Left atrial function across the heart failure continuum: Novel pathophysiologic insight into

diastolic dysfunction in women with ischemia but no obstructive coronary artery disease

by

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Abstract

Women with signs and symptoms of ischemia but no obstructive coronary artery disease (INOCA) are at increased risk of developing heart failure with preserved ejection fraction (HFpEF); however, the exact mechanism for HFpEF progression remains to be elucidated. Prior studies have focused specifically on impaired left ventricular diastolic function in INOCA. We hypothesized that extending our evaluation to include the left atrium (LA)— a key constituent of the transmitral pressure gradient and left ventricular filling— would provide additional, novel, pathophysiological insight. To extend our knowledge of the pathophysiologic mechanism(s) linking INOCA with HFpEF, we evaluated LA function across the heart failure continuum: (A) 12 reference controls, (B) 55 women with INOCA, and (C) 19 patients with HFpEF. In all subjects, LA function was analyzed (in duplicate) using commercially available software, with the strain profile divided into three distinct phases: (1) reservoir, passive expansion of the left atrium from the pulmonary circulation while the mitral valve is closed; (2) conduit, passive emptying of the atrium into the ventricle; and (3) booster, active emptying of the left atrium following atrial depolarization. Consistent with prior reports, left atrial reservoir strain was significantly lower in HFpEF vs. reference controls ($19.8 \pm 1.5\%$ vs. $25.8 \pm 1.3\%$, respectively; p=0.02) and tended to be lower compared to INOCA ($23.9 \pm 0.8\%$, p=0.09); however, we observed no group differences in conduit or booster strain. Interestingly, conduit strain rate tended to be depressed in both the INOCA and HFpEF groups (-1.6 ± 0.07 and $-1.5 \pm 0.1\%$, respectively) compared to reference controls ($-1.9 \pm 0.1\%$, p=0.07). To our knowledge, this is the first report of LA function in women with INOCA. That left atrial reservoir strain was reduced across the heart failure continuum, being lowest in HFpEF patients compared to either reference controls or INOCA supports our hypothesis that INOCA may contribute to HFpEF progression. The seemingly depressed conduit function

between in INOCA and HFpEF, may reflect a reduced transmitral pressure gradient, secondary to impaired ventricular diastolic function. More work is needed to better understand the role of chronic ischemia on left atrial function and the interaction between the atria and ventricles.

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Left atrial function across the heart failure continuum. LA reservoir strain was measured in all patients, while LA conduit and booster strain were measured in patients with sinus rhythm.

Left atrial function in INOCA subjects sub-classified according to their resting LV end-diastolic pressure.

Left atrial function across the heart failure continuum with INOCA subjects sub-classified according to their resting LV end-diastolic pressure.

Left atrial function in INOCA subjects sub-classified according to their coronary flow reserve. The results cannot be explained by differences in LVEDP (CFR>2.32 = 10.9 $\pm$ 0.9 mmHg; CFR$\leq$2.32 = 10.8 $\pm$ 1.6 mmHg, P = 0.97) or left atrial volume at end-systole (CFR>2.32 = 36.3 $\pm$ 0.9 mL/m^2; CFR$\leq$2.32 = 37.2 $\pm$ 2.1 mL/m^2, P = 0.69).

Left atrial function in INOCA subjects sub-classified according to presence or absence of vasospasm in response to intracoronary acetylcholine.

Left atrial function in INOCA subjects sub-classified according to their coronary blood flow.

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Dedication

I would like to dedicate my master's thesis to my beloved parents, my mother Shahin Deheshpoor and my father Hossein Zamani who themselves worked tirelessly and sacrificed their dreams, to provide me with a life filled with opportunity. I will never forget the mantra that you have taught me: "seek knowledge to acquire power, work and live to serve others, so you can make the world a little better than you found it; this is the only possible way you can find true happiness lifelong".

Chapter 1: Introduction

Cardiovascular disease is the leading cause of death worldwide (World Health Organization), driven by the rising rates of physical inactivity, hypertension, obesity, and metabolic syndrome (Shaw LJ. et al., 2006). The estimated cost associated with cardiovascular disease in America exceeds $555 billion, and is expected to double by 2035, to a staggering $1.1 trillion (American Heart Association). For decades, cardiovascular disease was largely ignored/overlooked in women; however, we now know that heart disease is the leading cause of death in women, more deadly than all forms of cancer combined (Nelson MD, 2017; Go AS. et al., 2014), afflicting as many as 2 million women in the United States (Shaw LJ. et al., 2008; Bairey Merz CN. et al., 2004).

Once thought to be the sole by-product of a major blockage of the coronary blood vessels (i.e. obstructive CAD), ischemic heart disease is now known to also share a non-obstructive origin (Bakir M. et al., 2016; Gulati M. et al., 2009), referred to from here on as ischemia but no obstructive CAD (INOCA, **Table 1.1**). In either case, the presence or absence of obstructive CAD is objectively assessed by gold-standard angiography, or non-invasive imaging techniques, like coronary computed tomography angiography. Seminal work from National Heart, Lung and Blood Institute (NHLBI)- sponsored Women's Ischemic Syndrome Evaluation (WISE) initiative, showed that more than half of women with INOCA have coronary vascular dysfunction; establishing coronary vascular dysfunction as a putative mechanism driving ischemic heart disease in INOCA.

Table 1.1 Overview of the two types of coronary artery diseases.

Patients in the United States with signs and symptoms of ischemic heart disease (IHD) **>3 million**	
Obstructive CAD **~50%**	**Non-obstructive CAD (INOCA)** **~50%**
Prevalence Men > Women	Prevalence Women > Men
Predominantly men & old women	Predominantly young/middle-age women
Reduced LV systolic function	Preserved LV systolic function
Blockage > 50%	Blockage < 50% with coronary vascular dysfunction
IHD diagnosis	IHD diagnosis is often delayed
Guideline-specific diagnosis, prevention, and treatment strategies	No guideline recommended assessment or management is available (except for symptom relief and CVD risk factor management). Need to develop effective prevention, diagnosis, and treatment approaches

Abbreviations: CAD = coronary artery disease; LV = left ventricular; CVD = cardiovascular disease.

Adapted from Pepine CJ. et al., 2015. Emergence of Nonobstructive Coronary Artery Disease. *Journal of the American College of Cardiology*.

The gold-standard approach to measure coronary vascular function is a technique called coronary reactivity testing; an invasive procedure which measures coronary lumen diameter (by angiography) and blood velocity in response to intra-coronary injection of known vasoactive substances (by a Doppler flow wire), including adenosine, acetylcholine, and nitroglycerine (Wei J. et al., 2016; Reis SE. et al., 2001). By evaluating the luminal diameter change, as well as the coronary flow, in response to intracoronary injection of each vasoactive substance, the presence or absence of macro/microvascular endothelial/non-endothelial dysfunction can be identified (**Table 1.2**) (Wei J. et al., 2016). Importantly, coronary reactivity testing has proven to be prognostic in INOCA, with reduced coronary flow reserve to adenosine or acetylcholine, and/or frank vasoconstriction to acetylcholine, being associated with higher rates of cardiovascular events and/or angina hospitalization (**Figure 1.1)** (AlBadri A. et al., 2019).

Table 1.2 Coronary reactivity testing to measure coronary vascular function.

	Macrovascular Dysfunction	**Microvascular Dysfunction**
Endothelial Dependent	Abnormal vasoreactivity to **Acetylcholine** (normal: > 5% chg)	Reduced coronary blood flow in response to **Acetylcholine** (normal: >50% chg)
Endothelial Independent	Abnormal vasoreactivity to **Nitroglycerine** (normal: > 20% chg)	Reduced coronary flow reserve in response to **Adenosine** (normal: > 2.3 CFR)

Abbreviation: CFR = coronary flow reserve.

Adapted from Wei J. et al., 2016. Diastolic dysfunction measured by cardiac magnetic resonance imaging in women with signs and symptoms of ischemia but no obstructive coronary artery disease. *International Journal of Cardiology*.
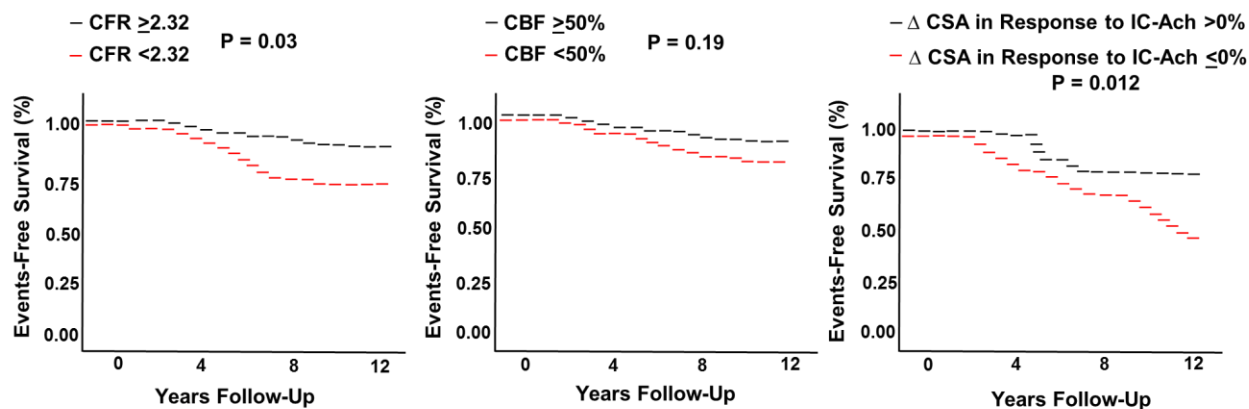
Figure 1.1 The association between coronary reactivity testing and major adverse cardiovascular events.

Adapted from Albadri A. et al., 2019. Impact of Abnormal Coronary Reactivity on Long-Term Clinical Outcomes in Women. *Journal of the American College of Cardiology.*

While INOCA is associated with all forms of major adverse cardiovascular events, including myocardial infarction, stroke and death, the most frequently observe adverse event is hospitalization for heart failure (Nelson MD, 2017; Bakir M. et al., 2016; Gulati M. et al., 2009). In the original WISE outcomes report by Gulati M. et al. (2009), the "type" of heart failure was not reported (nor measured). To address this limitation, Bakir, Nelson and colleagues (2016), completed a chart review in 223 INOCA patients, followed for 7-years. Remarkably, the results show that >92% of women hospitalized for heart failure had a preserved ejection fraction at the time of hospitalization. Taken together, these findings helped establish the hypothesis that INOCA may be a precursor to heart failure with preserved ejection fraction (HFpEF). In support of this hypothesis, INOCA and HFpEF often share many common unifying risk factors (e.g. hypertension, lipedema, hypercholesterolemia, dysglycemia; **Table 1.3**), and pathophysiologic

traits, including left ventricular diastolic dysfunction (Nelson MD. et al., 2017; Nelson MD. et al., 2014; Wei J. et al., 2016).

Table 1.3 Characteristic similarities between non-obstructive ischemic heart disease and heart failure with preserved ejection fraction.

Phenotype	Non-obstructive Ischemic Heart disease	HFpEF
Left ventricular ejection fraction	Normal	Normal
Sex predominance	Women > Men	Women > Men
Obesity	Prevalent	Prevalent
Diabetes	Prevalent	Prevalent
Hypertension	Prevalent	Prevalent
Hypercholesterolemia	Prevalent	Prevalent

Adapted from Nelson MD, 2017. Left ventricular diastolic dysfunction in women with nonobstructive ischemic heart disease: insights from magnetic resonance imaging and spectroscopy. *Am J Physiol Regul Integr Comp Physiol.*

Diastolic function across the heart failure continuum

Diastole is a coordinated physiologic process that allows the heart to fill sufficiently under low filling pressures. As systole ends, LV elastic recoil and active relaxation gives rise to an abrupt decline in LV pressure until the mitral valve opens, and blood flows along a pressure gradient toward the apex. Upon pressure equilibration between the left atrium and the LV (i.e., diastasis), the final component of ventricular filling occurs when the atrium contracts and systole resumes. Diastolic function is known to decline with age (Lakatta EG, 2015), and progress along the American College of Cardiology/American Heart Association (ACC/AHA) heart failure

continuum, from stage A (presence of cardiovascular risk factors with no structural adaptations) to stage C (structural adaptation and symptoms of heart failure) (Pandey A. et al., 2017). There are currently, 4 distinct phases of diastolic (dys)function, ranging from normal healthy filling of a compliant ventricle to impaired filling of a stiff ventricle with associated compensatory changes (i.e. elevated diastolic filling pressures) (**Table 1.4**).

Table 1.4 Different stages of diastolic dysfunction and common measurement approaches and outcomes.

Stage 1	Stage 2	Stage 3 (Pseudonormalization)	Stage 4
Normal healthy filling of a compliant ventricle	In early diastole, left ventricular filling is reduced while LV and LA pressures and compliance are normal	Increase in LA pressure; however, Doppler echocardiographic transmitral flow pattern is normal, which can be a concern to easily miss marked diastolic dysfunction	Severe restrictive diastolic filling with decrease in LV compliance
Low EDP (<12 mmHg) Early filling to late filling mitral inflow velocity (80/20)	Low EDP (< 12 mmHg) Early filling to late filling mitral inflow velocity (30/70)	Elevated EDP (>12 mmHg) Early filling to late filling mitral inflow velocity (80/20)	Elevated EDP (>12 mmHg) Early filling to late filling mitral inflow velocity (80/20)
Early filling to late filling annular tissue velocity (80/20)	Early filling to late filling annular tissue velocity (40/60)	Early filling to late filling annular tissue velocity (40/60)	Early filling to late filling annular tissue velocity (50/50)
Normal LAVi	Normal LAVi	Elevated LAVi	Elevated LAVi

Abbreviations: EDP = end-diastolic pressure; LAVi = left atrial volume index

Adapted from European Society of Cardiology (ESC) diastolic guidelines.

Balaney B. et al., 2018. Invasive Validation of the Echocardiographic Assessment of Left Ventricular Filling Pressures Using the 2016 Diastolic Guideline: Head-to-Head Comparison with the 2009 Guideline. *Journal of the American Society of Echocardiography.*

Hamlin SK. et al., 2004. Role of diastole in left ventricular function, II: diagnosis and treatment. *American Journal of Critical Care.*

Key mechanisms driving diastolic dysfunction

Given the complexity of diastole, and the various physiologic mechanisms that contribute to ventricular filling, there are numerous causes of diastolic dysfunction, many of which may co-exist. For example, diastole is a highly energy-dependent process, requiring sufficient delivery of oxygen for the generation of adenosine triphosphate (ATP). Unlike systole, which only requires ATP for the removal of troponin-C from actin, diastole requires ATP for the (1) reuptake of calcium into the sarcoplasmic reticulum via the sarco-endoplasmic reticulum calcium ATP-ase (SERCA), (2) dissociation of actin and myosin, and (3) uncoupling of calcium from troponin-C (Samuel TJ. et al., 2018; Nelson MD, 2017). Myocardial ischemia and/or metabolic dysfunction will therefore impair actin-myosin cross-bridge cycling in diastole, leading to a ventricular stiffness. In addition to "active" diastolic processes, the myocardial tissue properties themselves, may also contribute to diastolic dysfunction. Increased fibrosis and expansion of the extracellular matrix (i.e. increased collagen deposition) is indeed a major determinant of myocardial stiffness (Kwak HB, 2013; Goldsmith EC. Et al., 2002; Corda S. et al., 2000) and has been shown to increase with general aging (Lakatta EG, 2015; Arbab Zadeh A. et al., 2004), as well as various disease states, including hypertension (Wei J. et al., 2016), ischemic heart disease (Nelson MD, 2017), cardiomyopathies (Nelson MD. et al., 2018), as well as overt heart failure (Wei J. et al., 2018). At the molecular level, decreased titin phosphorylation—the giant elastic filament that connects the Z-disc to the M-line in the sarcomere— has also been shown to play a key role in ventricular stiffness/elastance. While the exact mechanisms controlling titin phosphorylation continue to be discovered, inflammation and oxidative stress appear to be key regulators.

Commons ways to assess *left ventricular* diastolic function

The "gold-standard" assessment of left ventricular diastolic function is the time constant for isovolumic pressure decay (i.e. tau), measured by a pressure catheter directly in the left ventricle (Yu WC. et al., 2004). However, this measure focuses entirely on early diastole, as the rate of isovolumic pressure decay is a primary constituent of the transmitral pressure gradient, driving early diastolic filling of the ventricle. In order to evaluate ventricular stiffness/compliance, one needs to evaluate the end-diastolic pressure-volume relationship, measured by a pressure catheter in the left ventricle (with measurements taken at end-diastole) and a simultaneous volume measurement (in this case, as end-diastole). Because pulmonary capillary pressure reflects LV pressure at end-diastole, many investigators and physicians may also use pulmonary capillary wedge pressure as a surrogate measure for LVEDP. However, these procedures are incredibly invasive, complex, and can only be done in a select number of medical centers/research groups. Accordingly, multiple different non-invasive surrogate measures have emerged to assess diastolic function indirectly.

Doppler ultrasound is by far the most widely used and recognized modalities for assessing diastolic function, given its high temporal resolution, accurate assessment of blood flow, and low cost/accessibility (Kebed KY. et al., 2018). As described in **Table 1.4**, Doppler measures of mitral inflow and annular tissue velocities, as well as echocardiography-derived measures of left atrial volume, are commonly used as guideline proven methods for assessing diastolic (dys)function. In addition to Doppler-derived indices of diastolic function, other imaging modalities and approaches are also commonly used both independently and in conjunction. For example, diastolic strain rate—the rate of regional tissue deformation in diastole—has recently emerged as a powerful, often more sensitive measure of tissue relaxation. Where strain is a fractional change in length of

a myocardial segment, strain rate reflects the rate of change in myocardial lengthening or shortening. Importantly, early diastolic strain rate has proven to be prognostically significant across a wide range of diseases (Ersboll M. et al., 2014; Dokainish H. et al., 2008). Diastolic strain rate can be measured by multiple imaging modalities; however, echocardiographic speckle-tracking and magnetic resonance tissue tagging and/or feature tracking are among the most widely used (Ersboll M. et al., 2014; Dokainish H. et al., 2008; Wang J. et al., 2007). Other less common imaging approaches for diastolic function include cardiac computed tomography and nuclear imaging modalities (Antonini-Canterin F. et al., 2018; Kalisz K. et al., 2017).

It is worth noting, that while left atrial volumes are often included as non-invasive imaging metrics (regardless of modalities), very few studies have focused on left atrial function, despite its important contribution to the transmitral pressure gradient and overall ventricular filling.

Left atrial function across the heart failure continuum

During the cardiac cycle, the left atrium first acts as a reservoir, receiving pulmonary venous return during LV systole (**Figure 1.2**). This passive process is determined by the pressure gradient between the pulmonary circulation and left atrium. Then, once the pressure in the atrium exceeds that of the left ventricle, the mitral value opens and blood flows passively from the atrium to the ventricle. In this way, the atrium acts as a conduit vessel. Finally, the atrium depolarizes, leading the atrial contraction, transferring a final bolus of blood to the ventricle. Described hereon in as the "booster phase".
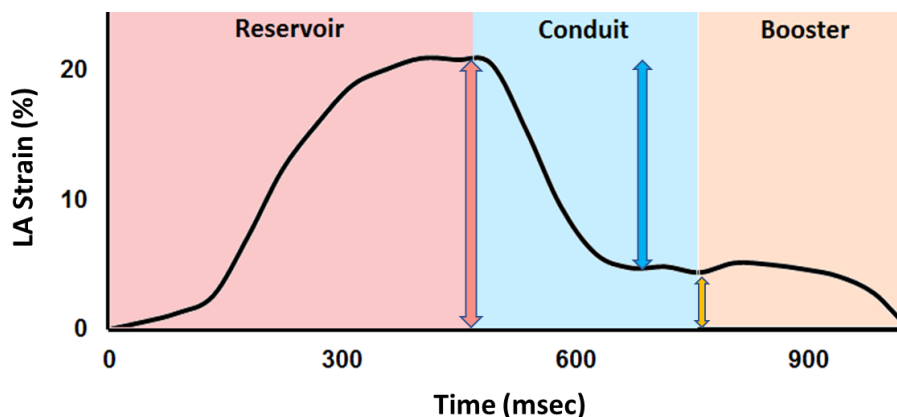
Figure 1.2 Representative strain curve illustrating the three atrial phases.

As with ventricular function, left atrial function can be assessed across a wide range of imaging modalities and endpoint measurements. For example, in the same way that maximal left atrial volume is often used as a diastolic index, volumetric assessment of each of the above phases can also be used to grossly assess left atrial function. Such volumetric approaches however are limited by user errors and can be quite time consuming. Accordingly, many investigators have incorporated strain imaging to assess atrial function (**Table 1.4**), given its high sensitivity and utility (AlSaikhan L. et al., 2018). Left atrial strain and strain rate represent the magnitude and rate, respectively, of atrium deformation (Jain S. et al., 2018). Much like ventricular diastolic strain/strain rate, LA strain is a powerful predictor of adverse cardiovascular outcomes, including stroke, development of atrial fibrillation, congestive heart failure, and death (Kebed KY. et al., 2018). Current left atrial strain publication, across the heart failure continuum, is summarized in **Table 1.5**.

Table 1.5: A summary of current left atrial strain publication.

Reference	Population	Sample Size	Imaging Modality	Key Findings
Aging				
Grootel RW. et al., 2018	Aging between 20-72 years of age	147	2D Speckle-Tracking Echo	50-72 yrs: ↓ Reservoir ↓ Conduit ↑ Booster
Obesity				
Chirinos JA. et al., 2018	Obesity based on different BMI ranges compare to normal BMI	1,531	2D Speckle-Tracking Echo	BMI >30: ↓ Reservoir ↓ Conduit ↑ Booster
Coronary Artery Stenosis				
Said KM. et al., 2018	Coronary Artery Stenosis (>50%) in high Syntax group in compare to low and moderate Syntax group	30	2D Speckle-Tracking Echo	↓ Reservoir ↓ Conduit ↑ Booster
Arrhythmia & Atrial Fibrillation				
Kurzawski J. et al., 2018	Atrial Fibrillation with LVEF <25% in compare to Preserved Sinus Rhythm	100	2D Speckle-Tracking Echo	↓ Reservoir ↓ Conduit ↓ Booster
Jarasunas J. et al., 2018	Paroxysmal Atrial Fibrillation with LV Diastolic Dysfunction compare to control	63	2D Speckle-Tracking Echo	↓ Reservoir ↓ Conduit ↔ Booster

Jarasunas J. et al., 2018	Paroxysmal Atrial Fibrillation without LV Diastolic Dysfunction compare to control	63	2D Speckle-Tracking Echo	↓ Reservoir ↓ Conduit ↓ Booster
Zghaib T., et al., 2018	Arrhythmogenic Right Ventricular Cardiomyopathy	90	Cardiac Magnetic Resonance	↓ Reservoir ↓ Conduit ↓ Booster
HFmEF				
AlSaikhan L. et al., 2018	HFmrEF compare to HFpEF	208	2D Speckle-Tracking Echo	↓ Reservoir ↔ Conduit ↓ Booster
HFpEF				
AlSaikhan L. et al., 2018	HFpEF compare to Control	208	2D Speckle-Tracking Echo	↓ Reservoir ↓ Conduit ↔ Booster
Melenovsky V. et al., 2015	HFpEF compare to Control	198	2D Speckle-Tracking Echo	↓ Reservoir ↓ Conduit ↓ Booster
Santos A. et al., 2014	HFpEF compare to Control	175	2D Speckle-Tracking Echo	↓ Reservoir ↓ Conduit ↓ Booster
Kowallick JT. et al., 2014	HFpEF compare to control	10	Cardiac Magnetic Resonance	↓ Reservoir ↓ Conduit ↓ Booster
Reddy YNV. et al., 2019	HFpEF compare to non-cardiac causes of dyspnea	363	2D Speckle-Tracking Echo	↓ Reservoir ↓ Conduit ↔ Booster
HFrEF				

Melenovsky V. et al., 2015	HFrEF compare to HFpEF	198	2D Speckle-Tracking Echo	↓ Reservoir ↓ Conduit ↓ Booster
Cardiac Hypertrophy				
Kowallick JT. et al., 2014	Hypertrophic Cardiomyopathy Compare to control	10	Cardiac Magnetic Resonance	↓ Reservoir ↓ Conduit ↑ Booster
Aortic Stenosis				
Mateescu AD. et al., 2019	HF symptomatic with severe Aortic Stenosis and preserved LVEF compare to asymptomatic	248	2D Speckle-Tracking Echo	↓ Reservoir ↓ Conduit ↓ Booster

Left atrial function in INOCA

To our knowledge, left atrial function has not been assessed in women with INOCA. Given the prior reports of _left ventricular_ diastolic dysfunction in INOCA, as well as the increased incidence of HFpEF in this patient population, we hypothesized that left atrial function would be impaired in INOCA compared to healthy reference controls. Moreover, we hypothesized that left atrial strain dysfunction would be more prominent in HFpEF, compared to INOCA, supporting a temporal hypothesis linking these two conditions. To test these hypotheses, we leveraged data from an ongoing trial (NCT02582021), and retrospectively analyzed cardiac magnetic resonance imaging data in three distinct groups (i.e. reference controls, INOCA and HFpEF), to evaluate left atrial strain across the heart failure continuum.

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Chapter 2: Diastolic dysfunction in women across the heart failure continuum: Novel pathophysiologic insight from left atrial feature tracking

1. Introduction

Ischemic heart disease is prevalent in women, accounting for increased death and health care costs (Nelson MD, 2017). Work from the NHLBI-Sponsored Women's Ischemia Syndrome Evaluation (WISE) suggests that most women presenting with signs and symptoms of ischemia have no obstructive CAD but have coronary vascular dysfunction (CVD) (Nelson MD, 2017; Bairey Merz CN. et al., 2017). We have consistently found these patients to have diastolic dysfunction (Nelson MD. et al., 2017; Nelson MD. et al., 2016, Wei J. et al., 2016), and to be at increased risk of heart failure with preserved ejection fraction (HFpEF) (Bakir M. et al., 2017). This has led to the hypothesis that CVD precedes HFpEF progression (Nelson MD, 2017; Gulati M. et al., 2009). Elucidating the pathophysiological mechanisms linking these two diseases is therefore important for future therapeutic discovery and heart failure prevention (Nelson MD. et al., 2018; Bairey Merz CN. et al., 2017).

To-date, our understanding of diastolic function in INOCA has been almost entirely derived from data focused on the *left ventricle* (LV), ignoring left atrial function altogether (a key constituent of ventricular filling). However, the left atrium plays an important role in both early and late diastolic ventricular filling. Indeed, the left atrium is more than just a simple passive filling chamber, with its function characterized by three distinct phases: First, during ventricular systole, the atrium passively receives blood from the pulmonary circulation, causing the atrium to swell/behave like a reservoir. Second, when the pressure in the atrium exceeds that of the ventricle, the mitral valve opens and the atrium functions as a conduit vessel, as the blood flows passively from the atrium to the ventricle due to the transmitral pressure gradient. Finally, following atrial depolarization, the atrium contracts, transferring blood into the ventricle and completing ventricular filling (i.e. LV end-diastolic volume). Strain imaging has emerged as a powerful tool

for quantitative evaluation of these three phases. Importantly, a growing body of literature supports the prognostic role of left atrial strain (Mateescu AD. et al., 2019; AlSaikhan L. et al., 2018; Chirinos JA. et al., 2018; Grootel RW. et al., 2018; Jarasunas J. et al., 2018; Kebed KY. et al., 2018; Kurzawski J. et al., 2018; Zghaib T. et al., 2018; Said KM. et al., 2018; Triposkiadis F. et al., 2016; Santos ABS. et al., 2014; Kowallick JT. et al., 2014; Hoit BD, 2014). To our knowledge, no study has evaluated left atrial strain in INOCA, or compared left atrial function between INOCA and HFpEF. As such, the aim of this project was to evaluate left atrial function in a group of well phenotyped women with INOCA and compare their atrial function to reference controls and patients with HFpEF. To accomplish this goal, we leveraged data from an ongoing trial (NCT02582021), and retrospectively analyzed cardiac magnetic resonance cine images, from three distinct groups of well phenotyped reference controls, INOCA and HFpEF, using novel feature tracking software.

2. Methods

2.1 Patient population

To test our hypothesis, we retrospectively analyzed 100 subjects from the WISECVD Continuation Trial (NCT02582021), including 58 women with suspected INOCA, 27 patients with HFpEF, and 15 reference controls. As previously published (Wei J. et al., 2016), all women with INOCA underwent angiography and coronary reactivity testing (CRT) and were found to have less than 50% stenosis in coronary arteries diameter. HFpEF patients were selected using a modified European Society of Cardiology criteria to diagnose HFpEF including (Ponikowski P. et al., 2016): symptoms of heart failure, left ventricular ejection fraction > 45% prior to study entry, structural evidence of cardiovascular abnormalities and evidence of elevated filling pressure. Angiography and CRT were not clinically warranted for the HFpEF patients, so obstructive CAD was ruled out using cardiac computed tomography angiography (Lee JH. et al., 2018). Reference control subjects did not have any symptoms, risk factors for, or evidence of ischemic heart disease, confirmed by a standardized 12-lead treadmill stress test that was within normal limits (Bairey Merz CN. et al., 2017; Jalnapurkar S. et la., 2017). All study participants gave written informed consent before undergoing evaluation and the study protocol was approved by the Institutional Review Board at Cedars-Sinai Medical Center (CSMC).

2.2 Coronary Angiography and reactivity testing in INOCA

As mentioned, clinically indicated invasive coronary angiography was performed to confirm the absence of obstructive CAD. In addition, left ventricular end-diastolic pressure was measured by a pressure catheter in the left ventricle. To assess coronary microvascular and macrovascular endothelial and non-endothelial dependent function, coronary flow was measured using a Doppler

flow wire in the proximal left anterior descending artery following intracoronary injection of adenosine and acetylcholine (Wei J. et al., 2016; AlBadri A. et al., 2019).

2.3 Cardiac magnetic resonance imaging

All subjects underwent cardiac magnetic resonance imaging, performed on a 3.0T MRI scanner (Siemens, Sonata, Erlangen, Germany), with ECG-gating and a phase-array surface coil. Heart rate and blood pressure were measured and recorded throughout the MRI.

To evaluate left atrial strain, cine images in the horizontal long axis plane (i.e. 4 chamber view), were obtained at rest, in all subjects. As illustrated in **Figure 2.1**, retrospective feature tracking analysis was then performed using commercially available software (CVI42, version 5.6.8; Calgary, Alberta). A single experienced observer, blinded to the clinical status of each subject, manually delineated the endocardial and epicardial borders of the left atrium, on a single cardiac phase at end-systole (just prior to mitral valve opening), before applying the feature tracking algorithm across the cardiac cycle. At a minimum, all strain measurements were performed in duplicate; however, if discrepancies between repeat measurements were identified, a third attempt was performed. An attempt was only included if it satisfied internal standards, primarily based on image quality and feature tracking quality. Insufficient tracking was defined as a visually apparent deviation of the contours from the endocardial and/or epicardial borders. In such a case, the contours were manually corrected and the tissue tracking algorithm re-applied. A second experienced observer, also blinded to the clinical status of the subject, reviewed and confirmed all included attempts. Reported strain measurements represent an average of each attempt for each subject. If both reviewers were not satisfied, data were excluded.
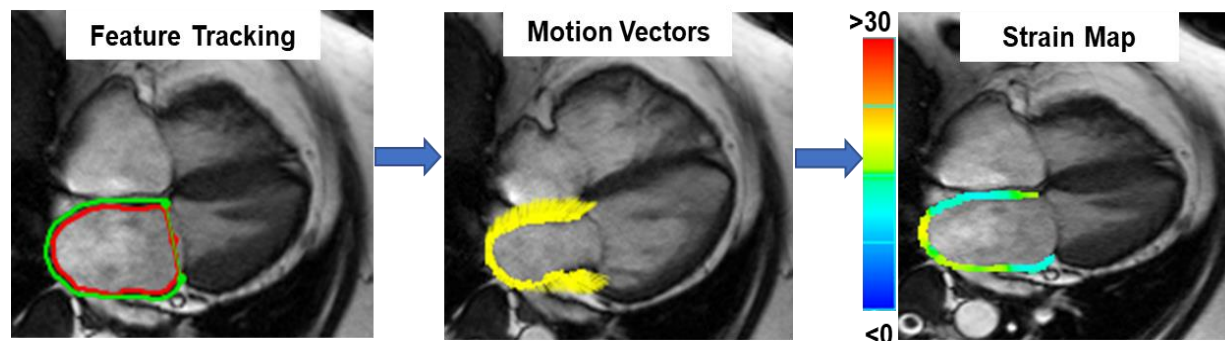
Figure 2.1 Left atrial (LA) tissue tracking in a representative horizontal long axis cine image. Left: Contours are drawn on the endo- and epicardial boarders at a single phase at end ventricular systole. Middle: Tissue tracking software propagates the contours automatically and follows the motion of the contour throughout the cardiac cycle; displayed as motion vectors across the atrial wall. Right: Longitudinal strain can then be displayed in the form of color maps throughout the cardiac cycle.

2.4 Data analysis and Statistical methods

Data are presented as median and interquartile range, unless otherwise specified. SigmaPlot 13.0 was used for all statistical analyses. To evaluate differences between reference controls, INOCA, and HFpEF, a one-way analysis of variance-ANOVA was performed. If data were not found to be normally distributed, a non-parametric test was performed. All probabilities were two-tailed and a p-value of less than 0.05 was considered statistically significant.

To evaluate specific mechanisms influencing left atrial strain in INOCA, we subdivided the INOCA group by: (1) LVEDP ($\leq$12 mmHg vs >12 mmHg), an established threshold signifying normal or abnormal diastolic function (Wei J. et al., 2018), respectively; (2) coronary flow reserve to adenosine ($\geq$ 2.3 vs. < 2.3), an established measure of coronary microvascular dysfunction (Wei J. et al., 2018; Pepine CJ. et al., 2015); (3) coronary blood flow response to acetylcholine (>50%

vs. <50%), an established measure of coronary microvascular dysfunction (Albadri A. et al., 2019; Wei J. et al., 2018); (4) coronary vasospasm to acetylcholine (positive change/negative change), an established measure of coronary macrovascular dysfunction (Albadri A. et al., 2019).

3. Results

3.1 Subject characteristics

As illustrated in **Figure 2.2**, 8 subjects were excluded from the final analysis, leaving a total of 12 reference controls, 55 INOCA and 25 HFpEF. To be consistent with recent LA strain investigations, LA reservoir strain was measured in all patients (regardless of cardiac rhythm), while LA conduit and booster strain were measured only in patients in normal sinus rhythm (Reddy YNV. et al., 2019). Subject characteristics of the final 92 subjects included in the data analysis are depicted in **Table 2.1**. Age, body mass index, and body surface area were different among the groups; with HFpEF patients being the oldest and having the highest body mass index.
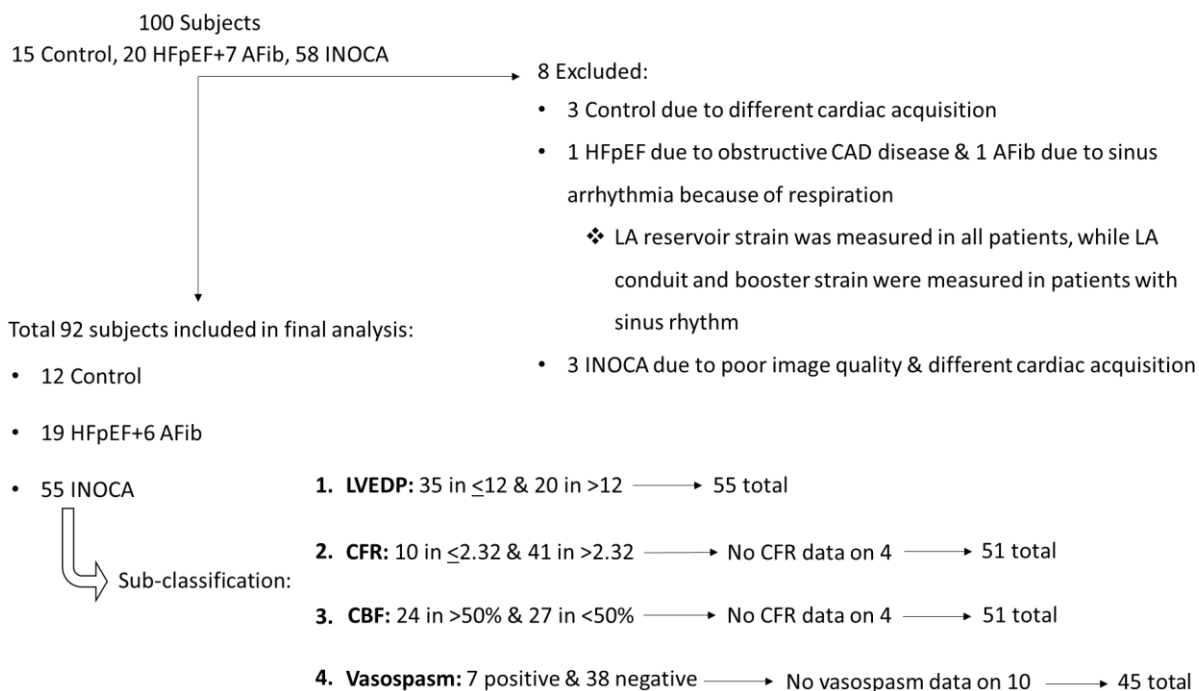
100 Subjects
15 Control, 20 HFpEF+7 AFib, 58 INOCA

8 Excluded:
- 3 Control due to different cardiac acquisition
- 1 HFpEF due to obstructive CAD disease & 1 AFib due to sinus arrhythmia because of respiration
  - ❖ LA reservoir strain was measured in all patients, while LA conduit and booster strain were measured in patients with sinus rhythm
- 3 INOCA due to poor image quality & different cardiac acquisition

Total 92 subjects included in final analysis:
- 12 Control
- 19 HFpEF+6 AFib
- 55 INOCA

Sub-classification:

1. **LVEDP:** 35 in ≤12 & 20 in >12 ⟶ 55 total
2. **CFR:** 10 in ≤2.32 & 41 in >2.32 ⟶ No CFR data on 4 ⟶ 51 total
3. **CBF:** 24 in >50% & 27 in <50% ⟶ No CFR data on 4 ⟶ 51 total
4. **Vasospasm:** 7 positive & 38 negative ⟶ No vasospasm data on 10 ⟶ 45 total

Figure 2.2 Flow chart illustrating the derivation of the final sample size.

Table 2.1: Subject characteristics.

Variable	Control	INOCA	HFpEF	P Value
Age, yr	47 ± 7	54 ± 12	64 ± 12	P <0.001
Heart rate, bpm	60 ± 6	61 ± 8	67 ± 12	P = 0.003
Systolic blood pressure, mmHg	110 ± 9	118 ± 13	129 ± 20	P = 0.008
Diastolic blood pressure, mmHg	60 ± 10	64 ± 9	69 ± 10	P = 0.07
Mean arterial pressure, mmHg	77 ± 7	82 ± 8	89 ± 11	P = 0.004
Height, cm	161 ± 7	160 ± 11	165 ± 10	P = 0.20
Weight, kg	65 ± 9	70 ± 13	83 ± 15	P <0.001
Body mass index, kg/m^2	25 ± 3	28 ± 6	30 ± 5	P = 0.01

Body surface area, m^2	2 ± 0.1	2 ± 0.2	2 ± 0.2	P <0.001

3.2 Left atrial function across the heart failure continuum

As illustrated in **Figure 2.3**, left atrial reservoir strain was significantly lower in HFpEF compared to reference controls, and tended to be lower compared to INOCA. No further group differences were observed for either conduit or booster strain.

Left atrial reservoir strain rate and booster strain rate were not difference between groups; however, we observed a strong trend for left atrial conduit strain rate to be depressed in both the INOCA and HFpEF groups, compared to reference controls (**Figure 2.3**).
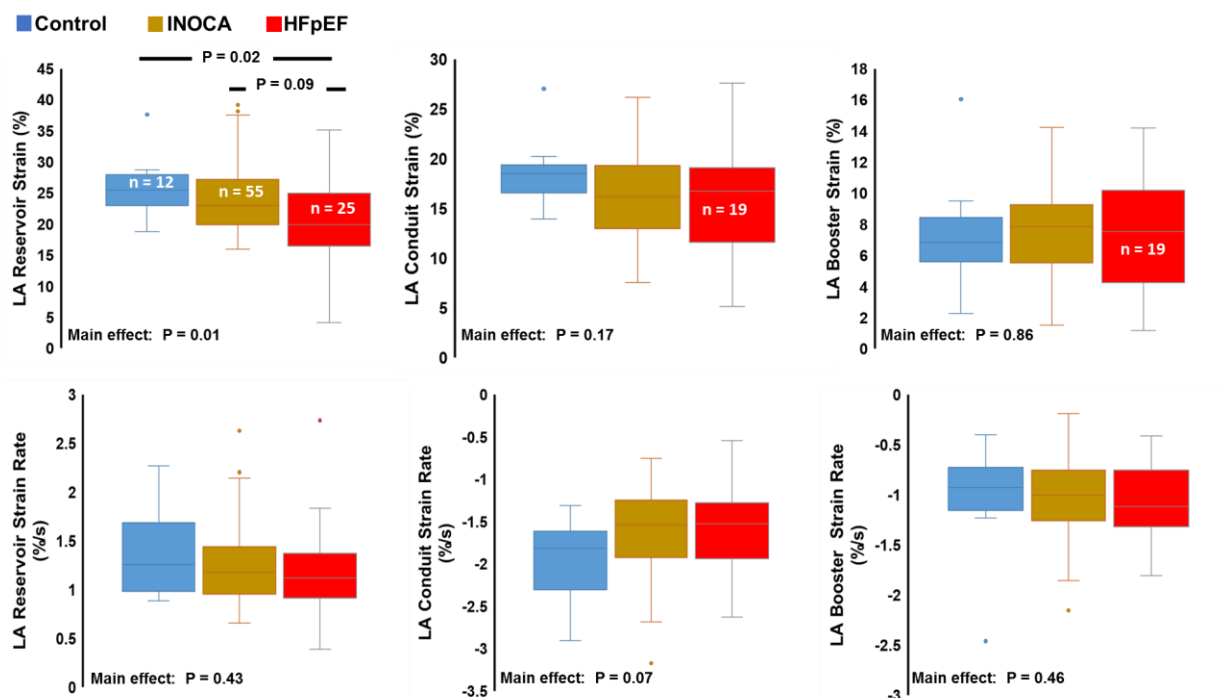
Figure 2.3 Left atrial function across the heart failure continuum. LA reservoir strain was measured in all patients, while LA conduit and booster strain were measured in patients with sinus rhythm.

3.2.1 Left atrial function in INOCA, Sub-Classified by LVEDP

To understand left atrial function in relation to the underlying pathophysiology of INOCA, we sub-divided INOCA subjects according to an established metric of diastolic (dys)function (<12>mmHg LVEDP). As illustrated in **Figure 2.4**, reservoir strain was higher in INOCA subjects with elevated LVEDP (n=20, 26.1 ± 1.3%) compared to those INOCA subjects who had normal LVEDP (n=35, 22.8 ± 0.9%, p=0.03). In contrast, we observed no group difference in conduit strain (16.5 ± 1.0% and 16.5 ± 0.7%, p=0.97, respectively), resulting in significantly higher atrial booster strain in the elevated LVEDP group (9.0 ± 0.6% and 7.3 ± 0.5%, p=0.03, respectively).
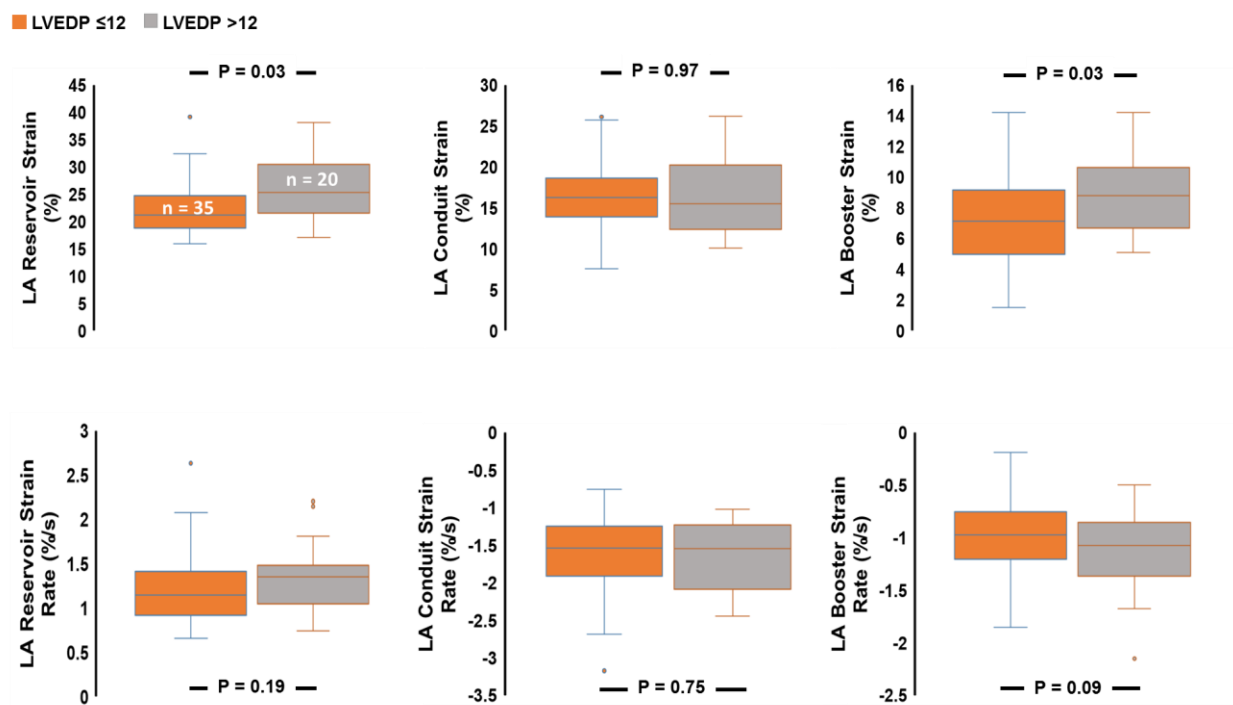
Figure 2.4 Left atrial function in INOCA subjects sub-classified according to their resting LV end-diastolic pressure.

To place this sub-classification into context, we also combined these new data with the reference controls and HFpEF patients, illustrated in **Figure 2.5**. Consistent with our prior observations, reservoir strain was lowest in the HFpEF subjects (n=19, 22.9 ± 1.1%), and highest in the reference controls and INOCA with LVEDP > 12 mmHg. Regardless of LVEDP, left atrial conduit strain rate tended to remain depressed, in INOCA and HFpEF. No other difference were observed.
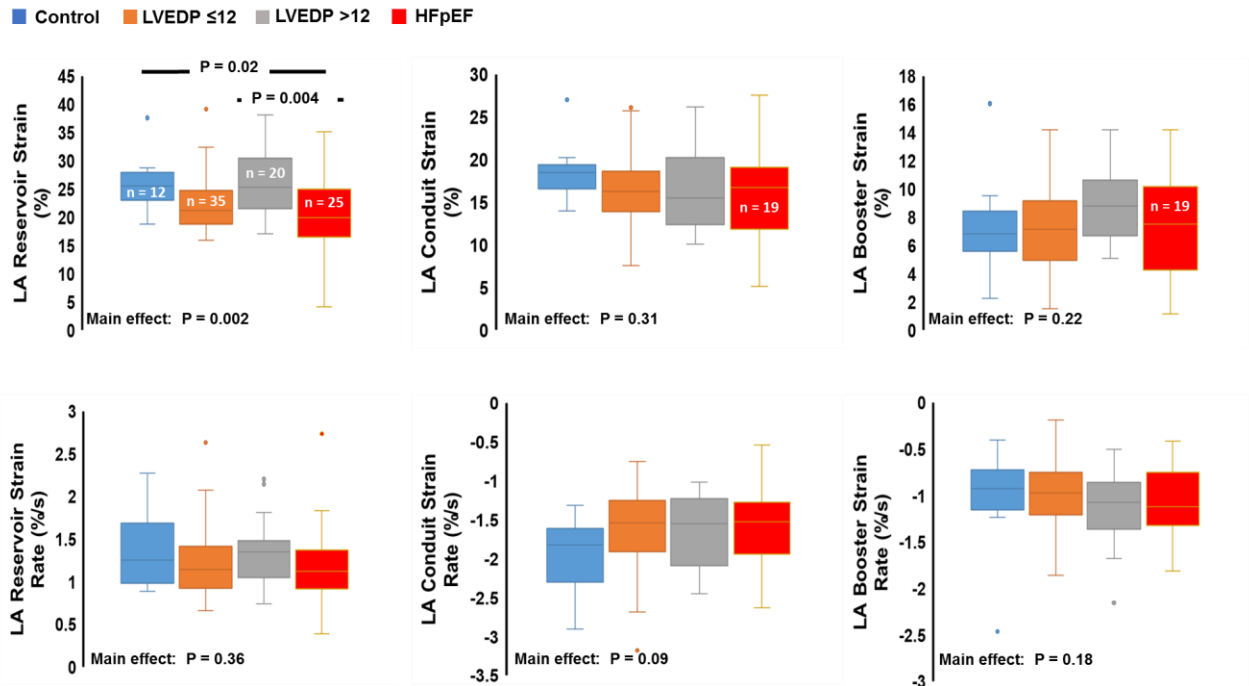
Figure 2.5 Left atrial function across the heart failure continuum with INOCA subjects subclassified according to their resting LV end-diastolic pressure.

3.2.2 Left atrial function in INOCA, Sub-Classified by Coronary Flow Reserve

To understand left atrial function in relation to coronary microvascular function in INOCA, we sub-divided INOCA subjects according to an established metric of coronary flow reserve (i.e. CFR ≤2.32>). As illustrated in **Figure 2.6**, there were no significant differences in any strain or strain rate value; however, left atrial reservoir strain tended to be elevated in patients with reduced CFR. This difference could not be explained by an elevated LVEDP, which was similar between groups (CFR >2.32, LVEDP = 10.9 ± 0.9 mmHg; CFR ≤2.32, LVEDP = 10.8 ± 1.6 mmHg, P = 0.97).
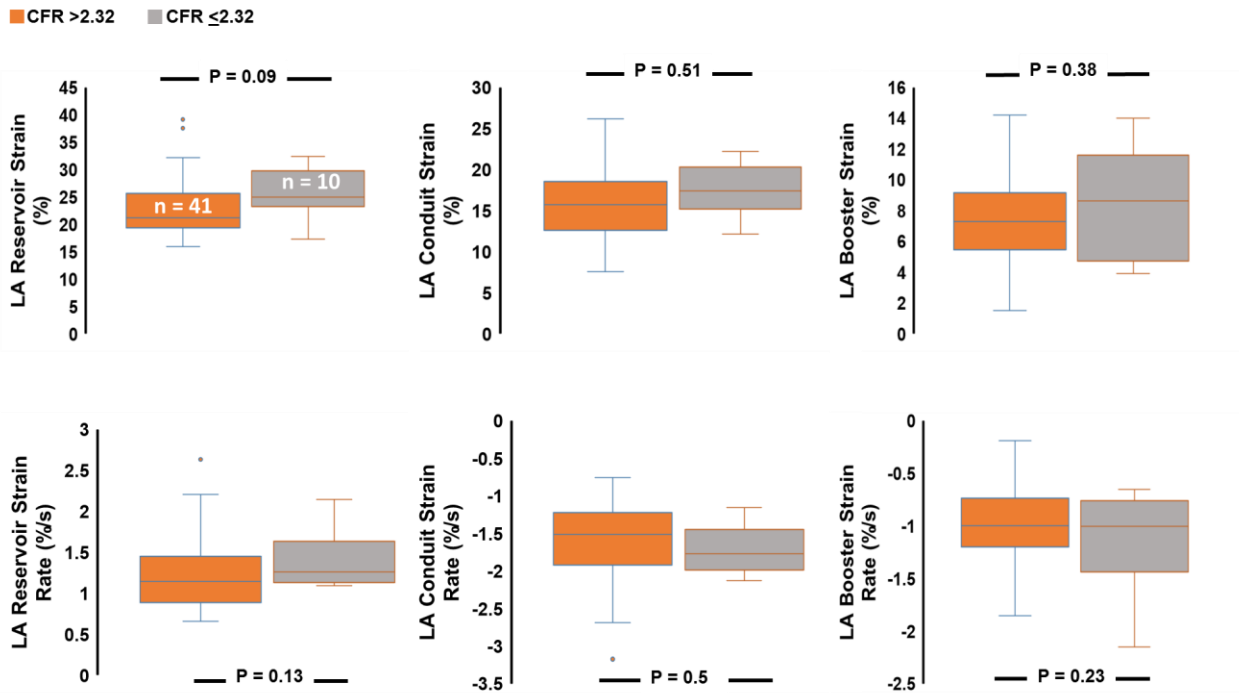
Figure 2.6 Left atrial function in INOCA subjects sub-classified according to their coronary flow reserve. The results cannot be explained by differences in LVEDP (CFR>2.32 = 10.9 $\pm$ 0.9 mmHg; CFR$\leq$2.32 = 10.8 $\pm$ 1.6 mmHg, P = 0.97) or left atrial volume at end-systole (CFR>2.32 = 36.3 $\pm$ 0.9 mL/m^2; CFR$\leq$2.32 = 37.2 $\pm$ 2.1 mL/m^2, P = 0.69).

3.2.3 Left atrial function in INOCA, Sub-Classified by presence or absence of vasospasm

To understand left atrial function in relation to coronary macrovascular dysfunction in INOCA, we sub-divided INOCA subjects according to the absence/presence of coronary vasospasm in response to intracoronary acetylcholine. As illustrated in **Figure 2.7**, there were no significant differences between groups in any of the strain or strain rate metrics; however, conduit strain rate tended to be reduced in the vasospasm positive cohort.
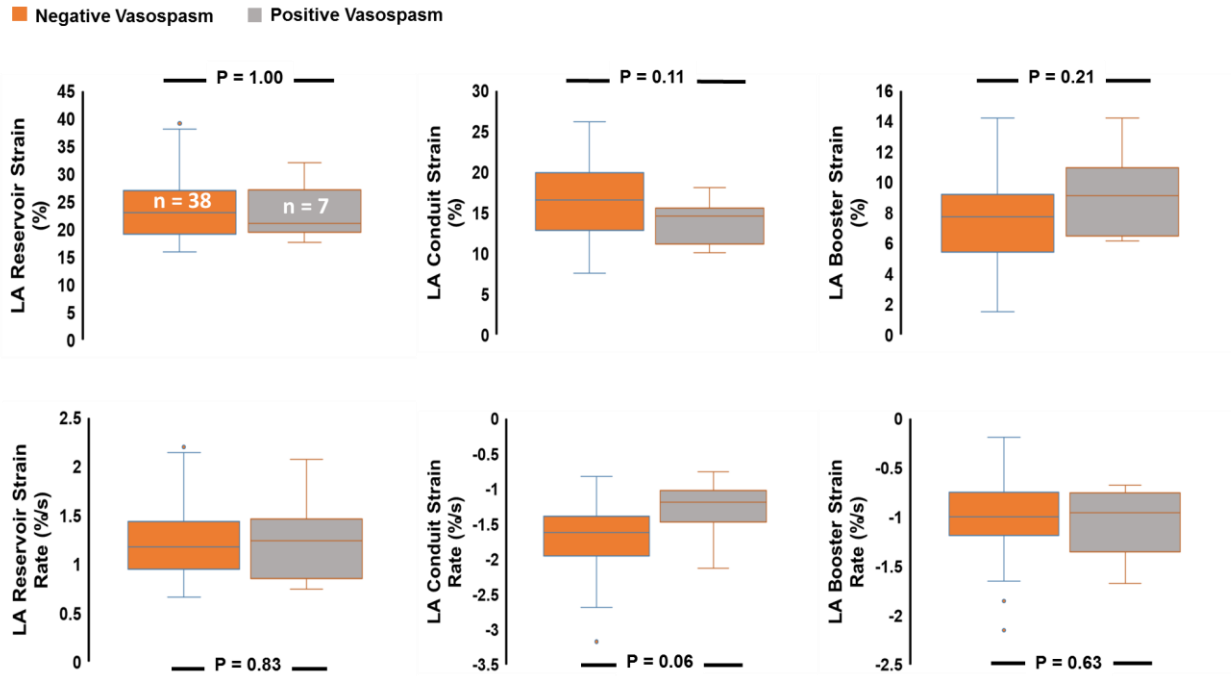
Figure 2.7 Left atrial function in INOCA subjects sub-classified according to presence or absence of vasospasm in response to intracoronary acetylcholine.

3.2.4 Left atrial function in INOCA, Sub-Classified by coronary blood flow

To understand left atrial function in relation to coronary microvascular function in INOCA, we sub-divided INOCA subjects according to an established metric of coronary blood flow (CBF <50%>). As illustrated in **Figure 2.8**, there were no significant differences in any of the strain or strain rate metrics.
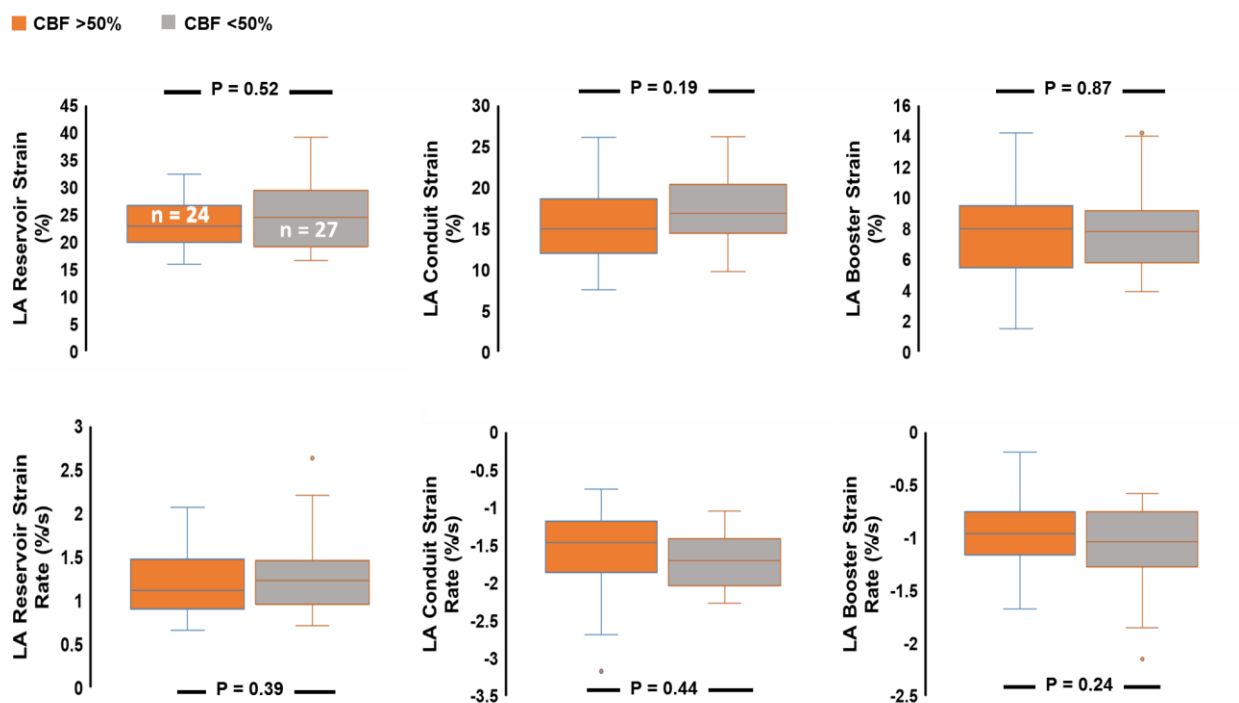
Figure 2.8 Left atrial function in INOCA subjects sub-classified according to their coronary blood flow.

4. Discussion

The major novel findings of this investigation are three-fold. First, left atrial reservoir strain is reduced across the heart failure continuum, being lowest in HFpEF patients compared to either reference controls or INOCA. Second, by subdividing INOCA subjects by LVEDP, we observe a paradoxical increase in left atrial reservoir strain, which we believe to be the first report of left atrial pseudo-normalization. Third, despite greater LA reservoir strain in INOCA subjects with elevated filling pressures, LA conduit function (strain and strain rate), tended to be depressed in INOCA and HFpEF relative to controls, consistent with ventricular diastolic dysfunction. Together, these data provide novel pathophysiological insight into heart failure progression in INOCA and support a role for analyzing LA function across the heart failure continuum.

To our knowledge, this is the first report of LA function in women with INOCA. Indeed, most of the work in INOCA to-date, has focused almost exclusively on left ventricle diastolic function (Wei J. et al., 2016; Nelson MD. et al., 2014). For example, Nelson et al. (2016), found left ventricular early diastolic circumferential strain rate to be reduced in INOCA, compared to reference controls, a finding which has since been confirmed across multiple difference investigations by this group, and in more than 150 patients (Nelson MD. et al., 2017; Wei J. et al., 2016). In addition, Nelson et al, in their seminal paper, observed a marked reduction in left ventricular untwisting rate, which is believed to be a key constituent of the transmitral pressure gradient (Nelson MD. et al., 2010). In this study, we extend these early observations, by evaluating LA function, another key constituent of diastolic filling and the transmitral pressure gradient. LA function is indeed prognostic (AlSaikhan L. et al., 2018; Kebed KY. et al., 2018), and has proven to be a useful discriminatory tool in a variety of different conditions, including heart failure (Melenovsky V. et al., 2015; Santos A. et al., 2014; Kowallick JT. et al., 2014), dilated cardiomyopathy (Kowallick JT. et al., 2014), and aortic stenosis (Mateescu AD. et al., 2019).

Consistent with recent reports, we observed a marked reduction in LA reservoir strain in HFpEF compared to reference controls (AlSaikhan L. et al., 2018; Melenovsky V. et al., 2015; Santos A. et al., 2014; Kowallick JT. et al., 2014). Indeed, LA reservoir strain has been shown to decrease as LV diastolic dysfunction worsens (Reddy YNV. et al., 2019; Singh A. et al., 2017) and provides incremental diagnostic information beyond left atrial volume alone (Reddy YNV. et al., 2019; Morris DA. et al., 2018). Considering these prior reports, we were surprised that LA reservoir strain was not significantly different between INOCA and controls, especially given our prior observations of LV diastolic dysfunction in this patient cohort. It is interesting to note however, that the LA conduit strain rate tended to be depressed in both the INOCA and HFpEF

groups, compared to reference controls, which we interpret as evidence of impaired LA-LV interaction (i.e. impaired transmitral gradient). While the exact mechanism driving this later finding is beyond the scope of this investigation, we hypothesize that impaired conduit strain and strain rate in INOCA is likely driven by ventricular diastolic dysfunction, given the seemingly normal reservoir strains compared to reference controls.

At least two prior investigations have attempted to normalize LA strain to estimated filling pressures (E/e', by echocardiography) (Reddy YNV. et al., 2019). In this investigation, by subdividing our cohort by invasively measured LVEDP, we observed a frank elevation in LA reservoir strain in INOCA patients with elevated LVEDP. This is somewhat contrary to what we expected, given that HFpEF patients often have reduced LA reservoir strain in the face of elevated LVEDP (AlSaikhan L. et al., 2018; Melenovsky V. et al., 2015; Santos A. et al., 2014; Kowallick JT. et al., 2014). We interpret this apparent discrepancy to reflect a "pseudo-normalized" pattern whereby a left atrium, which has not yet adversely remodeled, experiences greater strain in the presence of higher cardiac filling pressures. To our knowledge, this is the first report of such a finding, and supports the normalization of LA strain. Indeed, the conventional interpretation is that low LA reservoir strain reflects disease severity; however, our data suggest that this may be transient and dependent on the inherent LA tissue properties and cardiac hemodynamics.

Because coronary microvascular dysfunction is prevalent in INOCA, predictive of major adverse cardiovascular events (AlBadri A. et al., 2019; Wei J. et al., 2016), and believed to represent a key mechanistic pathway driving disease progression in these patients (AlBadri A. et al., 2019; Wei J. et al., 2016), we also evaluated LA function in relation to coronary vascular function. Despite its prognostic significance, we did not observe any major differences when INOCA patients were classified according to the three most common coronary pathways: (1)

coronary flow reserve to adenosine ($\geq$ 2.3 vs. < 2.3) (Pepine CJ. et al., 2015); (2) coronary blood flow response to acetylcholine (>50% vs. <50%), (Albadri A. et al., 2019); (3) coronary vasospasm to acetylcholine (positive change/negative change) (Albadri A. et al., 2019). An important consideration when interpreting these data, however, is that INOCA patients often have at least one impaired pathway and sometimes multiple vascular pathway impairments, which may have influenced these results. Despite the apparent lack of differences however, we find it interesting that LA conduit strain/strain rate tended to be depressed in patients with evidence of impaired coronary flow reserve and evidence of coronary vasospasm. We interpret this to reflect a reduced transmitral pressure gradient— secondary to impaired ventricular diastolic function— which may be mediated by chronic myocardial ischemia.

4.1 Study limitations

This study is not without limitation. The trial for which the data presented herein is ongoing, and therefore limited the total number of subjects included in final analysis. Moreover, the cohort of INOCA patients included in this investigation appear to be healthier, on average, compared to previously published reports. As a result, more subjects will be needed to help determine specific mechanistic pathways. While the LVEDP sub-classification proved to be useful for interpreting the INOCA data, we lack similar data in the controls and HFpEF groups, and thus could not apply this "correction" across all groups. Finally, the current study only focused on resting LA function. We, and others, have demonstrated the usefulness of assess cardiac function in response to physiologic stress (Samuel TJ. et al., 2018), and may prove useful moving forward. Future investigations are needed to address these limitations.

5. Conclusion

Despite these limitations, the results confirm recent reports of LA dysfunction in HFpEF, and show, for the first time, LA pseudo-normalization in women with signs and symptoms of ischemia in the absence of obstructive CAD. More work is needed to increase the overall sample size of this study, with further sub-classifications needed to help better understand key pathophysiologic mechanisms.

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Chapter 3: Discussion

Myocardial ischemia in the absence of obstructive coronary artery disease (INOCA) is prevalent in women and associated with coronary vascular dysfunction. Once thought to be a benign condition, we now know that INOCA is associated with major adverse cardiovascular events, including heart failure with preserved ejection fraction (HFpEF). The exact mechanism(s) driving heart failure progression in INOCA remains to be elucidated. For example, INOCA and HFpEF patients often share many common pathophysiologic traits, including a clustering of cardiovascular risk factors (hypertension, dyslipidemia, hyperglycemia, etc.) and left ventricular diastolic dysfunction (Nelson MD. et al., 2017; Wei J. et al., 2016). Despite these important observations, our understanding of the pathophysiology in INOCA remains limited. Indeed, our knowledge of diastolic dysfunction in INOCA remains almost entirely limited to the left ventricle. Left atrial function is equally important to overall diastolic filling and therefore represents an important unaddressed avenue for future investigation.

Herein, we assessed left atrial strain, and its time derivative, across the cardiac cycle in healthy reference controls, patients with INOCA, and patients with HFpEF using novel feature tracking software, retrospectively applied to an existing cardiac MRI database. Our results suggest that: (1) left atrial reservoir strain is reduced across the heart failure continuum, being lowest in HFpEF patients compared to either reference controls or INOCA; (2) INOCA subjects demonstrate a heterogenous response in LA reservoir strain, which appears to be driven by disease-related adaptations to cardiac filling pressure; and (3) LA conduit function (strain and strain rate) tends to be depressed in INOCA and HFpEF relative to controls, which we interpret to reflect left ventricular diastolic dysfunction. Taken together, these data provide novel pathophysiological insight into heart failure progression in INOCA and support a role for analyzing LA function across the heart failure continuum.

3.1 Lessons learned

Intra-rater reliability

At the onset of this project, several important unknown factors related to left atrial analysis first needed to be addressed:

1. What image is appropriate for the analysis (e.g. 4-chamber, 3-chamber, or 2-chamber)?
2. How can we use our existing, commercially available, software to assess left atrial strain?
3. If we use our existing feature tracking software (CVI42, Circle Cardiovascular Imaging, Calgary, Alberta), how can left atrial strain be assessed given that this is not a standard option?

To address these key questions, we first reviewed the literature and performed pilot testing of existing cine images. Based on our literature review and pilot testing, we agreed that evaluating left atrial strain from a 4-chamber (horizontal long axis), and "forcing" our software to work outside of its custom configuration (designed for left ventricular strain), was both appropriate and reproducible. Indeed, reproducibility was assessed by manually tracing, in triplicate, the endocardial and epicardial borders of the left atrium on a single cardiac phase just prior to mitral valve opening (LV end-systole), before applying the feature tracking algorithm across the cardiac cycle. This was performed in 15 randomly selected individuals, with the coefficient of variation calculated, resulting in 8.7$\pm$1.8% (1.3% absolute) for reservoir strain, 24.9$\pm$7.7% (1.8% absolute) for conduit strain, and 20.7$\pm$5.2% (1.6% absolute) for booster strain. Based on these data we felt it was appropriate to proceed with the current project.

With these pilot data forming our foundation, in the present study, we chose to perform strain measurements in duplicate for each subject; however, if inconsistencies between the first two

attempts were identified (either upon visual inspection or large variation in strain measurements), a third attempt was performed.

Clinical research

In this investigation I was fortunate to have access to a large de-identified database from an ongoing clinical trial (NCT02582021), to learn cardiac MRI analysis, cardiac physiology, and the challenges associated with clinical research. Particularly, this experience has taught me about subject heterogeneity. Indeed, many of our control participants has similar (if not worse) left atrial strain than some of our heart failure patients. To me, this exemplified the challenges associated with clinical research and highlights the need for careful patient phenotyping and large subject enrollment.

Another important lesson related to clinical research, was the challenges associated with obtaining gold-standard (often invasive) metrics. For example, based on my current data, I would love to know the pressure in the left atrium at the time of our imaging studies so that I could calculate left atrial compliance.

Despite these limitations, I remain fascinated by clinical research and committed to a career in clinical physiology.

Application of theory to practice

It is imperative to be able to put into practice what has been learned in the classroom and associate the theoretical concepts into practical value. To fuel my interest in cardiac physiology, I took Advanced Exercise Physiology and Cardiocirculatory courses which proved to be helpful throughout this project.

The objectives from the two courses that helped me to understand overall ventricular and atrial function and enable me to apply them throughout this project include: 1) The cardiac cycle, including the flow of blood through the heart, left ventricular pressure-volume loop, frank-starling law of the heart, pulmonary and systemic circulation, electrical activity and conduction system of the heart, basic interpretation of electrocardiogram, and pressure changes during cardiac cycle. 2) The structure of the cardiac myocyte excitation-contraction coupling concept in Advanced Exercise Physiology course helped me to understand the key mechanism driving diastolic dysfunction, which indeed is another key component of this project. 3) The electrocardiography and arrhythmia objective from Cardiocirculatory course helped me to understand arrhythmic mechanism of atrial fibrillation which enable me to recognize atrial fibrillation patients through their left atrial function. 4) Artery wall structure and function helped me to understand the role of vasoactive substances on coronary vascular function and enable me to differentiate macro/microvascular endothelial/non-endothelial dysfunction.

A remaining area which I plan to spend more time expanding my knowledge in, is statistics— the mathematical body of science— which I now appreciate more than ever is a critical component of research and study design.

3.2 Future directions

As mentioned, the lack of simultaneous left atrial pressure data challenges the interpretations made herein. Future research would greatly benefit with this important addition. Of course, this is not unique to this project, but rather is a limitation shared by all imaging modalities. For example, Doppler ultrasound provides useful information about mitral inflow velocities; however, these filling velocities are inherently dependent on cardiac filling pressure. Despite this limitation, Doppler ultrasound is used in echocardiography labs around the world each and every day,

providing, useful information, nevertheless. By extension, our left atrial strain data, despite not having simultaneous pressure data, still provides important insight. Likewise, in this study we used LVEDP (measured up to 1 month apart from the cardiac MRI) to categorize our patients; however, LVEDP does not determine left atrial strain, and therefore is not the ideal categorical discriminator for these types of studies.

The trial for which the data presented herein is ongoing, and therefore limited the total number of subjects included in final analysis. We performed power analysis of the existing data, and found small effect sizes for mean reservoir, mean conduit, and mean booster strain (0.36, 0.2 and 0.05, respectively). This is somewhat surprising given that our sample population is consistent with several recent publications in the area. While our intra-rater reliability was found to be acceptable, we observed high heterogeneity within each group, which likely played the biggest role in our power analysis. With greater sample sizes in each group, we could further separate those subjects with the highest and lowest response.

The current study only focused on resting LA function. Our lab has previously demonstrated the usefulness of assessing cardiac function in response to physiologic stress (Samuel TJ. et al., 2018). Whether diastolic stress testing, either using isometric handgrip (Samuel TJ. et al., 2017, Samuel TJ. et al., 2018) or dynamic leg exercise (Beaudry RI. et al., 2018) would help to differentiate left atrial strain dynamics remains incompletely understood and worth considering for future investigations.

3.3 Final thoughts and conclusions

This study tested the hypothesis that left atrial strain is impaired in INOCA, and represents a key pathophysiologic mechanism driving heart failure progression. To test this hypothesis, LA

function was measured by retrospective feature tracking analysis of horizontal long axis cine image from cardiac magnetic resonance imaging using CVi42 software. The data of this investigation show that: First, left atrial reservoir strain is reduced across the heart failure continuum, being lowest in HFpEF patients compared to either reference controls or INOCA. Second, by subdividing INOCA subjects by LVEDP, we observe a paradoxical increase in left atrial reservoir strain, which we believe to be the first report of left atrial pseudo-normalization. Third, despite greater LA reservoir strain in INOCA subjects with elevated filling pressures, LA conduit function (strain and strain rate), tended to be depressed in INOCA and HFpEF relative to controls, consistent with ventricular diastolic dysfunction. More work is needed to increase the overall sample size of this study, with further sub-classifications needed to help better understand key pathophysiologic mechanisms.