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

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Abstract

Background: Doppler studies at rest suggest left ventricular (LV) diastolic function rapidly improves from the neonate to infant. Whether this translates to its response to hemodynamic challenges is uncertain. We sought to explore the impact of early LV maturation on its ability to tolerate atrial tachycardia. As tachycardia reduces filling time, we hypothesized that the neonatal LV would be less tolerant of atrial tachycardia.

Methods and Results: Landrace cross piglets of two age groups (1-3days, NP; 14-17days, YP; n=7/group) were instrumented for an atrial pacing protocol (from 200 to 300bpm) and assessed by invasive monitoring and echocardiography. NP maintained their LV output and blood pressure, whereas YP did not. Although negative dP/dt in NP at baseline was lower than that of YP (-1599±83 vs -2470±226mmHg/s, respectively, p=0.007), with increasing tachycardia negative dP/dt converged between groups and were not different. Both groups had similar preload reduction during tachycardia however NP maintained shortening fraction (SF) while YP decreased (NP: 35.4 ± 1.4 vs 31.8 ± 2.2%, p=0.35; YP: 31.4 ± 0.8 vs 22.9 ± 0.8%, p < 0.001). Contractility measures did not differ between groups. Peak LV twist and untwisting rate also did not differ; however, NP tended to augment LV twist through increased apical rotation and YP through increasing basal rotation (p=0.009).

Conclusions: The NP appears more tolerant of atrial tachycardia than the YP. They have at least similar diastolic performance, enhanced systolic performance and different LV twist mechanics which may contribute to improved tachycardia tolerance of NP.
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50 New & noteworthy

The neonatal LV showed better tolerance to chronotropic stress compared to that of the young infant.

51 This was associated with at least similar diastolic reserve as found in young infants, an enhanced systolic reserve and a unique neonatal LV twist mechanics during tachycardia.
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, 12, 32), more translational and clinical studies are needed to provide insight into the functional capacity of the early postnatal heart. 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 (3, 27) 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 (16, 26, 31). These investigations have suggested that the neonatal LV myocardium has less robust diastolic relaxation (16) with greater dependency on atrial contraction for filling, much like that of the diseased adult heart with diastolic dysfunction (15). In the first few months of life, the increasing contribution of ventricular filling during early diastole and rapid decrease in isovolumic relaxation time is interpreted as evidence of improving ventricular relaxation (31). 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 (7), 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 (7, 23, 35). The resting LV twisting pattern in
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neonates differs from the more mature heart (24)(Figure 1A). Furthermore, the neonatal LV has a smaller twist amplitude coupled with slower and delayed untwisting when compared with older children and adults (23), 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, 27, 30) including its response to preload (2, 19, 28), 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 in part as tachycardia reduces diastolic filling time (36), 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 (29), 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, our initial hypothesis was that rapid atrial pacing would be less tolerated by the neonatal myocardium when compared that of the young infant.

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 at 14 to 17 days of age, (young piglet, YP group). These developmental stages were chosen because piglets are not yet weaned at that age and
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they have been previously used to describe infantile cardiovascular maturation (9, 13). In addition, the heart rate of the young piglet is relatively stable within the first 2 weeks after delivery (9). 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.

Anesthesia and Instrumentation

General anesthesia was induced with isoflurane 2-5% in nitrous oxide (5L/min) and oxygen (5L/min) via 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%). In order to minimize the effects of sedatives on the hemodynamic profiles of the piglets we used the lowest concentration of Isoflurane possible to achieve quiet sleep with some respiratory efforts. Although the level of isoflurane varied through the experiment within individual subjects, the level of sedation was similar between piglets.

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).
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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.).

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 (17). The animals were allowed to stabilize for 30 seconds (30) 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.

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:

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

**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 $247 \pm 7$ 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 (TDI) 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 were reported in degrees. All offline analysis was completed 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: \( \text{LVO} = (\text{TVI} \cdot \text{CSA} \cdot \text{HR}) / \text{Weight} \), 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 \( \text{CSA} = \pi \cdot \text{radius}^2 \)), 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 (11). Two nonconsecutive 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.

**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.

**RESULTS**

**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. Following the stabilization period,
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Baseline parameters were acquired during baseline echocardiogram, prior to pacing protocol. Table 1 summarized the findings between the groups, showing similar hemodynamic profiles and arterial blood gas (pH 7.40 ± 0.03 vs. 7.37 ± 0.03; PCO$_2$ 35 ± 3 vs. 40 ± 2mmHg; PO$_2$ 95 ± 11 vs. 121 ± 12mmHg, HCO$_3$ 22 ± 1 vs. 23 ± 1mmol/L of NP and YP groups, respectively; all p > 0.05). 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) in the NP group were higher than those of the YP group (Table 2), the baseline LVO and stroke volume (SV) were similar (p = 0.6 and 0.42, respectively).

All of the piglets remained normoxic throughout 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; PCO$_2$ 38 ± 5 vs. 43 ± 3mmHg; PO$_2$ 100 ± 10 vs. 118 ± 9mmHg; HCO$_3$ 20.4 ± 1.1 vs. 19.1 ± 0.8mmol/L for the NP and the YP groups, respectively; all p > 0.05).

**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 LVO in response to tachycardia from baseline to 220bpm (p < 0.001). However, this response was not linear and LVO then decreased at the higher heart rates (quadratic effect p = 0.003) to a level similar to baseline at 300bpm (p = 0.3). The YP piglets demonstrated worse tolerance to atrial tachycardia as LVO linearly decreased throughout the protocol to its lowest point at 300 bpm (p = 0.001) (Figure 2). SV decreased through the pacing protocol but more so in the YP group (interaction, p = 0.037), with a significant difference between groups at faster heart rates (NP 0.85 ± 0.05 vs YP 0.60 ± 0.06 ml/kg at 300bpm, p = 0.006).
Mean blood pressure varied differently with tachycardia (Interaction, p = 0.017). In the NP group there was an increase in mean BP from baseline to 230bpm (p=0.019), whereas in the YP group there was a progressive decrease in mean BP during the protocol (p=0.036 from baseline to 300bpm) (Figure 2). A similar pattern was seen for both systolic and diastolic BP (Interaction of 0.003 and 0.04, respectively) (Figure 3).

Parameters of LV diastolic function

There was significant interaction between groups regarding the changes of negative dP/dt (p = 0.011) (Figure 2). Although they had lower (i.e. less negative) values at baseline (table 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 300 bpm (-2468 ± 140 vs. -1838 ± 194mmHg/s, p = 0.002) suggesting less diastolic reserve.

There was no interaction between groups nor difference found between means at any heart rate for LV end-diastolic pressure (LVEDP) (Figure 2), tau or CVP (Figure 3). Given the similar values and trends between neonatal and young infant piglet groups, the data for LVEDP, tau and CVP were combined to enhance statistical power during analysis of trends during progressive tachycardia. All three variables 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). Changes in Tau and CVP 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 3).

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
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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.

**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 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).

**Parameters of systolic function**

Shortening fraction (SF) decreased significantly between baseline and 260bpm in the YP but not NP group (YP: 31.4 ± 0.8 vs 22.9 ± 0.8%, p < 0.001; NP: 35.4 ± 1.4 vs 31.8 ± 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 5). End diastolic dimension at 260bpm was similar between groups and reached 72 ± 2% 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 3). Non-invasive assessment of
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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).

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 atrial rates of 200 and 230bpm with a return to baseline values thereafter (p = 0.014, quadratic effect p = 0.015) (Figure 4). The most striking difference between the groups was in how twist was augmented and then maintained during atrial pacing. NPs 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 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 YPs increased the basal clockwise rotation in response to pacing more than the NPs (interaction p = 0.014) (Figure 4). Significant differences between groups were observed at 200 and 230 bpm (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 0.93 and 0.98 respectively, p < 0.05 for both) when analyzing the exact same heart beat (Figure 6).
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 NPs’ 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 enhancement of LV relaxation during tachycardia when compared to YPs. We observed similar acceleration of relaxation through shortening of both IVRT and tau in both groups. In the NP, negative dp/dt increased from baseline to levels similar to that of YPs soon after initiation of atrial tachycardia. The LVO in NPs was maintained from baseline, and this was associated with preserved FS and enhancement of its ejection phase through a progressive reduction of LV end systolic dimension (Figure 5) 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 (38) between groups. There was, however, an intriguing finding of significant differences in LV twist mechanics in response to tachycardia which potentially may contribute to NP’s tolerance of tachycardia.

Diastolic function of the neonatal heart

Contrary to our initial hypothesis that NPs would have impaired diastolic reserve during chronotropic stress, our study showed that NPs have similar acceleration of relaxation when compared to YP. This is demonstrated by comparable shortening of IVRT and decreasing tau, and appropriate enhancement of baseline negative dp/dt to levels similar to YPs during tachycardia. Given the observed response to tachycardia, despite worse baseline values, we conclude that the NP myocardium must have at least comparable diastolic reserve when compared to YPs. We speculate that the observed early enhancement of negative dP/dt likely contributed to NPs’ maintenance of LVO. This is supported by the
findings of an *in vitro* study of human ventricular muscle strips from neonates with congenital heart disease, that suggests preserved acceleration of relaxation (39) in neonatal hearts.

Early filling ventricular mechanics are affected by ventricular active relaxation through efficient sequestration of calcium into the sarcoplasmic reticulum by energy-dependent calcium 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 (25, 35). Although not reaching statistical significance, the NP untwisting velocity, a measure intimately linked to restoring forces (23), tended to further accelerate during increasing tachycardia as compared to YPs (Figure 4). This was consistent with the finding of a smaller LVESD in NPs, which may represent greater stored potential energy for LV relaxation. Interestingly, based on previous investigations, the titin isoform transitions to the stiffer adult variant would have already begun at 2 weeks of age in piglets (20) which should theoretically have favored potential energy storage in the YPs. One would have expected this evolution of titin expression to result in increased untwisting velocity in the YP as compared to the NP, however the lack of this enhancement in our findings suggest perhaps at this early age titin may play less of a role in diastolic function and ventricular untwisting. At a cellular level, the relative over expression of NCX (sarcolemmal gradient driven calcium exchanger) in the neonatal heart has been shown to decrease with maturation (37, 39). A larger number of NCX in the very young may allow for more efficient clearing of cytosolic calcium, and may favor NP LV relaxation performance during chronotropic stress, when compared with YP.

**Systolic function of the neonatal heart**

We found the neonatal heart to be more tolerant to tachycardia, contrary to our initial hypothesis where worse tolerance to tachycardia was expected as a consequence of presumed less
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robust diastolic function given the observations at rest in NPs and in human infants (16, 26, 31). Our findings are in keeping with those of Schmidt et al. who elegantly demonstrated the unique ability of the immature heart (9 ± 4 days of age) to adapt to tachycardia by optimizing its force-frequency relationship following sustained tachycardia, something that the adult heart could not manage (30). Our work suggests that even when compared to that of the YP, the neonatal heart has better tolerance to atrial tachycardia despite apparently reduced diastolic function at rest. The difference in LVO between groups is not explained by differences in preload as both LVEDD decreased similarly during atrial tachycardia. Stroke volume, however, was better maintained in our NP relative to YP groups, likely a function of better maintenance of FS, consistent with the finding of a lower relative LVESD during tachycardia in NPs. Given that we observed no difference in both invasive (positive dP/dt) and non-invasive (SR, VcFc) measures of contractility between the two groups, this response to atrial tachycardia in NPs may not be related to differences in LV contractility. What we did observe, were differences in LV deformation and twist mechanics in response to tachycardia, suggesting a potential role of differing LV mechanics that may have contributed to the observed better NPs systolic performance and maintenance of LVO during 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 (34). 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
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may reduce overall myocardial oxygen consumption (6, 35). 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) (34). Maturational studies that examine LV twist have shown that infants have different twist mechanics at rest compared to older children and adults (24). 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 at rest total LV twist compared to older children and adult hearts (24). We found baseline LV twist patterns in both NP and YPs to be similar to that of human infants (Figures 1A and 1B) with a prominent basal early counterclockwise rotation and delayed clockwise rotation. However, during atrial tachycardia, the LV twist response between the two groups differed despite generating similar peak LV twist. NPs increased 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 (33). 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 (8, 18). 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.

Clinical Implications:
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This study provides evidence of “normal” diastolic reserve in the neonate despite worse baseline diastolic measures. This finding is important to the clinician decisions making process when providing care for neonates. One has to consider the relative risk and benefits of inotropy versus the side effects of extreme tachycardia (HR > 180bpm) when using pharmacological means to support the patient’s cardiovascular circulation. Based on traditional concepts of LV maturation where neonates are viewed as having impaired diastolic performance compared to older infants, the clinician may choose to decrease a medication or even change the treatment because of the perceived risk of causing further ventricular inefficiency in an unstable patient when iatrogenically induced tachycardia is present. Our findings are novel and provide new insights to neonatal cardiac reserve during tachycardia. This not only alerts the research community to the limitations of extrapolating a patient’s diastolic reserve from at rest invasive and non-invasive assessment of diastolic function, it has the potential to influence future RCTs protocols on early hemodynamic management in neonates.

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 (5), as well as on the vascular system that may have impacted loading 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
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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 (21), and it is a well-established model for neonatal cardiovascular studies (3, 17, 30).

The effects of sedation on hemodynamics are always a concern as both isoflurane (14) and propofol (10) have been shown to negatively affect the myocardium. As this was a closed chest model, we were able to minimize the use of anesthetic to produce shallow anesthesia once instrumentation was completed. Levels of isoflurane fluctuated during the experiments, in order for the animal to be comfortable with pacing and echocardiography, a situation that more likely approximates the clinical setting with variable levels of sedation. However, such fluctuations would have been difficult to reproduce in a sham animal with only peripherally inserted catheters and without pacing or echocardiography as both of the latter would have required isoflurane to maintain quiet sleep.

Performing the experiment under deep sedation in order to have a fixed amount of sedation for all animals would have, in our opinion, greatly affected tolerance to tachycardia and made this study less informative. As we used low doses of sedatives through the protocol, the animals presented normal hemodynamic profiles after stabilization and each animal was his/her own control, we are confident that our results represent tolerance to tachycardia.

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
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Despite observed worse diastolic measures at rest, the neonatal LV may have better tolerance to chronotropic stress when compared to that of the young infant. The neonatal LV demonstrated better maintenance of cardiac output at extreme tachycardia that is supported by having at least comparable diastolic reserve and an enhanced LV ejection performance. The differences in observed LV ejection could relate to the 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 further highlight the necessary caution in the translation of findings from infants, children or adult literature to the neonate.

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FIGURE LEGENDS

Figure 1. Left ventricle twist motion and twist rate

Twist (A) and twist rate (C) of the piglet left ventricle. 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 on the twist rate figure (C) 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. Image B shows a human neonatal comparative with similar biphasic basal twist motion.

Figure 2. Evolution of invasive variables through the pacing protocol

* 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 3. Evolution of invasive variables through the pacing protocol (continued)

* 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 a a single group
|| As one group of 14 piglets, within group difference when compared to baseline
Figure 4. Rotation studies

* 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)

Figure 5. M-mode assessment of left ventricular dimensions and shortening fraction (SF)

M-mode assessment (A) of left ventricular end diastolic (LVED) dimension (solid line) and end systolic dimension (dashed line) expressed as a fraction of baseline LVED dimension. 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. SF evolution through pacing (B).

*Difference between the groups at that heart rate (p<0.05).
‡ Young infant group within group difference when compared to baseline (p<0.05)

Figure 6. Inter-observer variability assessed by Bland-Altman style plots for peak basal negative rotation and peak apical positive rotation

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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### Tables

#### Table 1: Baseline assessment of the two piglet groups

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

*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 fibre shortening corrected for heart rate, IVRT: isovolumic relaxation time. Values are means ± SE.
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Table 2. Baseline strain and rotation parameters

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

*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. SR: strain rate. Values are means ± SE.