Evolution of Left ventricular Function and Its Tolerance of Tachycardia in Early Infancy: a Simultaneous Invasive and Noninvasive Assessment in Neonatal and Young Infant Piglets

By

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Abstract

Background: The literature examining ventricular function of the neonate and young infant is fragmented. Furthermore, the clinical implications of the observed differences have been largely extrapolated from adult studies. Clinical echocardiography-based investigations at rest, for instance, have suggested the neonatal left ventricle (LV) rapidly improves its ability to relax within weeks of delivery. There is a paucity of data, however, on the functional reserve of the neonatal and young infant heart when facing hemodynamic stress. As reduced ventricular relaxation in the diseased adult heart is associated with poor tolerance to atrial tachycardia due in part to reduced filling time, it has been thought that the neonatal heart may be less tolerant to tachycardia than that of the infant. We sought to explore the impact of early LV maturation on its ability to tolerate atrial tachycardia. We hypothesized that the neonatal LV would be less tolerant of atrial tachycardia than that of the young infant equivalent.

Methods: Under general anesthesia (propofol, isoflurane), neonatal (1-3days, NP) and infant (14-17days, YP) Landrace cross piglets (n=7 each) were instrumented intravascularly with Millar® high-fidelity and pacing catheters in the left ventricle and right atrium, respectively. Invasive hemodynamic and basic echocardiography parameters were acquired at baseline, and at 200, 230 and 260bpm. Speckle tracking technique was then used for different left ventricular deformation studies (i.e. strain, strain rate and twist).

Results: At all rates, NP maintained their LV output and blood pressure, whereas YPs did not. Negative dP/dt was lower in NPs at baseline (-1599±83 vs. -2470±226mmHg/s, p=0.007) but increased with pacing (p=0.002). In contrast, YP baseline negative dp/dt

was greater and tended to decrease with pacing (p=0.056). All other invasive measures of diastolic function (left ventricular end-diastolic pressure, tau and isovolumic relaxation time) did not differ between groups. Except for fractional shortening which was reduced in YP at 260bpm; all invasive and noninvasive measures of contractility did not differ between groups. Twist studies demonstrated a similar increase in peak LV twist and untwisting rate in response to tachycardia (combined data: baseline -259±22; at 260bpm -498±59 deg/s, p=0.003); however, overall twist patterns differed between groups: NP tended to increase apical positive rotation (p=0.1) while YPs increased basal negative rotation (p=0.009) to augment LV twist. Finally, as it permitted testing of the effects of tachycardia on different noninvasive LV function markers, it was shown that echo-measured strain rate is not independent of heart rate in the immature heart. This is in contrast to what has been demonstrated for the more mature heart.

Conclusion: The neonatal piglet LV appears more tolerant of atrial tachycardia than that of the young piglet heart which could relate to better diastolic performance and differences in twist mechanics. Use of strain rate in the immature heart should take into consideration changes in heart rate.

Preface

This thesis is an original work by Étienne Fortin-Pellerin. The research project presented in this document has received ethics approval from the University of Alberta Research Ethics Board (Title: Evolution of Diastolic Function, AUP 00000920).

Data presented in this work have thus far been published as abstracts in several international meetings and one manuscript has been submitted to the American Journal of Physiology:

1. Fortin-Pellerin E, Mills L, Coe JY, Khoo NS, Cheung PY, Hornberger LK. "**Increased Diastolic Untwisting Velocity in Response to Tachycardia as Evidence of Diastolic Reserve in the Young Infant Heart: A Simultaneous Invasive and Noninvasive *in vivo* Swine Model."** American Heart Association, Chicago November 2014. (Appendix 2)

2. Fortin-Pellerin E, Mills L, Coe JY, Khoo NS, Cheung PY, Hornberger LK "**Decreased tolerance to tachycardia in the first weeks of life in a swine model, using speckle tracking echocardiography and invasive monitoring."** Pediatric Academic Societies, San Diego May 2015. (Appendix 3)

3. Etienne Fortin-Pellerin, Lisa K Hornberger, James Y Coe, Lindsay Mills, Jesus Serrano-Lomelin, Po Yin Cheung, and Nee S Khoo. **"Strain Rate is Affected by Heart Rate in Younger Heart: A Simultaneous Invasive and Noninvasive *In Vivo* Piglet Model."** American Heart Association, Orlando November 2015.

4. Fortin-Pellerin E, Khoo NS, Mills L, Coe JY, Serrano-Lomelin J, Cheung PY, Hornberger LK **"Postnatal Neonatal Myocardial Adaptation is Associated with Loss**

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Abbreviations

BP: Blood pressure

Ca: Calcium

CICR: Calcium-induced calcium release

CSA: Cross sectional area

CVP: Central venous pressure

FFR: Force frequency relationship. Also known as the Treppe or Bowditch phenomenon

FS: Fractional shortening

HR: Heart rate

IVRT: Isovolumic relaxation time

LV: Left ventricle

LVEDP: Left ventricular end diastolic pressure

LVEDD: Left ventricular end diastolic dimension

LVESD: Left ventricular end systolic dimension

LVO: Left ventricular output

MRI: Magnetic resonance imaging

NCX: Sodium-calcium exchanger

NP: Neonatal piglets

SERCA: Sarcoplasmic reticulum Ca++-ATPase

SR: Strain rate

SV: Stroke volume

TVI: Time velocity integral

VcFc: Rate-corrected velocity of fiber shortening

YP: Young piglets

Introduction

Hemodynamic management of the critically ill term newborn is complex. Understanding the functional nature of the neonatal myocardium and how it evolves through the first few weeks and months after birth is key to optimizing care. Although many authors have provided their opinions regarding clinical management strategies (1-3), more translational and clinical studies are needed to provide insight into the functional capacity of the early postnatal heart.

It has long been recognized that the diastolic or filling function of the newborn heart is different from that of the infant, the older child and even more so, of the healthy adult. Clinical investigations have suggested the neonatal left ventricle (LV) may have a reduced ability to relax relying more so on atrial contraction to fill at least at rest. The reserve of the neonatal LV when facing hemodynamic stress, including its response to chronotropic stress, which is poorly tolerated by the adult heart with diastolic dysfunction, however, remains inadequately explored.

At a cellular level, calcium (Ca) handling contributes critically to efficiency of ventricular relaxation. The body of literature exploring the evolution of cardiomyocyte cytosolic Ca handling from the neonatal to adult stages is extensive (4). However, this literature uses a wide range of models and is very fragmented. Furthermore, how changes in Ca handling translate to *in vivo* diastolic reserve has not been well explored. Some insightful animal models of cardiac function maturation are available (5-8) but tolerance to atrial tachycardia, a feature commonly observed in the critically ill neonate, has been

poorly explored. Most have either investigated differences between the fetus and the neonate or between the neonate and the adult. The subtle evolution of diastolic function in the first few weeks of life secondary to changes in cellular physiology are almost absent from the literature. As invasive studies are difficult to do in such a fragile and vulnerable patient population, our current understanding of the maturation of diastolic function in early infancy is strongly influenced by echocardiography-based observations. Most of these studies have been performed at rest and lack validation. Furthermore, many of the traditional echo based methods, particularly those examining diastolic function, have limited use at higher heart rates. Newer echocardiographic techniques that allow for direct assessment of the twist motion of the left ventricle (LV) open a new field of functional assessment at the bedside, and may prove to be more effective at teasing out differences in both systolic and diastolic function. However, the validity and specificities of this technique in the neonatal population needs to be further explored before it can be used clinically.

The overall purpose of the current research project is to explore differences in tolerance to atrial tachycardia in a piglet model from the first few days of life to the early infancy stage, focusing on diastolic function and twist mechanics. As a secondary objective, we will explore the impact of tachycardia using echocardiography-based deformation techniques (i.e. twist and strain rate) to validate their role in examining functional response to chronotropic stress noninvasively.

Chapter 1 - Background

Left Ventricular Diastolic Physiology of the Neonate and Its Assessment at Bedside and in the Laboratory

Normal diastolic function refers to the ability of the ventricle to adequately fill at low pressures in order to maintain forward flow. Diastole is divided into four phases: isovolumetric relaxation (events happening between aortic valve closure and mitral valve opening), early diastolic filling, diastasis and filling during atrial systole (9) (Figure 1.1). Two main features of the ventricular myocardium determine the performance of these phases: its ability to relax and its compliance (10). Traditionally, the early component of filling is associated with adequate relaxation of the ventricle, and late filling (secondary to atrial contraction) to passive compliance of the ventricle (10). Atrial tachycardia and exercise stress diastolic function as a reduction in cycle length affords the ventricle less time for filling.

In the following overview, the theoretical framework has been artificially divided between fundamental cellular properties and events and *in vivo* assessment of diastolic function. The ultimate goal of this work, however, is to elucidate how clinically applied noninvasive echo-based measures reflect functional response based on standard invasive measures that can facilitate translation to the bedside. This review focuses on functional processes described for the immature myocardium, particularly the left ventricle (LV) and its response to chronotropic stress. Strengths and weakness of the piglet model will also be outlined.

1.1 Cellular Components of Diastolic Function and Aspects Unique to the Neonatal Myocardium

1.1.1 Cellular Contraction & Relaxation Events

1.1.1.1 Systolic Calcium (Ca) Release

During systole, sarcolemmal depolarization leads to Ca entry into the myocyte through voltage-dependent L-type Ca channels. This triggers a large release of Ca from the sarcoplasmic reticulum by the ryanodine receptors, a process known as calcium-induced Ca release (CICR) (11) (Figure 1.2). The junctional sarcoplasmic reticulum is in close proximity with T-tubular membrane forming a dyadic cleft that is the basis for CICR (12) (Figure 1.3). This Ca in the cytosol interacts with troponin C allowing actin and myosin coupling which generates contraction. The mechanism that stops Ca entry in the cell is complex but likely involves a drop in sarcoplasmic reticulum Ca concentration (13) and inhibition of L-type Ca^{2+} channels by the calcium-calmodulin complex (14).

1.1.1.2 Diastolic Cytosolic Ca Clearing

In early diastole, myocardial relaxation requires Ca to be removed from the cytosol. First, Ca is actively pumped back into the sarcoplasmic reticulum by the sarcoplasmic reticulum Ca++-ATPase (SERCA). This pump is associated with phospholamban, a regulatory protein that inhibits SERCA activity when not phosphorylated (15). Phosphorylation of phospholamban is one of the end-results of beta-adrenergic stimulation of cardiomyocytes, which enhances Ca reuptake by the sarcoplasmic reticulum. Ca can also exit the cell through a gradient sensitive sodium-calcium exchanger (NCX) located on the myocyte membrane. The ratio of Ca extrusion for SERCA and NCX is species specific: roughly 7:3 in larger animals (e.g. rabbits) and 9:1 in smaller animals (e.g. rats) (16) . This extrusion ratio is not constant during diastole

and NCX becomes more important in the second half of diastole (17). The sodium gradient is maintained by the ubiquitous Na/K ATPase (Na pump) (18), which is thought to be partly regulated by phospholemman, an accessory protein that is an element within the Na pump complex (19). Phospholemman action is complex as its phosphorylation relieves the inhibition exerted on the Na pump, but it also inhibits NCX (20). These opposing effects on cytosolic Ca concentration have been hypothesized to prevent Ca accumulation thus reducing the risk of arrhythmia (19, 21), while maintaining contractility by preventing excessive Ca extrusion (21). Approximately 1-2% of Ca exits the cytosol either by an ATP-consuming sarcolemmal Ca pump to the extracellular space or into mitochondrial via a Ca uniport (16, 22).

Cytosolic Ca handling cannot be assessed only by its movement in and out of the cytosol. Cytosolic Ca transients are influenced by Ca buffers (23). Also, the complex spatial organization of regulatory proteins and organelles generates ionic microdomains in the cytosol (18, 22), such that the cytosol cannot be analyzed as a homogenous compartment.

1.1.1.3 Compliance

Compliance is the measure of change in volume per change in pressure. For the LV it is a passive property determined by the relative chamber volume and geometry, the ventricular muscle mass and the effective stiffness of a unit of the myocardium (myocardial tissue characteristic) (10). Normal LV compliance usually refers to the ability of the LV to accommodate blood at end diastole, during atrial contraction, without increasing left ventricular filling pressures. It is especially influenced by the nature of LV non-contractile elements. One such element is titin, an elastic molecule that supports

myosin. It is stretched at end diastole (24) but compressed at end systole, acting as a 'bidirectional spring' (25) (Figure 1.4). In early diastole, these large molecules can release the potential energy stored at end systole, referred to as "restoring forces," and this ultimately contributes to LV filling. Two major isoforms of titin are expressed in the adult heart, a stiff N2B variant and a more compliant N2BA variant (26).

1.1.1.4 Cellular Response to Tachycardia

As heart rate increases, contractility increases as well, a process known as the force-frequency relationship (FFR). It is thought that as the heart rate increases, the rapid entry of Ca in the cytosol overruns the ability of the NCX to extrude Ca out of the cell (known as the Na pump lag hypothesis). As already discussed, NCX depend on the Na pump to maintain a low intracellular Na concentration that drives the NCX. Aronsen and colleagues (18) suggested that the relative importance of SERCA activity in lowering cytosolic Ca would increase as diastolic time interval shortens with tachycardia, NCX being mostly a late contributor to Ca clearing (17). Ca is preferentially cleared by SERCA into sarcoplasmic reticulum and is then available for release in greater amounts during the next cardiac cycle, achieving higher contractility.

Catecholaminergic effects are usually present in patients with tachycardia. Most cardiac effects are mediated through beta1-adrenergic receptors on the cardiomyocyte cellular membrane. These receptors activate the GTP-cyclic AMP-protein kinase A pathway which leads to phosphorylation of phospholamban, L-type Ca channels, Ryanodine receptor, troponin I and myosin binding protein C (22). Acceleration of relaxation with adrenergic stimulation is mainly mediated by phosphorylation of phospholamban (27) which lifts the inhibition on SERCA activity. These adaptive

mechanisms are impaired in the failing heart which results in increased filling pressure during exercise, contributing to exercise intolerance (28, 29).

1.1.2 Ca Handling and Compliance of the Neonatal Cardiomyocyte

The neonatal cardiomyocyte is thought to rely on sarcolemmal Ca flux more than on CICR mechanism for contraction (30, 31). T-tubules are not well developed in the neonatal myocardium and the spatial configuration of the sarcoplasmic reticulum may be insufficient to fully accomplish CICR (32-34). The perinatal transition to the postnatal circulation, with acute changes in afterload and preload of the LV, sets off a maturational process leading to disproportionate, rapid LV growth (7). Already at 48 hours of life there is evidence for acceleration of ribosome formation and protein synthesis (35). With subsequent neonatal maturation, there is an increase in both SERCA (36-38) and phospholamban (39) levels. A higher SERCA activity, in theory, would increase the amount of Ca available for the next contraction (i.e. positive inotropy) and more phospholamban could increase the potential for adrenergic stimulation. Previous reports have provided evidence that with maturation there is progressive improvement in contractile response to adrenergic stimulation which is consistent with cellular changes in Ca handling (5, 6). Although there is evidence for an increase in 'contractility reserve' with maturation, the evolution of LV diastolic function, both relaxation and compliance, in early infancy, and the degree of diastolic reserve remains less clear.

Lower SERCA (36-38) and higher NCX (34, 40) expression in the neonatal period have been documented and engender legitimate questions about LV relaxation efficiency of the immature heart. Down regulation of SERCA and reliance on NCX for cytosolic Ca clearing are cellular features observed in adult heart failure models (41, 42)

and have been associated with relaxation abnormalities (43). Whether these observations in the diseased adult heart in fact can be translated to the functional capacity of the healthy developing neonatal myocardium is not certain particularly as there are some aspects of cellular events that could augment Ca handling in the neonatal heart. Over expression of the Na pump (40) in the neonate which could potentially drive the large number of NCX channels could actually be a very efficient route for Ca extrusion out of the cell during diastole. Furthermore, a recent elegant study using pathologic human neonatal cardiac tissue revealed adequate acceleration of relaxation with tachycardia as well as a normal amount and distribution of SERCA when compared to older children (34), suggesting a functional sarcoplasmic reticulum is present at birth in humans. This could support previous data showing SERCA protein expression levels at birth to be only slightly lower to adult levels in humans (44). Finally, phospholemman expression has been shown to decline with postnatal maturation (40) and at least one scientific article has described the evolution of intracellular Ca buffering capacity with maturation which could also contribute to changes in diastolic reserve (45). How these findings affect the overall clearing capacity of Ca to the extracellular space in the neonatal myocardium has not been fully explored.

It has been suggested, based on early studies of isolated muscle, that compliance of the LV myocardium increases with age, being the least in the fetus and improving through to the early adult period (7) (Figure 1.5). Lower compliance limits myocardial distensibility in diastole and tolerance to excessive preload, resulting in high filling pressures and signs of congestive heart failure under hemodynamic stress (46). Based on these early studies it has been suggested that differences in compliance may be due to the

greater collagen to total protein ratio and to the components of the extracellular matrix, particularly the type of collagen in the immature heart (47). However, some characteristics of the immature myocardium may in fact contribute to better compliance. For instance, the neonatal myocardium expresses a higher N2BA:N2B ratio than that of the adult, which includes a fetal variant N2BA subisoform that consists of a higher level of spring elements ultimately improving compliance (26). This fetal isoform has been shown to rapidly decrease in the first few weeks of life in the piglet model (half life for disappearance of 18 days) (26). In this particular study by Lahmers et al., the neonatal myocardium was indeed less stiff than that of the adult pig. Further studies are needed to confirm these changes and to understand whether these findings translate to human myocardium.

1.1.2.1 Limits of the cellular approach

Maturational aspects of cardiomyocyte Ca handling have been under scrutiny for decades. Although these findings can help generate hypotheses on neonatal tolerance to tachycardia in the critically ill patient, we must consider the numerous pitfalls associated with this approach. Cellular physiology literature relies on a wide range of animal models for cellular studies (i.e. rat, rabbit, swine, lamb, etc.). It is well known that some maturational processes are species specific, at least in their timing in relation to birth. For instance, although the piglet sarcoplasmic reticulum has been shown to be fully functional at birth, clearly contributing to the contraction-relaxation cycle (48), the opposite is true of smaller animal models (49). A recent study using pathologic human neonatal cardiac tissue revealed acceleration of relaxation with tachycardia as well as a normal amount and distribution of SERCA when compared to older child (34), suggesting at least some sarcoplasmic reticulum maturity at birth in humans; however,

pathological processes have been shown to have profound effects on Ca cellular handling (4, 41) and thus assuming observations in pathological human tissue accurately represent that of healthy myocardium is problematic. Still, these findings could be in keeping with the observations made in the piglet models. Finally, it is hard to predict the myocardial response to hemodynamic stress based on cellular studies as such response depends not only on intracellular calcium handling properties but also on cardiac micro and macro architecture, electrophysiology, loading conditions and so on. *In vivo* models are thus still essential to hemodynamic studies.

1.2 Assessment of Diastolic Function

Adequate LV diastolic function during tachycardia refers to the processes by which the ventricle can maintain filling in order to allow for sufficient cardiac output without increasing filling pressures. It cannot be analyzed as an isolated event as it is inherently affected by systolic function and loading conditions. As maturation also affects LV tolerance of loading which will ultimately impact diastolic function, a short review of how maturation affects the response to load is presented in this section. In the laboratory, heart function can be studied using isolated muscle strip/papillary muscle or the whole heart mounted on a Langendorff apparatus where concepts of preload, afterload and contraction rate can be adjusted. In the living human or mammalian models, these parameters can be assessed but rarely perfectly controlled. However, studying a living organism is what most resembles the clinical setting. The gold standard for *in vivo* assessment of diastolic function is the use of a high fidelity pressure catheter positioned within the ventricle. Unfortunately, clinically relevant occasions to insert such specialized catheters are rare in the neonatal population and invasive procedures cannot be done solely for research purposes. Hence, human neonatal diastolic function is now mostly studied using echocardiographic techniques.

1.2.1 Invasive *In Vivo* Models

1.2.1.1 Response to Preload

Tolerance to tachycardia is influenced by preload. Sugimoto & al. demonstrated decades ago using an adult dog model that variation in cardiac output with electrically induced tachycardia was greatly amplified by volume load (50). Although some controversies exist (8), it is generally accepted that the neonatal heart is able to increase

its output in response to increased preload (Franck-Starling law) (51-55). There is, however, evidence for a better response with maturation (51, 52). The optimal preload necessary to maximize cardiac output at different levels of tachycardia through the neonatal period has not been previously defined.

1.2.1.2 Tolerance to Afterload

Although not technically related to relaxation or compliance (i.e. diastolic properties), a low tolerance to afterload can generate low left ventricular output (LVO) and increase filling pressures, and must be taken into consideration when assessing response to tachycardia. Neonatal tolerance to afterload is believed to be low. As the low resistance placenta is removed from the circulation at birth, the neonatal LV must work against an acutely increased afterload in order to maintain output. Even though LV maturation occurs rapidly (see neonatal cellular Ca handling section), a low tolerance to a superimposed increase in afterload is probable. Eiby et al. demonstrated a decrease in LVO in both a preterm and term piglet isolated heart model in response to increased afterload (55). In an earlier study, Teitel et al. demonstrated an increase in 'contractility reserve' during the first 4 weeks of life in a lamb model (5).

1.2.1.3 Tolerance to tachycardia

Tachycardia reduces the ventricular filling period. If filling is not coupled in some way to heart rate, LVO would be expected to decrease as heart rate increases. Tachycardia induced by exercise and endogenous catecholamines have a variable effect on LV stroke volume (SV) (56, 57) as preload (58) and contractility (22) are also affected by the stimuli for tachycardia. A maintained or augmented SV in combination with tachycardia increases LVO; however, in order to precisely control heart rate and remove

the impact of exogenous catecholamines, an atrial pacing tachycardia model was chosen for the current work.

1.2.1.4 The Atrial Pacing Tachycardia Model

Tachycardia induced by atrial pacing is a distinct model. Pace-maker induced atrial tachycardia at lower rates has been shown to be associated with no significant change in mean arterial pressure, venous return, or circulating catecholamines (59). Right atrial pacing in healthy patients has been shown to reduce the SV, with a decrease in both end-diastolic and end-systolic volume while having little effect on neither LV ejection fraction nor LVO at low grade tachycardia (56, 57, 60, 61). LVO can however be increased by tachycardia if preload is significantly enhanced by volume infusion (50). LV end diastolic pressure (LVEDP) decreases with pacing in patients with normal diastolic function but this response is altered with diastolic dysfunction with progressively increasing LVEDP (Figure 1.6) (61).

Even in the absence of significant catecholaminergic stimulation during atrial pacing, some improvements in systolic mechanics may be observed. Anderson et al. found an increasing stroke volume with increasing levels of tachycardia when maintaining stable preload conditions (56).

Finally, atrial pacing might not be the best model to assess the impact of LV compliance on LVEDP as the heart cycle is artificially shortened and the ventricle is not stretched as it would normally be at end diastole.

1.2.1.5 Impact of Maturation on Tolerance to Tachycardia

The limits of tolerance to tachycardia of the immature heart through the first few weeks of life are poorly explored in the literature. In a lamb fetus model (0.75 to 0.85

gestation), Rudolph et al. demonstrated that spontaneous as well as pacemaker induced atrial tachycardia was associated with a stable or even slightly increased LVO (60), up to a rate of 270bpm. This suggests some level of diastolic reserve in the very immature heart and was later confirmed using a similar model (56). One must take into account the lower afterload and the existence of communications between systemic and pulmonary circulation in the fetus making it difficult to translate this data to the neonate. In contrast to the data in fetal animal models, using an isolated neonatal (1 to 3 days of age) piglet heart model, Joyce et al., observed an increase in LVEDP at 300bpm when compared to the baseline value obtained of 150bpm at a similar end diastolic volume (62). They also found tau to be similar at 150 and 300bpm. These findings would suggest the presence of diastolic failure or at least insufficient acceleration of relaxation at higher rates. The authors suggested that diastolic time might be insufficient for complete inactivation of cross-bridging of actin and myosin filaments (i.e. relaxation cannot keep up with heart rate). How this lack of tolerance to higher rates of tachycardia evolves with age, particularly within the first few weeks and months of life remains uncertain.

1.2.2 Echocardiographic Assessment of Diastolic Function in Humans

1.2.2.1 Mitral inflow studies

One frequently applied technique to study diastolic function at the bedside is the analysis of the Doppler signal of mitral inflow during diastole using echocardiography. As pressure decreases in the LV following contraction, an early 'E wave' is recorded, representing the rapid inflow of blood following mitral valve opening. A late 'A wave' represents the entry of blood with atrial contraction. Many markers of diastolic function using these Doppler studies (i.e. isovolumic relaxation time [IVRT], deceleration time,

E/A wave ratio) have been developed for adults. In the early phases of diastolic dysfunction, the transmitral Doppler interrogation shows a decrease in E/A wave ratio with an increase in dependency on atrial contraction for LV filling (9). This finding identifies the adult patient with poor tolerance to tachycardia (63). The transmitral flow pattern in the newborn (64, 65), obtained at rest by echocardiography, has a similar pattern. The neonatal LV also has a prolonged IVRT (66, 67) relative to the infant, child and adult, another marker of delayed or sluggish relaxation in adult patients. LV filling evolves rapidly such that by 3 months of age, the pattern is akin to that of an older child and young adolescent, suggesting significant improvement in the ability of the LV to relax (66). Partly based on these investigations, the newborn myocardium has been assumed to have less efficient relaxation; however, these studies have been performed at rest and as such it is difficult to know whether the neonatal heart has diastolic reserve in response to hemodynamic and chronotropic challenges. Furthermore, although it is reasonable to say that the relative contribution of the atrial contraction to LV filling is higher in the neonate, this cannot be linked directly nor solely to deficient relaxation, as other parameters such as the release of restoring forces are involved in the process of early LV filling. Few studies have examined the impact of tachycardia on diastolic echo-based parameters in the neonatal LV (68-70); however, these exams were conducted in otherwise healthy children with spontaneously occurring small increases in heart rate well within a normal range and with a paucity of data for heart rates above 160bpm. Finally, it has been well recognized that at fast heart rates, the E and A waves tend to fuse and this has made the interpretation of the findings a challenge (71).

1.2.2.2 Twist Assessment

Initially described by Lower in 1669 (72), LV twist has recently received heightened interest as advances in noninvasive imaging technology have made it possible for non-invasive assessment of this phenomenon (i.e. magnetic resonance imaging and echocardiography). LV twist is due to the geometrical distribution of muscle fibers of the LV myocardium. From a right-handed helix arrangement in the subendocardial region, muscle fibers smoothly transition to a left-handed helix in the subepicardial region (Figure 1.7A). During systole, the subepicardial fibers dominate and a wringing counterclockwise motion of the LV, as if viewed from the feet, occurs (73). As blood is ejected, the LV dimensions decrease under the LV resting volume (i.e. equilibrium volume) and potential energy (i.e. restoring forces) is stored in non-contractile elements of the LV wall. During diastole, a quick release of restoring forces is allowed by an adequate LV muscular relaxation and contributes to suction of blood within the LV chamber (74).

In the clinical setting, twist can be measured using two echocardiographic cross-section images of the heart, one obtained at the apex of the heart and the other at the base, using speckle-tracking analysis technique (Figure 1.7). By convention, counterclockwise is expressed as positive and clockwise as negative rotation. Twist refers to the net difference, in degree, between the apical and basal rotation motion. Torsion refers to twist divided by LV length. Of note, twist is not identical between the different segments as well as between the subendocardial and subepicardium regions (75, 76). The commercially available software used for the current work (i.e. EchoPac) averages the different LV segments as well as the rotation values from the whole thickness of the LV wall.

Scientific evidence relating to twist mechanics must be interpreted with caution as twist mechanics are influenced by many different hemodynamic parameters. Conceptually, LV twist can be viewed as an oscillation of the LV around the equilibrium volume, a parameter that is clinically difficult to assess and more than often poorly reported in the scientific literature. Alteration of end diastolic and/or end systolic LV dimensions will affect the oscillation pattern. Preload is crucial as it affects the relative position of the apex to the base of the heart at end systole and end diastole without necessarily affecting twist amplitude (77). The effects of increased afterload are even more complex. An acute increase in afterload on a single heart beat, at a fixed preload, will decrease apical rotation at end systole and thus lower twist amplitude (77). However, the impact of an increase in afterload should probably not be evaluated on a single beat as the subsequent heart cycles will be affected and adaptive mechanisms will occur. An increase in afterload using methoxamine in humans showed no reduction in global LV torsion (78). Positive inotropy increases twist (75, 76, 78), an effect partly mediated by lowering end-systolic volume, and secondarily affecting untwisting mechanics (79). Finally, increases in contractility or afterload have been associated with delayed untwisting (77).

In the mature heart the basal LV wall will, after a very short early systolic counterclockwise rotation motion, mostly rotate in a clockwise fashion during systole whereas the apex rotates counterclockwise. In the infant population, the basal counterclockwise initial motion is prominent during systole and LV twist increases with age as the basal component moves more clockwise (80). In a normal mature heart, most of the LV relaxation occurs before the opening of the mitral valve (80). This efficient

relaxation, combined with the release of restoring forces stored in the non-contractile elements of the myocardium following ventricular contraction, generates an intraventricular pressure gradient that "sucks" blood from the left atrium into the LV. This suction phenomenon is enhanced during exercise in the healthy heart (81, 82) and the inability to optimize LV relaxation with exercise is a characteristic of diastolic heart failure (9). The finding that the percentage of untwisting happening before mitral valve opening in a resting state is significantly less in an infant population than in adults has been used as an additional argument for reduced diastolic function in the immature heart relative to that of the mature heart (80); however, whether these observations are maintained with exercise or stress in the immature heart is not certain.

How twist is affected by acute exercise or chronic stress is of particular relevance to the current work. Notomi et al. demonstrated that the healthy adult increases both twist and untwisting velocity during sub maximal exercise, thereby increasing suction (82). In that population, both the negative basal component and the apical positive rotation components were increased. A recent study on LV twist during sub maximal exercise in children and adult subjects showed a blunted increase in peak twist and peak untwisting rates in the child group compared to the adults (83). The adults needed to increase basal rotation component more than the young patients. We are unaware of similar studies in the neonatal population. Patients with hypertrophic cardiomyopathy have an increased LV twist at rest but lose the ability to increase twist and untwisting velocity with exercise (82). An increase in baseline twist has also been described with aging (80), whereas it is decreased in highly trained hearts (84). The authors of this last study hypothesized that trained hearts simply do not need to twist as much at rest to maintain output. After an

intense exercise, the highly trained LV increases its torsion when compared to the resting state (85). Furthermore, chronic increase in afterload, such as with aortic coarctation or valvular aortic stenosis, will result in higher baseline LV torsion as a compensatory mechanism (86). Interestingly, a recent study from our team demonstrated a similar pattern of increased torsion amongst preterm neonates when compared to term neonates, both studied at 1 month of chronologic age, suggesting a chronic compensation for an early exposure to the postnatal circulation (i.e. high afterload) (87) (Poster presentation, see Appendix 1).

Based on this data, it is clear that twist mechanics change with age with, at least at rest, evidence of progressive improvement in function. However, the impact of stress including tachycardia, on twist mechanics in the neonatal and young infant heart has been underexplored. A more precise documentation of twist mechanics at rest and during exercise is warranted before functional markers of tolerance to tachycardia can be explored in the neonatal population.

1.3 The Piglet Model

The swine model has been gaining favor over canine models during the past couple of decades (88), and has been progressively used to study the neonatal cardiovascular function (89-94). The piglet heart anatomy is similar to that of the humans with only minor morphologic differences described (88, 95, 96). Its gross postnatal development has been documented (97, 98). The adult pig titin expression is much closer to what is seen in humans than with other species (26), an important parameter to consider when analyzing twist mechanics. Echocardiography in the swine is feasible but difficult because of narrow intercostal spaces and wide ribs (99). We have demonstrated the feasibility of twist studies in our model, and presented these preliminary results at the American Heart Association meeting in Chicago in November 2014 (100) (Appendix 2). We found the twist pattern of the neonatal piglet to be very similar to what we previously documented in healthy human neonates, with a similar biphasic pattern of basal rotation.

Defining corresponding developmental ages between the piglet and human is challenging. Direct correlation for each physiologic process is impossible. Domestic pigs are born at a lower weight (1-2kg) than humans but reach higher adult weights. From our own data set, the size of heart and great arteries are very similar between the piglet and the human neonate when matched for weight. Swines are weaned around 4-6 weeks of age (99). From birth to 4 months of age, the pig is said to have a cardiovascular size growth analogous to humans at mid-teens years (96). One way to try to correlate heart developmental stage between species is to look at the normal physiologic increase in binucleated myocyte ratio from birth to the adult age (101). In humans, binucleated myocytes increase from 9% +/- 5% at birth to 57% +/-17% at 1 year (102).

Unfortunately, studies on nucleation have not yielded consistent results, with human adult level of binucleation ranging from 25 to 60% and wide inter-species variation (103). Piglets have a similar percentage of binucleate myocytes at birth (i.e. just under 10%) (104), but adult pigs have been shown to have a very high level of myocytes with more than 2 nuclei (105) while human adults do not (106). Levels of binucleated cells in adult pigs have been reported as low as 12% (105) and neonatal changes in binucleated levels are not well described. Taken these inter-species differences and the lack of data, we cannot reasonably define an age correlation based on nucleation. For the purposes of the present investigation, we chose to utilize 2 week old piglets as a proxy for late neonatal to early infancy human period. At that age, they have not yet been weaned and have a similar size to human infants. More importantly, heart rate is relatively stable from birth to 2 weeks of life in the swine model (107). As this project focuses on tolerance to tachycardia within the first weeks of life, this particular characteristic simplifies data interpretation.

Figures

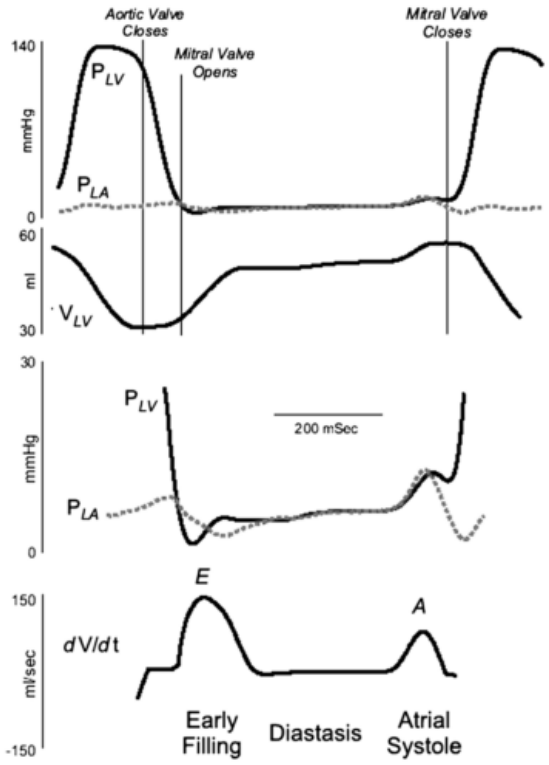

Figure 1.1: Left ventricular and left atrial pressure curves
Recording of left atrial pressure (P_{LA}), left ventricular pressure (P_{LV}), and the rate of change of left ventricular (LV) volume (dV/dt). The early diastolic pressure gradient is generated as LV pressure falls below LA pressure and the late diastolic gradient is generated as atrial contraction increases LA pressure above LV pressure. Reprinted with permission: Fukuta H, Little WC. The cardiac cycle and the physiologic basis of left ventricular contraction, ejection, relaxation, and filling. Heart failure clinics. 2008 Jan;4(1):1-11 (Data from: Little WC, Cheng CP. Left ventricular-arterial coupling in conscious dogs. Am J Physiol 1991;261:H70–H76.)

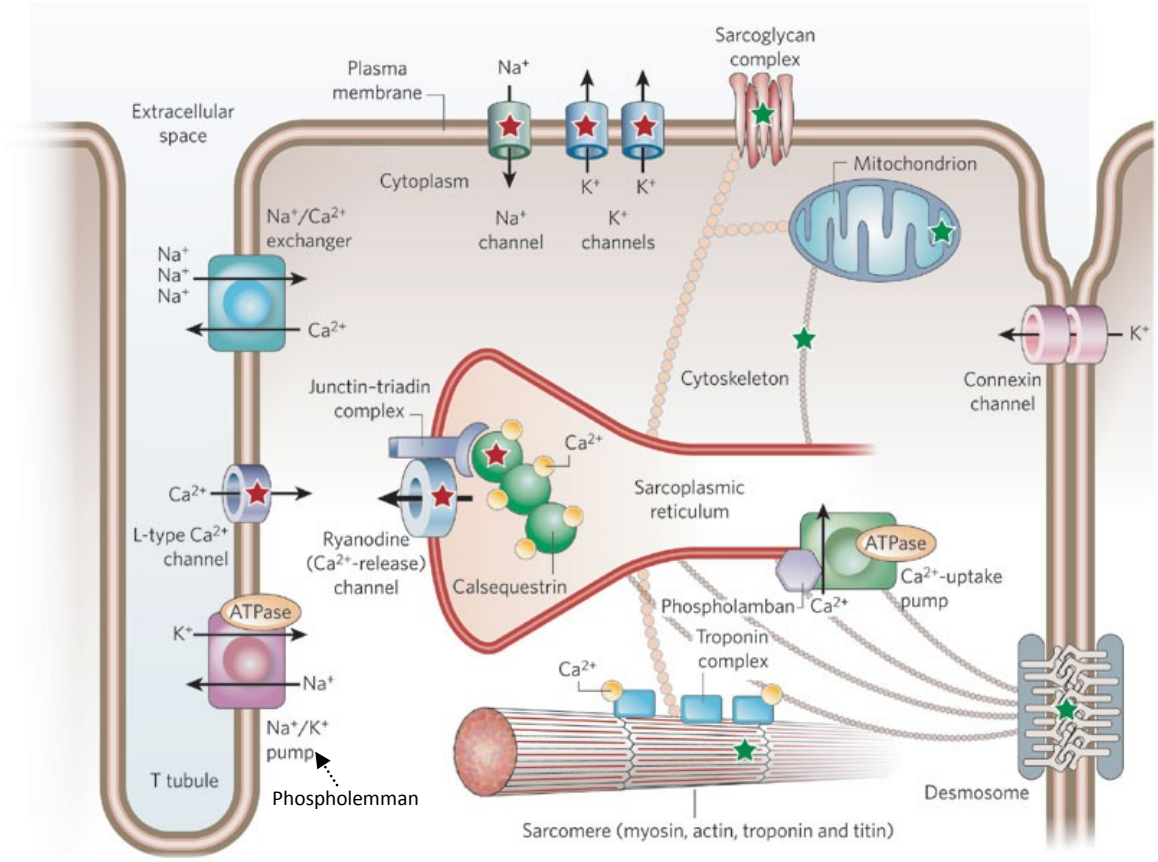

Figure 1.2: Ca handling by cardiomyocyte

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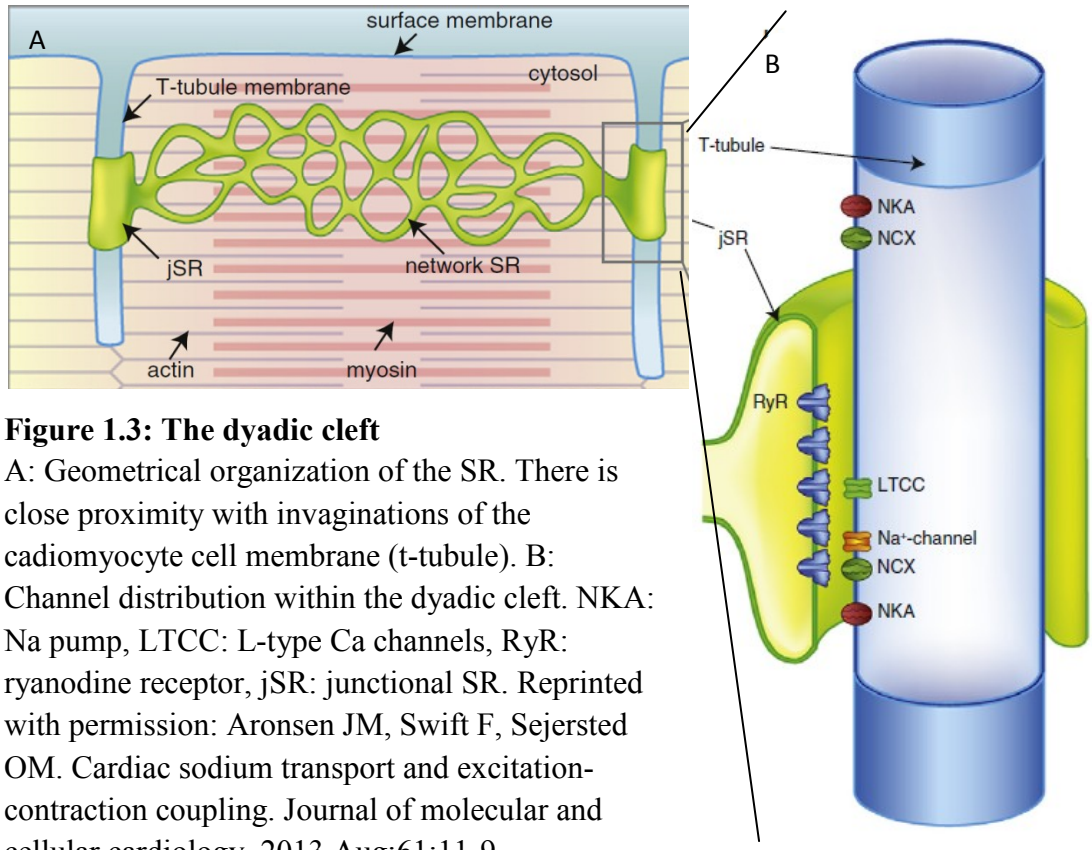

Figure 1.3: The dyadic cleft

A: Geometrical organization of the SR. There is close proximity with invaginations of the cadiomyocyte cell membrane (t-tubule). B: Channel distribution within the dyadic cleft. NKA: Na pump, LTCC: L-type Ca channels, RyR: ryanodine receptor, jSR: junctional SR. Reprinted with permission: Aronsen JM, Swift F, Sejersted OM. Cardiac sodium transport and excitation-contraction coupling. Journal of molecular and cellular cardiology. 2013 Aug;61:11-9.

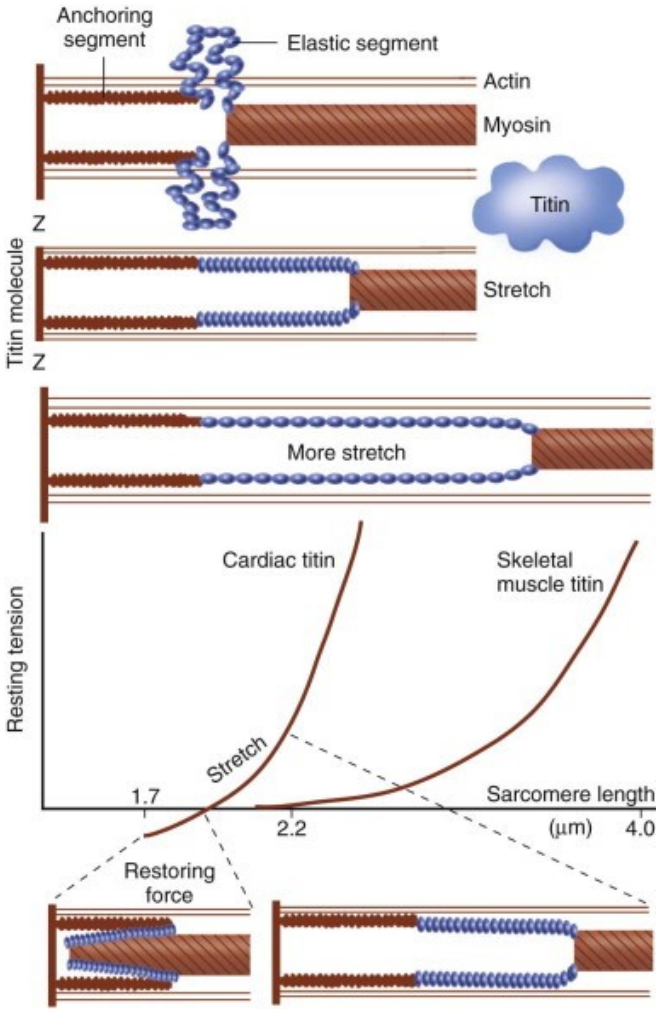

Figure 1.4. Titin localization and attachment

Titin molecule is a large protein extending from Z- to M-line of sarcomere with an elastic segment that contributes to LV stiffness when stretch and that accumulates potential energy when compressed under equilibrium volume, acting as a bidirectional spring. Image from: Bonow RO, Mann DL, Zipes DP, Libby P. Braunwald's Heart Disease: A Textbook of Cardiovascular Medicine. 9 ed: Elsevier Health Sciences; 2011. 2048 p.

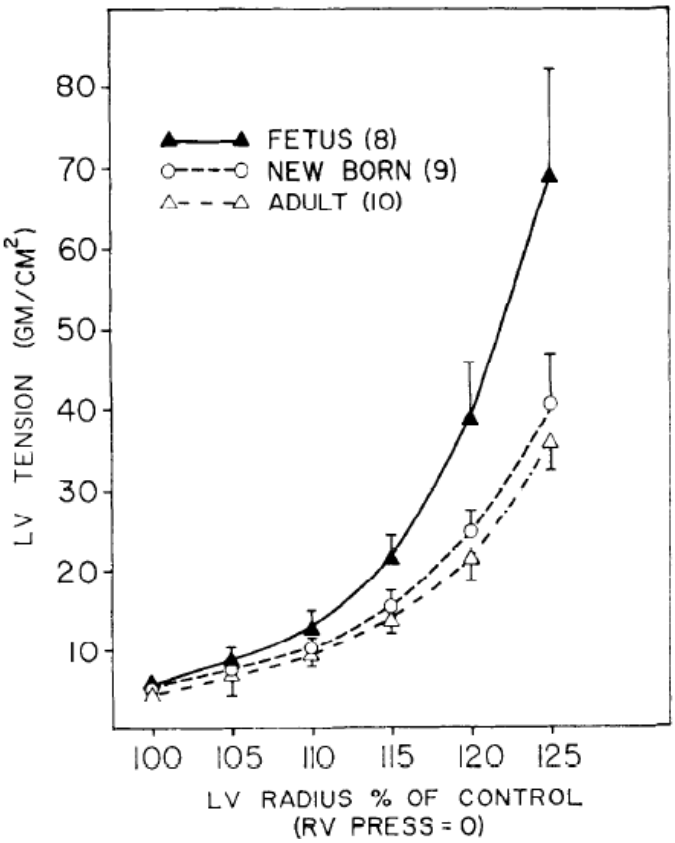

Figure 1.5 Left ventricular compliance in the fetus, neonatal and adult isolated heart Left ventricular tension-radius relations with right ventricle empty. Control left ventricular radius (100%) corresponds to radius at a calculated wall tension 5g/cm^2. At any radius, left ventricular wall tension was significantly greater in fetus than in newborn or adult ($p < 0.025$). No significant difference was observed between newborn and adult. Reprinted with permission: Romero T, Covell J, Friedman WF. A comparison of pressure-volume relations of the fetal, newborn, and adult heart. The American journal of physiology. 1972 May;222(5):1285-90.

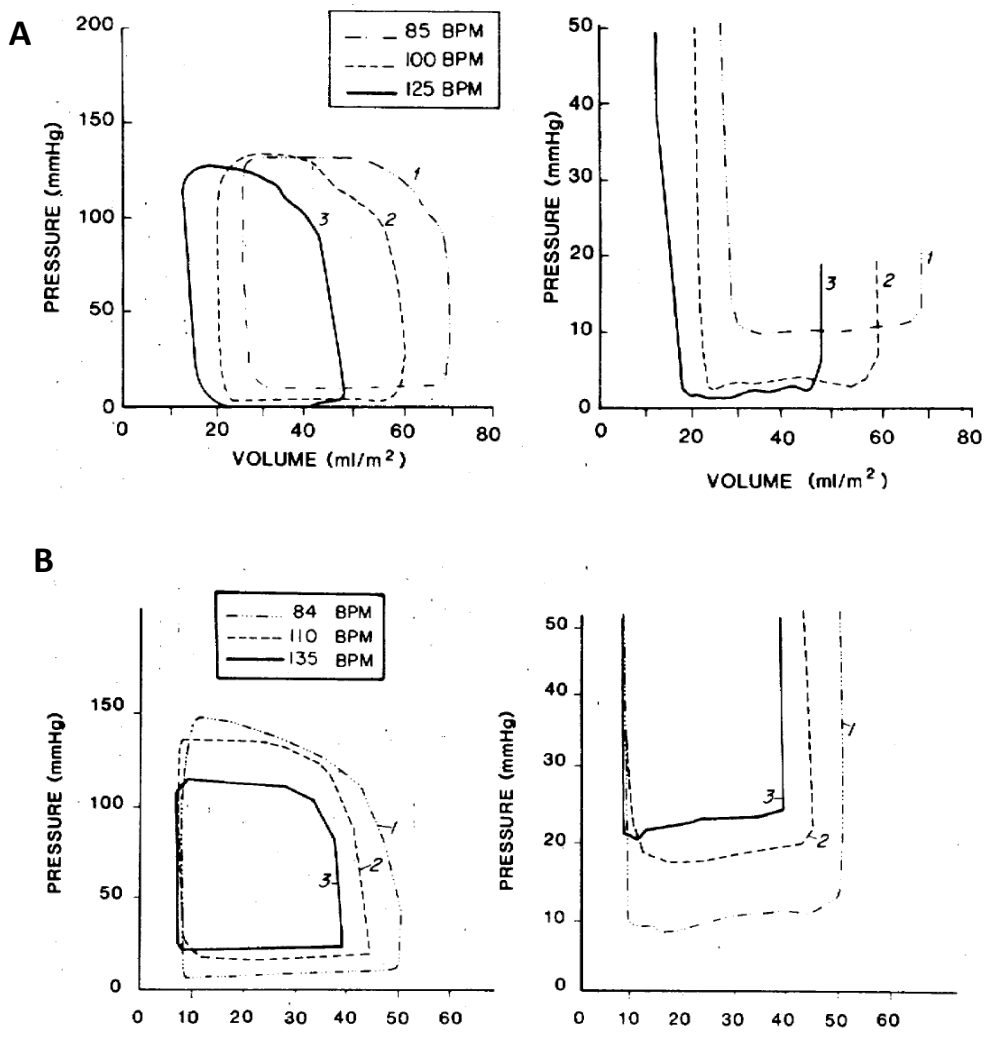

Figure 1.6 Pressure volume diagrams showing the response to atrial tachycardia in the healthy vs. the patient with diastolic dysfunction

A: Normal response to tachycardia. Note the progressive diminution of volumes as well as end-diastolic and end-systolic LV pressures. B: With diastolic dysfunction, end diastolic LV pressure increases with pacing. Reprinted with permission: Aroesty JM, McKay RG, Heller GV, Royal HD, Als AV, Grossman W. Simultaneous assessment of left ventricular systolic and diastolic dysfunction during pacing-induced ischemia. Circulation. 1985 May;71(5):889-900.

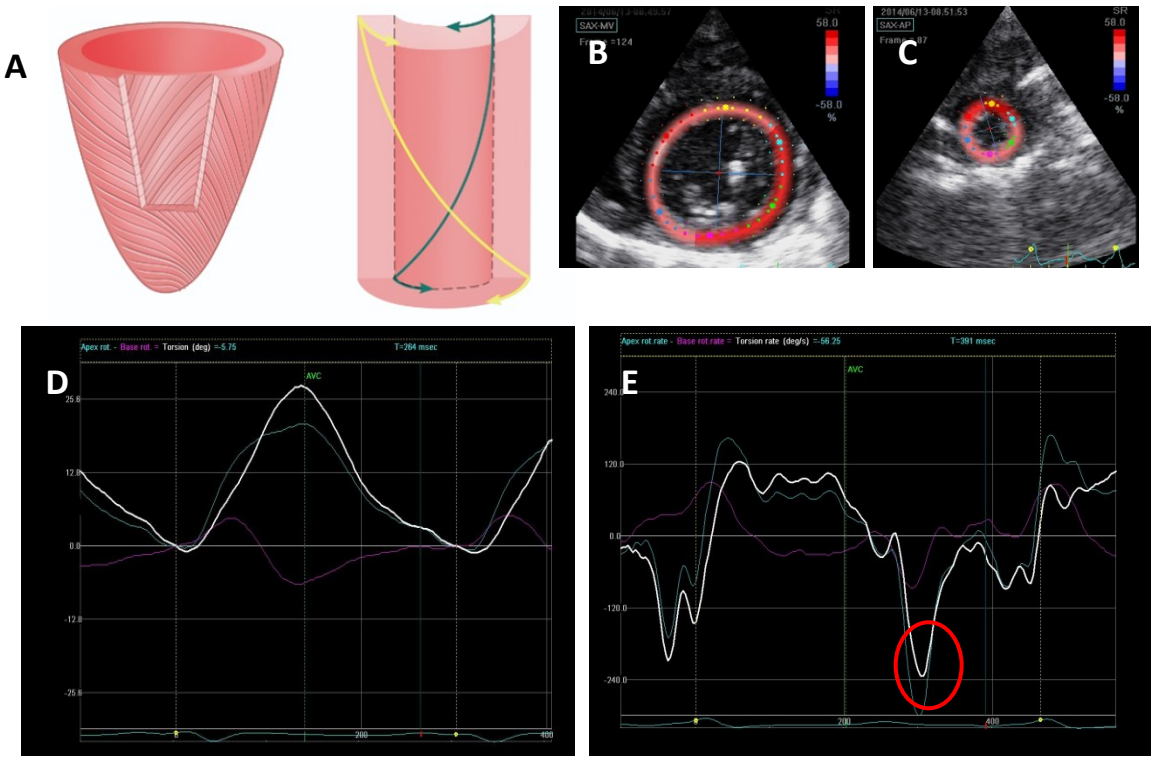

Figure 1.7 LV twist

A: LV muscle fibers arrangement (Reprinted with permission: Sengupta PP, Tajik AJ, Chandrasekaran K, Khandheria BK. Twist mechanics of the LV: principles and application. JACC Cardiovascular imaging. 2008 May;1(3):366-76.). B and C: echocardiographic short-axis basal and apical plane during speckle-tracking of rotation motion by EchoPac software. D: integration of both apical (green line) and basal (purple line) rotation motion into a global twist curve (white line). E: Derived rotation and twist curves from image D in order to obtain rotation (green and purple line) and twist (white line) rates. Peak untwisting rate is identified by a red circle.

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Chapter 2

Postnatal Neonatal Myocardial Adaptation is Associated with Loss of Tolerance to Tachycardia: a Simultaneous Invasive and Noninvasive Assessment in Neonatal and Young Infant Piglets

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2.1 Introduction

Hemodynamic management of the critically ill term newborn is complex. Understanding the functional nature of the neonatal myocardium and how it evolves through the first few weeks and months after birth is key to optimizing care. Although many authors have provided their opinions regarding clinical management strategies (1-3), more translational and clinical studies are needed to provide insight into the functional capacity of the early postnatal heart which can be used to optimize the neonatal care. In particular, changes in diastolic reserve during the first few weeks and months of life in relation to the known disproportionate left ventricular (LV) growth (4, 5) have been poorly explored.

In human infants, most of our understanding of the evolution of diastolic function has been derived from noninvasive Doppler-based studies (6-8). These investigations have suggested that the neonatal LV myocardium has less robust diastolic relaxation (6) with greater dependency on atrial contraction for filling, much like that of the diseased adult heart with diastolic dysfunction (9). In the first few months of life, the increasing contribution of ventricular filling during early diastole and rapid decrease in IVRT is interpreted as evidence of improving ventricular relaxation (7). While there is a wealth of literature that documents LV function at rest in infants and children, there is a paucity of data that examines diastolic and systolic LV function during hemodynamic stress which would be more relevant to the critically ill pediatric patient. This is in part due to technical difficulties at faster heart rates with ventricular inflow and tissue Doppler patterns frequently not interpretable due to fusion of the E (early diastolic filling) and A (late diastolic filling during atrial systole) waves. More recently, LV twist studied by

speckle tracking echo-based techniques has been explored in older children during exercise (10), and might allow us to circumvent challenges related to fast heart rates observed in infants. LV untwisting is demonstrated to be important for the generation of LV suction and contributes to efficient early diastolic filling (10-12). The resting LV twisting pattern in neonates differs from the more mature heart (13)(Figure 2.1A). Furthermore, the neonatal LV has a smaller twist amplitude coupled with slower and delayed untwisting when compared with older children and adults (11), which may reduce the contribution of LV untwisting/suction to early diastolic filling.

Invasive animal studies which have explored the functional maturation of the neonatal LV within the first weeks of life (4, 14, 15) including its response to preload (16-18), also support the current assumption that the neonatal LV may have relatively less diastolic reserve when faced with altered loading conditions. The impact of atrial tachycardia on neonatal LV function, a common finding in the critically ill neonate and a state that is poorly tolerated by the more mature heart with diastolic dysfunction (19), however, has been minimally examined. In a fetal lamb model, rapid atrial pacing has been shown to be associated with some augmentation of the LV output without an increase in left atrial pressure (20), which could suggest that the immature myocardium does have diastolic reserve; however translating these findings to the neonatal heart which faces different loading conditions and an in-series circulation is difficult.

In the present study, we sought to investigate the response of the neonatal and young infant myocardium to atrial tachycardia using a piglet model. We used invasive measures and noninvasive state-of-the-art echocardiography to better elucidate both LV systolic and diastolic hemodynamic and LV myocardial mechanical responses to atrial

tachycardia. Given the current understanding of the evolution of diastolic function, we hypothesized that rapid atrial pacing would be less tolerated by the neonatal myocardium when compared that of the young infant.

2.2 Methods

Landrace cross piglets of two different age groups were investigated. One group was studied as neonates (at 1 to 3 days of age, NP group) and the other in early infancy (at 14 to 17 days of age, YP group). Seven animals were studied for each group. This study was approved by the animal research ethics board at the University of Alberta and was designed in accordance with the Canadian Council on Animal Care guidelines.

2.2.1 Anesthesia and Instrumentation

General anesthesia was induced with isoflurane 2-5% in nitrous oxide (5L/min) and oxygen (5L/min) via a face mask. Tracheotomy and external jugular venous access were performed through a neck cutdown. Piglets were then mechanically ventilated (Ohio 30/70 Proportioner Anesthesia Machine, WI) with a peak inspiratory pressure between 20-25mmHg. Along with a D10W solution through the jugular venous catheter at 5ml/kg/min, a low dose infusion of propofol (85mcg/kg/min) was given and the desired level of anesthesia was adjusted with isoflurane (0.5 to 2%).

Vascular sheaths (5 to 6.5Fr) were introduced in both common carotids and external jugular veins and were then used to position the different catheters. Fluid filled catheters (3.5Fr) were positioned in the superior vena cava and the common carotid artery for central venous and arterial pressure monitoring, respectively. A pacemaker lead (4Fr) was introduced into the right atrial appendage and connected to an external pacemaker (Medtronic, MN). A high fidelity catheter (3.5Fr for neonatal piglets and a 5 Fr for young infant piglets, Millar Instruments, TX) was positioned in the LV mid cavity. Catheter placement was done under both fluoroscopic and echocardiographic guidance. Blood gas

analysis was performed before and immediately after the pacing protocol (istat system, Abbott Point of Care Inc., NJ). Rectal temperature and pulse oximetry were continuously monitored during the experiments. Following completion of the experiments, piglets were euthanized with an overdose of pentobarbital (100 mg/kg i.v.).

2.2.2 Pacing Protocol

After instrumentation, a 30 minute recovery period was allowed for the piglets to stabilize, defined by less than 10% variation in hemodynamic parameters and normal parameters in an arterial blood gas analysis. The baseline invasive parameters were then recorded with an arterial blood gas and detailed echocardiography for LV mechanics parameters performed. Atrial pacing was subsequently started at 200bpm and progressively increased up to 300bpm in increments of 10bpm. A maximum of 300bpm was chosen as it has previously been shown that the neonatal piglet heart demonstrates signs of failure at this rate (21). The animals were allowed to stabilize for 30 seconds (14) before invasive recording and echocardiography Doppler cardiac output was done at each heart rate increment. Detailed echocardiography to assess LV mechanics was repeated at 200, 230 and 260bpm.

2.2.3 Invasive Data Collection

Data from invasive monitoring was analyzed using Ponemah software (Data Sciences International, NW). Each datapoint for aortic blood pressure (systolic, diastolic and mean), dP/dt, negative dP/dt and tau were averaged from 20 to 25 heart cycles. Tau was generated by the software using the following formula:

$$\tau = -\frac{N \sum x^2 - \sum x * \sum x}{N \sum [x * \ln(p)] - \sum x * \sum \ln(p)}$$

where N is the number of points used in the calculation, x is the delta time in seconds at each sampled point (starting from the minimum dP/dt point) and p is the left ventricular pressure value at each sampled point. Left ventricular end diastolic pressure (LVEDP) was manually averaged from 7 to 10 heart beats to avoid where possible intrathoracic pressure variations related to mechanical ventilation. A very small number of nonconsecutive observations (5 out of 840 observations, one for each variable in one piglet) had to be generated by averaging of the values from the heart rate above and below the missing datapoint due to technical issues. Central venous pressure (CVP) was averaged from 5 cardiac cycles.

2.2.4 Echocardiography

Echocardiographic images were acquired with a Vivid 7 ultrasound machine (GE Healthcare, WI) and a 5 or 7 MHz probe by one of three pediatric cardiologists specialized in echocardiography (NK, LM LKH). Ductus arteriosus closure was confirmed before the beginning of the protocol. Frame rates (mean of 247 ± 7 frames/s [SEM]) were optimized in order to generate smooth curves even at faster heart rates. Measurements were done offline using EchoPAC BT12 software (GE Healthcare, WI) following standardized guidelines (22). Mitral inflow and Tissue Doppler Imaging parameters were averaged from 5 cardiac cycles. Strain and rotation studies using speckle tracking were done on a single heart beat per heart rate per animal. For rotation studies, the initiation of systole was set at mitral valve closure. By standard convention, counterclockwise motion, as if the heart was seen from the feet, is reported as positive and clockwise motion as negative rotation, all rotation and twist values are reported in

degrees. All offline analysis was done by the same investigator (EFP). Blinding was not possible due to obvious differences in cardiac chamber size. Inter-observer variability was performed on ten twist studies (LM) using the same cardiac loop used by the first examiner.

Left ventricular output (LVO) was calculated using the following formula: LVO = (TVI • CSA• HR) / Weight, where TVI is the time velocity integral obtained by placing the pulse wave Doppler sample at the level of the aortic valve from an apical 5-chamber view, averaged over 7 to 10 cycles, CSA is the cross sectional area of the aortic valve annulus measured from the parasternal long axis (calculated as CSA = π • radius^2), HR is heart rate in beats per minute (bpm) and weight of the animal in kilograms (kg). This method of LVO estimation has been validated against MRI in a neonatal population (23). Two non consecutive values out of 168 values for LVO had to be averaged from the HR over and under the missing value due to failure to record.

2.2.5 Statistical Analysis

Data was presented as mean ± SEM unless specified otherwise. We applied a statistical analysis for a mixed-anova design with repeated measures for two factors. Between-subject factor corresponds to the two groups of pigs and the within-subject factor corresponds to the atrial pacing protocol, from 200 to 300bpm in increments of 10bpm. Further tests (multiple contrast with repeated measures, or t-test when was acceptable) were applied according to the results and mixed-anova assumptions were also tested (sphericity, homocedasticity and normality). If there was no interaction and no significant difference between groups and distribution within both groups was judged to be similar, data was analyzed as a single group. At baseline and at the end of the

experiment, the 2 groups were compared using t-tests or Mann-Whitney U tests, depending on the nature of the variable distribution.

2.3 Results

2.3.1 Baseline assessment and tolerance to protocol

Both age (2.0 ± 0.2 vs. 15.0 ± 0.2 days of life, $p < 0.001$) and weight (1.89 ± 0.09 vs. 5.34 ± 0.41kg, $p<0.001$) differed between the NP and YP groups, respectively. At baseline (following instrumentation and stabilization), the groups had similar hemodynamic profiles (Table 2.1) and all had a normal arterial blood gas (pH 7.40 ± 0.03 vs. 7.37 ± 0.03, $p = 0.4$; pCO_2 35 ± 3 vs. 40 ± 2mmHg, $p = 0.2$; PO_2 95 ± 11 vs. 121 ± 12 mmHg, $p = 0.14$); HCO_3 22 ± 1 vs. 23 ± 1mmol/L, $p = 0.7$). Differences at baseline included a significantly lower blood pressure and a less negative value of the negative dP/dt in the NP group. Although basal strain and shortening fraction (SF) were higher in the NP group (Table 2.2), the baseline LVO and stroke volume (SV) were similar ($p = 0.6$ and 0.42, respectively). All of the piglets remained normoxic during the experiment and total time to protocol completion was the same for both groups (205 ± 15 vs. 202 ± 8 min, $p = 0.9$). Both groups tolerated the protocol well. Arterial blood gases taken 30 seconds after the pacemaker was disconnected were similar and did not show major metabolic abnormality (pH 7.35 ± 0.06 vs. 7.26 ± 0.03, $p = 0.2$; pCO_2 38 ± 5 vs. 43 ± 3mmHg, $p = 0.5$; PO_2 100 ± 10 vs. 118 ± 9mmHg, $p = 0.2$; HCO_3 20.4 ± 1.1 vs. 19.1 ± 0.8, $p = 0.4$ mmol/L, for the NP and the YP groups, respectively).

2.3.2 Left ventricular output and blood pressure

LVO varied differently with atrial pacing in the two groups (significant interaction, $p < 0.001$). Piglets in the NP group were able to increase baseline LVO in response to tachycardia from baseline to 220bpm ($p < 0.001$). However, this response was not linear and LVO decreased slightly at the highest heart rates (quadratic effect $p = 0.003$). At 300bpm, they demonstrated an LVO similar to baseline ($p = 0.3$). The YP

piglets demonstrated worse tolerance to atrial tachycardia as LVO linearly decreased throughout the protocol to its lowest point at 300bpm ($p = 0.001$) (Figure 2.2). SV decreased through the pacing protocol but more so in the YP group (interaction, $p = 0.037$) and SV was lower at 300bpm in the YP group (NP 0.85 ± 0.05 vs. YP 0.60 ± 0.06 ml/kg, $p = 0.006$).

There was no difference between group mean blood pressure (BP) values during the pacing protocol. There was, however, a significant difference in the response to tachycardia between groups (Interaction, $p = 0.017$). In the NP group, initially there was an increase in mean BP ($p = 0.019$) from baseline, whereas, in the YP group there was a progressive decrease in mean BP during the protocol ($p = 0.036$) (Figure 2.2). A similar pattern was seen for both systolic and diastolic BP (Interaction of 0.003 and 0.04, respectively) (Figure S2.1).

2.3.3 Parameters of systolic function

Shortening fraction (SF) decreased significantly between baseline and 260bpm only in the YP group (YP: 31.4 ± 0.8 vs. $22.9 \pm 0.8\%$, $p < 0.001$; NP: 35.4 ± 1.4 vs. $31.8 \pm 2.2\%$, $p=0.35$). There was a significant difference between groups in mean SF at 260bpm ($p = 0.007$). In the two groups, both end diastolic and end systolic dimensions decreased with pacing (no interaction) (Figure 2.3). End diastolic dimension at 260bpm was similar between groups and reached $73 \pm 3\%$ of baseline value ($n = 14$, $p < 0.001$), suggesting a reduction in preload during tachycardia. End systolic dimension of the NP group was lower than that of the YP group at 260bpm (NP 48 ± 2 vs. YP 57 ± 3 % of baseline value, $p = 0.03$).

Invasive measures of LV contractility showed increasing positive dP/dt with pacing in both NP and YP, with no significant difference between groups (Figure S2.1). Non-invasive assessment of contractility showed progressive increase of Vcfc from baseline to 260bpm pacing (3.22 ± 0.15 to 5.28 ± 0.37 circ/sec, p = 0.001), with a similar degree of enhancement between groups. Both basal and apical circumferential systolic strain rate was also similar between groups, demonstrating an increase with increasing HR (basal circumferential SR -1.53 ± 0.13 to -2.42 ± 0.27 1/s, p = 0.004; apical circumferential SR -2.79 ± 0.26 to -4.42 ± 0.49 1/s, p = 0.002).

2.3.4 LV systolic rotation and twist during pacing

Changes in peak systolic twist with pacing were similar between the NP and YP groups. LV twist increased at 200 and 230bpm pacing with a return to baseline values thereafter (p = 0.014, quadratic effect p = 0.015) (Figure 2.4). The most striking difference between the groups was in how twist was augmented and then maintained during atrial pacing. The NP tended to increase their LV twist by increasing apical rotation (p = 0.1) with significant difference between groups at 260bpm (18.4 ± 2.0 vs. 12.4 ± 1.5 deg, p = 0.04) (Figure 2.4). In contrast, YP augmented and maintained its LV twist by enhancing basal rotation. The early counter clockwise basal rotation was not different between groups and did not vary significantly during atrial pacing; however, the YP increased the basal clockwise rotation in response to pacing more than the NP (interaction p = 0.014) (Figure 2.4). Significant differences between groups were observed at 200 and 230bpm (p = 0.002 for both), but this response was not linear (quadratic effect p = 0.003) as the YP could not maintain the increased clockwise rotation amplitude at the highest heart rates. The difference in time between peak apical and peak basal rotation (synchrony of basal and apical rotation) was similar between groups and narrowed with increasing HR

(baseline 108 ± 25 vs. @260bpm 41 ± 11 ms, p = 0.013), a mechanism by which LV twist may be potentially augmented with pacing. The inter-observer variability was good for both the assessment of peak clockwise basal and peak counter clockwise apical rotation (intra class correlation coefficient of 0.94 and 0.98 respectively, $p < 0.05$ for both) when analyzing the exact same heart beat. Bland-Altman plots are available in the supplements for these two variables (Figure S2.2).

2.3.5 Parameters of LV diastolic function

There was significant interaction between groups regarding the evolution of negative dP/dt (p = 0.011) (Figure 2.2). Although they had lower (i.e. less negative) values at baseline (table 2.1), the NP were able to increase negative dP/dt (from -1598 ± 83 to a peak value of -2202 ± 139mmHg/s, p = 0.002) and maintain a similar value at 300bpm (-2202 ± 139 vs. -2051 ± 199 mmHg/s, p = 0.25). In the YP, negative dP/dt worsened with tachycardia from 200 to 300bpm (-2468 ± 140 vs. -1838 ± 194mmHg/s, p = 0.002) suggesting less robust diastolic reserve.

There was no interaction between groups nor difference between means at any heart rate for LV end-diastolic pressure (LVEDP) (Figure 2.2), tau or CVP (Figure S2.1). All three varied through the protocol ($p < 0.05$). LVEDP decreased between baseline and 300bpm (8.7 ± 1.1 vs. 5.9 ± 0.5mmHg, p = 0.025). Tau and CVP evolution through the protocol were not linear and adopted a U-shaped pattern (significant quadratic effect, $p < 0.005$) (Individual group data available in supplement Figure S2.1).

Doppler isovolumic relaxation time (IVRT) progressively shortened similarly in both groups ($p < 0.001$). It decreased from a baseline value of 56 ± 2 to 39 ± 2ms at 260bpm ($p < 0.001$). Diastolic assessment of mitral inflow pattern, LV wall tissue

Doppler and strain rate were not possible due to fusion of early and late diastolic events and thus could not be analyzed.

2.3.6 LV diastolic untwisting parameters

In early LV diastole, peak untwisting rate increased from baseline values with pacing when both groups were analysed together (baseline untwisting rate -259 ± 22 deg/s vs. -498 ± 59 deg/s at 260bpm; p = 0.003) (Figure 2.4). However, when examined as separate groups, although no statistical differences were detected, there was a trend towards a plateau in the peak untwisting rate in YPs with increasing HR, while NP untwisting rate continued to be augmented. Peak untwisting rate did not correlate with either tau or negative dP/dt. Peak LV untwist rate during isovolumic relaxation (before mitral valve opening) increased similarly in the 2 groups from a baseline value of -161 ± 27deg/s to a maximum of -273 ± 53deg/s, but did not reach statistical significance due to lack of power and wide distribution (p = 0.15).

2.4 Discussion

Our study suggests the neonatal LV may have better cardiac reserve in response to atrial tachycardia than that of the young infant as demonstrated by the NP's ability to maintain LVO which did not occur in YPs. With respect to diastolic function, despite a lower baseline negative dP/dt, we found NPs to have similar or even a relative enhancement of LV relaxation during tachycardia compared to the YP. We observed similar acceleration of relaxation through shortening of both IVRT and tau in both groups, and, in the NP, significant enhancement of negative dp/dt from baseline to similar YP level soon after initiation of atrial tachycardia. The maintained LVO in NPs was associated with preserved FS and enhancement of its ejection phase through a progressive reduction of LV end systolic dimension (Figure 2.3) when compared to YPs. Differences in LVO did not appear to be a function of differences in LV contractility given similar invasive LV dp/dt and non-invasive echo markers of contractility, Vcfc and LV strain rate (24) between groups. There was, however, an intriguing finding of significant differences in LV twist mechanics in response to tachycardia which potentially may contribute to its tolerance of tachycardia.

2.4.1 Diastolic function of the neonatal heart

Contrary to our initial hypothesis of impaired diastolic reserve in NP during chronotropic stress, our study showed that NPs have similar acceleration of relaxation when compared to YP with comparable shortening of IVRT and decreasing tau. There was also enhancement of baseline negative dp/dt in NPs, achieving similar levels to the YPs during tachycardia. Given the observed response to tachycardia and despite baseline values, the NP myocardium must have at least comparable diastolic reserve, and perhaps

an ability to enhance relaxation with the observed changes in negative dP/dt which likely contributed to maintenance of LVO. This supports the findings of an *in vitro* study of human ventricular muscle strips from neonates with congenital heart disease, that suggested preserved acceleration of relaxation (25).

Early filling ventricular mechanics are affected by ventricular active relaxation through efficient sequestration of calcium (Ca) into the sarcoplasmic reticulum by energy-dependent Ca exchangers. They are also impacted by mechanical restoring forces through "spring-like" myocardial proteins such as titin, when the LV is compressed beyond its "equilibrium volume" during LV chamber compression and twisting in systole (12, 26). Although not reaching statistical significance, the NP untwisting velocity, a measure intimately linked to restoring forces (11), tended to further accelerate during increasing tachycardia as compared to YPs (Figure 2.4). This was consistent with the finding of a smaller LVESD in NPs which may represent greater stored potential energy for LV relaxation. However, this complex interaction between end systolic dimension and restoring forces is probably also affected by many other maturational elements such as the titin isoform transition to the stiffer adult variant that has already begun at 2 weeks of age in piglets (27) or the concomitant rapid increase in LV mass (4). At a cellular level, the relative over expression of NCX (sarcolemmal gradient driven Ca exchanger) in the neonatal heart has been shown to decrease with maturation (25, 28). A larger number of NCX in the very young may allow for more efficient clearing of cytosolic Ca, and may favor NP LV relaxation performance during chronotropic stress, when compared with YP.

2.4.2 Systolic function of the neonatal heart

In human infants, LV systolic function at rest does not seem to vary importantly in the first few weeks of life (7, 29). Our study is the first to compare the LV systolic response of the neonatal and young infant heart to atrial tachycardia. The neonatal heart was more tolerant to tachycardia, contrary to our hypothesis where worse tolerance to tachycardia was expected as a consequence of less robust diastolic function given observations in diastolic function at rest in NPs and from human studies (6-8). *In Vivo* evidence of good neonatal tolerance to tachycardia is scarce but has been found in at least one other study. Schmidt & al. elegantly demonstrated the unique ability of the immature heart (9 ± 4 days of age) to adapt to tachycardia by optimizing force-frequency relationship (FFR) to higher heart rates following sustained tachycardia, something that the adult heart could not manage to accomplish . Our work suggests even compared to that of the YP, the neonatal heart has better tolerance to atrial tachycardia despite lower diastolic function parameters at rest. This is in keeping with rapidly evolving changes in myocardial reserve. That the LVEDD decreased similarly with atrial tachycardia in both groups, suggested no significant differences in LV preload. SV, however, was better maintained (data not shown) in our NP relative to YP groups as there was better maintenance of FS and a lower relative LVESD during tachycardia in NPs. Given that we observed no difference in both invasive (positive dP/dt) and noninvasive (SR, VcFc) measures of contractility between the two groups, this better response to atrial tachycardia in NPs may not have related to improved contractility.

We observed obvious and potentially relevant differences in LV deformation and twist mechanics in response to tachycardia, which we speculate could have contributed to the observed improved systolic performance and maintenance of LVO in NP with atrial

tachycardia. The LV twisting and wringing motion was first described in 1669, and vigorous LV twisting during cardiac surgery has long been recognized in the operating room as a sign of good intraoperative LV health (30). LV rotation by convention is described as being viewed from the apex of the heart. During LV systole, the base of the LV has an early counterclockwise motion followed by a more dominant clockwise rotation and the apex has a counter clockwise motion. This is followed by a rapid untwisting during the isovolumic relaxation period, a recoil motion from stored potential energy generated by LV systolic twist, contributing to early LV reformation and generation of LV suction during early diastolic filling. Hence LV twisting provides a coupled link between LV systole and diastole. In addition, LV twist helps redistribute transmural stress and tension from the endocardial to the epicardial myofibers, and this is believed to reduce myocardial oxygen consumption (12, 31). LV twist has also been postulated to have a role in aiding the development of efficient endocardial myofiber sheet rearrangement, a feature that is important during LV endocardial thickening (LV radial deformation) (30). Maturational studies that examine LV twist have shown that infants have different twist mechanics at rest compared to older children and adults (13). Infants have a more prominent basal early counterclockwise rotation and a delayed clockwise rotation component relative to the apical peak counterclockwise rotation. This results in a smaller total LV twist compared to older children and adult hearts (13) (Figure 2.1A). We found baseline LV twist patterns in both NP and YPs to be similar to that of human infants with a prominent basal early counter clockwise rotation and delayed clockwise rotation. However, during atrial tachycardia, the LV twist response between the two groups differed. NPs maintained peak LV twist by enhancing peak LV

apical rotation while the YPs achieved it through enhanced basal rotation. We now understand that the ventricular rotation and LV twist is the final outcome of opposing forces generated by subendocardial (right hand helix orientated) and subepicardial (left hand helix orientated) myofibers. In addition to the balance of endocardial and epicardial forces, rotation patterns are influenced by the electric activation sequence, with an earlier electrical depolarisation of the subendocardial fibers (32). LV geometry also likely plays a role in LV twist through relative change in LV fiber orientation secondary to LV remodelling as has been shown in adults with myocardial disease (33, 34).Given the observations of the current study, we postulate that the differences in LV twist mechanics between NP and YP would suggest fundamental differences in LV myocardial architecture which may contribute to the enhanced response of the NP LV to tachycardia. Whether this reflects differences in endocardial to epicardial fiber orientation angles, LV geometry and/or differences in LV electromechanical efficiency, is not certain and warrants further investigation.

2.4.3 Limitations

The main limitation for translation of the findings in this animal model is the use of atrial pacing to induce atrial tachycardia, which is physiologically different from sinus tachycardia secondary to endogenous or exogenous adrenergic stimulation from neonatal disease states such as sepsis or low cardiac output from cardiac dysfunction. However, the use of pacemaker induced tachycardia with relative absence of exogenous adrenergic stimulation serves to precisely control heart rate and limit the confounding properties of stress hormones on the observed acceleration of relaxation and diastolic function, such as

its potentiating properties on myocyte calcium handling capacities (35), as well as on the vascular system that may have impacted lading conditions. The use of an animal model is essential as it allows for invasive measurements as well as more extreme hemodynamic challenges that are not feasible in the human neonate. Comparison of noninvasive echocardiographic markers of cardiac function to invasive markers provides invaluable information that can facilitate translation of our findings to the clinical setting for further investigation of the critically ill neonate and the development of better management strategies. The piglet cardiac anatomy is very close to that of the human's (36), and it is a well-established model for neonatal cardiovascular studies (5, 14, 21).

We were not able to accurately assess end-diastolic LV compliance given a relative decrease in LV preload induced with our model (i.e. pacing tachycardia) as indirectly evidenced by a reduction in LV end diastolic dimension. As for any animal model, it is difficult to fully translate our findings to human neonates and infants. Furthermore, cellular processes that contribute to the transition of diastolic function in piglets are incompletely described. More in depth examination of calcium handling and evolution of titin and other myocardial elements during this period in the model to better establish their role in the evolution of systolic and diastolic reserve would be valuable.

2.5 Conclusion

Despite baseline findings suggestive of worse diastolic function, as shown through our piglet model and in keeping with clinical observations at rest, the neonatal LV may have better tolerance to chronotropic stress when compared to that of the young infant. This may relate to more robust diastolic and systolic reserve in the less mature hearts. Differences in observed LV ejection in response to atrial tachycardia could relate to unique patterns of LV twist which suggest the presence of fundamental differences in neonatal versus young infant myocardial architecture. Whether this reflects differences in endocardial to epicardial fiber orientation angles, LV geometry and/or other LV electromechanical efficiency or in unique cellular and molecular mechanisms is uncertain but warrants further study to further improve our understanding of its role in developmental changes in myocardial reserve. This study serves to highlight necessary caution in the translation of findings from infants, children or adult literature to the neonate.

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2.7 Acknowledgment

Dr Tze-Fun Lee and Min Lu for their technical assistance during the experiments.

Tables and Figures

Table 2.1: Baseline assessment of the two piglet groups

	Neonatal piglets (mean ± SE)	Young infant piglets (mean ± SE)	p value
Baseline characteristics			
Systolic blood pressure (mmHg)	66 ± 2	84 ± 3	0.001
Diastolic blood pressure (mmHg)	28 ± 3	44 ± 2	0.001
Mean blood pressure (mmHg)	45 ± 2	59 ± 4	0.01
Heart rate (bpm)	142 ± 8	159 ± 3	0.1
Cardiac output (ml/kg/min)	245 ± 16	257 ± 13	0.5
Stoke volume (ml/kg)	1.74 ± 0.12	1.62 ± 0.08	0.42
Invasive parameters			
dP/dt (mmHg/s)	1574 ± 182	1737 ± 148	0.5
Negative dP/dt (mmHg/s)	-1599 ± 83	-2470 ± 226	0.007
MinLVP (mmHg)	3.3 ± 1.9	3.1 ± 1.5	0.9
LVEDP (mmHg)	8.4 ± 2.2	9.0 ± 0.8	0.8
tau (ms)	24 ± 2	22 ± 2	0.6
CVP (cmH2O)	2.8 ± 0.8	3.6 ± 0.8	0.5
Echocardiography – m-mode			
LVEDD (cm)	1.59 ± 0.07	2.23 ± 0.12	0.001
Shortening fraction (%)	35.4 ± 1.4	31.4 ± 0.7	0.03
VcFc	3.1 ± 0.2	3.4 ± 0.17	0.3
Posterior wall thickness (mm)	2.9 ± 0.1	3.9 ± 0.2	0.005
Echocardiography – mitral inflow			
E/A ratio	0.81 ± 0.04	0.81 ± 0.04	0.98
Deceleration time (ms)	71 ± 4	78 ± 8	0.5
A filling fraction (%)	52 ± 2	50 ± 2	0.5
IVRT (ms)	57 ± 2	55 ± 3	0.7
Tissue Doppler imaging			
E/e'*	9.3 ± 0.4	10.0 ± 1.6	0.7
Lateral wall isovolumic acceleration (cm/s)	3.27 ± 0.26	4.31 ± 0.61	0.2
Twist and twist rates			
Peak twist (deg)	18.3 ± 2.0	14.7 ± 1.8	0.2
Peak twist rate (deg/s)	181 ± 29	234 ± 24	0.2
Peak untwisting rate (deg/s)	-243 ± 31	-267 ± 33	0.7
Peak untwisting rate before MVO(deg/s)	-152 ± 29	-170 ± 47	0.9

Legend: *e' was averaged from the LV free wall and the interventricular septum values. Values could only be measured for 6 neonatal and 5 infant piglets. MinLVP: minimum LV pressure reached during diastole, LVEDP: left ventricular end-diastolic pressure,

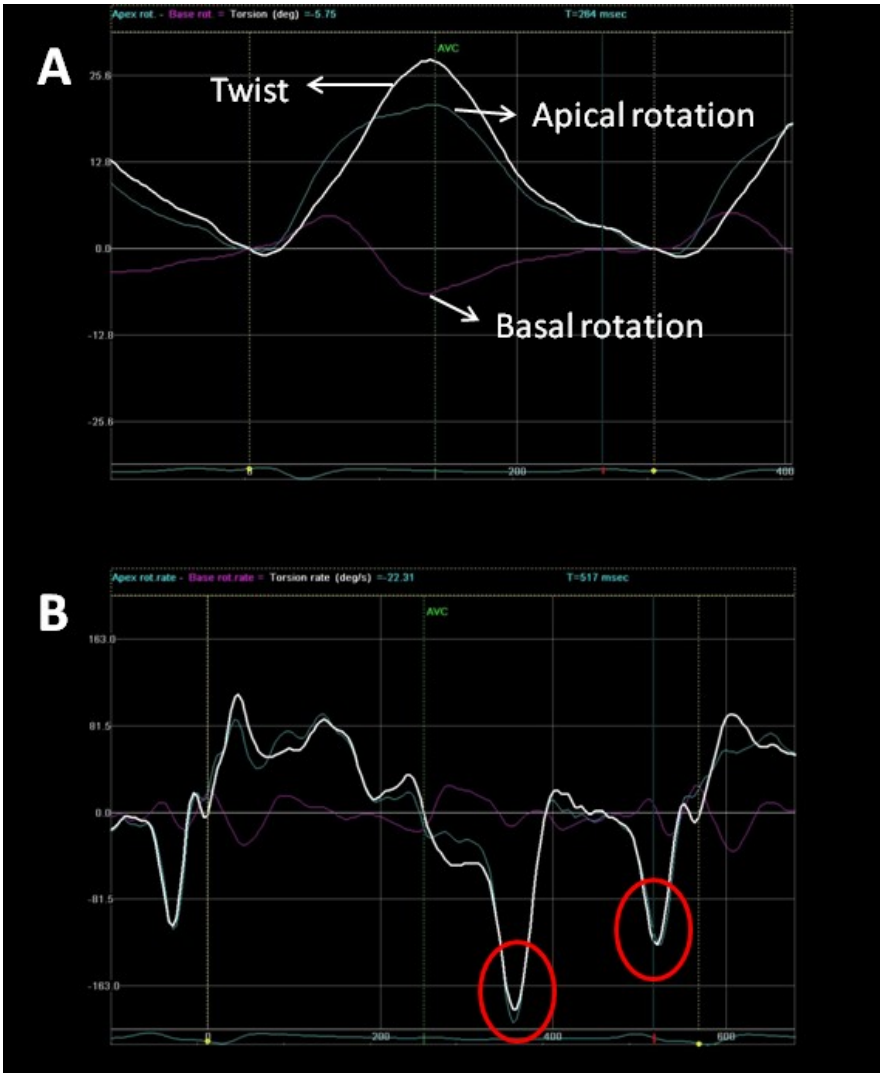
CVP: central venous pressure, LVEDD: left ventricular end-diastolic dimension, VcFc: velocity of circumferential fiber shortening corrected for heart rate, IVRT: isovolumic relaxation time.

Table 2.2. Baseline strain and rotation parameters

	Neonatal piglets (mean ± SE)	Young infant piglets (mean ± SE)	p value
Strain - Basal			
Peak strain (%)	-18.4 ± 1.7	-9.7 ± 0.74	0.001
Systolic SR (1/s)	-1.73 ± 0.21	-1.32 ± 0.10	0.1
Diastolic E SR (1/s)	2.25 ± 0.27	1.53 ± 0.11	0.029
Diastolic A SR (1/s)	2.04 ± 0.33	0.74 ± 0.12	0.006
Strain - Apical			
Peak strain (%)	-22.21 ± 1.89	-20.43 ± 2.43	0.57
Systolic SR (1/s)	-2.72 ± 0.34	-2.86 ± 0.41	0.80
Diastolic E SR (1/s)	4.65 ± 0.64	3.84 ± 0.51	0.34
Diastolic A SR (1/s)	3.24 ± 0.47	2.48 ± 0.42	0.24
Rotation - Basal			
Early Positive (deg)	5.3 ± 2.0	4.8 ± 1.0	0.81
Negative (deg)	-2.7 ± 1.1	-5.1 ± 1.2	0.15
Rotation – Apical			
Positive (deg)	19.3 ± 2.7	13.02 ± 1.6	0.07
Twist			
Peak twist (deg)	18.3 ± 2.0	14.7 ± 1.8	0.21
% untwist before MVO	33 ± 8	32 ± 13	0.93
Rotation rate - Basal			
Peak positive* (deg/s)	166 ± 54	137 ± 14	0.94
Peak Negative (deg/s)	-85 ± 14	-123 ± 19	0.17
Rotation rate - Apical			
Peak positive (deg/s)	223 ± 57	213 ± 37	1
Peak negative (deg/s)	-261 ± 57	-227 ± 30	0.46
Twist rate			
Peak Twist rate(deg/s)	181 ± 29	234 ± 24	0.21
Peak untwist rate (deg/s)	-243 ± 31	-267 ± 33	0.71
Peak twist rate before MVO (deg/s)	-152 ± 29	-170 ± 47	0.90

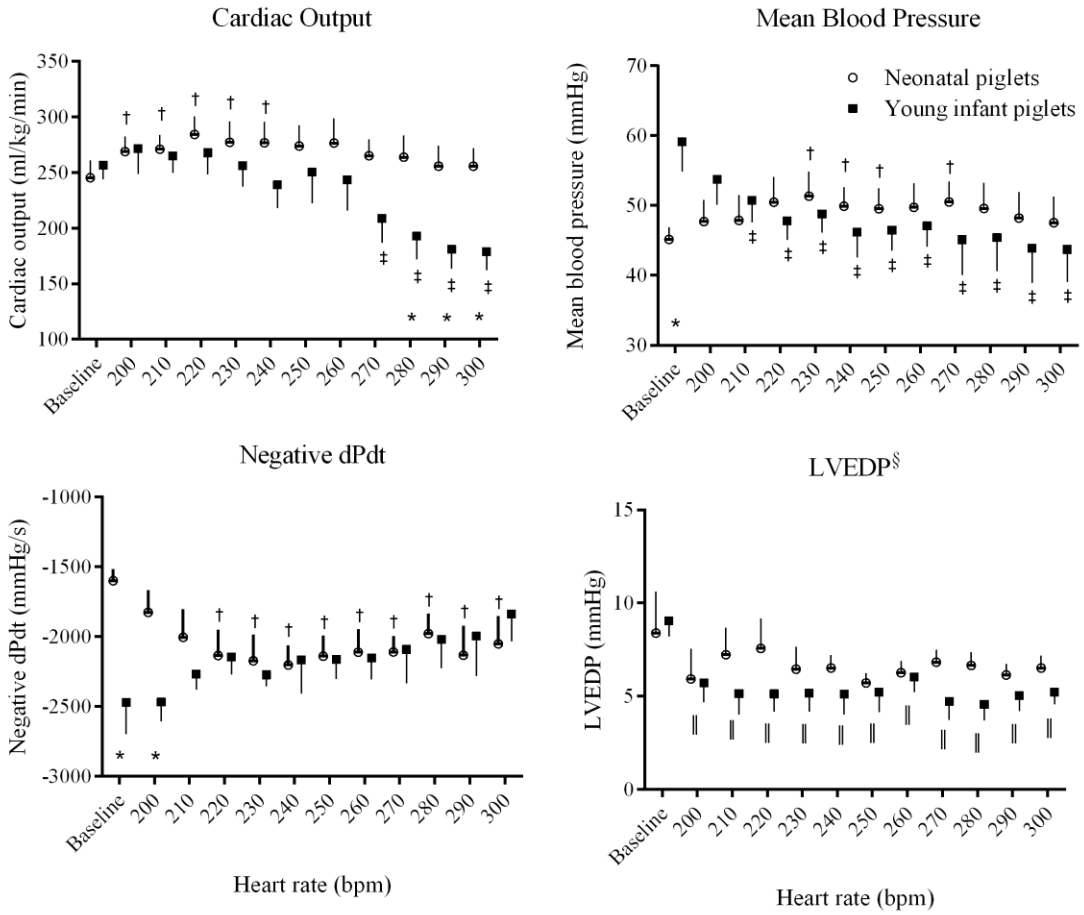
Legend: *One piglet in each group had no positive component to basal rotation at baseline heart rate. The positive rotation rate for these piglets was analyzed as a missing value.

Figure 2.1. Left ventricle twist motion and twist rate



Legend: Twist (A) and twist rate (B) of the LV. The white line represents twist of the LV as viewed from the feet. The green line is the apical rotation and the purple line is the basal rotation. By convention, a clockwise rotation is displayed as a negative value and counterclockwise rotation as a positive value. The two red circles (B) show the biphasic pattern of untwisting. The second untwisting velocity peak happens just after the p wave on the elongated ECG tracing at the bottom of the image.

Figure 2.2 Evolution of invasive variables through the pacing protocol



Legend: * Difference between the groups at that heart rate ($p<0.05$)

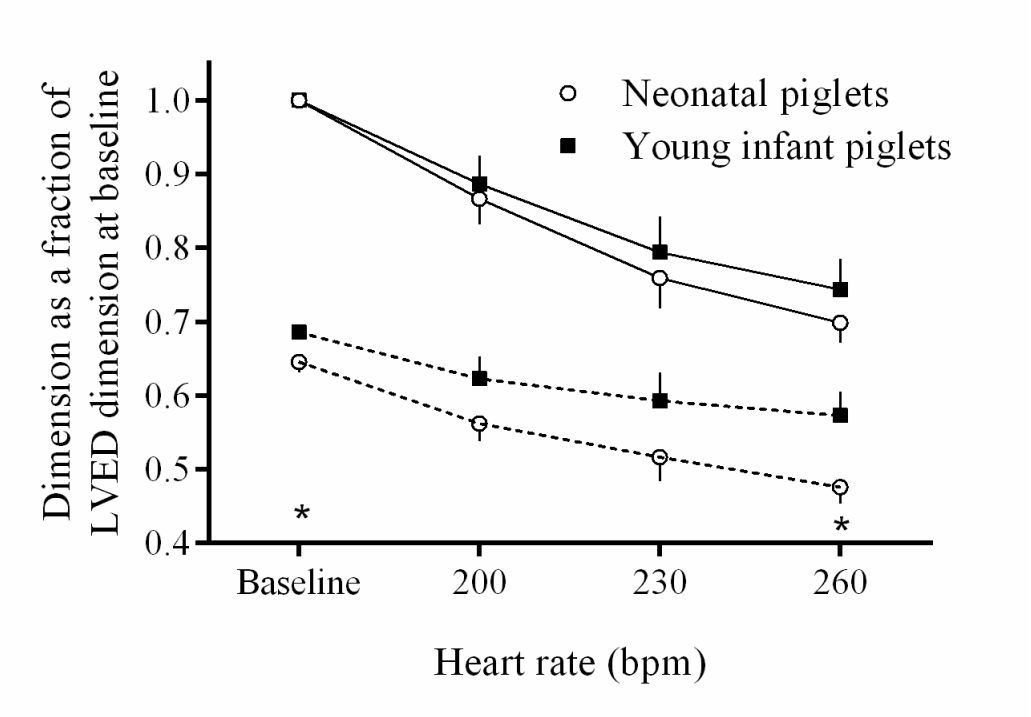
† Neonatal group within group difference when compared to baseline ($p<0.05$)

‡ Young infant group within group difference when compared to baseline ($p<0.05$)

§ Piglets were analyzed as a single group

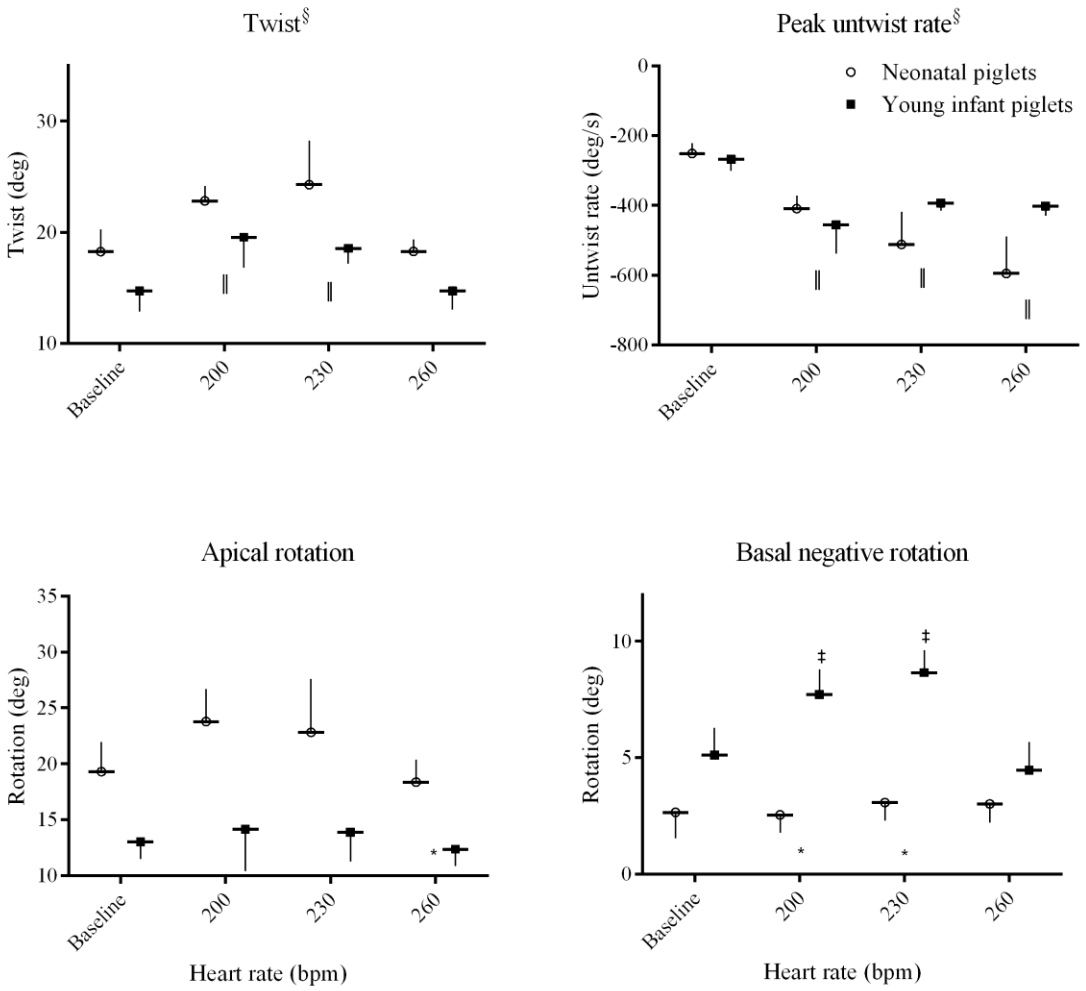
|| As one group of 14 piglets, within group difference when compared to baseline

Figure 2.3. M-mode assessment of left ventricular end diastolic (LVED) dimension (solid line) and end systolic dimension (dashed line) expressed as a fraction of baseline LVED dimension.



Legend: LVED dimension and end systolic dimension decrease with pacing (no interaction). However, end systolic dimension was significantly lower in the YP group at baseline and 260bpm (* in figure).

Figure 2.4. Rotation studies



Legend: * Difference between the groups at that heart rate ($p<0.05$)

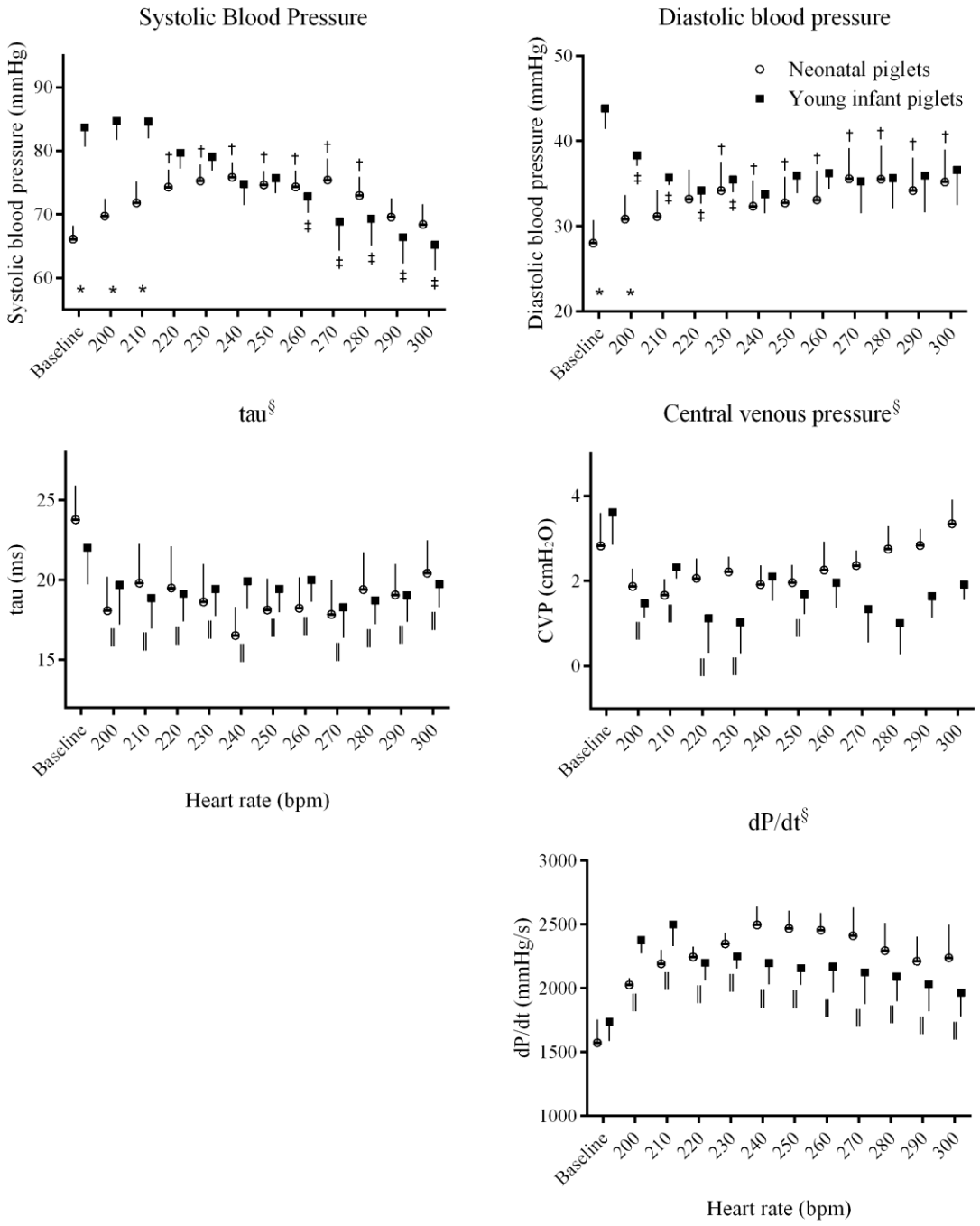
‡ Young infant group within group difference when compared to baseline ($p<0.05$)

§ Piglets were analyzed as a single group

|| As one group of 14 piglets, within group difference when compared to baseline ($p<0.05$)

Supplements

Figure S2.1. Evolution of invasive variables through the pacing protocol



Legend: * Difference between the groups at that heart rate ($p<0.05$)

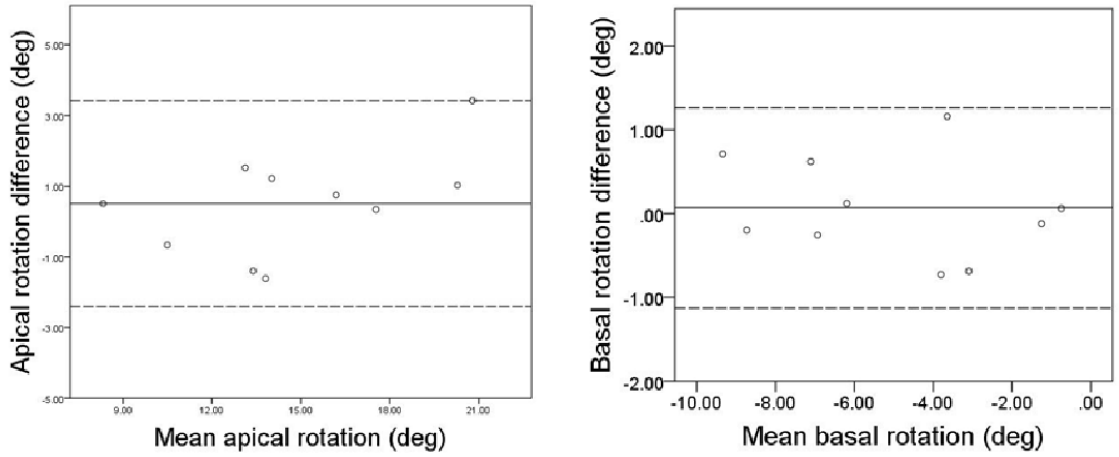
† Neonatal group within group difference when compared to baseline ($p<0.05$)

‡ Young infant group within group difference when compared to baseline ($p<0.05$)

§ Piglets were analyzed as a single group

|| As one group of 14 piglets, within group difference when compared to baseline

Figure S2.2. Inter-observer variability assessed by Bland-Altman style plots for peak basal negative rotation and peak apical positive rotation



Legend: Full lines represent mean difference between the 2 readings and dashed lines represent 1.96 x standard deviation of the difference between readings. For both variables, mean differences were not significant ($p > 0.05$).

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Chapter 3

Strain Rate is Affected by Heart Rate in Younger Heart: A Simultaneous Invasive and Noninvasive *In Vivo* Piglet Model

3.1 Introduction

Echocardiographic evaluation of systolic function is part of the follow-up of cardiovascular and noncardiovascular disease that potentially impacts myocardial function (i.e. cancer treatment). Noninvasive assessment of contractility has become an important part of the routine evaluation of pediatric cardiology patients. Ideally, a marker of contractility should be independent of changes in heart rate or loading conditions in order to reflect true changes in cardiac function from one examination to the other. Unfortunately, no echocardiographic marker is completely independent of these confounders. As newer markers of function based on tissue deformation studies make their way into clinical practice, with guidelines and standards for their use and application (1), one must carefully assess how robust they are to hemodynamic variations.

Strain and strain rate (SR) are noninvasive myocardial function measures acquired from myocardial deformation studies. Strain is the fractional change in the length of a myocardial segment during the cardiac cycle (unit less, expressed as a percentage) (2), which has been validated with sonomicrometry (3). The amount of deformation can be described in the longitudinal, circumferential and/or radial directions, the first two being more recognized (2). Strain analysis can be done by segment of the myocardium or may represent an average of all segments, so-called "global strain", using commercially available software. SR is the rate of change in strain and is a clinically-applied marker of contractility (4). SR analysis has been shown to detect subclinical changes in cardiac function in chronic conditions such as diabetes and hypertension (5) and changes following cancer treatment (6). Repeated assessment of heart function using these newer parameters is thus promising.

In adult dog and pig hearts, left ventricular (LV) systolic SR has been shown to be independent of heart rate (HR) during atrial tachycardia (4, 7). This characteristic, if validated, would be particularly useful in the pediatric patient as physiologic maturation and different arousal states often result in important variability in HR from one assessment to the next. One previous pediatric study has suggested that changes in heart rates naturally occurring with development are not associated with a change in systolic SR (8). No study to date, however, has determined if acute variation in heart rate affects SR in the pediatric patient.

In this study, we explore the impact of atrial tachycardia on systolic SR of the young infant heart using a simultaneous invasive and noninvasive piglet model to determine whether SR of the immature heart is influenced by increasing HR.

3.2 Methods

Strain rate analysis was done using standard echocardiographic 2D-loops obtained for a project on the effect of tachycardia on torsion (see Chapter 2). Landrace cross piglets of two different age groups were analyzed. One group was studied as neonates (at 1 to 3 days of age, NP group) and the other in early infancy (at 14 to 17 days of age, YP group). Seven animals were studied for each group. As data on SR and dP/dt was similar between groups when analyzed separately, they were combined to increase the power of the statistical analysis.

3.2.1 Anesthesia, instrumentation and pacing protocol

Animal model and surgical procedures have been detailed in chapter 2 of the present document. Briefly, under general anesthesia (propofol, isoflurane), piglets were instrumented intravascularly with Millar® high-fidelity and pacing catheters in the LV and right atrium, respectively. After stabilization, invasive hemodynamic and echocardiography parameters were acquired at baseline, and at 200, 230 and 260bpm. Invasive marker of contractility (i.e. dP/dt) was generated by the acquisition software (Ponemah, Data Sciences International, NW).

3.2.2 Echocardiography

Echocardiographic images were acquired at each HR with a Vivid 7 ultrasound machine (GE Healthcare, WI) and a 5 or 7 MHz probe. Global basal circumferential strain rate analysis was performed offline with EchoPAC BT12 software (GE Healthcare, WI) using the same 2D-loops as for twist analysis presented in chapter 2. Basal cut images were obtained perpendicularly to the long axis of the heart, at the level of the tip of the mitral leaflets. Frame rates were optimized in order to obtain adequate assessment at fast heart

rates (frame rates 247±7 Hz). LVED was obtained by m-mode using following recognized guidelines (9).

3.2.3 Statistical analysis

Data is expressed as mean ± SE. The same mixed-anova design, as previously described in this document (see chapter 2), was used. When values were similar between groups and response to pacing protocol was the same (i.e. no interaction), groups were combined for analysis. Direct comparison between groups mean at a specific heart rate were done with t-student test.

3.3 Results

Two groups of 7 piglets were used. Mean age (2.0 ± 0.2 vs. 15.0 ± 0.2 days of life, $p < 0.001$), weight (1.89 ± 0.09 vs. 5.34 ± 0.41kg, $p<0.001$) and LV end diastolic dimension (LVEDD) (table 3.1) differed between the NP and YP groups. However, baseline LV basal circumferential systolic SR, dP/dt and heart rate were similar between groups (Table 3.1).

SR was not different between groups at any heart rate with no interaction through the pacing protocol (Figure 3.1). Analyzed as a single group, LV systolic SR increased significantly with pacing ($p = 0.002$) (Figure 3.2).

There was marginal interaction between groups for dP/dt through the pacing protocol ($p = 0.06$). YP had a higher dP/dt value at 200 bpm ($p = 0.01$) (Figure 3.1); however, both groups increased there dP/dt value with pacing ($p < 0.001$ and 0.01 for group 1 and 2, respectively). As expected, the combined analysis also showed an increase in dP/dt with pacing ($p < 0.001$) (Figure 3.2).

LVEDD expressed as a percentage of baseline value decreased with atrial pacing. There was no interaction between groups and no statistical difference at any time point between groups (Figure 3.3). At 260bpm, the LVEDD reached 73 ± 3% of the baseline value ($p < 0.001$) consistent with reduced preload associated with pacing tachycardia. LV end diastolic pressure (LVEDP) evolved similarly with pacing in the two groups (no interaction) and there was no statistically significant difference at any heart rate. LVEDP decreased with pacing, reaching its nadir at 230bpm (8.72 ± 1.4 vs. 5.82 ± 0.78, at baseline and 230bpm, respectively, $p = 0.006$).

3.4 Discussion

Strain rate is a useful, sensitive marker of contractility in adults. It has been shown to be stable through increasing heart rates using right atrial pacing in two different adult animal models (4, 7). This is a surprising finding as one would expect an increase in contractility with pacing secondarily to the force-frequency relationship (FFR). As a consequence of this observation, it has been hypothesized that any increase in contractility related to the FFR may be balanced by a decrease in contractility due to reduced filling time and preload at higher HRs (4). This balance between positive and negative inotropy could vary with different populations. Our study suggests that in the immature heart, SR may be augmented by atrial tachycardia itself even in the presence of decreased preload. The increase in SR mirrored the increase in contractility assessed invasively by dP/dt. This is in keeping with preservation of the FFR and is in direct contrast to observations made in both adult animal models (4, 7) and in adult humans (10). One could argue the dominant effect of chronotropy over decreased preload during tachycardia could be specific to our piglet model and not to immaturity. To address this issue, our team has recently explored the effects of atrial tachycardia in a cohort of children with anatomically and functionally normal hearts undergoing electrophysiological procedures. The findings of this latter study again suggested that strain rate is dependent on heart rate, thus validating our animal findings (11). Exactly when, during maturation, SR becomes less affected by heart rate is unknown.

Systolic SR dependency on HR has implications for clinical evaluation of pediatric patients. Changing arousal state, and thus baseline HR, from one exam to the other must be taken into account when serially examining a critically ill neonate. HR will vary in

relation to medication choice and hemodynamic status in the sick patient. Our findings have relevance more so for the health myocardium. A distressed neonate with a healthy heart may raise his/her HR, and SR will be augmented as a consequence of maintenance of the FFR. This may not be true of a neonate or infant with baseline myocardial dysfunction secondary to myocardial or structural heart disease, particularly if diastolic dysfunction is present and hampers the ability to augment calcium handling in the face of increasing heart rates. The clinical utility of SR application in this setting and the impact of tachycardia require further exploration. Furthermore, SR should be interpreted in conjunction with other echocardiographic markers as well as the clinical examination.

3.4.1 Limits and perspective

The use of atrial pacing to induce tachycardia is different from acceleration in heart rate secondarily to exercise, which effects preload, afterload and contractility. However, to know that tachycardia in itself affects SR in a pediatric population is still relevant as it must be taken into consideration to avoid misinterpreting SR variations in the clinical setting. As this was a secondary analysis of previously acquired data, we had to combine the groups in order to increase the power of the statistical analysis. Although this approach is not perfect, we feel the two groups were very similar in their response and that the findings are accurate. The two groups were indeed at two very close developmental stages.

3.4.2 Conclusion

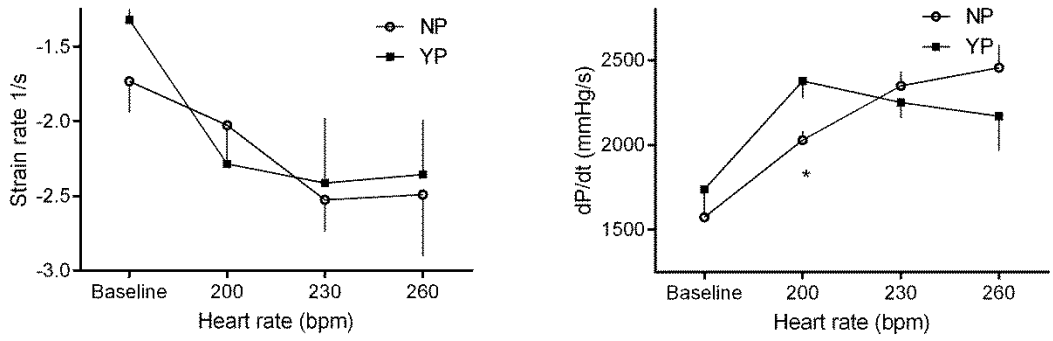
This study supports that heart rate should be taken into account when assessing contractility using SR in young patients.

Table and Figures

	Neonatal piglets (mean ± SE)	Young infant piglets (mean ± SE)	p value
Heart rate (bpm)	142 ± 8	159 ± 3	0.1
dP/dt (mmHg/s)	1574 ± 182	1737 ± 148	0.5
LVEDP (mmHg)	8.4 ± 2.2	9.0 ± 0.8	0.8
LVEDD (cm)	1.59 ± 0.07	2.23 ± 0.12	0.001
Systolic SR (1/s)	-1.73 ± 0.21	-1.32 ± 0.10	0.1

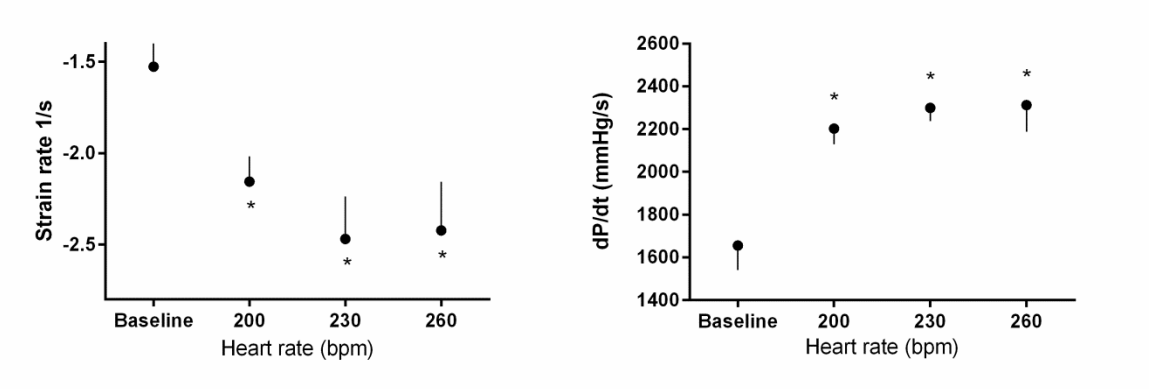
Table 3.1 Baseline characteristics
LVEDP: left ventricular end diastolic pressure; LVEDD: left ventricular end diastolic dimension

Figure 3.1 SR and dP/dt per group



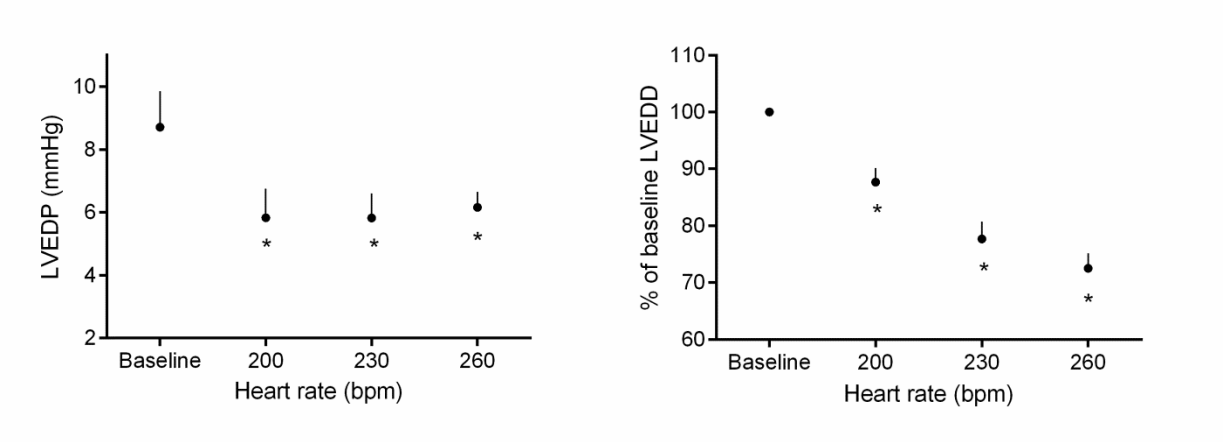
Legend: No interaction was found between groups through the pacing protocol for SR and dP/dt. As these two variables appear to evolve similarly between groups, it was decided that the data for both groups could subsequently be analyzed together. *Significant difference between groups ($p < 0.05$)

Figure 3.2 Combined analysis for SR and dP/dt



Legend: Both SR and dP/dt are optimized with higher atrial rates through pacing. Note here that, by convention, SR is expressed as a negative value whereas dPt/dt is expressed as a positive value during systole. *Significant difference to baseline value ($p < 0.05$)

Figure 3.3 LV preload as assessed by LVEDP and LVEDD



Legend: LVEDP and LVEDD decrease with pacing. This suggests that ventricular preload is likely reduced at end diastole despite which there is maintenance of FFR. *Significant difference to baseline value ($p < 0.05$).

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Chapter 4

Conclusions

4.1 Summary

Assessment of cardiac function in the neonatal population is limited by the rare opportunities for invasive monitoring and has traditionally been largely based on echocardiographic studies performed at rest (1-5). Although valuable, using the noninvasive data to predict myocardial behavior under stress by extrapolating from the adult literature is not always accurate. Previous animal studies do provide insight into the function of the immature heart (6, 7) but most have focused on differences between the fetus or neonate and the adult, leaving the subacute transition to extra-uterine life poorly explored. Our group developed a piglet model of neonatal tachycardia in order to test the tolerance to hemodynamic challenges, beginning with atrial tachycardia, through neonatal development, as well as to explore how baseline echocardiography state of the art markers of function would be affected by this hemodynamic challenge.

In our initial study, we discovered the newborn heart to be highly tolerant to tachycardia. We were able to demonstrate both invasively and non-invasively an acceleration of relaxation of the neonatal LV which permitted maintenance of LVO. It also became obvious that the low untwisting velocity at rest, also previously documented in infants, could be significantly accelerated during tachycardia, a sign of good diastolic reserve. The tolerance to tachycardia was lower in YP, but the reasons for this are still elusive. Both groups seem to have very similar systolic function, but a few differences suggested late-systolic and early diastolic coupling may change even within this short developmental period. Indeed, end systolic dimensions and early peak negative dP/dt were better in the NPs. Interestingly, twist mechanics were also different between these two groups, which could suggest differences in LV geometry/architecture or

electromechanical coupling. These findings contradict clinical observations in patients at rest that suggest diastolic function improves over the first few months of life and thus should have theoretically advantaged the YP at fast heart rates (4). It is, however, in keeping with emerging *in vitro* evidence that suggests acceleration of relaxation does occur in human neonatal LV tissue (8). It is also supported by the known existence of alternative routes for cytosolic Ca^{2+} clearing in immature myocardial tissue (8, 9).

Echocardiographic myocardial deformation studies are emerging into clinical practice. As they are applied to the management of patients, it is critical that the limitations of these markers are understood particularly with respect to their relevance in different patient populations. Previous studies in adult animal models and adult patients have suggested systolic basal SR is not impacted by heart rate (10, 11); however, this had not been as well-documented in the pediatric population. Using our piglet model and exploring SR in pediatric patients, we have now demonstrated that SR increases with HR and this increase parallels increasing dP/dt suggesting true increase in contractility, evidence of the FFR. From the neonatal period, HR changes substantially. Furthermore, in neonatal and older pediatric patients, HR varies frequently during and between exams. Given our findings, HR must be taken into consideration when interpreting changes in SR.

Echocardiography is a valuable tool for bedside assessment of function. This project however highlights the many limits of the 'newer' deformation studies and challenges some generally accepted ideas on neonatal heart function.

4.1.1 Limitations

The piglet is known for its cardiovascular anatomical proximity with humans (12). Direct age comparison between the two species is, however, not perfect. Considering the available evidence, the 2 week old piglet approximates the cardiovascular maturity of a young human infant. Correlations with histological and cellular data will help us establish physiological mechanisms responsible and their correlation with human myocardial data will provide relevance of our animal model to human development.

We used right atrial pacing to generate tachycardia. This is different from the naturally occurring tachycardia with exercise or acute illness. One of the main differences is the lowered LV end-diastolic volume (low preload). Although we believe our conclusions regarding early diastole and LV relaxation properties are valid, it is harder to draw conclusions on the effect of developmental changes in LV compliance on late diastolic events as the LV may not be stretched as much as would normally occur with exercise or hemodynamic stress. Further animal studies that examine the impact of changing loading conditions with the use of high fidelity indwelling catheters for the generation of pressure volume curves will help us explore the additional impact of evolving ventricular compliance on diastolic and systolic function.

4.1.2 Future directions

The piglet model we have established will allow us to continue to explore the unique functional properties of the neonatal and early infant myocardium in health and disease and the relevance of noninvasive echo-based measures of ventricular function. Further investigations are currently underway to explore the impact of loading conditions on tolerance of tachycardia that will provide additional insight into the limits and

evolution of both systolic and diastolic reserve in this early developmental period. Concomitant histological and cellular correlations in the piglet model will elucidate the physiological and cellular mechanisms responsible for these changes and will allow us to correlate findings in the piglet model with findings in human myocardial tissue, where available. This will elucidate how closely our model relates to the human.

Further investigations that include parallel invasive and noninvasive assessments will provide opportunities to examine how noninvasive markers of systolic and diastolic function are impacted by loading conditions. We intend ultimately to use our experience to identify the best noninvasive echo-based measures of function that can be applied at the bedside for the management of the critically ill neonate. For instance, identifying the level of tachycardia where untwisting evolves from an active mechanism with suction of blood into the LV chamber to a passive ventricular event with filling occurring as a consequence of atrial contraction is a promising avenue (see figure 4.1).

While we are gaining experience with our animal model, we also have parallel clinical studies relevant to this research direction. In addition to studying the impact of atrial tachycardia through invasive pacing on LV SR in pediatric patients, we are exploring the evolution of myocardial function in the preterm infant including the evolution of twist mechanics (appendix 1).

Our understanding of the evolution of diastolic function and tolerance to tachycardia has an impact on clinical decision making. Tailoring heart rate, either pharmacologically or electrically, during acute illness or post-operative hospitalization in order to optimize cardiac output depending on LV maturational state is a valuable target.

Individualized care is however an elusive goal as we do not currently have early markers of tolerance to tachycardia. We believe a better understanding of the evolution of cardiac mechanics and the relevance of our noninvasive clinical tools are the first steps towards that goal.

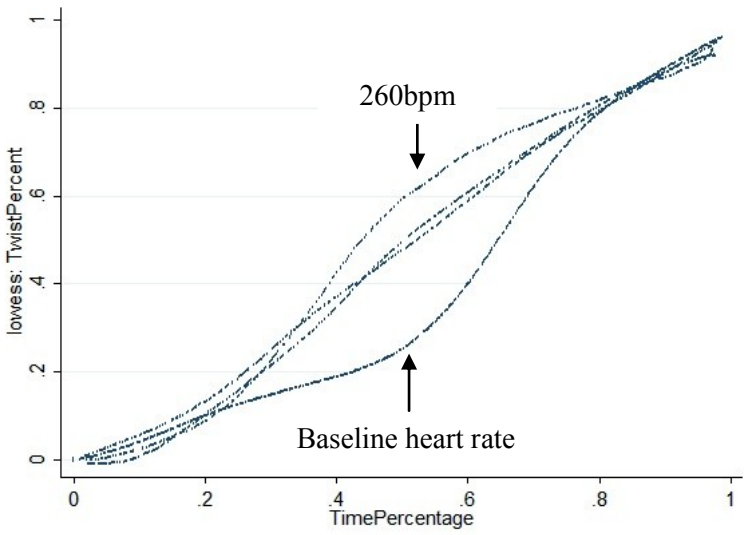

Figure 4.1 Impact of tachycardia on LV diastolic untwisting

Lowess analysis showing the effect of heart rates on twist curves. As heart rate increases, the percentage of untwisting happening in the first half of diastole decreases. Untwisting becomes a passive motion of the LV secondary to blood flow from the atrial contraction. In this graph, TimePercentage = 1 is the beginning of diastole and TimePercentage = 0 is the end of diastole. Lowess: TwistPercent = 1 represents the LV fully twisted at the beginning of diastole and lowess: TwistPercent = 0 represents the return to baseline at the end of diastole, before the next heart cycle. Arrows identify baseline heart rate and 260bpm. The twist lines in the middle are at 200 and 230bpm.

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Appendices

Appendix 1

Premature Infants have Increased Left Ventricular Twist from Enhanced Apical Rotation and Deformation

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BACKGROUND

Premature hearts have altered myocardial cellular properties compared to term infants, and we have previously shown they have altered diastolic function and impaired relaxation[1,2]

Preterm infants are exposed to higher afterload in the postnatal circulation relative to fetal intrauterine circulation

In studies of chronically pressure loaded ventricles the LV compensates by augmenting twist to improve ejection [3]

AIM

To determine if preterm infants have different LV twist mechanics compared to post-natal age matched term infants

METHODS

- Transthoracic echocardiograms were prospectively performed on healthy preterm and term (> 37 weeks GA) infants with structurally normal hearts at approximately 4 weeks of age
- Frame rate optimized LV basal and apical parasternal short-axis echocardiography images were analyzed by speckle-tracking imaging on EchoPac software:
  - Circumferential strain and strain rate, peak rotation and rotation rate, twist and twisting rates were recorded
  - Rotational and twist values were normalized to LV length measured at end-diastole from the mitral valve hinge points to apex
  - Time to peak for each parameter was normalized to the systolic interval and expressed as a percentage of systole
  - Untwist performance in diastole was determined as a ratio of the twist value at mitral valve opening (MVO), 110%, 120%, and 130% of systole compared to the peak twist
- Parasternal long axis M-mode dimensions were recorded to obtain LV dimensions and ejection fraction
- Diastolic parameters were were recorded: mitral valve E and A waves, and septal and lateral LV annular pulse wave tissue Doppler imaging

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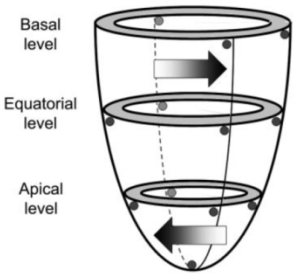
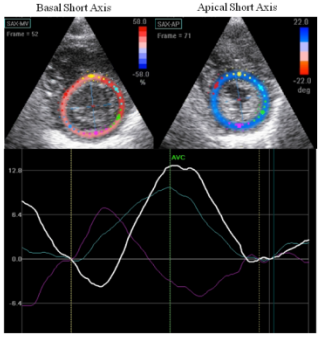


Figure 1. LV twist is the wringing motion of the LV and is the net result of basal clockwise and apical counterclockwise rotation, as viewed from apex

Figure 2. LV Twist and Rotation Profile Curves



Typical LV twist curve of preterm infant. Purple line denotes basal roation (°), blue line denotes apical rotation (°), and white lines denotes twist (°). AVC is aortic valve closure. Both groups had similar rotational deformation delay between the base and apex and there was no difference in the time to peak twist or peak untwist rate.

RESULTS

Table 1. Basic Characteristics of Subjects

	Preterm (n=30)	Term (n=28)	P
Gestational age at birth (weeks)	27.8 ± 1.9	> 37	p<0.01
Age at study (days)	29 ± 4	29 ± 10	NS
Weight (kg)	1.43 ± 0.28	4.3 ± 1	p<0.01
Length (cm)	38.4 ± 2.9	54.4 ± 3.4	p<0.01
Heart rate (beats/min)	152 ± 14	143 ± 13	p=0.01
Systolic blood pressure (mmHg)	70 ± 8	78 ± 7	p<0.01
Diastolic blood pressure (mmHg)	44 ± 10	45 ± 8	NS
Mean blood pressure (mmHg)	52 ± 8	56 ± 6	p=0.07

Table 2. Echo Measurements

	Preterm	Term	P
LV length end-diastole (cm)	2.65 ± 0.45	3.15 ± 0.34	p<0.01
LV end-diastolic dimension (cm)	1.7 ± 0.3	2.0 ± 0.2	p<0.01
LV end-diastolic dimension z-score	-0.6 ± 1.6	-0.2 ± 1.2	NS
LV posterior wall dimension diastole	2.8 ± 0.6	3.3 ± 0.57	p<0.01
LV posterior wall dimension diastole z-score	1.9 ± 0.5	0.40 ± 0.8	p<0.01
Ejection Fraction (%)	66 ± 6	65 ± 5	NS
End-Systolic Wall Stress (g/cm2)	29.3 ± 8.2	31.4 ± 6.9	NS
E/A ratio	0.89 ± 0.14	1.03 ± 0.14	p< 0.01
E/e' (septal wall)	11.6 ± 1.6	10.4 ± 1.9	NS
E/e' (lateral wall)	11.3 ± 3.2	9.4 ± 2.1	p=0.03
Isovolumic relaxation time (ms)	39 ± 16	44 ± 13	NS

Table 3. Systolic Parameters

	Preterm	Term	P
Basal peak strain(%)	-16.8 ± 4.2	-15.4 ± 4.6	NS
Basal systolic strain rate (1/sec)	-1.8 ± 0.4	-1.7 ± 0.3	NS
Apical peak strain (%)	-24.3 ± 7.1	-20.0 ± 5.5	p=0.01
Apical systolic strain rate (1/sec)	-2.4 ± 0.7	-1.9 ± 0.9	p=0.06
Normalized basal early systolic rotation (°/cm)	1.4 ± 0.8	1.2 ± 0.5	NS
Normalized basal peak rotation (°/cm)	-2.6 ± 1.2	-2.1 ± 1.6	NS
Normalized basal early systolic rotation rate (°/cm/s)	34.5 ± 14.6	27.7 ± 13.8	NS
Normalized basal rotation rate (°/cm/s)	-43.2 ± 16.4	-34.6 ± 17.6	NS
Normalized apical peak rotation (°/cm)	2.9 ± 1.4	1.7 ± 0.8	p<0.001
Normalized apical rotation rate (°/cm/s)	46.6 ± 18.1	33.1 ± 17.1	p=0.02
Normalized peak twist (°/cm)	5.1 ± 1.9	3.4 ± 1.5	p=0.01
Normalized peak twist rate (°/cm/s)	50.4 ± 27.9	41.7 ± 18.9	NS
Time to apical peak rotation- percent systole (%)	82.3 ± 22.0	79.1 ± 33.2	NS
Time to basal peak rotation- percent systole (%)	115.6 ± 11.6	110.1 ± 12.3	NS
Time to peak twist- percent of systole (%)	101 ± 9	95 ± 3	NS

Table 4. Diastolic Parameters

	Preterm	Term	P
Percent untwist at MVO (%)	19.2 ± 17.8	29.8 ± 38.1	NS
Percent untwist at 110% systole (%)	11.3 ± 12.1	17.2 ± 20.3	NS
Percent untwist at 120% systole (%)	20.7 ± 17.9	36.9 ± 49.4	NS
Percent untwist at 130% systole (%)	34.2 ± 23.0	48.4 ± 43.5	NS
Normalized peak untwist rate (°/cm/s)	-78.4 ± 23.1	-56.5 ± 25.4	p<0.01
Time to peak untwist rate- percent systole (%)	136.2 ± 20.6	132.6 ± 17.6	NS
Time to peak untwist rate- percent MVO (%)	120 ± 18.1	119.0 ± 16.7	NS

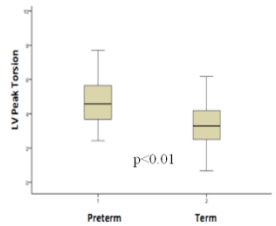


Figure 3. Box-Plot for LV peak twist normalized for LV end-diastolic length ("LV Torsion")

CONCLUSIONS

- Preterm infants have relative LV hypertrophy and greater LV peak twist generated mainly through increased apical rotation and deformation
- This enhanced LV twist may represent a compensatory mechanism in premature hearts adapting to the increase in afterload of the postnatal circulation for their gestational maturity

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Appendix 2

Increased Diastolic Untwisting Velocity in Response to Tachycardia as Evidence of Diastolic Reserve in the Young Infant Heart: a Simultaneous Invasive and Noninvasive In Vivo Swine Model

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BACKGROUND

- In response to exercise, the healthy adult left ventricle (LV) augments its filling through early untwisting, creating suction even before the AV valve opens.
- The role of untwisting in the immature heart remains controversial.
- Older infants have delayed and decreased LV untwisting rates at baseline, suggesting untwisting may play less of a role in LV filling, at least at rest.
- This could be interpreted as additional evidence of impaired diastolic function in the early infancy.
- However, at least one in vitro investigation of human infant myocardium found that relaxation may be augmented during tachycardia which could suggest an element of diastolic reserve.

OBJECTIVE

In the present study, we sought to explore the effect of tachycardia on left ventricular untwisting mechanics, correlating our observations with invasive markers of diastolic function in a young piglet model.

This project was supported by the Women and Children's Health Research Institute and the Canadian Institutes of Health Research

METHODS

- 1-15 day old healthy piglets were employed
- Studies performed under general anesthesia (propofol & isoflurane)
- Instrumentation: Millar high-fidelity catheter in LV and pacing catheter in right atrium (RA), respectively.
- After stabilization, invasive hemodynamic and echo parameters were acquired at baseline and at 230bpm (30-40% above baseline). LV twist was analyzed off-line by speckle tracking (226 ± 55 frames/s).

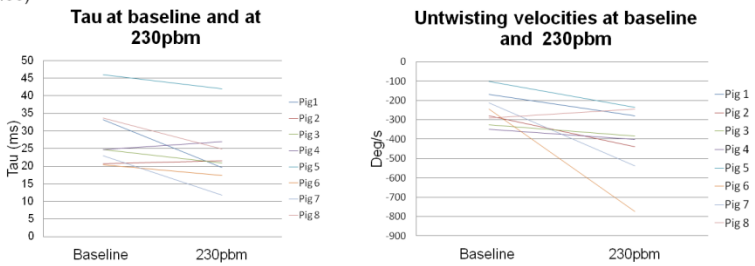
Statistical analysis:
Paired t-tests were used for comparisons with the animals as their own control after confirmation of normal distribution for each variable. Values were expressed as mean ± SD.

RESULTS

Eight piglets were assessed (mean age 8.6 ± 6.8 days, weight 3.6 ± 2.2 kg, baseline heart rate 157 ± 18 bpm).
With tachycardia:

- Tau decreased from 28 ± 9 ms to 23 ± 9 ms (p = 0.03)
- There was a trend towards reduction in LV end diastolic pressure from 13 ± 6 to 10 ± 5 mmHg (p = 0.067)
- Peak untwisting rate increased from -247 ± 83 to -412 ± 179 degrees/s (p = 0.04) and the change correlated with tau (r=0.45, p=0.04)
- Untwisting rate during isovolumic relaxation increased from -146 ± 74 deg/s to -335 ± 205 deg/s (p = 0.03)

Figure 2 and 3 Individual pig data through the pacing protocol



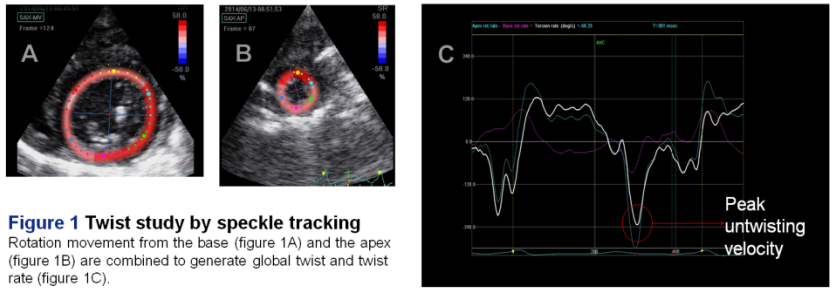


Figure 1 Twist study by speckle tracking
Rotation movement from the base (figure 1A) and the apex (figure 1B) are combined to generate global twist and twist rate (figure 1C).

CONCLUSIONS

- The early infant heart has the capacity to maintain normal LV filling pressures during atrial tachycardia, and this is associated with increased LV untwisting suggesting diastolic reserve.
- The boundaries of this diastolic reserve, and whether this knowledge can be exploited to augment LV filling in the critically ill infant is the subject of ongoing investigations.

Appendix 3

Decreased Tolerance to Tachycardia in the First Weeks of Life in a Swine Model, Using Speckle Tracking Echocardiography and Invasive Monitoring

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BACKGROUND

- The evolution of left ventricular (LV) diastolic function in early life is incompletely described.
- Neonatal diastolic echocardiographic studies are usually performed at rest[1-3] and suggest diastolic function improves in early infancy[2].
- Tolerance to tachycardia documented by the ability to maintain adequate cardiac output without pulmonary vascular congestion is the definite proof of 'diastolic reserve'.
- Traditional echocardiographic markers of diastolic function (i.e. mitral inflow patterns) are hard to interpret at fast heart rates. Newer echocardiographic techniques could help us to better understand the diastolic function of the neonate.

OBJECTIVE

- We sought to explore the limits of LV relaxation in response to atrial tachycardia at two early developmental stages in a closed chest infant swine model using non-invasive and invasive techniques.

METHODS

- Two groups of 7 piglets (mean age 2.0±0.2 days, newborns vs 15.0±0.2 days, young infants p<0.001) were used.
- Under GA (propofol and isoflurane), piglets were instrumented via a neck cut down (tracheotomy, pacing wire in right atrium, LV Millar Hi-fi catheter and aortic catheter).
- Heart rate (HR) was increased by 10 bpm increments to 300bpm.
- Measurements: LV output (LVO) was measured by Doppler echocardiography and dP/dt using the Millar catheter. Speckle tracking twist studies were done at rest, 200, 230 and 260bpm (Fig 1).
- Repeated measure ANOVA, student t-tests, paired t-tests and non-parametric equivalents were used where appropriate. Values are expressed as mean±SEM.

RESULTS

- Baseline hemodynamic characteristics were similar with the exception of negative dP/dt (ndPdt) which was lower in the younger pigs (Table 1).
- As HR increased, the small pigs maintained their LVO better ($p < 0.01$), demonstrated greater increase in ndPdt (149±7 vs 118±8 % of baseline, p=0.01) and maintained their maximum ndPdt better at 300 bpm (-21±7 vs -40±5%, p=0.05) (Fig.2).
- There was an increase in peak negative apical rotation rate in the small group (-577±108 vs -326 ± 30 deg/s, p = 0.01) and a trend toward higher peak negative twist rate at 260bpm (-595±106 vs -401±27 deg/s, p = 0.07).

Table 1 Baseline characteristics

	Young pigs	Older Pigs	p value
Heart rate (bpm)	142±8	159±5	0.1
LVO (ml/kg/min)	245 ±16	257 ± 36	0.6
dP/dt (mmHg/s)	1574±182	1737±148	0.5
ndP/dt (mmHg/s)	-1599±83	-2470±226	0.007
Twist (deg)	18±2	15±2	0.2
Peak untwist rate (deg/s)	-243±31	-267±33	0.7

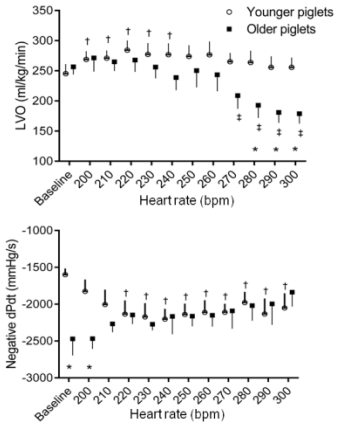

Figure 2 Evolution of LVO and ndP/dt through the pacing protocol * Difference between the groups at that heart rate (p<0.05) † Younger group within group difference when compared to baseline(p<0.05) ‡ Older group within group difference when compared to baseline (p<0.05)

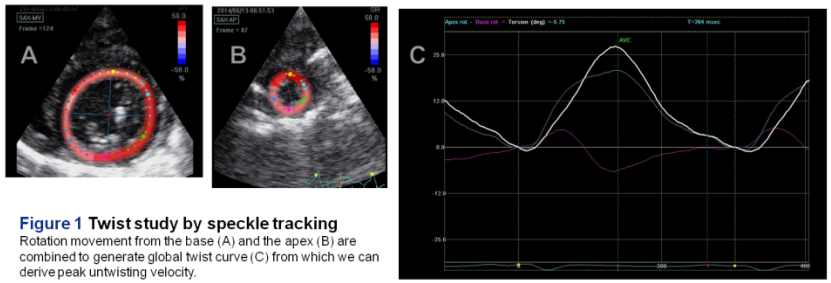

Figure 1 Twist study by speckle tracking
Rotation movement from the base (A) and the apex (B) are combined to generate global twist curve (C) from which we can derive peak untwisting velocity.

CONCLUSIONS

Although not apparent at rest, our study suggests better diastolic reserve of the neonatal as compared to the early infant myocardium.

This project was supported by the Women and Children's Health Research Institute and the Canadian Institutes of Health Research

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