Age-specific cardiac remodeling outcomes induced by isoproterenol

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Abstract

Purpose of Study: Heart failure (HF) impacts patients of all ages and is an enormous public health problem. Historically, HF has been treated with a single, multi-purpose approach, despite the observation that biological differences such as age influence the pathogenesis and thus treatment of this disease. We hypothesized that molecular mechanisms of HF pathogenesis differ across the life-course, a hypothesis which we tested with a mouse model of cardiac dysfunction at three distinct stages of life.

Methods Used: C57BL/6 mice at pediatric (5 weeks; n=12), adult (3-5 months; n=12), and old (18 months, n=10) ages were treated with a subcutaneous mini-osmotic pump that eluted isoproterenol (ISO; 30mg/kg/hour), a non-selective β-adrenergic receptor agonist commonly used to induce acute cardiomyopathy in mice. Following 6 days, we performed echocardiography, biochemical assessments, and RNA sequencing of the left ventricle (LV).

Summary of Results: Both the pediatric and adult groups underwent hypertrophic remodeling in response to ISO, as evident by higher LV weight relative to tibia length (TL). However, ISO exposure did not increase LV/TL in old mice. Echocardiographic imaging demonstrated thickening of the ventricular wall in ISO mice compared to control. Expression of pro-fibrotic mediators also differed across the life-course in response to ISO, with adults inducing a pro-fibrotic transcriptional program (α-smooth muscle actin, fibronectin, collagen, periostin) that was attenuated in old and absent in pediatric animals. RNA-sequencing identified that 119, 1515, and 33 genes were significantly differentially expressed in pediatric, adult, and old mice exposed to ISO, respectively ($p<0.05$). Of these genes, only 2 transcripts were differentially expressed across all three ages.

Conclusions: Biological age significantly impacts the molecular mechanisms of ISO-induced cardiac remodeling. Ongoing analysis of these molecular targets will inform HF therapies using age as a biological variable.

Background

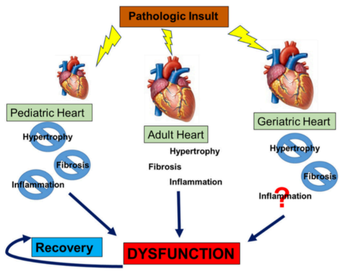


Figure 1: Age-specific changes in heart remodeling

Woulfe and Bruns, *Journal of Molecular and Cellular Cardiology*, 2019

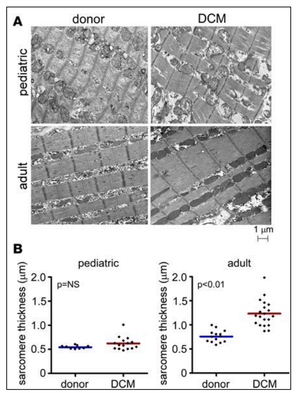


Figure 2: (A) SEM of pediatric and adult cardiac sarcomeres. (B) Quantification of sarcomere thickness

Patel et al , *JCI*, 2017

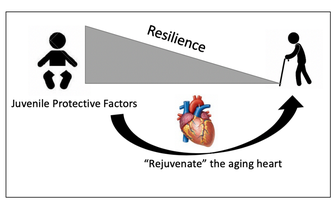


Figure 3: Identification of juvenile protective factors to rejuvenate the aging heart.

Woulfe and Bruns, *Journal of Molecular and Cellular Cardiology*, 2019

Pediatric Heart Failure	Heart Failure in the Aged
• Rare • Some chance of recovery • No specific targeted therapies	• Common • Little chance of recovery • Targeted therapies

Figure 4: Differences in heart failure based on age

The overall goal of this project is:

- To understand age-specific cardiac remodeling to inform heart failure therapy based on age of patient
- To identify specific juvenile protection factors to "rejuvenate" the aged heart

Methods and Experimental Design

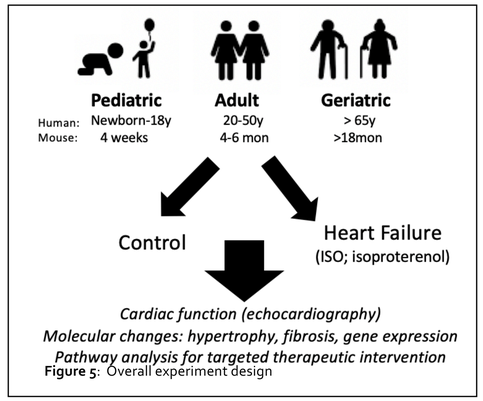


Figure 5: Overall experiment design

Results

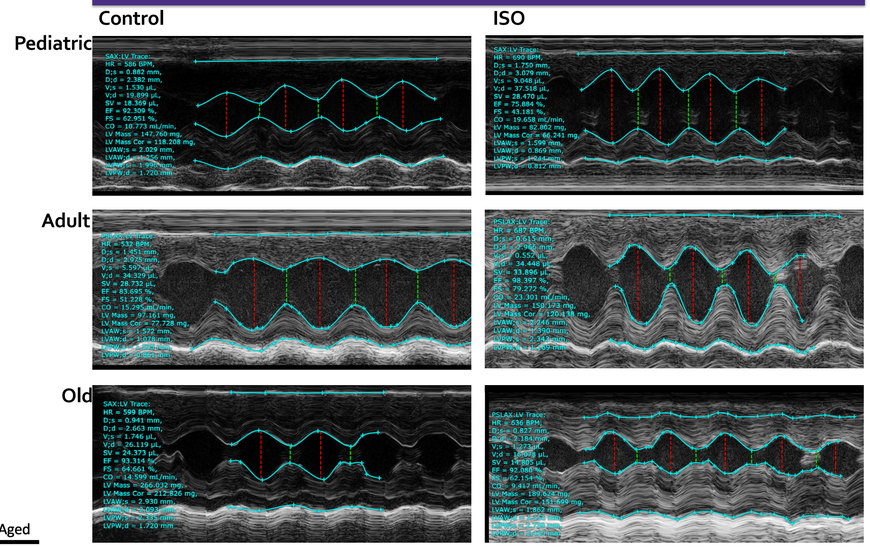


Figure 5: Echocardiography analysis of heart function viewed from the parasternal short axis in M mode

Table 1: Summary of echocardiography data means

	Volume, systole (μL)	Volume, diastole (μL)	Stroke Volume (μL)	Ejection Fraction (%)	Fractional Shortening (%)
Pediatric Control (n=8)	11.90 ± 2.20	36.96 ± 2.99	25.06 ± 1.35	69.55 ± 4.34	39.11 ± 4.02^
Pediatric ISO (n=7)	12.01 ± 4.00	38.24 ± 6.66	26.24 ± 2.80	71.85 ± 3.77	48.45 ± 6.09^
Adult Control (n=8)	18.26 ± 4.96#	52.57 ± 6.73	34.31 ± 1.93	69.66 ± 5.20#	40.00 ± 4.36#
Adult ISO (n=8)	5.86 ± 3.70#	38.94 ± 5.80	33.08 ± 2.75	88.88 ± 4.60#	62.03 ± 5.30#
Old Control (n=8)	11.10 ± 4.07	51.72 ± 10.14	40.63 ± 6.45	83.09 ± 3.80	53.01 ± 4.27
Old ISO (n=6)	6.37 ± 3.26	38.16 ± 11.34	31.79 ± 8.25	86.50 ± 3.95	57.37 ± 5.59

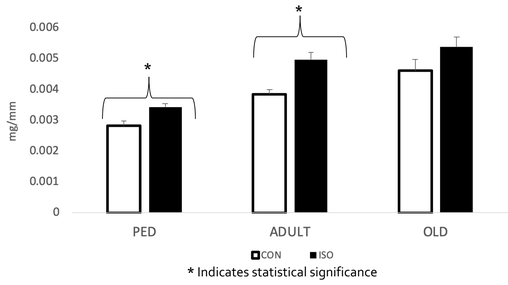
^ indicates statistical significance within pediatric-aged mice
# indicates statistical significance within adult-aged mice

Table 2: Summary of Morphometric Means for Mice Cohort

^ indicates statistical significance within pediatric-aged mice
# indicates statistical significance within adult-aged mice

	Body Weight (g)	Heart Weight (mg)	Left Ventricle Weight (mg)	Right Ventricle Weight (mg)	Tibia Length (mm)
Pediatric Control Male N=5	17.02 ± 0.58	99.20 ± 9.3	51.90 ± 2.2^	15.16 ± 2.3	18.83 ± 0.27
Pediatric Iso. Male N=6	15.34 ± 0.90	134.2 ± 18.2	66.70 ± 3.4^	14.95 ± 1.8	18.82 ± 0.52
Pediatric Control Female N=6	14.02 ± 0.7	91.60 ± 8.2	47.30 ± 3.4^	8.95± 0.5	19.54 ± 1.0
Pediatric Iso. Female N=6	13.88 ± 0.2	116.4 ± 3.7	59.40 ± 1.7 ^	11.32 ± 2.6	17.97 ± 0.29
Adult Control Male N=4	24.21 ± 1.1	135.3 ± 6.0	91.33 ± 2.7#	20.40 ± 3.9	22.16 ± 0.40
Adult Iso. Male N=6	29.02 ± 1.3	195.6 ± 20.0	117.9 ± 9.7#	28.79 ± 2.9	23.05 ± 1.2
Adult Control Female N=5	21.55 ± 0.8	116.7 ± 8.9	80.60 ± 5.1#	17.98 ± 1.0	22.30 ± 0.46
Adult Iso. Fem N=6	24.76 ± 1.0	165.3 ± 7.2	107.0 ± 4.6#	26.97 ± 1.7	22.17 ± 0.25
Old Control Male N=6	32.19 ± 0.64	230.7 ± 19.0	117.9 ± 6.8	21.90 ± 3.3	21.66 ± 0.54
Old Iso. Male N=4	30.75 ± 0.81	214.2± 19.78	127.3 ± 3.9	26.20 ± 2.6	22.29 ± 0.42
Old Control Female N=6	25.96 ± 0.70	176.6 ± 17.27	84.50 ± 5.7	18.90 ± 4.7	22.46 ± 0.40
Old Iso. Female N=6	26.49 ± 0.71	182.2 ± 6.5	106.4 ± 5.1	19.78 ± 1.8	25.72 ± 1.3

Figure 6: Left ventricular weight relative to tibia length

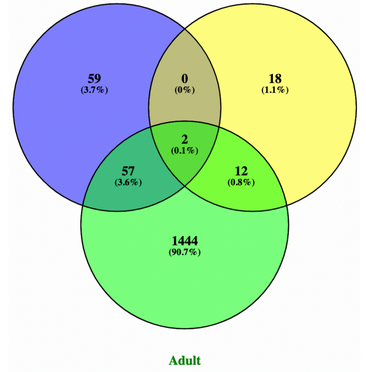


* Indicates statistical significance

Table 3: Changes in pro-fibrotic gene expression based on age

	Pediatric	Adult	Old
αSMA	=	↑	=
Fibronectin	=	↑	↑
Collagen	=	↑	↑
Periostin	=	↑	=

Figure 7: Results of preliminary RNA sequencing demonstrating number of genes expressed in different ages of mice exposed to ISO



Summary

- Pediatric and adult mice underwent hypertrophic remodeling in response to isoproterenol, whereas old mice did not.
- Echocardiography data demonstrated thickening of ventricular wall in ISO mice compared to control.
- Pro-fibrotic genetic expression differed between age groups in response to isoproterenol.

Ongoing Questions

- How does sex, in addition to age, affect cardiac remodeling?
- Can we utilize the differences in the pro-fibrotic gene expression due to age and sex to inform new heart failure therapies?

Acknowledgements

The authors thank: University of Wyoming College of Health Science Faculty Aid; Institutional Development Award (IDeA) from NIGMS/ NIH #2P20GM103432; University of Washington School of Medicine WWAMI Program- Laramie Foundation Site