± 2.49° (45–55 years) vs. −7.07 ± 1.25° (55–65 years)].

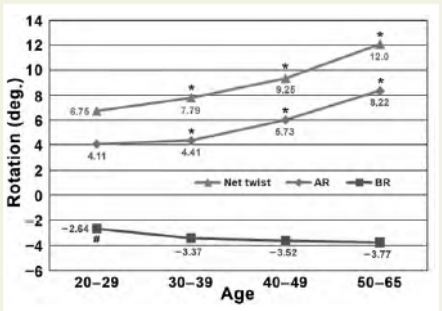


Figure 2 Age-related values in peak rotation and twist parameters. *$P < 0.0001$ when compared with the age 20–29 group. #$P = 0.0005$ when compared with the age 30–39 group. AR, apical rotation; BR, basal rotation.

LV twist characteristics undergo substantial changes during the ageing process. The increase in LV twist can be partially explained by lessened opposition to AR, which results from a gradual decrease in subendocardial function with ageing.[19–22] Impairment of subendocardial contractile function may be on the basis of ischaemia or fibrosis and results in diminished clockwise systolic BR rotation, which leads to less opposition of counterclockwise systolic AR, thereby causing net twist to increase.[19–22]

An analysis of other aspects of myocardial mechanics revealed a concurrent reduction in longitudinal, circumferential, and radial strain with increasing age. Whereas some researchers described reduced strain with increasing age[28–30] or increasing strain with ageing,[11] others found no correlation between strain and age[31] in other populations. Moreover, some studies using speckle tracking found some strain parameters (circumferential and radial) increased,[10,11] whereas longitudinal strain decreased.[10] Another possible rationale for the increase in twist with ageing is a compensatory mechanism for the decline in other strain parameters.

LV twist is an effective method to preserve LV systolic function, as the helical fibre architecture of the heart doubles the ejection fraction.[26,27] We postulate that increased net twist with advancing age may demonstrate a possible contribution of net twist to preserving ejection fraction and maintaining normal systolic function of the left ventricle.

AR is the primary contributing factor of LV twist, with an increase in AR resulting in an increase in twist. This implies that apical function is perhaps the most important determinant for LV twist, and one would expect that pathological states that impair apical function to a greater degree than other anatomic sites may result in greater LV dysfunction. However, age was the main independent predictor of net LV twist in our study. Furthermore, despite an increase in relative wall thickness, LVMI, systolic blood pressure, and changes in diastolic indices with advancing age in

Table 4 Univariate linear regression analysis for net twist

Variable	β coefficients ± standard error	R^2	P-value
Age	0.164 ± 0.007	0.80	<0.0001
Sex (F:M ratio)	0.476 ± 0.392	0.01	0.23
Body surface area (m^2)	−2.654 ± 1.340	0.03	0.05
Systolic blood pressure (mmHg)	0.066 ± 0.039	0.02	0.09
Heart rate (b.p.m.)	0.003 ± 0.020	0.0002	0.87
LVMI (g/m^2)	0.037 ± 0.010	0.09	0.0006
Ejection fraction (%)	0.063 ± 0.028	0.04	0.02
ESVI (ratio)	−0.067 ± 0.042	0.02	0.11
Longitudinal strain (%)	0.577 ± 0.031	0.74	<0.0001
Circumferential strain (%)	0.59 ± 0.029	0.77	<0.0001
Radial strain (%)	−0.524 ± 0.033	0.67	<0.0001

ESVI, end-systolic volume index; F:M, female to male; LVMI, left ventricular mass index.

Table 5 Multivariate linear regression analyses for net twist

	β coefficients ± standard error	P-value
$R^2 = 0.81$; $P < 0.0001$		
Age (years)	0.167 ± 0.008	<0.0001
Sex (F:M ratio)	0.382 ± 0.176	0.03
Pulse pressure (mmHg)	0.004 ± 0.011	0.73
LVMI (g/m^2)	−0.003 ± 0.005	0.57
$R^2 = 0.82$; $P < 0.0001$		
Age (years)	0.159 ± 0.007	<0.0001
Sex (F:M ratio)	0.491 ± 0.177	0.006
Body surface area (m^2)	−1.230 ± 0.617	0.05
ESVI (ratio)	−0.031 ± 0.021	0.14
Ejection fraction (%)	0.011 ± 0.014	0.45
$R^2 = 0.81$; $P < 0.0001$		
Age (years)	0.133 ± 0.034	0.0002
Sex (F:M ratio)	0.376 ± 0.177	0.04
Longitudinal strain (%)	−0.002 ± 0.108	0.99
Circumferential strain (%)	0.127 ± 0.127	0.32
Radial strain (%)	0.009 ± 0.072	0.90

F:M, female to male; R^2, multiple correlation coefficient.

our study, conventional echocardiographic and clinical variables were not able to predict net LV twist. Cameli *et al.*[32] showed an independent correlation of indexed end-systolic LV volume, LV relaxation, heart rate, gender, and age with LV torsion mechanics in normal individuals. However, echocardiographic and clinical variables were not independent predictors of LV torsion mechanics.[32] Moreover, strain parameters showed an independent correlation with net LV twist in our study but were unable to predict net LV twist.

Limitations

The technique of speckle tracking used in this study only provides a two-dimensional analysis, and the consistency of these findings using other two-dimensional vendor technology is uncertain. MRI tagging or sonomicrometry was not employed as the gold standard to compare speckle-tracking findings.

Normal deformation values vary among publications and depend on the vendor used, suggesting that reference values have to be obtained for each method and software.[33] The interpretation of functional data across a spectrum of ages requires consideration of the ventricular loading conditions and vascular impedance. Further research is required to assess the fibre structure and loading conditions that change with ageing between different populations, as the response of the left ventricle in black patients in pathological states does differ.[34,35] Further research is required to determine the effect of ageing on strain and rotation using three-dimensional speckle tracking, preferably in an international multicentre cohort.

Conclusions

The data reported in this study establish the normal values and patterns for LV strain and rotational mechanics in a normal black-African adult population and can be used as a baseline for future studies. Ageing is associated with increased net LV twist, whereas decreasing strain parameters are associated with advancing age. Age is the major determinant of increased LV twist in this population.

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APPENDIX c - Time to twist: marker of systolic dysfunction in Africans with hypertension

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Time to twist: marker of systolic dysfunction in Africans with hypertension

Nirvarthi Maharaj[1], Bijoy K. Khandheria[2]*, Ferande Peters[1], Elena Libhaber[1], and Mohammed R. Essop[1]

[1]Division of Cardiology, Chris Hani Baragwanath Hospital and University of the Witwatersrand, Johannesburg, South Africa; and [2]Aurora Cardiovascular Services, Aurora Sinai/Aurora St Luke's Medical Centers, University of Wisconsin School of Medicine and Public Health, 2801 W. Kinnickinnic River Parkway, #845, Milwaukee, WI, USA

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Aims Speckle-tracking echocardiography (STE) has emerged as a quantitative technique to assess left ventricular (LV) function via twist mechanics. Hypertension-associated changes in LV twist have not been previously evaluated in African patients with LV systolic dysfunction. This study evaluates LV twist dynamics in hypertensive patients with a low ejection fraction (EF).

Methods and results Forty-one hypertensive patients with EF <45% and normal coronary angiography (HTLEF group) were compared with 41 control subjects using STE. Peak apical rotation (AR) and isochronous basal rotation (BR) were measured, as was net twist (maximal value of simultaneous AR − BR). Rigid body rotation (RBR) was defined as AR and BR occurring in the same direction. Of 41 HTLEF patients, 28 (68%) had normal twist pattern, whereas 13 (32%) exhibited RBR; 11 of those 13 exhibited clockwise rotation. AR ($1.89^\circ \pm 0.99$ vs. $7.15^\circ \pm 2.26$; $P < 0.0001$), BR ($1.44^\circ \pm 0.78$ vs. $-3.75^\circ \pm 1.61$; $P < 0.0001$), and net twist ($3.3^\circ \pm 1.17$ vs. $10.9^\circ \pm 2.7$; $P < 0.0001$) were lower in HTLEF patients. The subgroup with clockwise RBR showed greater LV dysfunction (EF: 27.9 ± 5.8 vs. $35 \pm 7.5\%$; $P = 0.005$) and more spherical LV geometry in both diastole and systole ($P = 0.0009$ and $P = 0.0002$, respectively) compared with those who had a normal pattern of twist. In the HTLEF subgroup with normal twist pattern, AR ($r = 0.57$; $P = 0.002$) and net twist ($r = 0.57$; $P = 0.002$) correlated with EF.

Conclusions LV twist in patients with hypertension and EF <45% is diminished. Apical rotation and net twist are good correlates for EF in hypertensive patients with EF <45%.

Keywords Twist • Speckle tracking • Hypertension • Rotation • Ejection fraction • Imaging • Echocardiography

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Introduction

Hypertension is a well-documented precedent to left ventricular (LV) dysfunction and congestive heart failure. In the Framingham Heart Study, lifetime risk for congestive heart failure was 20% for both men and women.[1] Even after the exclusion of patients with antecedent myocardial infarction, risk for heart failure remains significant.[2] Although the contribution of hypertension to heart failure is known, the mechanism is multifactorial and unclear.[2] It is generally postulated that the development of systolic dysfunction in hypertensive patients is either due to a myocardial infarction or as a consequence of concomitant ischaemic heart disease.[2] Hypertension may cause systolic dysfunction as a consequence of adverse remodelling, but this has not been clearly documented.[2] LV twist may be a contributing factor to this dysfunction independent of ischaemic heart disease, thus, a focus on abnormalities in the cardiac mechanics of twist and torsion in the left ventricle may be helpful in understanding the pathogenesis behind the transition from compensated to decompensated heart failure.

LV twist occurs when the LV apex moves in a counterclockwise direction during systole, whereas the base moves in a clockwise direction. LV twist can now be accurately assessed using speckle-tracking echocardiography (STE), which has been validated against magnetic resonance imaging (MRI).[3,4] Evaluation of LV twist has proved a sensitive technique to assess cardiac performance.[5–7] We hypothesized that LV rotational parameters and net LV twist are diminished in hypertensive black patients with a low

* Corresponding author. Tel: +1 414 649 3909; fax: +1 414 649 3529. Email: publishing22@aurora.org

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ejection fraction (EF) in the absence of ischaemic heart disease. In a population with no concomitant coronary disease, we compared LV twist and its components in hypertensive patients who presented with heart failure and low EF to healthy, normal subjects.

Methods

This cross-sectional study was approved by the University of Witwatersrand Ethics Committee and the Institutional Review Board. Subjects were recruited from the Chris Hani Baragwanath Hospital Cardiac Clinic from January 2011 to October 2011. Inclusion criteria were: documented prior diagnosis of hypertension (measurements on three separate occasions where the systolic blood pressure was $\geq$140 mmHg, or the diastolic blood pressure was $\geq$90 mmHg taken over a period of 2 months[8]) at the Hypertension Clinic, documented heart failure [past or on presentation as evidenced by symptoms (New York Heart Association functional class), clinical examination and/or admissions for heart failure], sinus rhythm, EF $<$45% on echocardiography, and normal coronary angiography. Exclusion criteria were: previous myocardial infarction, anaemia (haemoglobin $<$12 g/dL in females, $<$13 g/dL in males), excess alcohol intake (maximum alcohol intake no $>$40 g per day in males and 20 g per day in females with two alcohol-free days per week), renal dysfunction (glomerular filtration rate $<$60 mL/min/1.73 m^2), documented diagnosis of diabetes and/or glycated haemoglobin $>$7%, organic valvular disease, dilated cardiomyopathy of any aetiology, cardiac infiltrative diseases, post-viral myocarditis, any systemic illness [e.g. human immunodeficiency virus (HIV)], thyroid disease, and any primary organ dysfunction or failure (e.g. chronic renal disease). Subjects who fulfilled the criteria and provided voluntary informed consent were enrolled and underwent a detailed clinical and echocardiographic evaluation at baseline. A total of 50 patients were screened for inclusion in the study. Nine patients were excluded as a result of inadequate image quality that did not allow for complete segmental assessment of LV rotation at both the basal and apical left ventricle. The remaining 41 hypertensive patients with low ejection fraction (HTLEF) made up the study group.

The control group was recruited from the staff at Chris Hani Baragwanath Hospital, patient escorts, and community members from Soweto. All members of the control group were unrelated to the patients in the HTLEF group. Individuals younger than 50 years were matched with a tolerance of 5 years in terms of age, whereas individuals older than 50 years were allowed a tolerance of up to 10 years. All members of the control group were asymptomatic, normotensive, had no evidence of cardiovascular or systemic diseases and had a normal 12-lead electrocardiogram prior to undergoing echocardiography for the study. The control group was age- and sex-matched with the HTLEF cohort. Echocardiographic evaluation of the control group is part of an ongoing prospective study to provide normal reference ranges for echocardiographic measurements in subjects of African ancestry.

Echocardiography

Comprehensive transthoracic echocardiography was performed using a commercially available system (iE33 xMATRIX, Philips Healthcare, Andover, MA, USA) equipped with an S5-1 transducer (frequency transmitted 1.7 MHz, frequency received 3.4 MHz) according to a standardized protocol. All data were transferred to an Xcelera workstation (Philips Healthcare) and analysed offline. All echocardiographic measurements were averaged from three heartbeats. Measurements relating to chamber size and function were performed in accordance with the American Society of Echocardiography (ASE) chamber quantification guidelines of 2006[9] and the ASE 2010 guidelines on right heart assessment.[10] The severity of mitral and tricuspid regurgitation was analysed in accordance with the ASE guidelines on native valvular regurgitation.[11] EF was calculated from LV volumes by the modified biplane Simpson rule in accordance with the guidelines.[9] From the mitral-inflow pattern, peak early (E) and late (A) filling velocities, *E/A* ratio, and E-velocity deceleration time were measured. Tissue Doppler was applied end-expiratory in the pulsed-wave Doppler mode at the level of the inferoseptal side of the mitral annulus from an apical four-chamber view. To acquire the highest wall tissue velocities, the angle between the Doppler beam and the longitudinal motion of the investigated structure was adjusted to a minimal level. The spectral pulsed-wave Doppler velocity range was adjusted to obtain an appropriate scale. The time interval between the peak of the R-wave on the electrocardiogram and the aortic valve opening and closure, as well as the time interval between the R-wave and the mitral valve opening and closure, was measured using pulsed Doppler acquired from LV outflow and inflow, respectively. The LV mass was calculated using the formula: LV mass $= 0.8 \times \{1.04[(\text{LVIDd} + \text{PWTd} + \text{SWTd})^3 - (\text{LVIDd})^3]\} + 0.6$ g, where LVIDd, PWTd, and SWTd are LV internal diameter at end diastole, posterior wall thickness at end diastole, and septal wall thickness at end diastole, respectively.[9,12] Relative wall thickness (RWT) was calculated using the formula: $(2 \times \text{PWTd})/\text{LVIDd}$.[9,12] Concentric hypertrophy was defined as RWT $>$0.42 and left ventricular mass index (LVMI) (g/m^2) $>$95 in females and $>$115 in males, whereas eccentric hypertrophy was defined as RWT $<$0.42 and LVMI $>$95 in females and $>$115 in males.[9,12] The LV sphericity index was calculated by dividing the LV maximal long-axis internal dimension by the maximal short-axis internal dimension at end diastole and end systole[13] using the apical four-chamber view.

Speckle-tracking analysis

Two-dimensional images were obtained at a rate of 50–80 frames per second. Parasternal short-axis images at the LV basal level showing the tips of the mitral valve leaflets were obtained with the cross-section as circular as possible. To obtain a short-axis image at the true LV apical level, the transducer was positioned one or two intercostal spaces more caudal and acquired in a manner described by van Dalen *et al.*[14] Analysis of data sets was performed using QLAB advanced quantification Version 8.0 software (Philips Healthcare, Best, Netherlands) by a physician experienced in speckle tracking. The assessment of LV twist using QLAB software had previously been validated against MRI tagging.[15]

To assess LV rotation, six tracking points were placed on the myocardium,[16] avoiding the pericardium on an end-diastolic frame in each parasternal short-axis image as determined automatically by the nature of the software algorithm. At the base, tracking points were separated about 60° from one another to fit the total LV circumference as described by van Dalen *et al.*[16] Repositioning of tracking points was allowed provided the position was moved no $>$30°. At the apex, tracking points were placed per the software algorithm from the endocardium to epicardium. After positioning the tracking points, the programme tracked these points. The operator ensured that cardiac cycles with heart rate variability $<$10% were selected for the measurements of LV rotation parameters. Manual correction for ROI repositioning was required in six (14.6%) control subjects and 10 (20%) hypertensive patients screened for inclusion in the study. Time needed for analysis was, on average, 2–3 min per patient.

Counterclockwise rotation, as viewed from the apex, was expressed as a positive value; clockwise rotation was expressed as a negative value.[17] End systole was defined as the point of aortic valve closure. Analysis was performed to evaluate the peak apical rotation (AR)

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during the ejection phase, the basal rotation (BR) at a time isochronous with that of peak AR during the ejection phase and the net instantaneous twist of the left ventricle (net twist), which was calculated as peak AR minus the BR at a time isochronous with peak AR.

In addition to quantifying AR and BR, we analysed the direction of rotation in systole. We focused on the pattern of rotation during the ejection phase of systole and identified rotation as either normal or having evidence of rigid body rotation (RBR), which was either counterclockwise or clockwise at both the basal and apical levels. Normal patterns evaluated were characterized by clockwise systolic BR and counterclockwise systolic AR.[18] The untwisting rate was defined as the mean diastolic untwisting velocity from instantaneous peak systolic twist to mitral valve opening and calculated thusly: (twist at mitral valve opening − peak systolic twist)/(time interval from peak systolic twist to mitral valve opening).

Statistical analysis

Database management and statistical analyses were performed with the SAS Version 9.2 software (SAS Institute, Inc., Cary, NC, USA). Data were presented as mean ± standard deviation. Continuous variables were compared using an independent *t*-test or the Wilcoxon–Mann–Whitney test when the distribution was non-normal. Spearman correlation coefficients for the relationships between LV rotation parameters against conventional echocardiographic parameters were determined. The interobserver variability was assessed for twist measurements in 20 randomly selected patients, calculated as the standard deviation of the differences between the measurements of two independent observers who were unaware of the other patient data, and expressed as a percentage of the average value. The intraobserver variability was calculated as the standard deviation of the differences between a first and second determination of a single observer and expressed as a percentage of the average value. Intraobserver and interobserver variability for LV rotation parameters in our centre varies from 2 ± 3 to 10 ± 9%, and from 4 ± 4 to 12 ± 8%, respectively. *P*-values <0.05 were considered statistically significant.

Results

Patient selection

A total of 50 patients with HTLEF were screened for inclusion in the study. Inadequate visualization of the endocardial border precluded correct wall-motion tracking in nine patients, who were then excluded from the analysis. The remaining 41 patients showed adequate tracking for apical and basal short-axis views and were consecutively included (*Table 1*). The control group was composed of 41 individuals. All patients from the HTLEF group were on optimal doses of beta-blocker (carvedilol) and the angiotensin-converting enzyme inhibitors, spironolactone and furosemide.

Baseline and echocardiographic characteristics

Baseline characteristics of the study population are represented in *Table 1*. Patients with HTLEF differed significantly from the patients in the control group with respect to age, body mass index, and baseline blood pressure. Adjustments were made in the data analysis to accommodate for these differences. As expected, patients with HTLEF differed significantly from the control group with regard to all echocardiographic measurements (*Table 1*). Two major geometric patterns were noted in subjects with HTLEF: concentric hypertrophy in 22 patients (54%) and eccentric hypertrophy in 19 patients (46%).

Table 1 Baseline clinical and echocardiographic characteristics

	Control group	HTLEF group
No. patients	41	41
Age, years	50.1 ± 9.3	55.0 ± 9.1#
Male gender, *n* (%)	19 (46.3)	19 (46.3)
Body mass index, kg/m^2	24.7 ± 1.8	29.5 ± 5.0*
Body surface area, m^2	1.72 ± 0.14	1.86 ± 0.17*
NYHA functional capacity, *n* (%)		
Class I	41 (100)	12 (29)*
Class II		14 (34)*
Class III		15 (37)*
Duration of hypertension, years	—	12.2 ± 6.5
Duration of heart failure, years	—	3.2 ± 1.6
Medication (ACE/ARB, diuretics, beta-blocker, spironolactone)	—	41
Systolic blood pressure, mmHg	120 ± 7	156 ± 8*
Diastolic blood pressure, mmHg	71 ± 6	89 ± 10*
Heart rate, bpm	73 ± 10	81 ± 10*
EDD, mm	43 ± 5	58 ± 5†
IVSD, mm	10 ± 1	12 ± 2†
IVPWD, mm	8 ± 1	12 ± 2†
End-diastolic volume, mL	80.6 ± 13.8	133.2 ± 41.2†
End-diastolic volume index, mL/m^2	46.8 ± 7.7	72.3 ± 23.6†
End-systolic volume, mL	32.6 ± 9.8	91.1 ± 38.7†
End-systolic volume index, mL/m^2	18.9 ± 5.6	49.4 ± 21.4†
Ejection fraction, %	69.3 ± 9.5	33.0 ± 7.6†
Relative wall thickness ratio	0.38 ± 0.03	0.41 ± 0.06#
Left ventricular mass index, g/m^2	73.8 ± 13.9	161.4 ± 32.8†
Left atrial volume index, mL/m^2	23.0 ± 2.2	31.0 ± 16.3#
Functional mitral regurgitation	—	1.83 ± 0.8
End-diastolic LVSPHI ratio	1.88 ± 0.30	1.36 ± 0.12†
End-systolic LVSPHI ratio	2.13 ± 0.54	1.39 ± 0.10†
Peak E velocity (cm/s)	80.2 + 7.8	65.1 + 8.2†
Peak A velocity (cm/s)	49.5 ± 8.4	85.3 ± 7.6†
Deceleration time (ms)	170 ± 31	245 ± 44†
E/*A* ratio	1.70 ± 0.34	0.77 ± 0.11†

#$P < 0.05$.
*$P < 0.0005$.
†$P < 0.0001$.
A, late (atrial) ventricular filling velocity; ACE/ARB, angiotensin-converting enzyme inhibitors/angiotensin receptor blockers; E, early ventricular filling velocity; EDD, end-diastolic diameter; HTLEF, patient study group with hypertension and low ejection fraction (<45%); IVPWD, interventricular posterior wall at end-diastolic dimension; IVSD, interventricular septal thickness at end-diastolic dimension; LVSPHI, left ventricular sphericity index; NYHA, New York Heart Association.

Left ventricular rotation analysis

All subjects in the control group had a normal pattern of rotation (*Figure 1*). Three distinct rotational patterns were identified in the

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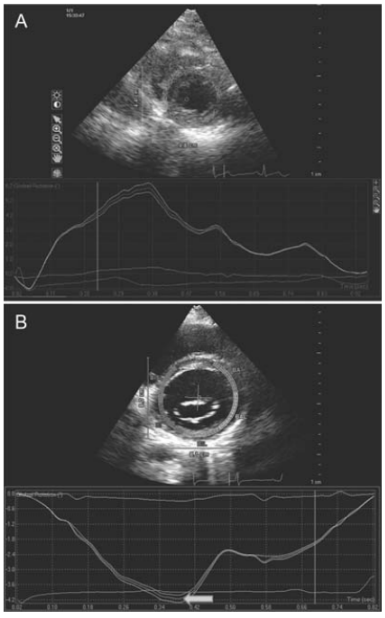


Figure 1 (*A*) Normal apical rotation in control patients. Tracking points at the left ventricular apical level. The left ventricular rotation–time curve (*x*-axis: time in ms; *y*-axis: left ventricular apical rotation in degrees). An electrocardiogram is displayed at the bottom. (*B*) Normal basal rotation in control patients. Tracking points at the left ventricular basal level. The left ventricular rotation–time curve (*x*-axis: time in ms; *y*-axis: left ventricular basal rotation in degrees). An electrocardiogram is displayed at the bottom (arrow indicates peak global basal rotation at time of aortic valve closure).

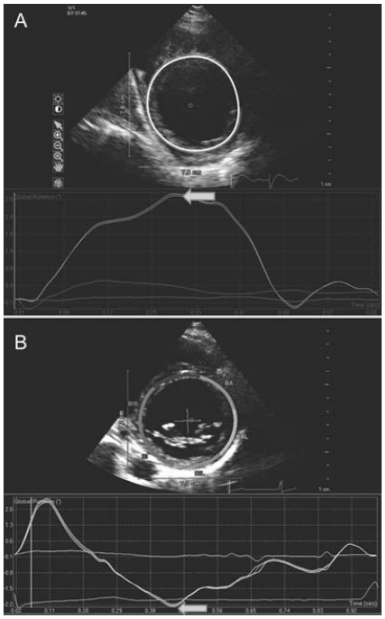


Figure 2 (*A*) Apical rotation with normal pattern in hypertensive patients with ejection fraction <45%. Tracking points at the left ventricular apical level. The left ventricular rotation–time curve (*x*-axis: time in ms; *y*-axis: left ventricular apical rotation in degrees). An electrocardiogram is displayed at the bottom (arrow indicates peak global apical rotation at time of aortic valve closure). (*B*) Basal rotation with normal pattern in hypertensive patients with ejection fraction <45%. Tracking points at the left ventricular basal level. The left ventricular rotation–time curve (*x*-axis: time in ms; *y*-axis: left ventricular basal rotation in degrees). An electrocardiogram is displayed at the bottom (arrow indicates peak global basal rotation at time of aortic valve closure).

HTLEF group. Of the 41 patients with HTLEF, 28 patients (68%) had the normal pattern of twist (*Figure 2*, *Table 2*), whereas 13 (32%) exhibited RBR. Eleven of the 13 patients had evidence of clockwise RBR (*Figure 3* and *Table 2*) and two had counterclockwise RBR. AR ($1.89^\circ \pm 0.99$ vs. $7.15^\circ \pm 2.26$; $P < 0.0001$), BR ($1.44^\circ \pm 0.78$ vs. $-3.75^\circ \pm 1.61$; $P < 0.0001$), and net twist ($3.3^\circ \pm 1.2$ vs. $10.9^\circ \pm 2.7$; $P < 0.0001$) were lower in HTLEF patients compared with controls (*Table 3*). The untwisting rate was reduced in HTLEF patients compared with controls ($P = 0.03$) (*Table 3*).

Comparison of HTLEF patients with different patterns of twist

Patients with HTLEF exhibited three different patterns of twist (normal, clockwise RBR, and counterclockwise RBR). Since only two patients were observed to have counterclockwise RBR, they were not included in the analysis. There were no significant differences between the normal pattern and the clockwise RBR subgroups in terms of age, sex, body surface area, baseline blood pressure, and duration of hypertension (*Table 2*). NYHA class was statistically significant ($P = 0.017$). No patients in the HTLEF subgroup who had clockwise RBR were NYHA class I, 64% were NYHA class III, and 36% were NYHA class II. In contrast, 43% of patients in the HTLEF subgroup who had the normal pattern of twist were NYHA class I, and 28% were NYHA class II or III (*Table 2*). Patients with clockwise RBR had significantly larger LV volumes ($P = 0.02$ and $P = 0.008$ for end-diastolic volume and end-systolic volume, respectively), lower EF ($P = 0.005$), and a more spherical LV geometry in both diastole and systole ($P = 0.0009$ and $P = 0.0002$, respectively) (*Table 2*).

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Table 2 Comparison between HTLEF patients with different patterns of twist

	Normal pattern (n = 28)	Clockwise RBR (n = 11)	P-value
Age, years	55.0 ± 9.7	56.0 ± 8.2	0.61
Male gender, n (%)	16 (57.1)	2 (18.1)	0.07
Body mass index, kg/m^2	28.8 ± 5.2	31.4 ± 4.6	0.17
Body surface area, m^2	1.86 ± 0.17	1.88 ± 0.18	0.99
Systolic blood pressure, mmHg	156 ± 8	154 ± 9	0.49
Diastolic blood pressure, mmHg	89 ± 10	93 ± 11	0.35
Heart rate, bpm	78 ± 10	87 ± 8	0.02
NYHA functional capacity, n (%)			
Class I	12 (43)	0 (0)	0.017
Class II	8 (28.5)	4 (36)	
Class III	8 (28.5)	7 (64)	
Duration of hypertension, years	11.3 ± 6.9	13.5 ± 4.2	0.06
Duration of heart failure, years	3.1 ± 1.6	3.2 ± 1.1	0.60
End-diastolic diameter, mm	58 ± 6	59 ± 3	0.15
IVSD, mm	12 ± 2	13 ± 2	0.01
IVPWD, mm	12 ± 2	12 ± 1	0.86
End-diastolic volume, mL	122.8 ± 36.1	159.1 ± 45.7	0.02
End-diastolic volume index, mL/m^2	66.8 ± 22.1	85.2 ± 23.3	0.03
End-systolic volume, mL	79.2 ± 29.5	119.6 ± 45.9	0.008
End-systolic volume index, mL/m^2	43.0 ± 17.2	64.2 ± 23.8	0.009
Ejection fraction, %	35.0 ± 7.5	27.9 ± 5.8	0.005
Relative wall thickness ratio	0.41 ± 0.07	0.40 ± 0.04	0.31
Left ventricular mass index, g/m^2	152.4 ± 32.6	176.1 ± 30.1	0.08
Left atrial volume index, mL/m^2	33.2 ± 15.0	43.9 ± 12.2	0.04
End-diastolic LVSPHI ratio	1.40 ± 0.09	1.26 ± 0.11	0.0009
End-systolic LVSPHI ratio	1.43 ± 0.08	1.29 ± 0.07	0.0002

HTLEF, patient study group with hypertension and low ejection fraction (<45%); IVPWD, interventricular posterior wall at end-diastolic dimension; IVSD, interventricular septal thickness at end-diastolic dimension; LVSPHI, left ventricular sphericity index; NYHA, New York Heart Association; RBR, rigid body rotation.

Correlation analysis

Significant positive correlations were noted between net twist and EF ($r = 0.83$, $P < 0.0001$) and AR and EF ($r = 0.80$, $P < 0.0001$) in both groups (control and HTLEF with the normal pattern of twist). A negative correlation was seen between BR and EF in both groups (control and HTLEF with the normal pattern of twist) ($r = -0.59$, $P < 0.0001$). Subanalysis of the control group showed no significant correlation between LV rotation parameters and EF (AR: $r = -0.02$, $P = 0.88$; BR: $r = -0.19$, $P = 0.23$, net twist: $r = 0.08$, $P = 0.60$). However, there was a significant correlation between AR and EF ($r = 0.57$, $P = 0.002$) (*Figure 4*, *A*) and between net twist and EF ($r = 0.57$, $P = 0.002$) (*Figure 4*, *C*) in the subset of the HTLEF group with the normal pattern of rotation; BR showed no correlation to EF ($r = -0.14$; $P = 0.49$) (*Figure 4*, *B*). There was no association between RWT and LV rotational parameters and net twist (net twist: $r = 0.12$, $P = 0.58$; AR: $r = 0.32$, $P = 0.13$; BR: $r = 0.24$, $P = 0.25$) in both groups (control and HTLEF with the normal pattern of twist). There was no association between LVMI and net twist ($r = -0.069$, $P = 0.66$ in controls; $r = -0.015$, $P = 0.94$ in HTLEF with the normal pattern of twist). Furthermore, there was no relationship between the LVMI and untwist rate ($r = -0.037$, $P = 0.82$ in controls; $r = -0.31$, $P = 0.11$ in HTLEF with the normal pattern of twist).

Discussion

Main findings

In this study of patients with hypertension and low EF, we found that (i) net instantaneous twist and its determinants are diminished; (ii) patients exhibiting RBR have more advanced LV dysfunction and adverse remodelling; and (iii) AR and net twist correlate well with EF.

This is the first study to our knowledge that has evaluated twist mechanics in hypertensive patients with a low EF. The clinical profile of this cohort with HTLEF is unique in that all subjects had no angiographic evidence of epicardial coronary artery disease (CAD) and no evidence of previous myocardial infarction on electrocardiography, echocardiography, or LV angiography. This challenges the long-held postulate that progression to

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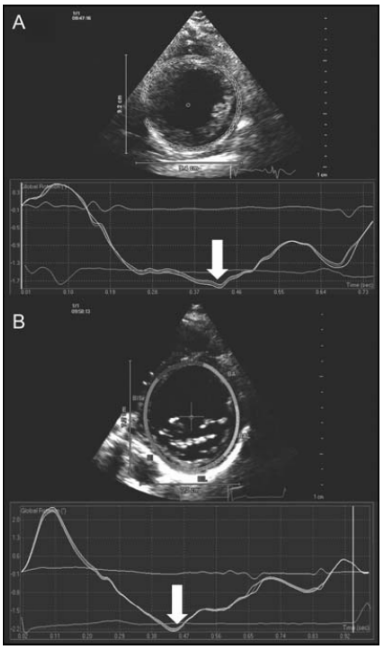


Figure 3 (*A*) Apical clockwise rotation in hypertensive patients with ejection fraction <45%. Tracking points at the left ventricular apical level. The left ventricular rotation–time curve (*x*-axis: time in ms; *y*-axis: left ventricular apical rotation in degrees). An electrocardiogram is displayed at the bottom (arrow indicates peak global apical rotation at time of aortic valve closure). (*B*) Basal rotation with normal pattern in hypertensive patients with ejection fraction <45%. Tracking points at the left ventricular basal level. The left ventricular rotation–time curve (*x*-axis: time in ms; *y*-axis: left ventricular basal rotation in degrees). An electrocardiogram is displayed at the bottom (arrow indicates peak global basal rotation at time of aortic valve closure).

Table 3 Comparison of left ventricular twist between control and HTLEF patients with normal pattern of twist

	Control group ($n = 41$)	HTLEF group ($n = 28$)	*P*-value
Apical rotation	$7.15 \pm 2.26^{\circ}$	$1.89 \pm 0.99^{\circ}$	<0.0001
Basal rotation	$-3.75 \pm 1.61^{\circ}$	$-1.44 \pm 0.78^{\circ}$	<0.0001
Time to peak apical rotation, ms	348 ± 120	332 ± 70	0.52
Net twist	$10.90 \pm 2.70^{\circ}$	$3.30 \pm 1.17^{\circ}$	<0.0001
Untwisting rate, degrees/s	-45 ± 17	-30 ± 15	0.03

HTLEF, patient study group with hypertension and low ejection fraction (<45%).

severe systolic dysfunction in patients with hypertension is associated with ischaemia secondary to CAD or previous myocardial infarction. In addition, the degree of remodelling and LV dysfunction in this cohort of patients is not influenced by other comorbidities that may cause LV dysfunction such as diabetes, atrial fibrillation, and volume-overloaded conditions such as renal failure and anaemia. Thus, hypertension is more likely to be the sole initiator of the remodelling process. The second important finding of this study is the fact that both concentric and eccentric hypertrophy were present in patients with HTLEF.

Twist is determined by the rotation of the apex with respect to the base along its long axis and is produced by the contraction of the heart's obliquely spiraling myocardial fibre layers. Owing to their longer radii of rotation, subepicardial fibres are thought to be the predominant cause of the net counterclockwise rotation at the apex and the net clockwise rotation at the base.[19] We noted that diminished net twist occurred in all HTLEF patients, caused by both a reduction in AR and BR. However, only AR correlated positively with the degree of LV dysfunction, suggesting that the degree of apical dysfunction is more important in LV dysfunction than the degree of basal dysfunction. The absence of ischaemia or infarction in our study population is important, as previous studies have shown impaired AR and LV twist deformation in patients with myocardial infarction.[20–24] The reduction in both AR and BR may imply that both areas are affected by a more global abnormality that affects both subendocardial and subepicardial fibres. One such process may be microvascular ischaemia, either from microcirculatory dysfunction or due to the oxygen supply/demand mismatch. All are well documented as occurring in hypertensive patients in the absence of epicardial CAD.

In this study, we have documented RBR in patients with HTLEF for the first time. The predominant pattern was clockwise, implying that the apex was moving in a completely opposite direction to normal. In the patients with clockwise RBR, left ventricles were significantly more dilated, spherical, and dysfunctional. We postulate that ventricular remodelling and abnormal fibre orientation[25] may contribute to change in the normal twist pattern. In severely dilated ventricles, increased LV sphericity may lead to widening of the apex resulting in significant changes in LV AR values.[26,27] Consequently, widening of the apex brings about the loss of the oblique architecture of the apical loop fibres, which become more transverse and resemble the horizontal fibre orientation of the basal loop.[25] Thus the apex moves in a reverse direction, similar to the base, resulting in clockwise RBR. However, this postulate does not explain the mechanism of counterclockwise rotation observed in two patients.

RBR and reversed systolic AR have been documented in patients with isolated LV noncompaction and idiopathic dilated cardiomyopathy.[26–29] Some of these reports suggest patients with abnormal

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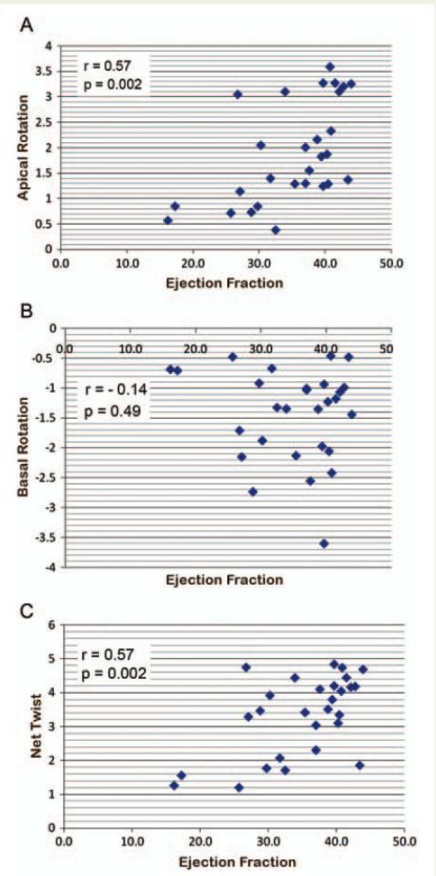


Figure 4 Correlation between rotational parameters and ejection fraction in subgroup of hypertensive patients with ejection fraction <45% and a normal pattern of twist ($n = 28$). Shown are correlations between (*A*) apical rotation and ejection fraction; (*B*) basal rotation and ejection fraction; and (*C*) net twist and ejection fraction.

twist patterns have more severe LV remodelling than patients with normal patterns of twist.[26–29] We propose that the presence of RBR, especially in a clockwise direction, represents a novel assessment of more severe LV remodelling in hypertensive patients with LV systolic dysfunction in the absence of ischaemia. The loss of LV torsion may result in further decline in LV function and initiate a continuous, vicious cycle of remodelling. This raises the question whether RBR represents a more advanced state of remodelling or if it is the precipitant to the development of more advanced remodelling.

Study limitations

Our study has a number of limitations. This study cohort represents a select population, rare in Western populations where comorbidities do exist. Moreover, as this study was cross-sectional, further follow-up and prospective research is required to determine the progression of the disease and long-term outcome in patients with decreased LV twist and RBR associated with hypertension and LV systolic dysfunction. The technique of speckle tracking utilized in this study only provides a two-dimensional analysis; reproducibility of these findings using other technology is uncertain. In addition, the gold standards of MRI tagging or sonomicrometry were not employed to confirm speckle-tracking findings in our study. Rotational values are lower using this specific vendor technology, and reproducibility of these findings using other vendors needs to be assessed.

Further prospective studies with larger sample sizes are needed to confirm the clinical implications of our findings. Finally, a three-dimensional speckle-tracking analysis may offer new insight on patients with HTLEF and provide a good comparison with the reported findings using two-dimensional STE.

Conclusion

This study provides important insight into the LV twist characteristics of hypertensive patients with systolic dysfunction and African ancestry. We found patients with hypertension and low EF in the absence of CAD had reduced net twist as a result of diminished apical and BR. Rigid body rotation can occur in association with hypertension and low EF and may result in adverse remodelling and a greater degree of LV dysfunction.

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APPENDIX d - Relationship between Left Ventricular Twist and Circulating Biomarkers of Collagen Turnover in Hypertensive Patients with Heart Failure

Clinical Study

Relationship Between Left Ventricular Twist and Circulating Biomarkers of Collagen Turnover in Hypertensive Patients With Heart Failure

Nirvarthi Maharaj, MD, Bijoy K. Khandheria, MD, Elena Libhaber, PhD, Samantha Govender, BSc, Raquel Duarte, PhD, Ferande Peters, MD, Mohammed R. Essop, MD

From the *Division of Cardiology, Chris Hani Baragwanath Hospital and University of the Witwatersrand, Johannesburg, South Africa (N.M., E.L., S.G., R.D., F.P., M.R.E.); and Aurora Cardiovascular Services, Aurora Sinai/Aurora St. Luke's Medical Centers, University of Wisconsin School of Medicine and Public Health, Milwaukee, Wisconsin (B.K.K.).

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Abstract:

Left ventricular twist may be a compensatory mechanism to preserve ejection fraction. In hypertensive patients, twist varies depending on the left ventricle's degree of remodelling and systolic function; it is increased in those with preserved ejection fraction and diminished in those with low ejection fraction. The ratio of collagen-degradation biomarkers in hypertensive patients is higher in those with low ejection fraction than those with preserved ejection fraction and may contribute to remodelling and systolic dysfunction. This study evaluated the relationship between these biomarkers and left ventricular twist in 82 hypertensive patients – 41 with ejection fraction <50% (HTLEF group) and 41 with ejection fraction ≥50% (HTNEF group). Net left ventricular twist was measured using speckle-tracking echocardiography. Markers of collagen turnover, including serum concentrations of matrix metalloproteinase-1 (MMP1), tissue inhibitor of MMP1 (TIMP1) and ratio of MMP1:TIMP1, were measured. Log TIMP1, Log MMP1 and Log MMP1:TIMP1 ratio levels were higher in the HTLEF group versus the HTNEF group (12.32 ± 0.25 vs. 11.81 ± 0.13, $p<0.0001$; 9.08 ± 0.32 vs. 8.00 ± 0.18, $p<0.0001$; -3.25 ± 0.30 vs. -3.81 ± 0.18, $p<0.0001$; respectively). Net left ventricular twist was lower in the HTLEF group versus the HTNEF group (3.34 ± 1.10 vs. 11.70 ± 0.67, $p<0.0001$). An inverse correlation existed between Log MMP1:TIMP1 and net left ventricular twist after adjusting for ejection fraction ($r=-0.41$, $p<0.0001$). This inverse correlation between twist and loss of myocardial collagen scaffolding suggests the integrity of the extracellular matrix plays an important role in preserving myocardial deformation.

Key Words: twist ■ speckle tracking ■ heart failure ■ hypertension ■ matrix metalloproteinases ■ tissue inhibitor of matrix metalloproteinase-1 ■ strain

Introduction

Left ventricular (LV) twist is defined as the wringing motion of the heart during systole whereby the apex rotates in a counterclockwise direction with respect to the base rotating in a clockwise direction.[1-5] LV twist is an important contributing factor to the systolic function of the left ventricle in health and disease.[1-5] Evaluation of LV twist using speckle tracking is a sensitive technique used to assess cardiac performance[1-5] and can be a better index of systolic function than ejection fraction (EF) in hypertensive patients. In hypertensive patients with preserved EF, LV twist is increased while longitudinal strain is diminished, suggesting that LV twist may be a compensatory mechanism to preserve ejection fraction.[6] LV twist is diminished in hypertensive patients with low EF who initially present with heart failure[7] and has been found to be more diminished in hypertensive patients with eccentric LV hypertrophy compared to concentric hypertrophy.[8] This suggests LV twist varies with the degree of remodelling and systolic function caused by hypertension.

The remodelling process of the left ventricle in hypertension entails a complex interplay between myocyte hypertrophy and dysfunction, with qualitative changes in the extracellular matrix contributing to progressive dysfunction.[9-12] Adverse LV remodelling and hypertrophy in hypertensive patients is associated with derangements in the dynamic balance between the accumulation and breakdown of collagen in the cardiac extracellular matrix.[9-12] Furthermore, increased matrix metalloproteinase-1 (MMP1) levels – reflecting collagen degradation – may contribute to the development of LV dilatation and failure in hypertensive patients.[9-13] Lopez et al.[13] found that a greater excess of MMP1 to the tissue inhibitor of matrix metalloproteinase-1 (TIMP1) occurred in the myocardium of hypertensive patients with low EF than those with

normal EF. Moreover, Lopez et al. found that the circulating MMP:TIMP ratio was associated with greater LV dilatation and systolic dysfunction.[13] This landmark study implied the varying morphology and function in hypertensive heart disease was related to the equilibrium of MMP1 and TIMP1 in maintaining collagen homeostasis.[13] Hypertension can cause systolic dysfunction as a consequence of adverse remodelling and LV hypertrophy, but given the multitude of factors involved in LV decompensation mediated by mechanical, neurohormonal and cytokine routes, the exact mechanisms that contribute to the adverse remodelling and EF deterioration are not fully elucidated.[14,15]

We postulate that changes in the extracellular matrix as reflected by MMP1:TIMP1 ratio account for the varying morphology, EF and LV twist in hypertensive patients who present with heart failure. The aim of this study was to evaluate LV twist mechanics and their relationship with biomarkers of collagen degradation in hypertensive patients.

Methods

This cross-sectional study was approved by the University of Witwatersrand Ethics Committee and the Institutional Review Board. Subjects were consecutively recruited from the Chris Hani Baragwanath Hospital Cardiac Clinic from January 2011 to June 2012. Inclusion criteria were: documented prior diagnosis of hypertension (measurements on three separate occasions where systolic blood pressure was ≥140 mmHg or diastolic blood pressure was ≥90 mmHg taken over a period of two months[16] at the Hypertension Clinic), documented heart failure using Framingham Study criteria,[17] sinus rhythm, and normal coronary angiography. Exclusion criteria were: previous myocardial infarction, previous arrhythmia, anemia (hemoglobin <12 g/dL in females,

<13 g/dL in males), excess alcohol intake (maximum alcohol intake no more than 40 grams per day in males and 20 grams per day in females with two alcohol-free days per week), renal dysfunction (glomerular filtration rate <60 mL/min/1.73 m^2), documented diagnosis of diabetes and/or glycated hemoglobin >7%, organic valvular disease, dilated cardiomyopathy of any etiology, cardiac infiltrative diseases, post-viral myocarditis, any systemic illness (e.g. human immunodeficiency virus [HIV]), thyroid disease, any primary organ dysfunction or failure (e.g. chronic renal disease), and patients with evidence of rigid body rotation on speckle tracking.

A total of 107 subjects who fulfilled the criteria and provided voluntary informed consent were enrolled and underwent a detailed clinical and echocardiographic evaluation at baseline. After echocardiography, 25 patients were excluded as a result of inadequate image quality that did not allow for complete segmental assessment of LV rotation at both the basal and apical left ventricle. The remaining 82 hypertensive patients, 41 of whom had EF <50% (HTLEF group) and 41 of whom had EF ≥50% (HTNEF group), made up the study group and were given adequate doses of heart failure therapy as per individual patient's requirements.

The control group (n=41) was recruited from staff at Chris Hani Baragwanath Hospital, patient escorts and community members from Soweto. All controls were unrelated to patients in the study groups and were asymptomatic, normotensive, had no evidence of cardiovascular or systemic disease, and had a normal 12-lead electrocardiogram prior to undergoing echocardiography for the study. The control group was age- and sex-matched with the HTLEF and HTNEF cohorts. Individuals younger than 50 years were matched with a tolerance of 5 years

in terms of age, whereas individuals older than 50 years were allowed a tolerance of up to 10 years.

Echocardiography

Comprehensive transthoracic echocardiography was performed using a commercially available system (iE33 xMATRIX, Philips Healthcare, Best, The Netherlands) according to a standardized protocol. All echocardiographic measurements were averaged from three heartbeats. Measurements relating to chamber size and function were performed in accordance with the American Society of Echocardiography (ASE) chamber quantification guidelines of 2006[18] and the ASE 2010 guidelines on right heart assessment.[19] Severity of mitral and tricuspid regurgitation was analyzed in accordance with ASE guidelines on native valvular regurgitation.[20] EF was calculated from LV volumes by the modified biplane Simpson rule in accordance with guidelines.[18]

From the mitral-inflow pattern, peak early (E) and late (A) filling velocities, E/A ratio, and E-velocity deceleration time were measured. Tissue Doppler was applied end-expiratory in the pulsed-wave Doppler mode at the level of the inferoseptal side of the mitral annulus from an apical four-chamber view. The time interval between the peak of the R wave on the electrocardiogram and the aortic valve opening and closure, as well as the time interval between the R wave and the mitral valve opening and closure, was measured using pulsed Doppler acquired from LV outflow and inflow, respectively. LV mass was calculated using the formula: LV mass = $0.8 \times [1.04[(\text{LVIDd} + \text{PWTd} + \text{SWTd})^3 - (\text{LVIDd})^3]] + 0.6$ g, where LVIDd, PWTd and SWTd are left ventricular internal diameter at end diastole, posterior wall thickness at end

diastole and septal wall thickness at end diastole, respectively.[18,21] Relative wall thickness was calculated using the formula: (2 × PWTd)/LVIDd.[20,23] Concentric hypertrophy was defined as relative wall thickness >0.42 and LV mass index (g/m^2) >95 in females and >115 in males, whereas eccentric hypertrophy was defined as relative wall thickness <0.42 and LV mass index >95 in females and >115 in males.[18,21] LV sphericity index was calculated by dividing the maximal long-axis internal dimension by the maximal short-axis internal dimension at end diastole and end systole[22] using apical four-chamber view.

Speckle-Tracking Analysis

Two-dimensional images were obtained at a rate of 50 to 80 frames/s. Parasternal short-axis images at the LV basal level showing the tips of the mitral valve leaflets were obtained with the cross-section as circular as possible. To obtain a short-axis image at the true LV apical level, the transducer was positioned one or two intercostal spaces more caudal and acquired in a manner described by van Dalen et al.[23] LV longitudinal strain analysis was performed using apical chamber views. From short-axis views, radial and circumferential strain values were averaged across six wall segments (anteroseptal, anterior, lateral, posterior, inferior and septal) at the papillary muscle level. Analysis of datasets was performed using QLAB advanced quantification software (Version 8.0, Philips Healthcare) by a physician experienced in speckle tracking. The assessment of LV twist using QLAB software has been validated against tagged magnetic resonance imaging.[24]

To assess LV rotation, six tracking points were placed on the myocardium,[25] avoiding the pericardium on an end-diastolic frame in each parasternal short-axis image as determined

automatically by the nature of the software algorithm. At the base, tracking points were separated about 60 degrees from one another to fit the total LV circumference as described by Van Dalen et al.[25] Repositioning of tracking points was allowed provided the position was moved no more than 30 degrees. At the apex, tracking points were placed from the endocardium to epicardium per the software algorithm. The operator ensured that cardiac cycles with heart rate variability <10% were selected to measure LV rotation parameters. Manual correction for ROI repositioning was required in 6 controls and 10 hypertensive patients. Time needed for analysis was, on average, 2-3 minutes per patient.

Counterclockwise rotation, as viewed from the apex, was expressed as a positive value; clockwise rotation was expressed as a negative value.[5] End systole was defined as the point of aortic valve closure. Analysis was performed to evaluate the peak apical rotation (AR) during the ejection phase, the basal rotation (BR) at a time isochronous with that of peak AR during ejection, and the net instantaneous twist of the left ventricle (calculated as peak AR – isochronous BR).

In addition to quantifying AR and BR, the direction of systolic rotation was analyzed. Normal patterns were characterized by clockwise systolic BR and counterclockwise systolic AR.[5] The untwisting rate was defined as the mean diastolic untwisting velocity from instantaneous peak systolic twist to mitral valve opening and calculated thusly: (twist at mitral valve opening − peak systolic twist)/(time interval from peak systolic twist to mitral valve opening).

Biomarkers

All blood samples were taken at the time of echocardiographic examination. Samples were collected in a serum separator tube, which allowed samples to clot for 30 minutes before centrifuging for 15 minutes at 1000 × gravity. Serum was removed immediately and samples stored at less than or equal to -20°C. Total serum levels of TIMP1 and MMP1 were determined using the Fluorokine® MAP multiplex kits (R&D Systems, Minneapolis, MN) designed for use with the Luminex® 100 dual-laser, flow-based analyzers (Luminex Corp., Austin, TX), which detect antibodies to human TIMP1 and MMP1. The inter- and intra-assay coefficients of variation were 12.7% and 5.9% for MMP1 and 10.1% and 6.5% for TIMP1, respectively. Lower detection limits were 4.40 pg/ml of MMP1 and 1.54 pg/ml of TIMP1. Because the actual activity of MMP1 depends on the balance between active enzyme and inhibitor (i.e. TIMP1), the serum MMP1:TIMP1 ratio was considered an index of MMP1 activity.

Statistical Analysis

Database management and analyses were performed with SAS software (Version 9.2, SAS Institute Inc., Cary, NC). Data was presented as mean ± standard deviation. Continuous variables between groups were compared using ANOVA, or Kruskal-Wallis test when the distribution was non-normal. Two-by-two comparisons were then performed applying Bonferroni correction with $p<0.0083$ denoting significance. Biomarker levels (TIMP1, MMP1 and MMP1:TIMP1 ratio) were log-transformed before analysis when distribution was non-normal. Pearson correlation coefficients were determined for the relationships between LV myocardial deformation parameters and biomarker levels. Interobserver variability was assessed for twist and strain measurements in 20 randomly selected patients and calculated as the standard deviation of the

differences between measurements of two independent observers who were unaware of the other patient data, then expressed as a percentage of the average value. Intraobserver variability was calculated as the standard deviation of the differences between a first and second determination of a single observer, and expressed as a percentage of the average value. Intra- and interobserver variability for LV rotation parameters in our center varies from 2 ± 3% to 10 ± 9%, and from 4 ± 4% to 12 ± 8%, respectively.[26]

Results

Baseline and Echocardiographic Characteristics

There were no statistically significant differences in age, sex or body mass index between the HTLEF and HTNEF groups ($p>0.05$) (Table 1). Mean duration of hypertension was similar between the two groups (12.2 ± 6.5 years in HTLEF and 15.5 ± 8.4 years in HTNEF, $p>0.05$) (Table 1), but mean duration of heart failure was higher in the HTLEF group (3.2 ± 1.6 years) than the HTNEF group (1.8 ± 1.0 years), $p<0.005$. All patients in the HTLEF group had LV hypertrophy compared with 85% in the HTNEF group. The HTLEF group had significantly higher LV mass index and end-diastolic volume index and lower sphericity index ($p<0.01$) (Table 2).

Myocardial Deformation and Biomarkers Analysis

Net LV twist was significantly higher in the HTNEF group (11.70° ± 0.67) compared to controls (10.9° ± 2.7) and the HTLEF group (3.34° ± 1.10) [$p<0.001$] (Table 3). AR was higher in the HTNEF group than in controls ($p<0.001$), while basal rotation was similar ($p=0.36$) (Table 3). AR and BR values were significantly higher in HTNEF compared with HTLEF ($p<0.0001$)

(Table 3). Longitudinal, circumferential and radial strain were diminished in the HTNEF and HTLEF groups compared with controls, with a greater decrement in the HTLEF group ($p<0.0001$) (Table 3). Log TIMP1, Log MMP1 and Log MMP1:TIMP1 ratio were increased in HTLEF compared with HTNEF (12.32 ± 0.25 vs. 11.81 ± 0.13; 9.08 ± 0.32 vs. 8.00 ± 0.18; and -3.25 ± 0.30 vs. -3.81 ± 0.18, $p<0.0001$; respectively) (Table 3).

Relationship Between Biomarkers and LV Twist

As longitudinal and circumferential strain and BR decreased (increasing to a positive value), Log MMP1:TIMP1 ratio increased in the HTNEF and HTLEF groups (Table 4). Log MMP1:TIMP1 ratio inversely correlated with net LV twist and EF ($r=-0.74$, $p<0.0001$; $r=-0.67$, $p<0.0001$, respectively) in hypertensive patients (Table 4). However, after adjusting for EF, Log MMP1:TIMP1 ratio explained 16.8% of the net LV twist variation ($p<0.0001$) (Table 4).

Discussion

This study's main findings were: 1) LV twist is increased in HTNEF patients, whereas all other strain parameters are diminished compared with controls; 2) In HTLEF patients, LV twist is decreased while all strain parameters are diminished to a greater extent than that observed in HTNEF patients; 3) Circulating biomarkers of collagen turnover are increased to a greater degree in HTLEF patients than in HTNEF patients; and 4) There is a modest inverse correlation between LV twist and Log MMP1:TIMP1 ratio even when adjusted for EF.

LV twist originates from the dynamic interaction between oppositely wound subepicardial and subendocardial myocardial fiber helices and has an important role in LV ejection.[7] The

contraction of the subepicardial fibers rotates the apex in a counterclockwise direction and the base in a clockwise direction. The contraction of the subendocardial region rotates the apex and base in exactly the opposite directions.[7] When both layers contract simultaneously, a larger radius of rotation for subepicardial fibers results in a mechanical advantage determining the overall direction of rotation at apex (counterclockwise) and base (clockwise).[7]

This study confirms that LV twist varies with the degree of remodelling and dysfunction in hypertensive heart disease, with similar abnormalities reported in prior studies of hypertensive patients with either normal or low EF.[6-8] Studies[6,7,22,27,28] show a positive correlation between LV twist and EF, reflecting that reduction in LV systolic function is accompanied by reduction of twist. In hypertensive patients with normal EF, longitudinal strain decreases while an increment in LV twist ensures preservation of normal EF.[6,8] HTNEF patients have less adverse remodelling; consequently their subendocardial and subepicardial fibers are less affected. Thus, while there is a decline in all strain modalities compared with controls, LV twist increases to preserve EF in these patients,[6] especially those with preserved apical function.

However, LV twist is decreased in hypertensive patients with low EF, in keeping with the overall decrease in other strain parameters. The major mechanism for this appears to relate to apical function.[7] A prior study depicted that AR correlated positively with the degree of LV dysfunction, suggesting degree of apical dysfunction is more important than the degree of basal dysfunction.[7] The absence of ischemia or infarction in our study population is important, since these confounding factors may decrease apical function and, consequently, LV twist.[28-31] It

cannot be ascertained whether the difference in LV twist observed in normal versus low EF represents a cause or consequence of a decline in EF in hypertensive-associated heart failure.

The second finding of this study is that circulating biomarkers of collagen are elevated to a greater degree – with decrements in myocardial deformation – in hypertensive patients with low EF compared to those with normal EF. These findings are similar to those documented by Lopez et al.,[13] who demonstrated an excess of MMP1 relative to TIMP1 in the myocardium of hypertensive patients with low EF compared with those with preserved EF in 39 patients with no coronary artery disease (16 with low EF, 23 with preserved EF). Their study documented that hypertensive patients with low EF had a lower volume of myocardial tissue occupied by endomysial and perimysial collagen but increased perivascular and scar-related collagen deposits compared with hypertensive patients with normal EF.[13] They also found that the serum MMP1:TIMP1 ratio was inversely correlated with EF, whereas the degree of LV dilatation was positively correlated.[13] Thus, the phenotype of LV dilatation associated with reduced EF may be a consequence of this remodelling that occurs in the extracellular matrix due to excessive MMP1 activity relative to TIMP1.

Our study shows an inverse relationship between MMP1:TIMP1 ratio and LV twist even when adjusting for EF, implying that the decline in LV twist may be related to the remodelling process of the extracellular matrix. Hypertensive patients with normal EF have lower MMP1:TIMP1 ratios, suggesting less extracellular remodelling that does not affect all regions of the myocardium to the same extent. Prior studies have suggested the major pathophysiological abnormality in HTNEF patients is that of subendocardial ischemia,[32] which occurs in the absence

of coronary disease and initially affects subendocardial fibers.[33] Subendocardial ischemia results from diminished coronary flow autoregulation in the subendocardium compared to the subepicardium with increased transmural pressure loss within the microvasculature contributing to subendocardial underperfusion and vulnerability to ischemic injury.[32]

In hypertensive patients with normal EF, the degree and location of collagen degradation may relate to the myocardial deformation indices, i.e. interstitial and perivascular fibrosis and collagen degradation is likely to initially affect the subendocardial fibers in the presence of subendocardial ischemia,[33] with decrement in longitudinal strain due to their prominent subendocardial location,[33] and thereafter the development of circular fiber dysfunction in the midwall (decreases in radial and circumferential strain).[33] If the greatest area of involvement is the subendocardium and perhaps some degree of involvement in the midmyocardium, then longitudinal strain will be most affected with varying decrements of circumferential and radial strain noted. Subendocardial fiber dysfunction will cause less opposition to the subepicardial fibers, thus the predominant rotatory effects of the subepicardial fibers dominate during systole and result in enhanced BR, AR and net LV twist. In our study, this postulate would explain the strain, AR and net LV twist observed in HTNEF. A nonsignificant trend of increased BR also was noted.

Higher MMP1:TIMP1 ratio was detected in hypertensive patients with low EF compared with HTNEF patients. This finding is similar to Lopez et al.'s[13] and suggests this process is characterized by more extensive extracellular remodelling. We propose that perhaps this adverse remodelling is a more diffuse process that affects all regions of the myocardium to a similar

degree, such that the relatively preserved subepicardial fiber function seen in HTNEF is abolished, resulting in impairment of all myocardial strain and LV twist. As collagen degradation increases, there is global dysfunction of both the subendocardial and subepicardial fibers, with diffuse collagen degradation in subepicardial and subendocardial fibers resulting in greater impairment in AR and LV twist. This accompanies greater decrements in strain parameters in hypertensive patients with low EF.

The decline in LV twist may relate both to factors that cause a global reduction in systolic function and specific remodelling factors that decrease LV twist. The loss or disruption of this collagen network may compromise systolic function by three mechanisms. Firstly, the loss of mysial collagen results in discontinuity of the extracellular matrix, compromising the alignment and support of the matrix.[13] A second factor relates to the possible loss of synchronized sarcomere contraction.[13] The final mechanism occurs as a consequence of increased MMP1 activity where there is myocardial slippage and, consequently, a decreased number of muscle layers in the left ventricle.[13] Collagen loss promotes myocyte slippage, thereby altering LV structure and function with ventricular dilatation, increasing LV sphericity and greater systolic dysfunction if the process is exaggerated.[9-13] The loss of the oblique architecture of loop fibers and abnormal muscle fiber orientation[34] responsible for the wringing motion results in a decline in LV twist.[22]

Limitations

This study cohort represents a select population, rare in Western populations where comorbidities do exist. Speckle-tracking echocardiography software used only provides a two-

dimensional analysis, and the consistency of these findings using other two-dimensional vendor technology is uncertain. Tagged magnetic resonance imaging or sonomicrometry were not employed as the gold standard to compare speckle-tracking findings. We did not histologically investigate the correlation between echocardiographic parameters and the collagen volume fraction in myocardium or assessment of tissue MMP activity (i.e. zymography). Moreover, as this study was cross-sectional with a small sample size, further follow-up and prospective research is required to determine how the changes in LV twist, extracellular collagen degradation and remodelling affect progression of hypertensive heart disease.

Perspectives

Hypertensive patients with low EF have more collagen degradation and impaired twist compared to hypertensive patients with normal EF. Log MMP1:TIMP1 ratio inversely correlates with net LV twist even when adjusted for EF. These preliminary findings may indicate that increased loss of myocardial collagen scaffolding not only accompanies more adverse remodelling but also contributes to the differences in LV twist mechanics observed between HTLEF and HTNEF patients. Alterations in collagen turnover can affect the oblique spiral myocardial fiber architecture, resulting in abnormalities in LV twist mechanics.

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Tables

Table 1. Clinical Characteristics

	Control Group	HTNEF Group	HTLEF Group
No. patients	41	41	41
Age, years	50.1±9.3^	55.5±8.4	55.0±9.1*
Gender, F:M	22:19	22:19	22:19
Body mass index, kg/m^2	24.7±1.8^	30.2±4.9	28.9±4.2$^{\#}$
Body surface area, m^2	1.72±0.14^	1.86±0.15	1.85±0.16$^{\#}$
NYHA functional capacity, no. (%)			
Class I	41 (100%)	26 (63%)$^{\#\#}$	12 (29%)$^{\#}$
Class II		15 (37%)	14 (34%)
Class III			15 (37%)
Duration of hypertension, years	---	15.5±8.4	12.2±6.5
Duration of heart failure, years	---	1.8±1.0$^{\#\#}$	3.2±1.6
Systolic blood pressure, mmHg	128±10^	141±14$^{\#\#}$	156±8$^{\#}$
Diastolic blood pressure, mmHg	79±8	84±11	89±10$^{\#}$
Heart rate, bpm	73±10	75±11$^{\#\#}$	81±10$^{\#}$
Medication, no. (%)			
Furosemide	0	41 (100%)	41 (100%)
ACE-I or ARB	0	41 (100%)	41 (100%)
Beta blockers	0	28 (68%)	41 (100%)
Amlodopine	0	41 (100%)	41 (100%)
Spironolactone	0	0	41 (100%)

Values are mean ± standard deviation. $^{\#}p<0.001$ for comparison among all groups; *p=0.03 for age among all groups; $^{\#\#}p<0.005$ for comparison between HTNEF and HTLEF. ^p<0.001 for comparison between controls and HTNEF.

ACE-I indicates angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; F:M, female-to-male ratio of number of patients; HTLEF, patient study group with hypertension and low ejection fraction (<50%); HTNEF, patient study group with hypertension and normal ejection fraction (≥50%); NYHA, New York Heart Association.

Table 2. Echocardiographic Data

	Control Group	HTNEF Group	HTLEF Group
No. patients	41	41	41
End-diastolic diameter, mm	43±5	45±1$^{\#\#}$	58±5$^{\#}$
IVSD, mm	10±1^	13±2$^{\#\#}$	12±2$^{\#}$
IVPWD, mm	8±1^	10±1	11±1$^{\#}$
End-diastolic volume index, ml/m^2	46.8±7.7	45.5±13.8$^{\#\#}$	71.7±21.6$^{\#}$
End-systolic volume index, ml/m^2	18.9±5.6	18.4±7.3$^{\#\#}$	47.1±17.0$^{\#}$
Ejection fraction, %	69.3±9.5^	60.9±7.3$^{\#\#}$	33.4±7.1$^{\#}$
Left ventricular mass index, g/m^2	73.8±13.9^	106.3±21.3$^{\#\#}$	152.0±25.3$^{\#}$
Left atrial volume index, ml/m^2	23.0±2.2^	26.8±3.8$^{\#\#}$	31.0±16.3$^{\#}$
LVSPHI (end diastole), ratio	1.9±0.3^	1.7±0.1$^{\#\#}$	1.4±0.1$^{\#}$
LVSPHI (end systole), ratio	2.1±0.5^	1.8±0.2$^{\#\#}$	1.4±0.1$^{\#}$
E/E` ratio	5.6±1.8^	11.6±4.6$^{\#\#}$	18.7±12.1$^{\#}$

Values are mean ± standard deviation. $^{\#}p<0.001$ for comparison among all groups; $^{\#\#}p<0.01$ for comparison between HTNEF and HTLEF. ^$p < 0.001$ for comparison between controls and HTNEF.

HTLEF indicates patients with hypertension and ejection fraction <50% on echocardiography; HTNEF, patients with hypertension and ejection fraction ≥50% on echocardiography; IVPWD, interventricular posterior wall at end-diastolic dimension; IVSD, interventricular septal thickness at end-diastolic dimension; LVSPHI, left ventricular sphericity index.

Table 3. Myocardial Deformation and Biomarkers Comparison Between Controls and Hypertensive Patients

	Control Group	HTNEF Group	HTLEF Group
No. patients	41	41	41
Apical rotation, degrees	6.8±1.5^	7.7±0.4##	1.7±1.0#
Basal rotation, degrees	-3.8±1.6	-4.0±0.6##	-1.6±0.9#
Time to peak apical rotation, ms	348±20	356±14##	326±6*
Net twist, degrees	10.6±2.3^	11.7±0.7##	3.3±1.1#
Untwisting rate, degrees/s	-50.6±4.3^	-39.1±3.4##	-26.8±6.4#
Longitudinal strain, %	-14.6±2.2^	-11.4±0.2##	-8.7±1.2#
Circumferential strain,%	-14.6±2.3^	-11.5±0.3##	-9.0±1.4#
Radial strain, %	54.6±2.3^	42.2±2.4##	29.0±1.3#
TIMP1, pg/ml	86240.2±12405.2^	135969.3±20767.1##	232612.2±66117.2#
Log TIMP1	11.4±0.1^	11.8±0.1##	12.3±0.3#
MMP1, pg/ml	894.8±213.1^	3036.8±553.7##	9203.1±2981.0#
Log MMP1	6.8±0.2^	8.0±0.2##	9.1±0.3#
MMP1:TIMP1 ratio	0.011±0.003^	0.023±0.004##	0.041±0.014#
Log MMP1:TIMP1 ratio	-4.6±0.3^	-3.8±0.2##	-3.3±0.3#

Values are mean ± standard deviation. #p<0.0001 for comparison among all groups; *p<0.01 for comparison among all groups; ##p<0.005 for comparison between HTNEF and HTLEF. ^p<0.001 for comparison between controls and HTNEF.

HTLEF indicates patients with hypertension and ejection fraction <50% on echocardiography; HTNEF, patients with hypertension and ejection fraction ≥50% on echocardiography; MMP1, matrix metalloproteinase-1; TIMP1, tissue inhibitor of matrix metalloproteinase-1.

Table 4: Correlation of Log MMP1:TIMP1 With Myocardial Deformation Parameters in Hypertensive Patients

	Unadjusted	Adjusted for ejection fraction
Ejection fraction, %	r=-0.67 p<0.0001	---
Twist, degrees	r=-0.74 p<0.0001	**r=-0.41** **p<0.0001**
Apical rotation, degrees	r=-0.74 p<0.0001	r=-0.40 p=0.0002
Basal rotation, degrees	r=0.64 p<0.0001	r=0.30 p=0.006
Longitudinal strain, %	r=0.53 p<0.0001	r=0.08 p=0.50

MMP1:TIMP1 indicates ratio of matrix metalloproteinase-1 to tissue inhibitor of matrix metalloproteinase-1.

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