THE IMPACT OF AGE AND FRAILTY ON THE FISCHER-344 RAT HEART

by

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ABSTRACT

The purpose of this study was to develop a frailty index (FI) for rats, and characterize the impact of age and frailty on cardiac parameters at baseline and following arrest with del Nido cardioplegia. Male F344 rats were followed in a longitudinal study from youth into adulthood, and middle age into old age. Data on survival and 45 variables in health systems known to change with age were serially collected to develop a 30-item FI. Mean FI scores increased with age, and a high FI score was associated with an increase in mortality. Cardiac function was assessed *in vivo,* and in the isolated working heart. We developed a novel method to assess diastolic function in the isolated working heart by measuring changes in end-diastolic volume at a fixed LV filling pressure. Aged and frail hearts were more arrhythmic at baseline, however neither age nor frailty were associated with statistically significant changes in cardiac structure, inotropic, hemodynamic, or diastolic function *in vivo* or in the isolated working heart. Following arrest with del Nido cardioplegia, functional recovery and cardiac troponin-I release into the coronary effluent were similar when compared by age and frailty. Potential differences in recovery from cardioplegic arrest may have been mitigated by the use of del Nido cardioplegia, which has been shown to be very effective in aged hearts.

LIST OF ABBREVIATIONS AND SYMBOLS USED

°C	degrees Celsius
2-D	Two-dimensional
A wave	late transmitral flow velocity
AF	aortic flow
ANOVA	analysis of variance
bpm	beats per minute
BUN	blood urea nitrogen
Ca^{2+}	calcium ion
$CaCl_2$	calcium chloride
CF	coronary flow
Cl-	chloride ion
cm	centimeter
CO	cardiac output
CO_2	carbon dioxide
cTnI	cardiac troponin-I
CVR	coronary vascular resistance
dL	deciliter
E wave	early transmitral flow velocity
E/A	early transmitral flow velocity to late transmitral flow velocity ratio
ECG	electrocardiogram
EDTA	ethylenediaminetetraacetic acid
EDV	end diastolic volume
EF	ejection fraction
ELISA	enzyme linked immunosorbent assay
ESV	end systolic volume
F344	Fischer-344
FI	frailty index
FS	fractional shortening
g	gram

H^+	hydrogen ion
H_2O	water
Hb	hemoglobin
HR	heart rate
HW/TL	heart weight to tibia length ratio
IL-10	interleukin-10
IL-10$^{tm/tm}$	interleukin-10 knockout
IL-6	interleukin-6
I_{Na}	sodium current
IVS	Interventricular septum
IVSd	interventricular septum at diastole
IVSs	interventricular septum at systole
K^+	potassium ion
KCl	potassium chloride
kg	kilograms
KH_2PO4	monopotassium phosphate
KHB	Krebs-Henseleit buffer
L	liter
LV	left ventricle
LVDP	left ventricular developed pressure
LVEDP	left ventricular end diastolic pressure
LVEDPVR	left ventricular end diastolic pressure-volume relationship
LVID	left ventricular internal diameter
LVIDd	left ventricular internal diameter at diastole
LVIDs	left ventricular internal diameter at systole
LVPW	left ventricular posterior wall
LVPWd	left ventricular posterior wall at diastole
LVPWs	left ventricular posterior wall at systole
LVV	left ventricular volume
MAP	mean aortic pressure
mg	milligram

$MgSO_4$	magnesium sulphate
MHz	megahertz
min	minutes
mL	milliliters
mm	millimeter
mM	millimolar
mmHg	millimeters of mercury
ms	millisecond
Na^+	sodium ion
NaCl	sodium chloride
$NaHCO_3$	sodium bicarbonate
NCX	sodium-calcium exchanger
ng	nanogram
NS	not significant ($p<0.05$)
O_2	oxygen
pH	power of hydrogen
PVC	premature ventricular complex
RPP	rate pressure product
SD	standard deviation
SEM	standard error of the mean
SP	systolic pressure
SV	stroke volume
SW	stroke work
VF	ventricular fibrillation
VT	ventricular tachycardia
μm	micrometer

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CHAPTER 1: INTRODUCTION

1. General Overview

In Canada, population aging is becoming a primary concern for cardiovascular care providers (Forman et al., 2011). During the past century, the average life expectancy has lengthened, resulting in a steady growth in the proportion of Canadians aged 65 years and older. When the first of the baby boomers turned 65 in 2011, this demographic entered a period of unprecedented growth. Indeed, it is projected that in the next 50 years the proportion of Canadians aged 65 and older will increase from 15.7% to 26% (Statistics Canada, 2014). The incidence of acute and chronic cardiovascular disease increases with age (Forman et al., 2011). It is therefore expected that as the Canadian population ages the number of seniors requiring cardiovascular care will all also rise considerably (Wiedemann et al., 2010).

Advanced age is frequently associated with greater cardiovascular disease severity (Wiedemann et al., 2010). As such, there are an increasing number of elderly patients referred for cardiac surgery (Nicolini et al., 2014). Advanced age increases a patient's risk for post-operative complications, including an increased risk of morbidity, mortality and institutionalization (Alexander et al., 2000). As such, surgeons may withhold interventions in certain elderly patients. Nevertheless, elderly patients have been shown to derive sizeable benefits from cardiac surgery, including improved quality of life and freedom from cardiac limitations (Fruitman et al., 1999; Zingone et al., 2009). Understanding which elderly patients will derive benefit from cardiac surgery is of increasing concern for surgeons assessing perioperative risk.

People age at different rates. As such, physiological age can vary widely for a given chronological age (Mitnitski et al., 2001). For example, a cardiac surgeon can have two patients of the same age referred for surgery where one patient appears significantly younger and more robust than the other. Frailty, a geriatric syndrome associated with impaired resiliency to acute stress, is a concept increasingly explored in the literature as an approximate measure of biological age (Mitnitski et al., 2013). While there is no

universally accepted operational definition of frailty, it can be theoretically defined as a state of increased vulnerability to adverse health outcomes for two individuals of the same age (Rodriguez-Mañas et al., 2013).

In the past decade, frailty has been increasingly explored as a predictor of adverse health outcomes following cardiac procedures (Afilalo et al., 2014). In a review examining the impact of frailty on cardiac surgery outcomes, it was found that frail elderly patients have an increased risk of mortality and institutionalization relative to their non-frail counterparts (Sepehri et al., 2014). To elucidate why frail patients are at greater risk of adverse outcomes following cardiac interventions, there is a need to better understand the impact of frailty on cardiac physiology.

Until recently, there was little known about the biology of frailty and its effects on the heart because it had not been quantified in an animal model of aging (Howlett, 2015). In 2012, Parks *et al.* demonstrated that frailty could be quantified in aging C57BL/6J mice with an invasive frailty index (FI) using deficit accumulation. With this model, a high FI score was associated with hypertrophy and reduced contractility in ventricular myocytes (Parks et al., 2012). More recently, Whitehead *et al.* (2014) developed a non-invasive FI for the C57BL/J mouse, allowing for the longitudinal study of frailty. In a follow-up study, it was demonstrated that a high FI score better predicted changes in cardiac morphology and function than chronological age (Sun, 2014). Together, the efforts of these authors have demonstrated that the relationship between cardiac physiology and frailty can be studied in an aging rodent model.

Nonetheless, there are limitations to the indexes used to quantify frailty in mice. Briefly, the invasive nature of the index developed by Parks *et al.* (2012) only allows for cross-sectional studies of frailty. The clinical index developed by Whitehead *et al.* (2014) does not measure changes in physical activity levels or cognition; two key components of frailty (de Vries et al., 2011). Furthermore, performing cardiac physiological assessments at the organ level is difficult in the C57BL/6 mouse given the technical challenge of working with a small heart. The goal of this thesis is to expand on the work of Parks *et al.* (2012) and Whitehead *et al.* (2014) by (1) developing an FI that addresses the aforementioned limitations in a larger rodent model, the Fischer 344 rat; (2) use this index to determine the impact of age and frailty on baseline cardiac function; and (3) determine

the impact of age and frailty on functional recovery from arrest with del Nido cardioplegia.

The first part of this introduction will focus on a brief review of what is currently known about the effects of aging on cardiac structure and function in humans and animals. The second part will review the concept of frailty, and the different methods used to operationalize the assessment of frailty in humans and animals. The third part of the introduction will explore the relationship between frailty and cardiovascular care, as frailty is becoming increasingly relevant for assessing perioperative risk in elderly patients.

2. Age-related changes in cardiac structure and function

Age is a known risk factor for the development of cardiovascular diseases that require surgical treatment, including coronary artery disease and heart failure (Nicolini et al., 2014). To understand why aging increases the risk of developing cardiovascular diseases, it is important to understand age-related changes in cardiac structure and function in the absence of disease.

2.1 Age-related changes in cardiac structure

It is generally accepted that changes in cardiac structure occur with age in the absence of cardiovascular disease. In humans, there is an increase in left ventricular wall thickness with age (Cheng et al., 2009). Cardiac and left ventricular mass is preserved or decreased, which has been attributed to a decline in the total number of cardiac myocytes (Cheng et al., 2009; Olivetti et al., 1991). The loss of cardiac myocytes is accompanied by cellular hypertrophy (Olivetti et al., 1991). In addition, there is increased myocardial collagen deposition and enhanced collagen cross-linking throughout the lifespan (de Souza, 2002). Thus, age-related changes are characterized by the development of cellular hypertrophy and fibrosis.

While the age-associated remodeling of the myocardium does not directly cause cardiovascular disease, it does lower the threshold for disease manifestation. For example, left ventricular hypertrophy and fibrosis reduce ventricular compliance, which impairs

relaxation during early diastolic filling (de Souza, 2002). This leads to a left and upward shift in the end-diastolic pressure volume relationship. Under these circumstances, a relatively small increase in central blood volume, venous tone, or arterial stiffness can lead to a substantial rise in left atrial and pulmonary venous pressure (Aurigemma & Gaasch, 2004). In turn, this can lower the threshold for pulmonary congestion or edema, two prominent pathological features of heart failure (Aurigemma & Gaasch, 2004; Zile & Brutsaert, 2002).

Aged rodent models have also been utilized to investigate changes in cardiac morphology, as aged rats exhibit similar changes in cardiac structure and function as humans (Lakatta & Sollott, 2002). Animals are typically considered senescent at the age where 50% mortality occurs. In rats, this typically occurs between 20-24 months of age (Lakatta & Sollot, 2002). In aged rats, the left ventricle is thickened and composed of a smaller number of hypertrophied cells relative to adults (Anversa et al., 1986). Collagen deposition markedly increases with age (Anversa & Capasso, 1991; Walker et al., 2006). Unlike humans however, there is an increase in cardiac mass throughout the lifespan (Walker et al., 2006). Still, the morphological remodeling observed with age in rats is thought to be fairly similar to humans (Lakatta & Sollott, 2002; Nadon, 2007). It is possible that these structural changes also increase the susceptibility of the aged heart to cardiovascular disease.

2.2 Age-related changes in cardiac function

In addition to studying changes in cardiac structure, there has been extensive study regarding age-related changes in cardiac function in the absence of cardiovascular disease. In humans, changes in systolic function with age appear to be gender-dependent. There is evidence that myocardial contractility declines with age in males, but is preserved or modestly increased with age in females (Claessens et al., 2007). Furthermore, there is a decline in peak ejection fraction with age (Stratton et al., 1994). There is a progressive decline in diastolic function throughout the lifespan, as evidenced by changes in the peak early to late diastolic transmitral flow velocity. With aging, there is a reduction in the early diastolic transmitral flow velocity (E wave) and a compensatory

increase in late diastolic transmitral flow velocity (A wave). This results in a decline of the E/A ratio with age (Benjamin et al., 1992; Watanabe et al., 2005).

Resting heart rate (HR) remains relatively consistent throughout the lifespan, however there is a decline in maximal heart rate (Strait & Lakatta, 2012). The cardiac conduction system is also affected by age. For example, there is an increase in the P-R interval, a leftward shift of the QRS axis and a minor increase in the Q-T interval with age (Fleg et al., 1990; Strait & Lakatta, 2012). The prevalence of arrhythmia, including ventricular ectopy and ventricular tachycardias also increase with age (Strait & Lakatta, 2012). Although these changes in cardiac electrophysiology do not affect mortality in healthy elderly persons, they generally increase one's risk of cardiovascular disease (Strait & Lakatta, 2012).

Age-related changes in cardiac function have also been investigated in rats. Some studies report that ejection fraction, a commonly used measure of systolic performance, is preserved in female rats, but decline in males (Fannin et al., 2014; Hacker et al., 2006; Walker et al., 2006). Most studies agree that diastolic function declines with age, however there are some reports that suggest it is maintained (Fannin et al., 2014; Hacker et al., 2006; Walker et al., 2006). Clearly, there is some disagreement with regards to age-related changes in the rat heart. Many of these studies however are not directly comparable. Differences between studies in age, body weight, strain and anesthesia protocol all could have contributed to a difference in echocardiography outcomes (Watson et al., 2004). Aged rat hearts also tend to be more arrhythmic at rest relative to adult rats (Walker et al., 2006).

In summary, when changes in cardiac structure and function are assessed throughout the lifespan of humans and rats in the absence of cardiovascular disease, increased LV wall thickness, collagen deposition, impaired diastolic filling and altered heart rhythm represent the most consistent changes observed with age. While these changes do not directly cause cardiovascular disease, they compromise the cardiovascular reserve capacity (Lakatta & Sollott, 2002; Strait & Lakatta, 2012). Thus, age-associated changes in cardiac structure and function reduce the threshold for developing the clinical signs and symptoms of cardiovascular disease (Lakatta & Levy, 2003).

3. Frailty

As discussed in the previous section, several age-related changes in structure and function increase the susceptibility of the heart to cardiovascular disease. It is important to note however that these characteristics are not universal among all aged hearts. People age at different rates; therefore two persons of the same chronological age can have a wide variation in biological health (Mitnitski et al., 2001). For example, a 70 year-old individual that lives an active lifestyle, has a low burden of disease and requires little assistance with activities of daily living has a vastly different health status from a 70 year-old who lives a sedentary lifestyle, suffers from numerous diseases and is dependent on others. Thus, the health status of older individuals varies from fit to frail (Mitnitski et al., 2013).

Frailty is a concept increasingly explored in the geriatric literature to account for the heterogeneity of health outcomes in elderly persons of the same age (Rockwood & Mitnitski, 2007). It refers to a multidimensional physiological syndrome where individuals experience a loss in one or more domains of human functioning (de Vries et al., 2011). As these functional losses (called deficits) accumulate, the individual's ability to maintain homeostasis in the face of acute or chronic stress is diminished (Rockwood & Mitnitski, 2007). Consequently when the frail individual is exposed to a stressor, they are more vulnerable to adverse health outcomes such as postoperative complications, admission to a long-term care facility and death relative to fitter individuals of the same age (Lee et al., 2015).

The ability to recover from exposure to stress depends on an individual's reserve capacity (Mitnitski et al., 2013). Since early publications, frailty has been considered to represent a loss of physiologic reserve (Rockwood et al., 1994). This leads to deterioration in the maintenance of homeostatic processes over time, which ultimately leads to functional decline and an increased risk of disease and death (Mitnitski et al., 2013). Aging itself is associated with a decline in physiologic reserve that impairs one's resiliency to stress (Fulop et al., 2010). Indeed, a pathway that is similar to the aging process has been proposed as contributing to the development of frailty (de Vries et al., 2011).

Aging is characterized by chronic inflammation, impaired immunity, neuroendocrine dysregulation and alterations in metabolism (de Vries et al., 2011). Elevated levels of inflammatory biomarkers such as IL-6 and C-reactive protein suggest that aging is associated with a chronic, low-grade inflammation (Franceschi & Campisi, 2014). Chronic inflammation has also been implicated in the pathogenesis of frailty. For example, it has been demonstrated that community-dwelling frail elderly persons have significantly higher levels of IL-6 and neopterin (a biomarker of immune system activation) relative to non-frail age-matched controls (Leng et al., 2002; 2011). Data from the Cardiovascular Health Study and Longitudinal Aging Study Amsterdam have also demonstrated that elevated C-reactive protein is associated with frailty in age-matched controls without cardiovascular disease (Puts et al., 2005; Walston et al., 2002).

With aging, there is also significant neuroendocrine dysregulation. For example, in men there is a decline in testosterone throughout the lifespan (Harman et al., 2001). Low testosterone levels are associated with sarcopenia, a key determinant of frailty (Fried et al., 2001). While cross-sectional studies have demonstrated that low bioavailable testosterone is associated with frailty; results from longitudinal studies are inconclusive (Afilalo, 2014). Aging is also associated with insulin resistance (Fulop et al., 2010). Results from the Cardiovascular Health Study suggest that frail patients are more likely to be insulin-resistant relative to their non-frail counterparts (Barzilay et al., 2007). Thus, it is possible that metabolic alterations, such as insulin-resistance, also contribute to the development of frailty (Barzilay et al., 2007).

While the exact biology of frailty remains unclear, chronic inflammation, impaired immunity, neuroendocrine dysregulation and metabolic alterations have all been implicated in its pathogenesis (de Vries et al., 2011). One of the key limitations for researchers studying the biology of frailty is the lack of a standardized method for quantifying the geriatric syndrome. As such, an individual deemed frail by one frailty instrument might be considered non-frail (or robust) by another. The subsequent sections will discuss the different methods used to quantify frailty in humans and animal models.

3.1 Quantification of frailty in humans

While the theoretical definition of frailty is almost universally accepted, there is little consensus regarding how to best operationalize the assessment of frailty. Twenty-seven different frailty scales have been described in the literature (Bouillon et al., 2013). The next section will focus on the two major operational definitions that have emerged in the past decade: the FI, and the frailty phenotype, also known as the Fried phenotype or the Cardiovascular Health Study definition.

In 2011, de Vries *et al.* identified eight risk factors important for the quantification of frailty. In the physical domain, measures of nutritional status, physical activity, mobility, strength and energy should be considered. In the psychological domain, cognition and mood should be measured; and in the social domain, measures of social contact and support should be included. Frailty is a multidimensional syndrome; therefore a comprehensive frailty instrument should consider all eight factors in its assessment (de Vries et al., 2011).

In 2001, Mitnitski *et al.* developed an FI based on the principle of deficit accumulation (Mitnitski et al., 2001). An FI can be created by counting the accumulation of deficits in health across multiple systems, such as clinical signs and symptoms, laboratory abnormalities, or diseases, and expressing them as a ratio of the total number of deficits measured. Individual items are evaluated using a combination of self-report, clinical assessment, specific tests, and laboratory evaluation; they are then assigned a score between 0 (no deficit) and 1 (sever deficit). The number of deficits present is then summed and expressed as a ratio of the total number of deficits measured. This will yield an FI score between 0 (no deficits present) and 1 (all deficits present) (Rockwood & Mitnitski, 2007). For example, if an individual possessed 10 of 30 measured deficits; they would have an FI score of 0.33.

Previous studies in humans have demonstrated that an FI should measure a minimum of 30 deficits in health, as this increases the likelihood that the index would predict adverse health outcomes (Peña et al., 2014). Furthermore it has been shown that when greater than 30 deficits are measured, the specific deficits evaluated are not as important because the predictive power of the index lies in the number of deficits measured (Peña et al., 2014). Searle *et al.* (2008) published four criteria that deficits must

satisfy to be included in a human FI: (1) the deficit's prevalence must generally increase with age; (2) the deficit's prevalence must not saturate at too early an age; (3) the deficit must be associated with an adverse health outcome; and (4) as a group, the candidate deficits must cover a wide range of health systems. Furthermore, if serially tracking changes in frailty, the deficits comprising the FI must be consistent from one measurement to the next (Searle et al., 2008).

There are multiple advantages to assessing frailty with an FI. It is currently the most comprehensive frailty instrument available, assessing all eight factors deemed critical for the quantification of frailty (de Vries et al., 2011). It assesses deficits across multiple physiological systems, and has good reliability and predictive validity (Bouillon et al., 2013; Howlett, 2015). Furthermore, the FI allows for more than one phenotypic presentation of frailty. This is important because while people accumulate deficits as they age, they do not necessarily accumulate the same deficits at the same rate (Rockwood & Mitnitski, 2011). That being said, some frailty indices measure upwards of 40 variables, which can be time-consuming for the clinician (Rockwood & Mitnitski, 2007; Rockwood et al., 2005). There are also no clear cut-off points as to what constitutes frail versus non-frail, so it can be difficult to select at what score a patient is considered frail (Bouillon et al., 2013).

The other major method for assessing frailty is the frailty phenotype. It was developed by Fried *et al.* in 2001 and tested in the Cardiovascular Health Study, a large cohort study with 5,317 men and women greater than 65 years of age. The frailty phenotype defines frailty as clinical syndrome in which three of more of the following criteria are present: unintentional weight loss (>10 lbs. in the past year), self-reported exhaustion, weakness (grip strength), slow walking speed, and self-reported low levels of physical activity. Individuals with three factors are considered frail, while those with one or two are classified as pre-frail. Individuals with none of these factors are considered fit, or non-frail (Fried et al., 2001).

There are many advantages for clinicians assessing frailty with the frailty phenotype: the tests comprising the phenotype are easily administered, inexpensive and allow frailty to be assessed relatively quickly with good reliability (Bouillon et al., 2013). The tests do not require specialized equipment; therefore frailty assessments can be

performed in the community relatively easily. While the Fried phenotype is the most commonly used frailty assessment for patients with cardiovascular disease (CVD), it is fundamentally flawed as it attempts to measure a multidimensional syndrome with a one-dimensional assessment tool (Bouillon et al., 2013; Afilalo et al. 2014). It ignores functional losses in the psychological and social domains and focuses solely on deficits in the physical domain (de Vries et al., 2011). Thus, an obese individual with cognitive decline, depressed mood and a lack of social support is not considered frail by the Fried definition.

There is currently no consensus regarding a gold standard for frailty assessment. With respect to the two most cited instruments, the FI and the frailty phenotype, there are advantages and disadvantages to both. Briefly, while the frailty phenotype is the most commonly used instrument for studying frailty in patients with CVD, it attempts to measure a multidimensional syndrome with a one-dimensional instrument (Afilalo et al., 2014; Bouillon et al., 2013). While somewhat inefficient, the FI is the only instrument that assesses all eight factors important for the quantification of frailty (de Vries et al., 2011). Because the FI provides the most comprehensive assessment of frailty, it is perhaps the more suitable of the two methods. Developing animal models of frailty would help us to further understand the biology of frailty and its relationship to disease (Howlett, 2015). In turn, this could help to identify a gold standard of assessment. The subsequent section will review how frailty has been quantified using animal models.

3.2 Quantification of frailty in animals

Animal models are frequently used for investigating the physiology, cell biology, genetics and behavioural biology of normal aging and age-associated diseases (Nadon, 2007). Rodent models are often used to study the aging process because of their short lifespan and similarity in physiology and disease pathogenesis to humans (Demetrius, 2006; Nadon, 2007). Furthermore, the genetic background, diet, environment and health status of rodents can be controlled, thereby helping researchers distinguish between the functional changes associated with healthy aging and pathologic conditions associated with old age (Nadon, 2007). Rodent models have been integral to understanding the

biology of aging, however until recently there was little known about the biology of frailty in these animals (Howlett, 2015).

In 2008, Walston *et al.* proposed the first animal model of frailty, the IL-10 knock out mouse. These mice carry a homozygous deletion of the IL-10 gene (IL-$10^{tm/tm}$); therefore they cannot produce the anti-inflammatory cytokine IL-10. Genetic deletion of IL-10 mimics several aspects of human frailty. When compared with wild-type C57BL/6 mice, IL-$10^{tm/tm}$ mice have higher levels of inflammation, decreased skeletal muscle strength, lower physical activity and increased mortality (Ko et al., 2012; Walston et al., 2008). Thus, the IL-$10^{tm/tm}$ mouse has a similar phenotype to that proposed by Fried *et al.* (2001) in humans. A unique advantage of this animal model is that animals are considered "old" when they are greater than 9 months of age (Sikka et al., 2013). Frailty studies can therefore be completed relatively quickly and inexpensively. This model is limited however in that it has yet to be determined if this genetically altered mouse mimics natural aging (Howlett, 2015). For example, these mice were initially developed as an animal model for Crohn's disease, irritable bowel disease, anemia and growth retardation (Howlett, 2015; Walston et al., 2008). Together, these limitations may make it difficult to translate frailty findings in the IL-$10^{tm/tm}$ mice to humans (Howlett, 2015).

In 2012, Parks *et al.* took a bedside to benchside approach to quantify frailty in a natural animal model of aging. Using the same theory of deficit accumulation as that used to develop the FI for humans, they developed an FI for C57BL/6 mice in a cross-sectional study. During the course of the study, 31 health-related variables linked to the function of different systems known to change with age were measured. Parameters measured included changes in activity levels, hemodynamic status, body composition, basic metabolism and organ function. Each potential deficit was assigned a score between 0 (no deficit) and 1 (severe deficit) using a graded system based on the number of standard deviations away from a mean reference value. The total number of deficits was then summed and divided by the total number of deficits measured to yield an FI score between 0 (no deficits present) and 1 (all deficits present) for each animal. A higher FI score indicated a frailer animal (Parks et al., 2012).

This study was the first to successfully demonstrate that the principle of deficit accumulation could be applied to quantify frailty in aging mice. Frail mice exhibited

similar characteristics to frail humans. For example, a high FI score in mice was associated with a greater likelihood of adverse cardiac health outcomes (Parks et al., 2012). A key limitation of this study included the small sample size (n=12), however statistical differences in FI score and ventricular myocyte function were still observed with age. The objective nature of the tests used to quantify each deficit is an advantage, however many of these tests are invasive or require specialized equipment. This unfortunately renders the Parks *et al.* (2012) FI appropriate for cross-sectional studies of frailty only (Whitehead et al., 2014).

To address these limitations, Whitehead *et al.* (2014) developed a non-invasive FI based on a clinical assessment of 31 potential deficits in health in C57BL/6 mice. Signs of clinical deterioration were evaluated in multiple health systems. Each deficit was assessed and given a score between 0 and 1 using a graded scoring system: 0 = no deficit; 0.5 = mild deficit; and 1 = severe deficit. The total number of deficits was then summed and divided by the total number of deficits measured (31) to yield an FI score between 0 and 1. Again, a higher FI score indicated a more frail animal (Whitehead et al., 2014).

This simplified index is extremely beneficial for the geriatric researcher because it allows frailty to be assessed in a rapid and non-invasive manner, thus allowing for multiple frailty assessments across the lifespan. It does not require specialized equipment; therefore multiple labs will be able to use this FI without having to purchase additional equipment. Frailty scores increased with age in mice in a similar pattern to that observed in humans and frail mice were at greater risk of adverse cardiac outcomes (Sun, 2014). Thus, this index has promising predictive validity. Furthermore, this FI has been shown to have good inter-rater reliability (Feridooni et al., 2015). A key limitation of this index is that it does not evaluate changes in cognition or physical activity, two key components of frailty in the elderly (de Vries et al., 2011).

4. Frailty and cardiovascular care

Frailty is becoming increasingly relevant to the field of cardiovascular medicine. Improvements in cardiovascular disease treatment coupled with population aging have changed the epidemiology of CVD to elderly persons (Yazdanyar & Newman, 2009).

Twenty-three percent of Canadian seniors indicate they are living with one or more cardiovascular diseases (Public Health Agency of Canada, 2009). A significant number of these patients are also frail, with estimates ranging from 25-50% depending on the population and assessment tool used (Afilalo, 2011). Thus it is important to understand frailty as it pertains to cardiovascular care in the elderly (Afilalo et al., 2009).

As discussed previously, chronological age is not sufficient to characterize health status in the elderly given the heterogeneity of the patient population (Mitnitski et al., 2001). Measures of physiological age, such as frailty, may therefore be helpful for predicting patient outcomes. Frailty reflects a dynamic state where elderly individuals suffer from impaired resilience to stress; thus it is well-suited to predict the elderly patient's response to acute or chronic cardiovascular stress (Afilalo, 2011). Indeed, one of the most promising clinical applications of frailty is its incorporation into perioperative risk assessment. Existing risk scores, such as the Society of Thoracic Surgeons (STS) and European System for Cardiac Operative Risk Evaluation II (EuroSCORE II), do not evaluate frailty or other measures of biological age, and typically over- or underestimate perioperative risk (Rowe et al., 2014). Multiple studies have shown that the incorporation of frailty into existing risk scores improves perioperative risk assessment, regardless of the operational frailty definition used (Sepehri et al., 2014). For example, frailty was demonstrated to be an independent predictor of adverse health outcomes after cardiac surgery, including increased short- (30-day) and mid-term (1-year) mortality, prolonged institutional care and functional decline (Lee et al., 2010; Schoenenberger et al., 2013; Sündermann et al., 2014). Consideration of frailty status is therefore beneficial for cardiac surgeons assessing whether or not an elderly patient will benefit from surgery.

While there is sufficient evidence to demonstrate that differences in cardiac surgery outcomes exist between frail and non-frail elderly patients, there is little evidence as to how frail patients referred for cardiac surgery should be managed. For example, it remains unknown if frail patients would have a better prognosis if they underwent a less-invasive procedure (Afilalo et al., 2014). The search for these answers is limited by a lack of standardized frailty assessment and minimal information regarding differences in cardiac physiology between frail and non-frail populations.

Recent studies have attempted to investigate baseline differences in cardiac structure and function between frail and non-frail populations. In humans, Gharacholou *et al.* (2015) demonstrated that frail patients had a diminished cardiovascular reserve capacity. For example, frail patients had lower stroke volumes, decreased diastolic function and increased pulmonary artery systolic pressures relative to less frail patients of the same age. The Cardiovascular Health Study demonstrated that subclinical cardiovascular disease abnormalities, such as LV hypertrophy, hypertension and electrocardiographic abnormalities are associated with frailty (Newman et al., 2001). In animals, Parks *et al.* (2012) demonstrated in a pilot study that a high FI score was associated with hypertrophy and reduced contractility in ventricular myocytes from C57BL/6 mice. Results from Sun (2014) also suggest that frailty better predicts changes in cardiac structure than chronological age. Together, these data suggest that there are differences in baseline cardiovascular reserve capacity that render the frail heart more vulnerable to adverse health outcomes when exposed to stressors such as cardiovascular disease or cardiac surgery. Still, whether frailty better predicts changes in cardiovascular structure and function in a larger rodent model, the Fischer-344 rat, remains to be elucidated. Furthermore, whether frail hearts have impaired functional recovery after exposure to acute stress (i.e. cardioplegia-induced ischemia) has yet to be determined.

5. Research Objectives

The first objective of the present study was to develop an index to quantify frailty in the F344 rat using the principle of deficit accumulation. To develop the FI, we serially collected data on 45 variables in health known to change with age in a longitudinal study of aging male F344 rats. From these results, we developed a 30-item FI that allows for multiple frailty assessments throughout the lifespan.

The second objective of this study was to determine the impact of frailty and chronological age on baseline cardiac function. For this study, we performed serial echocardiography to assess changes in cardiac structure and function *in vivo* on two cohorts of aging rats: a "young" cohort aged from 3 – 9 months and an "old" cohort aged from 13 – 21 months. Echocardiography variables were then compared by age and FI

score. There are limitations to evaluating changes in cardiac function with echocardiography in small animals, as resolution and image quality can make it difficult to define endocardial borders. Furthermore, loading conditions, anesthesia and neurohormonal influences can complicate comparisons in the living animals. Therefore we further examined the effects of age and frailty on baseline cardiac function in the isolated working rat heart. Additionally, we developed a novel technique to examine diastolic function using the isolated working heart in conjunction with echocardiography.

The third objective of this study was to determine the impact of chronological age and frailty on functional recovery from arrest with cardioplegia. For this study, we arrested hearts with a single dose of del Nido cardioplegia, a hyperkalemic solution that contains lidocaine, less blood, and less calcium relative to standard cardioplegia (Matte & del Nido, 2012). Previous work from our lab has demonstrated that del Nido cardioplegia protects aged hearts against ischemia reperfusion injury better than standard cardioplegia in both isolated myocytes and in the isolated working heart (O'Blenes et al., 2011; Govindapillai et al., 2016). It was our intent to expand on this work to determine the impact of age and frailty on recovery from arrest with del Nido cardioplegia. For this study, we collected several working heart measurements after cardioplegic arrest. Post-reperfusion working heart measurements were compared with baseline values; percent recovery was compared by age and FI score.

6. Hypotheses

1. An FI can be created for the F344 rat based on the principle of deficit accumulation.
2. Mean FI scores increase with age, and a high FI score is associated with an increased risk of mortality.
3. Frailty and age will be associated with changes in baseline cardiac morphology and function *in vivo*.
4. Frailty and age will be associated with changes in baseline cardiac function in the isolated working heart.

5. Frailty will predict changes in functional recovery from arrest with del Nido cardioplegia independently of age.

CHAPTER 2: METHODS

1. Experimental animals

Male F344 rats (n=44) purchased from Harlan Laboratories (Maine, USA) were aged from middle age (13 months, n=44) into old age (21 months) in a longitudinal study. A second cohort of rats was aged from youth (3-6 months, n=12) into adulthood (9 months) to serve as a young control group for experiments (Yu et al., 1982). Rats were fed *ad libitum* and housed in isolation on a 12-hour light/dark cycle. All experiments were approved by the Dalhousie University Committee on Laboratory Animals and performed in accordance with guidelines published by the Canadian Council on Animal Care (CCAC; Ottawa, Ontario: Vol 1, 2nd edition, 1993; Vol 2, 1984).

2. Frailty Index

2.1 Potential deficits

To develop the FI, 45 health-related variables linked to the function of different systems known to change with age in the F344 rat were serially measured in a young (n=4) and old cohort of rats (n=36). Additional rats were added later in the study to the young (3 months, n=2; 6 months, n=6) and old group (17 months; n=8) for additional experiments (outlined in methods section 2.4-2.5). Deficits related to changes in nutritional status, health of the integument, pain and discomfort, the musculoskeletal system, ocular and nasal systems, auditory system, vestibulocochlear system, neurological system, respiratory system, digestive system, urogenital system, sensorimotor function, basic metabolic status and hematology were all considered for the FI.

Tests for potential deficits were conducted at approximately the same time of day during the specified test interval (e.g. every week, or every two months). In the event that an animal was terminally ill, all tests were conducted prior to euthanasia (if possible) so that a terminal FI score could be generated. The severity of each deficit was scored using a scale previously described in mice by Whitehead *et al.* (2014): 0 = absent, 0.5 = mild, 1

= severe. Details about how each deficit was coded to fit this scoring system are described subsequently.

2.2 Nutritional

Food was weighed twice weekly to assess changes in food intake. Biweekly measurements were summed and divided by 7 to calculate daily food intake. A decline in food intake of <15% reflected the absence of a deficit (0), a 15-29% decline in food intake reflected a mild deficit (0.5) and a >30% decline in food intake reflected a severe deficit (1). Rats were weighed once a week to evaluate changes in body weight. An increase/decline in body weight of <10% reflected the absence of a deficit (0), an increase/decline in body weight of 10-19% reflected a mild deficit (0.5) and an increase/decline in body weight of >20% reflected a severe deficit (1). Cut-points for both food intake and body weight were determined through consultation with a veterinarian.

2.3 Clinical signs

Rats were observed for 23 clinical signs of deterioration once a week using a modified set of methods from previously described studies in the C57BL/6 mouse (Whitehead et al., 2014). This included assessment of the integument, discomfort, and the following systems: musculoskeletal, ocular, nasal, auditory, vestibulocochlear, neurological, respiratory, digestive and urogenital. Clinical signs were selected through a review of the literature on age-related pathological changes in the F344 rat and through consultations with a veterinarian (table 1). Clinical assessment techniques were developed by modifying methods previously described for clinical frailty assessment in the C57BL/6 mouse, and in consultation with a veterinarian (Whitehead et al., 2014). Young rats were observed in comparison with older rats to refine assessment techniques.

To assess clinical signs, rats were moved to an assessment room in the animal care facility and allowed 5 minutes to acclimatize to their new surroundings. Animals were then briefly observed in their home cage prior to undergoing examination for clinical signs of deterioration. Scoring of clinical signs is described in Appendix A.

Table 1: Clinical signs of deterioration in aging Fischer-344 rats.

System/Variable	Description	Ref.
Alopecia	Acquired hair loss due to inflammation, endocrine disorder, or idiopathic disease.	50
Fur colour	Change in fur colour from white to yellow.	66
Skin lesions	Excessive scratching, self-mutilation, or skin conditions leading to open sores on the body.	107
Coat condition	Ungroomed appearance: fur appears ruffled and matted.	107
Tumours	Presence of neoplastic growths.	50
Hunched posture	Presence of hunched posture (head down, feet together); reduced mobility.	107
Gait disorders	Abnormal locomotion: slow movement, lack of coordination, stumbling, falling, or limping.	107
Tremor	Involuntary shaking at rest.	107
Body condition	Visual signs of emaciation or obesity. Based on the amount of flesh covering the vertebral column and dorsal pelvis.	107
Distended abdomen	Asymmetric/enlarged abdomen. May be due to neoplastic growths, organ enlargement or peritoneal fluid accumulation.	99
Head tilt	Abnormal/asymmetric head position associated with a central nervous system disturbance.	50,99
Hearing loss	Impaired acoustic startle reflex; associated with loss of hearing sensitivity.	99
Cataracts	Opaque spot in the center of the eye; clouding of the lens.	99
Chromodacryorrhea	Porphyrin staining around the eyes/nose.	50
Exophthalmos	Abnormal protrusion of the eye.	93
Microphthalmos	Abnormally small eye. Sunken in appearance.	20
Corneal opacity	Cornea appears white or clouded.	99
Malocclusion	Abnormal occlusion due to uneven or overgrown incisors.	50
Rectal prolapse	Tissue protruding from the rectum.	99
Diarrhea	Increased frequency and decreased consistency of bowel movements. Fecal smearing in cage.	99,107
Jaundice	Yellowing of the feet, nose, ears and tail associated with accumulation of bilirubin.	99,107
Breathing rate/depth	Bradypnea, tachypnea, dyspnea, or snuffling.	50,107
Unusual sounds	Acute vocalization in response to touch.	50
Body temperature	Increase or decrease in body temperature.	107

2.4 Sensorimotor function

Sensorimotor function was evaluated once a month using a battery of four tests: the blind alley test, plank walk test, prehensile strength test and inclined plane test. Longitudinal analysis of performance was conducted in animals followed from youth (3 months, n=6) into adult hood (9 months, n=12), and from middle age (13 months, n=36) into old age (21 months, n=18). Cross-sectional analysis of first exposure to each test was performed at 3- (n=6), 6- (n=6), 13- (n=36), and 21-months (n=5).

2.4.1 Blind alley test

The blind alley test was used to evaluate contextual processing (Markowska et al., 1998). For this test, the rat was placed facing the back wall of an alley (29 cm x 9.5 cm with walls 24 cm high) (figure 1). The time it took the rat to turn around and face the open end of the alley was recorded to a maximum of 5 minutes. Each rat was given 1 trial in the blind alley test with no pretrial.

To score the blind alley test, we divided the time to turn around in the blind alley at 13 months into tertiles. Times to turn around in the lowest tertile (<10 sec) reflected good contextual processing (0), the middle tertile (11 – 19 sec) reflected a mild loss of contextual processing (0.5) and the highest tertile (>20 sec) reflected a severe decline in contextual processing (1).

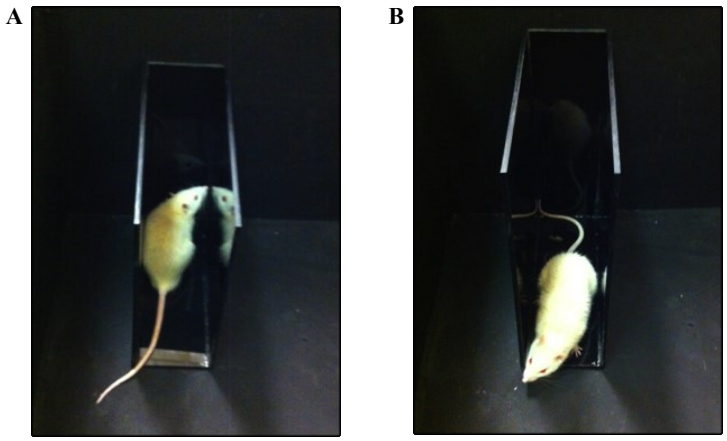

Figure 1. The blind alley test was used to evaluate contextual processing. (A) Rats were placed face first into an enclosed alley with walls on three sides of the apparatus. (B) The time it took for the rat to turn and face the open end of the alley was recorded to a maximum latency of 5 minutes.

2.4.2 Plank walk test

The plank walk test was used to assess balance and coordination by exposing rats to planks of different widths: narrow (NP) = 13 mm, medium (MP) = 25 mm, and wide (WP) = 38 mm) (Joseph et al., 1983). Each plank was 100 cm long and elevated 34 cm above a 5 cm foam pad (figure 2). The rat was placed with all four paws on the center of the plank and latency to fall was recorded to a maximum of 5 minutes. Each rat was given 1 trial on each plank width with no pretrial. The order of plank width exposure was randomized for each rat.

To score the plank walk test, latency to fall measurements for the narrow plank width at 13 months were divided into tertiles. Latency to fall scores in the upper tertile (>54 sec) reflected good balance and coordination (0), the middle tertile (23 – 53 sec) reflected a mild loss of balance and coordination (0.5) and the lower tertile (<22 sec) reflected a severe decline in balance and coordination (1). For the medium and wide plank tests, successfully completing the test indicated good balance and coordination (0); while failure to stay on the plank indicated a severe loss of balance and coordination (1).

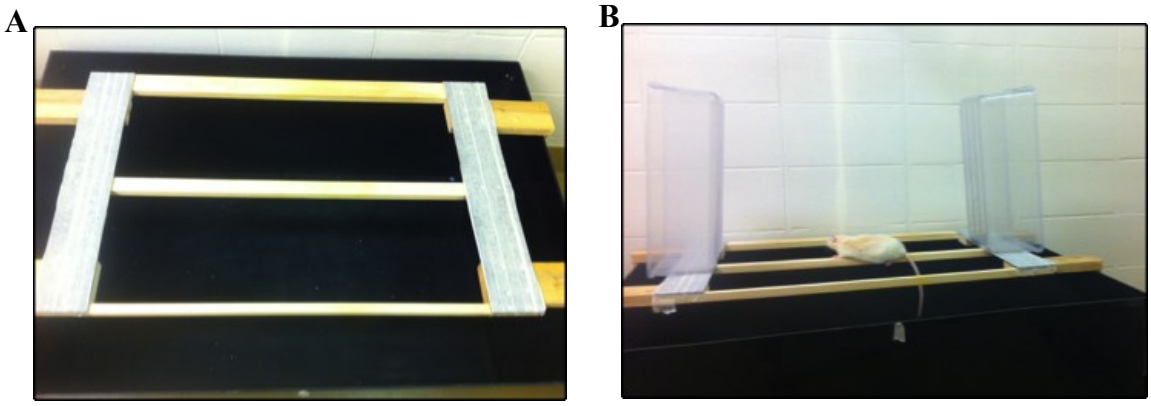

Figure 2. The plank test was used to evaluate balance and coordination. (A) Rats were randomly exposed to planks of three different widths: narrow, medium, and wide. (B) The latency to fall from each plank width was recorded to a maximum latency of 5 minutes.

2.4.3 Prehensile strength test

The prehensile strength test was used to evaluate muscular grip strength (Markowska et al., 1998). In this test, the rat's forepaws were placed on a narrow wooden beam elevated 34 cm above a 5 cm foam pad. The animal was then released and time to

fall was recorded (figure 3). All rats were given 1 trial on the grip strength test with no pretrial unless they fell immediately following release. In this situation, the animal was given a second exposure to the test.

To score the prehensile strength test, latency to fall measurements obtained at 13 months were divided into tertiles. Latency to fall scores in the upper tertile (>9 sec) reflected good prehensile strength (0), the middle tertile (6 – 8 sec) reflected a mild loss of prehensile strength (0.5) and the lower tertile (<5 sec) reflected a severe decline in prehensile strength (1).

Figure 3. The prehensile strength test was used to evaluate muscular grip strength. Rats' forepaws were placed on a narrow wooden beam and latency to fall was recorded to a maximum of 30 seconds.

2.4.4 Inclined plane test

The inclined plane test was used to measure muscle tone and stamina (Joseph et al., 1983; Markowska et al., 1998). In this test, the rat is placed facing upward in the middle of a 60° tilted, 0.5 cm mesh screen (37 cm x 65 cm) elevated above a 5 cm foam pad (figure 4). The time to fall from the inclined plane was recorded with a maximum latency of 5 minutes. Each rat was given one trial on the inclined plane test with no pretrial.

To score the inclined plane test, the latency to fall measurements obtained at 13 months were divided into quartiles. Latency to fall times above the 50th percentile (>228 sec) reflected good muscular stamina (0), times in the 25th to 49th percentile (142 – 227

sec) reflected a mild loss of muscular stamina (0.5) and times below the 25th percentile (<141 sec) reflected a severe decline in muscular stamina (1).

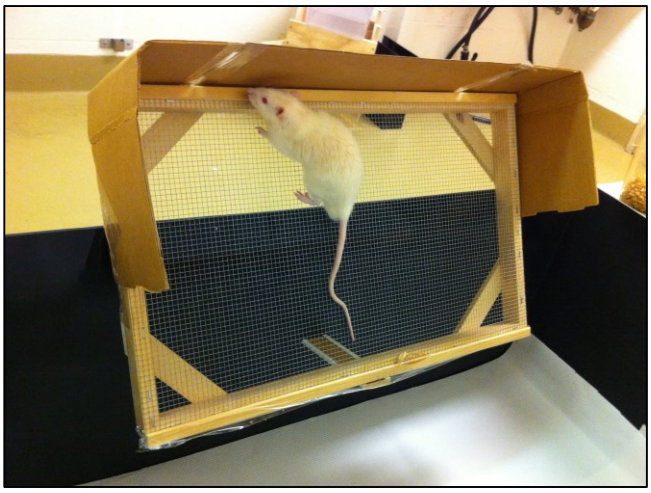

Figure 4. The inclined plane test was used to evaluate muscular strength and stamina. Each rat was placed in the middle of a 60° tilted, 0.5 cm mesh screen. Latency to fall was recorded to a maximum of 5 minutes.

2.5 Exploratory activity

Exploratory activity was assessed every two months using an open-field test (Markowska et al., 1998). Each rat was placed in a black open-field (119 cm x 83cm) and recorded for 15 minutes using video-tracking software (EthoVision 3, Noldus Information Technology). By tracking the contrast of the white rat against the black open field, the Ethovision software was able to provide estimates of total distance moved, maximal distance moved, total duration of movement, percent duration of movement, mean velocity of movement and rearing frequency (figure 5). Each rat was given one trial in the open field test each month with no pretrial.

Performance was examined in a longitudinal analysis of animals aged from youth (3 months, n=6) into adult hood (9 months, n=12), and from middle age (13 months, n=36) into old age (21 months, n=18). Performance with first exposure to the test was examined in a cross-sectional analysis at 3- (n=6), 6- (n=6), 13- (n=36), and 21-months (n=5). To score exploratory activity, movement characteristics for each rat were compared to a mean reference value. Mean reference values were generated by

calculating the arithmetic mean for each parameter measured at 13-months. Values that were <1 standard deviation (SD) away from the mean were given a score of 0, values that were 1 – 2 SD away from the mean reflected a mild deficit (0.5) and values that were >2 SD away from the mean reflected a severe deficit.

Figure 5. Exploratory activity was assessed using the open field test. Each rat was placed in an open field and recorded for 15 minutes. Video-tracking software was used to measure various movement characteristics.

2.6 Basic metabolic status and hematology

Basic metabolic status and hematology were evaluated every 3 months in the young rats (3, 6 and 9 months) and every 4 months in the older rats (13, 17 and 21 months) using an i-STAT portable clinical analyzer and Chem8+ cartridges (Abbott Diagnostics, Canada). After the animals were anaesthetized with isoflurane (5% induction dose, 1.5 – 2% maintenance dose), blood samples (0.2 ml) were obtained from the saphenous vein. Levels of sodium, potassium, chloride, blood urea nitrogen, creatinine, carbon dioxide, hematocrit and hemoglobin were subsequently analyzed from the blood sample with the i-STAT.

To score basic metabolic status and hematological parameters, values for each rat were compared to a mean reference value. Mean reference values were generated by calculating the arithmetic mean for each parameter measured at 13-months. Values that were <1 standard deviation (SD) away from the mean were given a score of 0, values that

were 1 – 2 SD away from the mean reflected a mild deficit (0.5) and values that were >2 SD away from the mean reflected a severe deficit.

2.7 Deficit selection criteria

Following conclusion of the longitudinal study, potential deficits were included in the FI if they satisfied the following criteria (adapted from Searle *et al.* 2008):

1. The deficit must be associated with a change in health status.
2. The deficit's prevalence must not saturate at too early an age.
3. As a group, the candidate deficits must cover a wide range of health systems.
4. If serially tracking changes in frailty, the deficits comprising the FI must be consistent from one measurement to the next.

We did not include the criterion that a deficit's prevalence must generally increase with age because frailty is age-independent. Furthermore, previous murine frailty indices have excluded this criterion (Parks et al., 2012; Whitehead et al., 2014). We additionally stipulated that if a deficit were to be included in the FI, the test measuring the deficit had to have good construct validity. To satisfy this criteria, the test (1) had to have been proven valid in previous studies, and (2) could not display a significant learning effect, whereby performance improved with each exposure to the test.

3. Quantification of *in vivo* cardiac structure and function with echocardiography

Rats participating in the longitudinal study underwent serial transthoracic echocardiography to assess *in vivo* heart function. In the older group of animals, echocardiographic studies were conducted at 13, 17 and 21 months. In the younger group of animals, studies were conducted at 3, 6 and 9 months. All studies were conducted using a Vivid 7 cardiac ultrasound system (GE Medical Systems, Norway) equipped with a 10- to 14- MHz linear transducer (il3L, GE Medical Systems, Norway) and a 4.0- to 10.5- MHz apical transducer (10s, GE Medical Systems, Norway). Measurements of chamber dimensions and cardiac function were made offline by a single experienced echocardiographer blinded to the FI score of each rat.

Rats were anesthetized with inhaled isoflurane in oxygen (5% induction, 1.5-2% maintenance dose) and placed supine on a warming pad (37°C). The isoflurane dose was titrated such that the minimum concentration of isoflurane required to immobilize the rat was used. Following anesthesia, the ventral thorax was shaved from the left sternal border to the left axillary line. Depilatory cream was applied to remove hair from the chest area of the rat. Gold-plated electrocardiography electrodes were inserted subcutaneously into the right forelimb, left forelimb and left hind leg to obtain electrocardiographic measurements. Ultrasonic transmission gel was then applied to this area.

Two-dimensional guided M-mode measurements were used to characterize LV structure and systolic function with a 10- to 14- MHz linear transducer (il3L, GE Medical Systems, Norway) in the parasternal short-axis. LV structural parameters were assessed at the tips of the papillary muscles in M-mode using the leading edge-to-leading edge convention. Structural measurements included LV interventricular septal thickness at systole (IVSs) and diastole (IVSd), LV internal diameter at systole (LVIDs) and diastole (LVIDd), and LV posterior wall thickness at systole (LVPWs) and diastole (LVPWd).

Indices of systolic function assessed included ejection fraction (EF) and fractional shortening (FS). EF, which provides a measure of the fraction of blood pumped from the heart with each beat, was calculated using the following equation: $EF\ (\%) = [(LVIDd^3 - LVIDs)^3)/LVIDd^3] \times 100$. FS, which measures the percent change in ventricular diameter between systole and diastole, was calculated as: $FS\ (\%) = [(LVIDd - LVIDs)/LVIDd] \times 100\%$.

In the apical four-chamber view, pulsed wave Doppler imaging was used to characterize LV diastolic function with a 4.0- to 10.5 MHz apical phased array transducer (10S, GE Medical Systems, Norway). The sample volume was placed at the tips of the mitral leaflets, and transmitral flow velocities were recorded to assess diastolic function. From the mitral valve inflow velocity curve, the following measurements were made: peak early diastolic transmitral flow velocity (E), peak late diastolic transmitral flow velocity (A) and early filling deceleration time (DT). The E/A ratio was calculated from the E and A velocities. Mitral DT was measured as the time from the peak to the end of the Doppler E wave.

HR was calculated from the R-R interval on ECG traces. Following echocardiography, rats were returned to their home cage and monitored to ensure appropriate recovery of motor function from anesthesia. Rats that underwent echocardiography were given minimum 5 days to recover before undergoing other testing for the FI.

4. The isolated working rat heart

4.1 Setup

For the isolated working heart studies, we utilized a modified Langendorff apparatus. In contrast to a standard Langendorff heart preparation, where the heart is perfused retrogradely through the cannulated aorta, the working heart apparatus perfuses the left atrium through a secondary cannula inserted into the pulmonary vein. Perfusate flows into the left ventricle and is ejected out of the heart through the aorta (figure 6). Thus, the isolated working heart model allows perfusate to follow normal circulatory dynamics through the left ventricle.

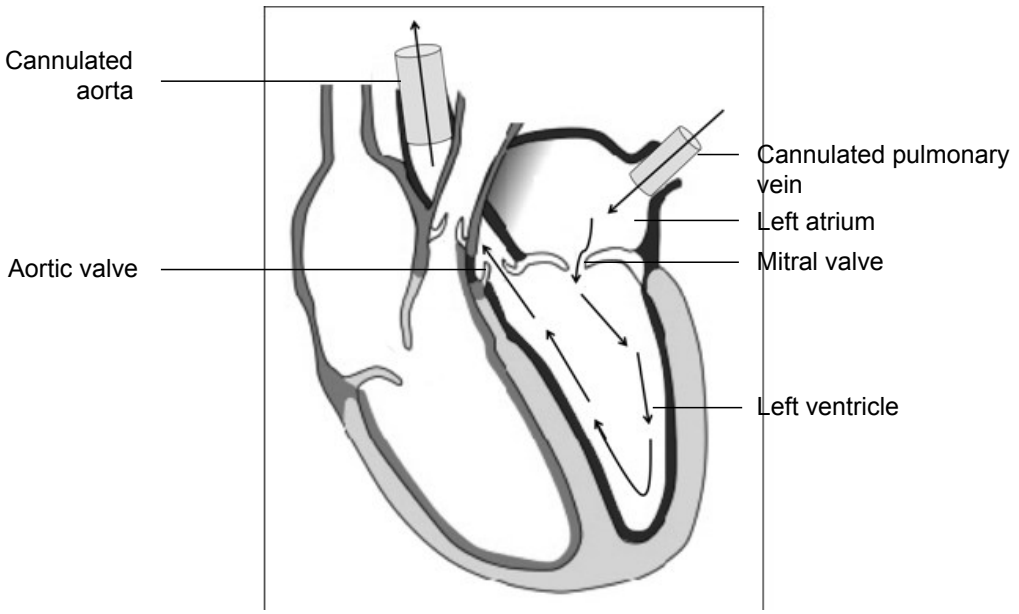

Figure 6. Direction of flow in working heart mode. Perfusate enters the left atrium via the pulmonary vein, circulates through the left ventricle, and is ejected out through the aorta.

In our lab's working heart setup, perfusate is drawn from a water-jacketed reservoir by a peristaltic pump and is routed to the atrial bubble trap. It is then directed into the left atrium through the cannulated pulmonary vein at a fixed preload pressure (atrial pressure). Preload is determined by the height of the overflow from the atrial perfusion bubble trap relative to the heart. In our lab setup, the height of the atrial bubble trap is fixed at 20 cm. The perfusate then passes into the left ventricle and is pumped out of the heart through the cannulated aorta against a fixed afterload pressure. Afterload pressure (aortic resistance) is determined by the height of the afterload compliance reservoir relative to the aortic cannula. The reservoir is pre-filled with perfusate, such that the heart is ejecting against a fixed hydrostatic pressure head. In our lab setup, the height of the afterload reservoir is 100 cm. Within the compliance loop, there is a pre-filled aortic bubble trap compliance chamber with a 2 mm diameter bubble of air to mimic vascular elasticity (figure 7) (Radnoti Working Heart Manual, 2013).

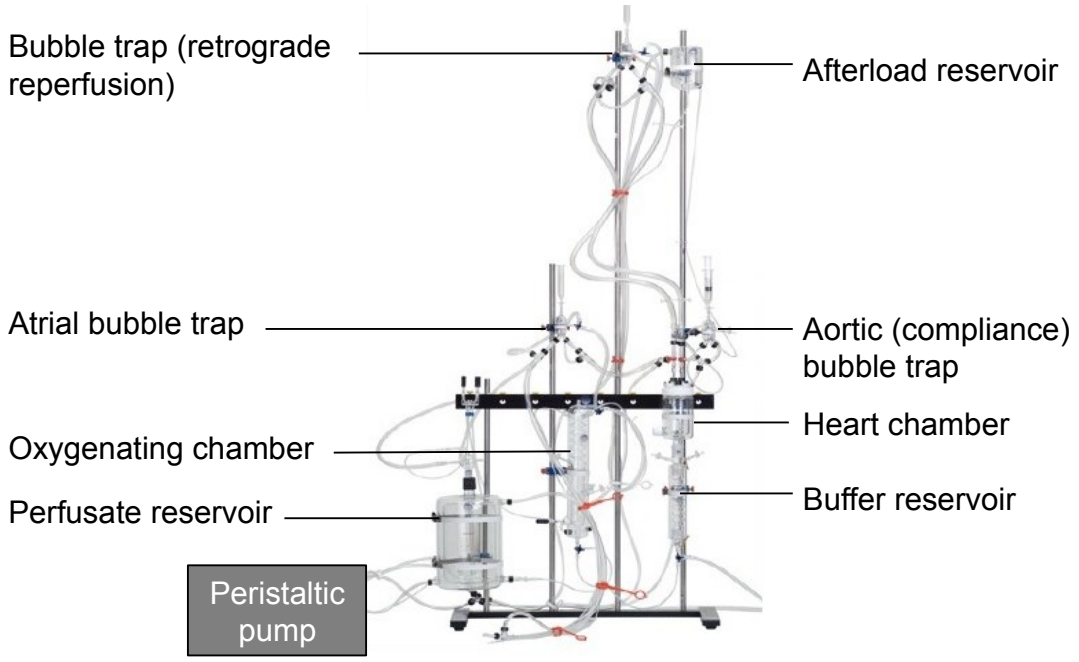

Figure 7. Diagram of the isolated working heart set-up.

During the course of ejection, a small amount of perfusate enters the coronary arteries via the ostia at the base of the aortic root. After passing through the coronary circulation, the perfusate drains into the right atrium via the coronary sinus. It is then filtered (5-µm, Pall Life Sciences, Cornwall, UK) and recirculates back into the water-

jacketed reservoir. Temperature of the system is maintained by a thermal circulating pump that warms water contained in the water-jacketed reservoir to 37°C. This warms perfusate in the reservoir, which in turn will warm the system (and the heart) as it circulates through the apparatus (Radnoti Working Heart Manual, 2013).

4.2 Isolated heart preparation

Hearts were isolated from adult (9 months, n=12) and aged (21 months, n=23) rats. Animals were considered "old" at 21 months as this is the age where 50% mortality occurred (Yu et al., 1982; Lakatta and Sollot, 2002). Rats were anesthetized for the isolated working heart studies with sodium pentobarbital (160 mg/kg; CDMV; Saint-Hyacinthe, QC) via intraperitoneal injection. A second intraperitoneal injection of heparin (3000 U; Pharmaceutical Partners of Canada, Richmond, ON) was subsequently administered to prevent coagulation. Adequate surgical anesthesia was ensured through the toe pinch withdrawal reflex test. The absence of foot withdrawal following toe pinch indicated that the anesthesia was deep enough to commence with surgery.

To expose the heart, a thoracotomy was performed to open the chest. Specifically, a skin and muscle incision was made from the lower abdomen to the throat. The diaphragm was dissected from the ribs, and the thorax opened by cutting the ribs. To expose the heart, the anterior thoracic wall was turned upwards over the head. Following removal of the pericardium, the ascending aorta, vena cava and pulmonary vessels were separated from the surrounding connective tissue. Care was taken to ensure that the aorta was long enough to allow for cannulation.

The excised heart-lung mass was then placed immediately into a petri-dish containing pre-chilled Krebs-Henseleit buffer (KHB; NaCl 118.0 mM, $NaHCO_3$ 25.0 mM, KCl 4.7 mM, $MgSO_4$ 1.2 mM, KH_2PO_4 1.2 mM, glucose 11.0 mM, $CaCl_2$ 2.5 mM, EDTA 0.5 mM, equilibrated with 95% O_2/5% CO_2, pH = 7.4; all chemicals obtained from Sigma-Aldrich Co. LLC.). The heart was isolated from the lungs and the aorta cannulated onto the modified Langendorff apparatus. After proper insertion of the cannula, sutures were placed to secure the cannula to the aorta. Care was taken to ensure that the cannula was positioned superior to the coronary ostia such that perfusion of the coronary arteries

was not disrupted. A secondary cannula was then inserted into the pulmonary vein and secured with multiple sutures. Following successful cannulation of the aorta and pulmonary vein, the heart was suspended in a warmed-air heating chamber (37°C).

4.3 Perfusion protocol

Following successful cannulation of the aorta and pulmonary vein, the heart was stabilized in standard Langendorff mode using constant flow and constant pressure. Hearts were first retrogradely perfused with KHB (37°C; gassed with 95% O_2/5% CO_2) in constant flow mode for 5 min. In this mode, perfusate is delivered at a constant rate of flow (10 mL/min) to the coronary ostia at the base of the aortic root using the peristaltic roller pump (Radnoti Working Heart Manual, 2013).

The heart was then switched into constant pressure mode for 5 min, where it was retrogradely perfused at a constant hydrostatic pressure. A constant hydrostatic pressure is obtained by positioning a perfusate reservoir and its fluid meniscus a known distance above the aortic cannula. KHB (37°C; gassed with 95% O_2/5% CO_2) is pumped to the perfusate reservoir using a peristaltic roller pump. It is then routed to the coronary arteries via the ostia at the base of the aortic root at a constant pressure (100 cm H_2O) (Radnoti Working Heart Manual, 2013).

The heart was then switched into working heart mode for approximately 10 minutes to collect baseline hemodynamic measurements. In this mode, perfusate follows normal circulatory dynamics through the left heart. KHB enters the left atria at an experimentally controlled preload (20 cm H_2O; 37°C; gassed with 95% O_2/5% CO_2), passes into the left ventricle, and is ejected through the aorta against an experimentally controlled afterload (100 cm H_2O; 37°C; gassed with 95% O_2/5% CO_2).

Hearts were then briefly switched back into constant pressure and constant flow modes, and arrested with del Nido cardioplegia (subsequently described) for 60 minutes. Following the ischemic period, hearts were gradually reperfused with KHB (37°C; gassed with 95% O_2/5% CO_2) in constant flow mode for a total of 10 minutes. Reperfusion started at 4 mL/min and was increased in 30-second increments by 0.5 mL/min to a maximum flow rate of 10 mL/min (5 minutes). The heart was perfused in constant flow

mode for an additional 5 minutes at 10 mL/min and then switched into constant pressure mode for 5 minutes. Hearts were then switched into working heart mode for 60 minutes for the collection of post-reperfusion hemodynamic measurements.

At the conclusion of the protocol, the atria were dissected from the ventricles. Ventricles were blotted dry, weighed (wet weight) and desiccated at 80°C for 24 hours. The hearts were then reweighed (dry weight).

4.4 Preparation and delivery of cardioplegia

Hearts were arrested with del Nido cardioplegia (Matte & del Nido, 2012). To prepare the cardioplegia, we supplemented 8 mL cardioplegia base solution from the Queen Elizabeth II hospital (Halifax, Canada) with sodium bicarbonate, mannitol and lidocaine. Autologous blood collected immediately following excision of the heart-lung mass was diluted with diluent to simulate hemodilution during cardiopulmonary bypass. Cardioplegia base solution (8 mL) was mixed with diluted blood (2 mL) to simulate the ionic composition of del Nido cardioplegia used in clinical practice at Dalhousie University (table 2). Cardioplegia was then gently bubbled with oxygen and cooled in an ice bath.

Prior to delivery, the temperature of the cardioplegia was measured. Mean temperature was 0.8±0.1°C. Cardioplegia was filtered using a 20-μm filter (EMD Millipore, Maryland, USA) and delivered by hand injection as a single induction dose (15 mL/kg body weight). Care was taken to ensure that aortic pressure was <50 mmHg during delivery. No additional doses were administered during the 60-minute ischemic period.

Table 2. Composition of del Nido cardioplegia.

Base Solution (1L) (mmol/L)	
Na+	140
K+	5
Mg2+	1.5
Cl-	98
Acetate	27
Gluconate	23
Glucose	--
Additives (ml/L)	
KCl (2 mEq/mL)	13
$NaHCO_3$ (1 mEq/mL)	13
$MgSO_4$ (0.2g/mL)	10
Lidocaine (1%)	13
Mannitol (25%)	13
Dilution (blood:cardioplegia)	1:4
Estimated Final Composition (mmol/L)	
Na^+	143
K^+	24
Mg^{2+}	7
Ca^{2+}	0.24
Glucose	1
Lidocaine	0.36
Estimated Final Hematocrit (%)	6

4.5 Measurements

4.5.1 Calculated hemodynamic variables

Several hemodynamic measurements were made during the working heart period. Following a 2-minute period of stabilization, each variable was measured once during the baseline working heart period (t = 4 min) and at several time points post-reperfusion (t = 0, 5, 10, 15, 30, 45 and 60 min). The following hemodynamic variables were then calculated for each time point:

Cardiac output (CO), the volume of blood pumped by the heart each minute, was calculated by summing coronary flow (CF) and aortic flow (AF): CO = CF + AF. Stroke

volume (SV), the amount of blood ejected by the left ventricle during a single contraction, was calculated with the following equation: SV = CO/HR. Stroke work (SW) refers to the work required by the left ventricle to eject a given volume (i.e., SV) into the aorta. It was calculated using SV and maximum systolic pressure (SP): SW = SV*SP. Rate pressure product (RPP), a measure of the total workload of the heart and an indicator of hemodynamic stress, was calculated with the formula: RPP = HR*SP. Left ventricular developed pressure (LVDP), which refers to the pressure developed in the left ventricle during the course of one cardiac cycle, was determined as follows: LVDP = SP – LVEDP (left ventricular end diastolic pressure).

During constant pressure mode, coronary vascular resistance (CVR) was calculated at baseline and during reperfusion. CVR provides a measure of the resistance to blood flow through the coronary arteries and was determined using the following equation: CVR = MAP/CF, where MAP = mean aortic pressure.

4.5.2 Heart Rhythm

During the baseline working heart period, hearts were analyzed for the incidence of arrhythmia. To evaluate heart rhythm, we looked for premature ventricular complexes (PVCs), ventricular tachycardia (VT) and ventricular fibrillation (VF) on the ECG. The aortic pressure trace was also analyzed for irregularities. Hearts were classified as arrhythmic if they had three or more bouts of arrhythmia lasting >10 cardiac cycles, or if they had one sustained period of arrhythmic activity lasting >10 seconds.

Following arrest with cardioplegia, we evaluated the return of normal sinus rhythm. The length of time it took for the first heartbeat to occur following the start of reperfusion in constant flow mode was recorded. ECG tracings were also examined throughout the reperfusion period for the presence of arrhythmia. The same criteria used to assess arrhythmia in baseline working heart mode was applied during reperfusion.

4.5.3 Cardiac troponin-I release into the coronary effluent

The release of cardiac troponin-I (cTnI) into the coronary effluent following cardioplegic arrest was used to evaluate myocardial damage. During the reperfusion

period, retrograde perfusion was increased from a constant flow rate of 4 mL/min to 10 mL/min in increments of 0.5 mL/min every 30 seconds. When flow reached 10 mL/min, coronary effluent was collected for 1 minute. The effluent was then aliquoted into 2 mL tubes and frozen at -80°C.

After all the isolated working heart experiments were completed, the frozen coronary effluent samples were thawed and the concentration of cTnI in each sample was analyzed using a high-sensitivity ELISA (rat serum cTnI ELISA kit, CTNI-2-HS, Life Diagnostics, Inc.). All samples were analyzed at the same time according to the procedures outlined in the ELISA kit.

4.5.4 Myocardial edema and cardiac hypertrophy

Myocardial edema, a measure of fluid accumulation in the interstitial compartment of the heart, was determined by calculating the indexed myocardial water content of the heart. The following equation was used: indexed mass of myocardial water = (wet weight – dry weight)/tibia length. Cardiac hypertrophy was evaluated by evaluating changes in the dry heart weight to tibia length ratio.

4.5.6 Diastolic function

We developed a novel method to quantify diastolic function using the isolated working heart in conjunction with 2-D echocardiography (figure 8). In diastolic dysfunction, there is a left and upward shift in the left ventricular end diastolic pressure-volume relationship (LVEDPVR) as the left ventricle becomes less compliant. Thus, for a given pressure there should be a reduction in left ventricular EDV. In the isolated working heart, hearts are perfused at a constant filling pressure. Changes in EDV should therefore reflect changes in diastolic function. For example, a reduction in EDV would indicate impaired relaxation.

To quantify EDV, we used echocardiography to determine both ESV and EDV using the area-length method (bullet formula): LVV = ⅝ Area x Length. In this formula, area was calculated in the parasternal short-axis at the level of the papillary muscles while length measurements were collected in the parasternal long-axis. Measurements taken

during systole were used to calculate ESV; measurements collected during diastole were used to quantify EDV. Stroke volume was then calculated using the formula: SV = EDV – ESV.

Echocardiography-derived volumes are determined indirectly using a geometric formula. The isolated working heart provides a more direct measure of SV, using measured parameters. We therefore developed a correction factor for volume measurements obtained with echocardiography using SV obtained with the isolated working heart apparatus. Briefly, we compared the SV measured by the isolated working heart with the SV obtained with echocardiography to develop a correction factor for echo volumes. This correction factor was applied to EDV measurements to determine changes in diastolic function. EDV was measured once during the baseline working heart period (t = 8 min) and once during reperfusion (t = 15 min).

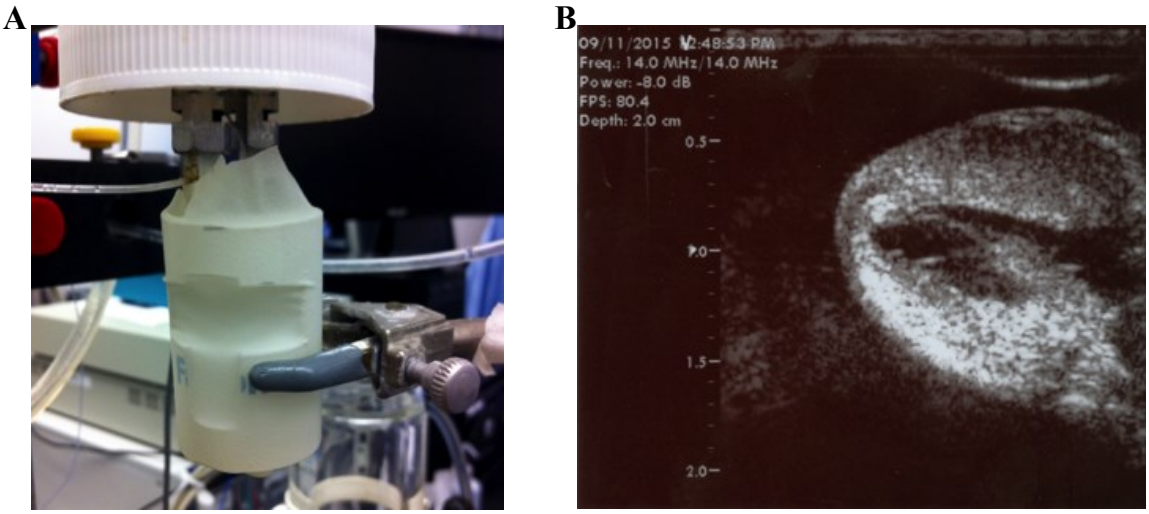

Figure 8. Quantifying diastolic function using the isolated working heart in conjunction with echocardiography. (A) The apparatus used to echo the isolated working heart. (B) Representative image take in the parasternal long-axis during an isolated working heart experiment.

4.6 Inclusion criteria

Hearts that satisfied the following criteria during baseline working heart measurements were included in our analysis: (1) coronary flow >10mL/min; (2) cardiac

output >25 ml/min; and (3) consistent HR (<10% change from beginning to end of the baseline working heart period).

5. Statistical Analysis

All data are presented as mean±SEM. Survival data was illustrated using a Kaplan-Meier survival curve and analyzed using the log-rank test. Mean FI scores, body weight, food intake, sensorimotor function, ethovision data, and age-related differences in echocardiography were evaluated using one-way repeated measures ANOVA with Tukey's post-hoc testing for multiple comparisons. Frailty-related differences in echocardiography were compared with unpaired t-tests. Age- and frailty-related differences in hemodynamic parameters, CVR and EDV obtained at baseline were compared with unpaired t-tests. These variables were compared using two-way repeated measures ANOVA with Tukey's post-hoc testing following reperfusion. Baseline and reperfusion arrhythmia incidence were assessed with the Fisher's exact test by both age and FI score. cTnI and myocardial edema were compared by age and frailty using unpaired t-tests.

CHAPTER 3: RESULTS

1. Potential deficits

1.1 Nutrition

Figure 9 depicts changes in body weight and daily food intake with age. Body weight increased from youth into adulthood months (3 months, 310.9±10.7; 6 months 375.6±8.4; 9 months, 404.3±8.7 g; $p<0.05$), and remained relatively consistent until old age (13 months, 453.1±5.1; 17 months, 451.4±3.7; 21 months 426.8±5.6 g; $p<0.05$). There was no significant effect of age on food intake in the young cohort of rats (3 months, 16.5±0.7; 6 months 17.6±0.4; 9 months, 17.7±0.5 g; $p=0.40$); however it steadily declined throughout the lifespan in the older group of animals (13 months, 19.3±0.2; 17 months, 17.8±0.4; 21 months 14.2±1.1 g; $p<0.05$).

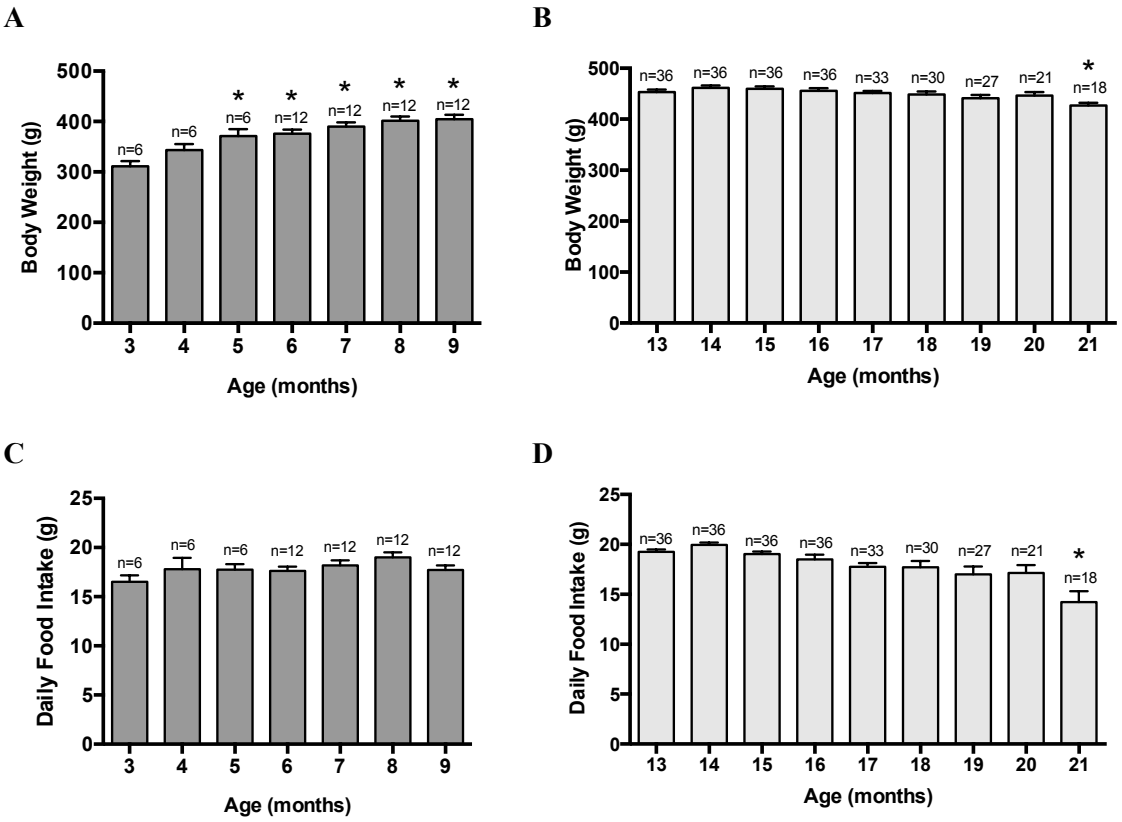

Figure 9. Changes in nutritional status as a function of age. (A) Body weight increased from youth into adulthood ($p<0.05$). (B) Body weight was fairly consistent throughout middle age, but significantly declined with old age ($p<0.05$). (C) Food intake was consistent in the young cohort of rats ($p=0.40$). (D) Food intake steadily declined between 13 and 21 months ($p<0.05$). *Denotes $p<0.05$ relative to baseline.

1.2 Clinical signs of deterioration

Twenty-three potential clinical signs of deterioration were evaluated once a week. We incorporated 18 of these clinical deficits into our final FI (table 3). In addition to meeting the criteria outlined in section 2.3, clinical signs of deterioration included in the FI were easily observed and fairly invariable. Excluded deficits were difficult to observe or transient in nature. For example, fur colour, the presence of tremor and how vocal the rat was varied from week to week. Abdominal distention was difficult to measure given the rats were averse to abdominal exposure and palpation. Rectal prolapse was not observed in this group of rats.

Table 3. Clinical signs of deterioration included and excluded from the frailty index.

Clinical signs of deterioration evaluated	
Included in frailty index	Excluded from frailty index
Alopecia	Change in fur colour
Skin lesions	Tremor
Coat condition	Distended abdomen
Tumours	Rectal prolapse
Hunched posture	Unusual sounds
Body condition score	
Gait disorder	
Hearing loss	
Cataracts	
Chromodacryorrhea	
Corneal opacity	
Microphthalmos	
Exophthalmos	
Head Tilt	
Breathing rate/depth	
Malocclusion	
Jaundice	
Diarrhea	

1.3 Sensorimotor Function

Once a month, rats underwent a battery of tests to evaluate sensorimotor function. The young cohort of rats underwent serial testing from youth into adulthood (3 to 9 months) while the old cohort was tested from middle age into old age (13 months to 21 months).

1.3.1 Blind alley test

Contextual processing was evaluated using the blind alley test. In the young cohort of rats, time to turn around tended to increase with age, however this was not statistically significant (3 months, 6.2±1.6; 6 months 50.9±22.3; 9 months, 115.8±39.6 sec; $p=0.23$). In the older cohort of rats, time to turn around increased until 16 months and then plateaued for the remainder of the experiment (13 months, 31.0±11.1; 17 months, 121.7±22.3; 21 months 123.7±34.1 sec; $p<0.05$). A cross-sectional analysis of each rat's first exposure to the test revealed there was no significant effect of age on time to turn around (3 months, 6.2±1.6; 6 months, 16.2±2.9; 13 months; 31.0±11.1; 21 months 10.8±2.3 sec; $p=0.68$) (figure 10).

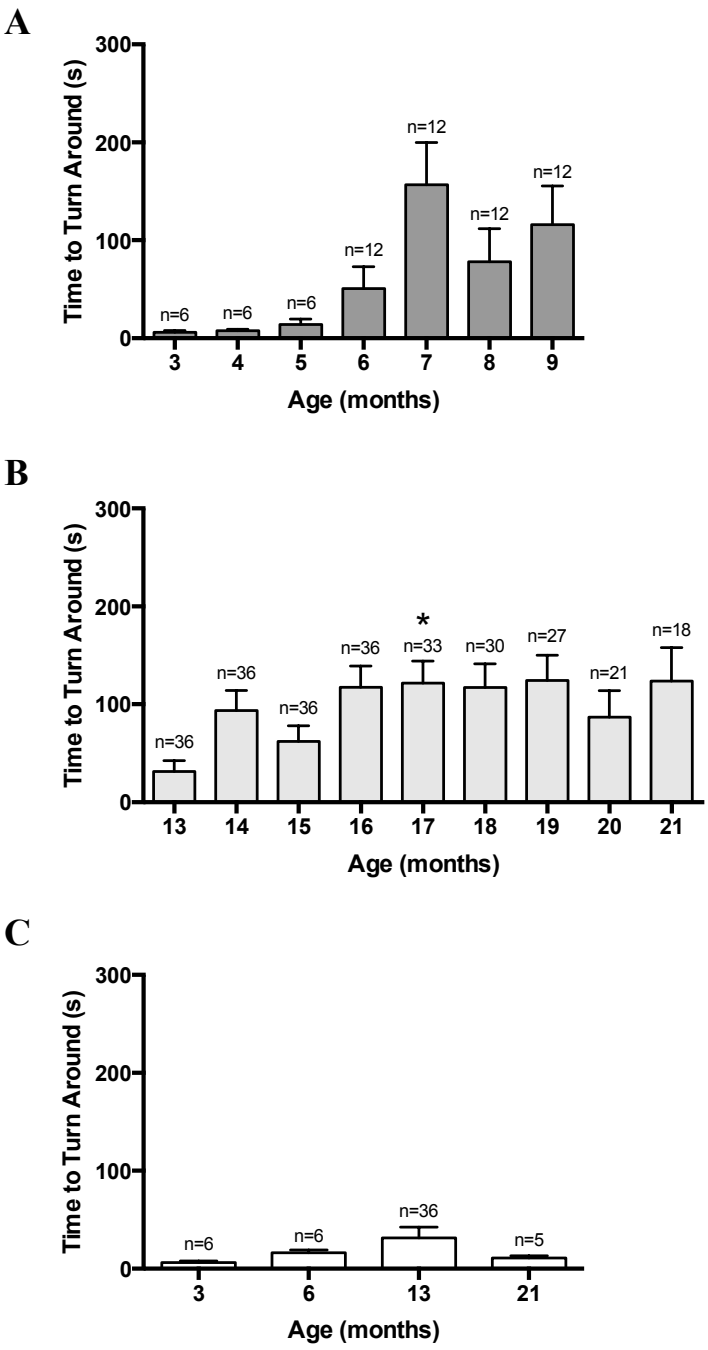

Figure 10. Blind alley test. An increase in time to turn around reflected a decline in contextual processing. (A) In the young cohort of rats, time to turn around tended to increase with age, however this was not statistically significant ($p=0.23$). (B) In the older cohort of rats, time to turn around increased until 16 months, then plateaued for the remainder of the lifespan ($p<0.05$). (C) First exposure to the test was compared in a cross-sectional analysis. Performance was relatively consistent from youth into old age ($p=0.68$). *Denotes $p<0.05$ relative to baseline.

1.3.2 Prehensile strength test

Grip strength was evaluated using the prehensile strength test. In the young cohort of rats, latency to fall had a parabolic relationship with age (3 months, 23.2±4.6; 6 months 11.5±2.2; 9 months, 21.2±3.8 sec; $p=0.13$). In the older cohort of rats, latency to fall decreased until 17 months, and plateaued for the remainder of the lifespan (13 months, 11.1±1.2; 17 months, 6.2±0.7; 21 months 6.8±2.6 sec; $p<0.05$). In a cross-sectional analysis of first exposure to the test, latency to fall declined after 3 months and was relatively consistent between 6 and 21 months of age (3 months, 23.2±4.6; 6 months, 9.0±2.6; 13 months; 11.0±1.3; 21 months 9.8±2.0 sec; $p<0.05$) (figure 11).

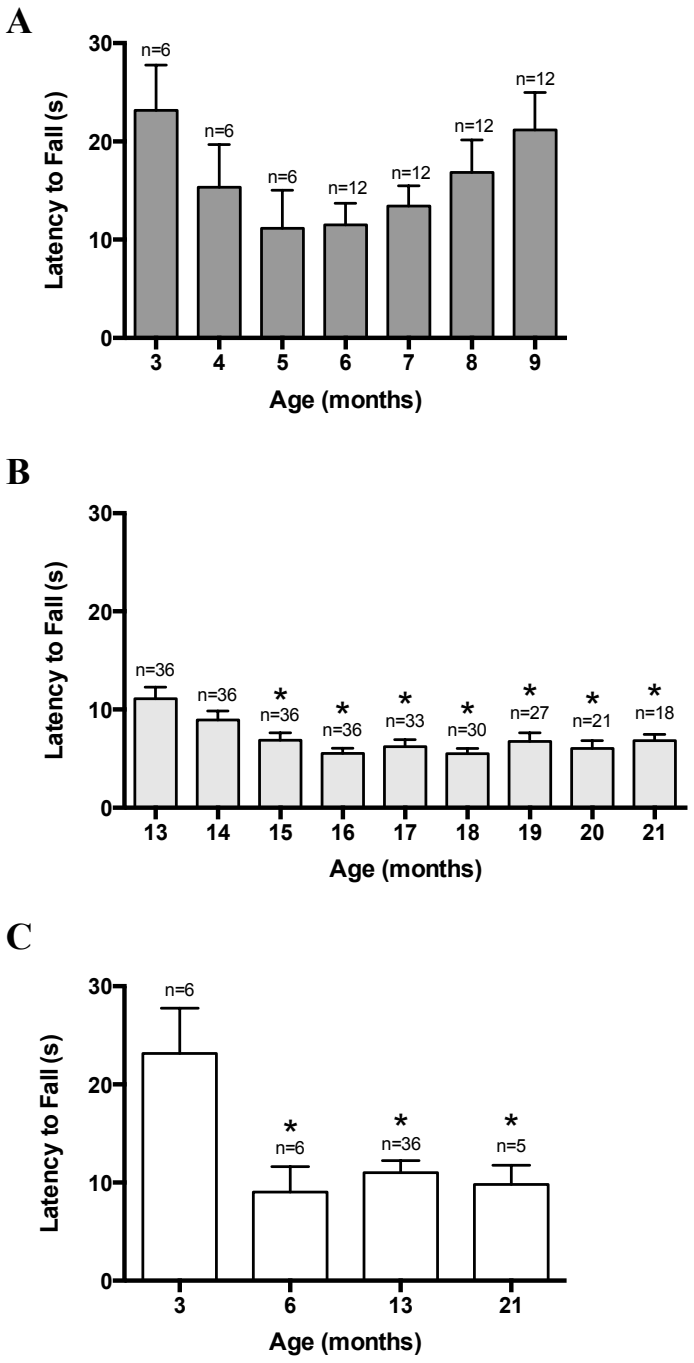

Figure 11. Prehensile strength test. A decrease in latency to fall reflects a decline in grip strength. (A) In the young cohort of rats, there was no relationship between age and grip strength ($p=0.13$). (B) In the older cohort of rats, grip strength declined with age ($p<0.05$). (C) First exposure to the test was compared in a cross-sectional analysis. Grip strength declined with age ($p<0.05$). *Denotes $p<0.05$ relative to baseline.

1.3.3 Inclined plane test

Figure 12 depicts the results of the inclined plane test, which was used to evaluate muscular stamina. There was no significant effect of age in the young cohort of rats (3 months, 300.0±0.0; 6 months 278.9±20.6; 9 months, 286.2±10.3 sec; $p=0.70$). There was a significant effect of age on latency to fall in the older cohort of rats, declining between 13 and 21 months (13 months, 218.9±15.0; 17 months, 258.9±10.9; 21 months 139.6±22.1 sec; $p<0.05$). The sharp increase in latency to fall at 17 months can be attributed to a change in the test: previously, the inclined plane was oriented such that the base of the plane touched the floor. Animals were learning to climb off the plane by 16 months; to circumvent this we elevated the base of the plane off of the floor. Rats that had learned to climb off the plane at 16 months were unable to do so at 17 months, thus increasing the latency to fall. A cross-sectional analysis of first exposure to the test revealed a decline in latency to fall with age, however this did not attain significance (3 months, 300.0±0.0; 6 months, 298.2±1.8; 13 months; 218.9±15.0; 21 months 242.2±30.5 sec; $p=0.08$) (figure 12).

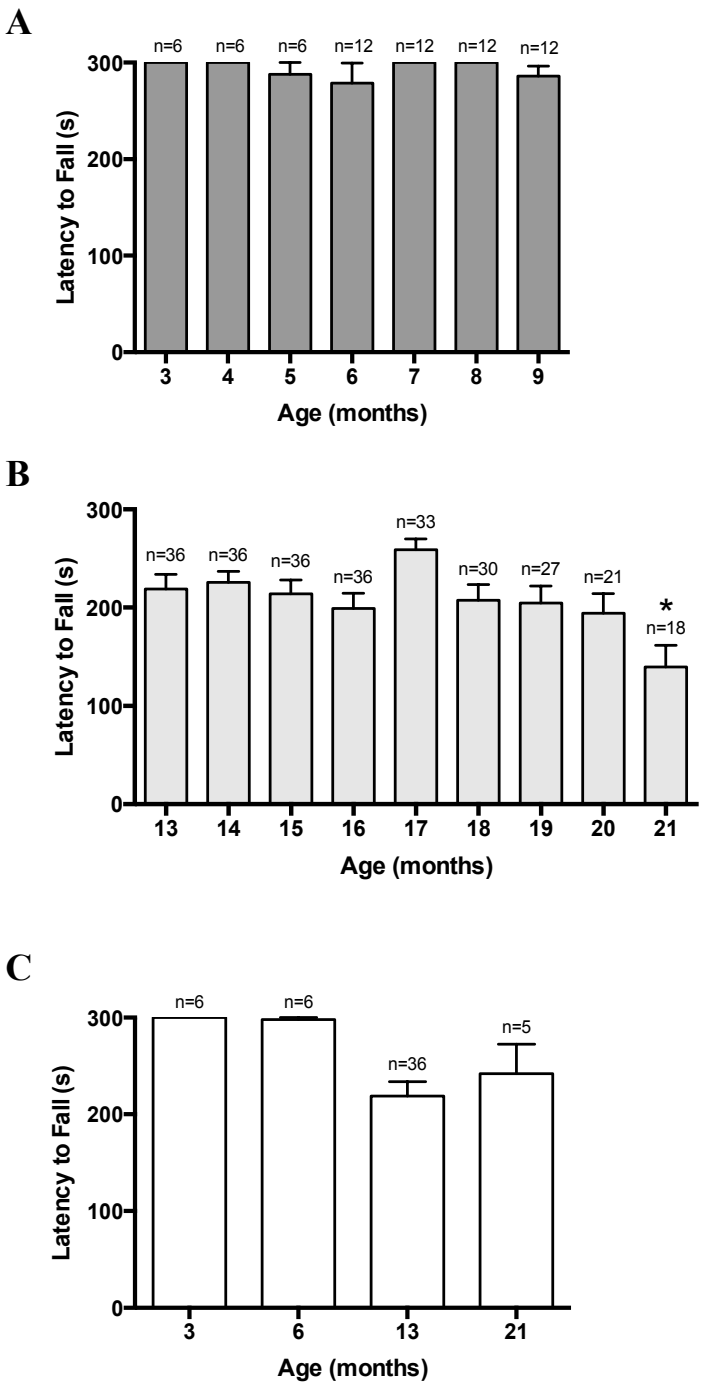

Figure 12. Inclined plane test. A decrease in latency to fall reflects a decline in muscle stamina. (A) In the young cohort of rats, performance was consistent from youth to adulthood ($p=0.70$). (B) In the older cohort of rats, performance declined with age relative to 13 months ($p<0.05$). (C) First exposure to the test was compared in a cross-sectional analysis. There was a trend for performance to decline with age, however this did not attain significance ($p=0.08$). *Denotes $p<0.05$ relative to baseline.

1.3.4 Plank tests

Balance and coordination were evaluated by exposing rats to planks of various widths. On the wide plank width, latency to fall was consistent from youth into adulthood (3 months, 300.0±0.0; 6 months 300.0±0.0; 9 months, 300.0±0.0 sec; p=0.62). There was no significant effect of age in the older cohort of rats (13 months, 295.4±4.6; 17 months 300.0±0.0; 21 months, 300.0±0.0 sec; p=0.59). When first exposure to the test was compared in a cross-sectional analysis, performance was consistent across the lifespan (3 months, 300.0±0.0; 6 months, 300.0±0.0; 13 months; 295.4±4.6; 21 months 300±0.0 sec; p=0.92) (figure 13**)**.

When exposed to the medium plank width, there was no significant effect of age in the young cohort of rats (3 months, 300.0±0.0; 6 months 300.0±0.0; 9 months, 278.3±21.8 sec; p=0.75), or in the old group (13 months, 258.0±14.3; 17 months 283.7±11.4; 21 months, 288.0±12.0 sec; p=0.17). While there was some decline with age when first exposure to the medium plank test was compared in a cross-sectional analysis, this did not attain significance (3 months, 300.0±0.0; 6 months, 300.0±0.0; 13 months; 258.0±14.3; 21 months 249.4±50.6 sec; p=0.43) (figure 14).

There was greater variation in the latency to fall times when rats were exposed to the narrow plank test. Performance was relatively consistent in the young cohort of rats (3 months, 300.0±0.0; 6 months 238.1±32.4; 9 months, 280.0±20.0 sec; p=0.40). In the older cohort of rats, latency to fall increased from 13 to 17 months, and remained relatively consistent throughout the remainder of the lifespan (13 months, 65.7±13.6; 17 months 183.2±22.3; 21 months, 171.8±30.3 sec; $p<0.05$). A cross-sectional analysis of first exposure to the narrow plank width suggested there was a significant effect of age (3 months, 300.0±0.0; 6 months, 257.0±43.0; 13 months; 65.7±13.6; 21 months 49.4±18.3 sec; $p<0.05$) (figure 15).

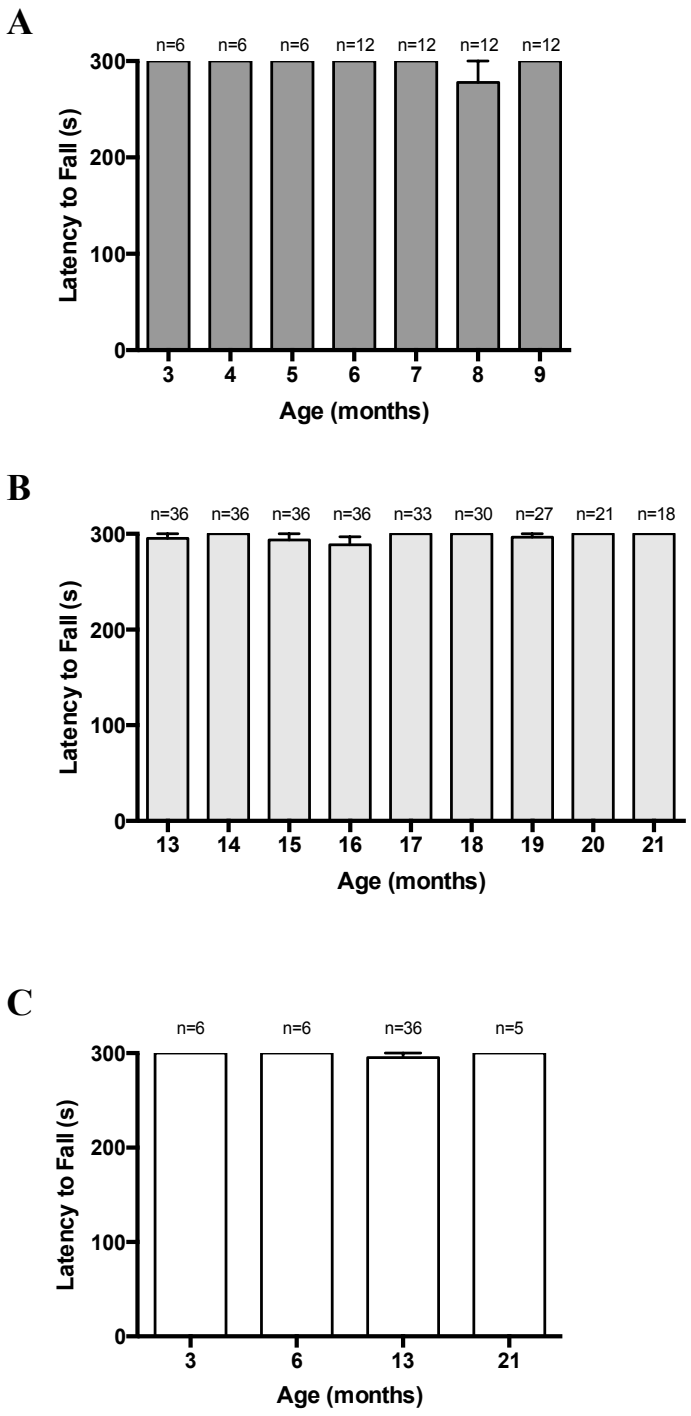

Figure 13. Wide plank test. A reduction in latency to fall reflected a decline in balance and coordination. (A) Performance was consistent in the young cohort of rats from 3 months to 9 months ($p=0.62$). (B) Performance was consistent throughout the lifespan in the older cohort of rats ($p=0.59$). (C) First exposure to the test was compared in a cross-sectional analysis. Latency to fall was consistent from youth into old age ($p=0.92$).

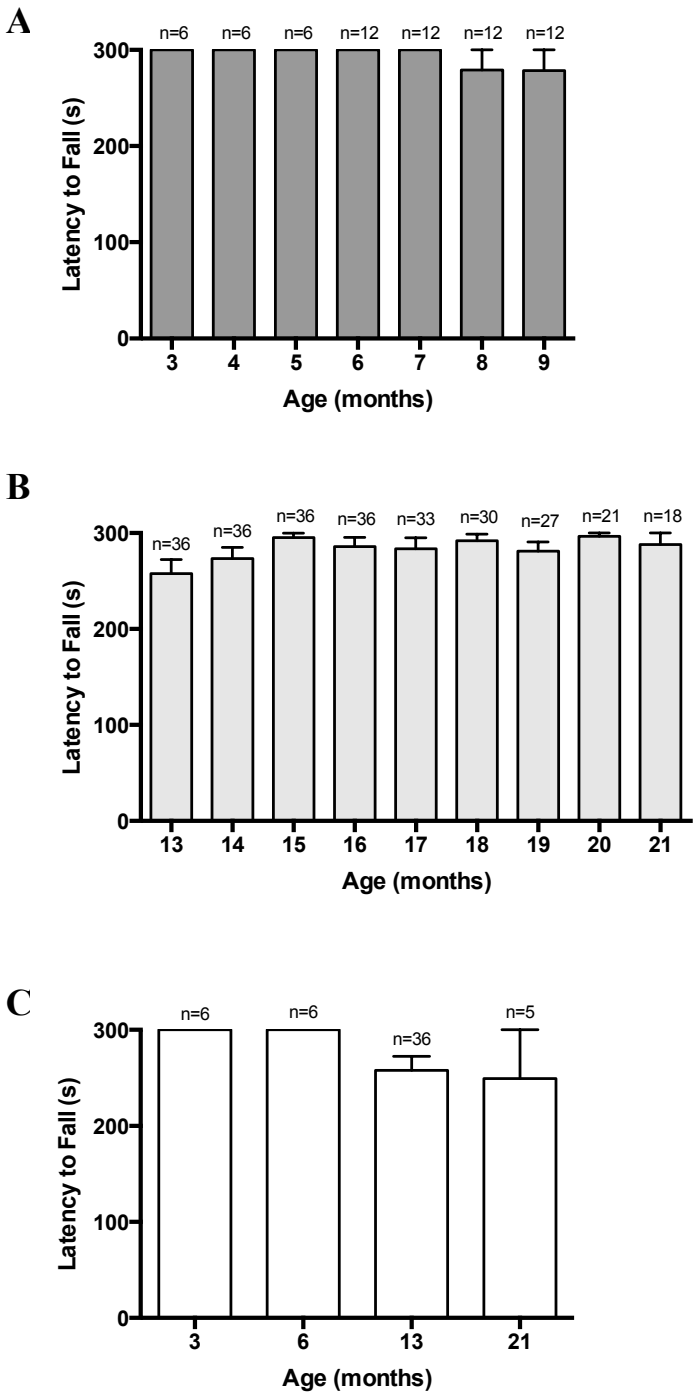

Figure 14. Medium plank test. A reduction in latency to fall reflected a decline in balance and coordination. (A) Performance was consistent in the young cohort of rats from 3 months to 9 months (p=0.75). (B) Latency to fall was consistent throughout the lifespan in the older cohort of rats (p=0.17). (C) First exposure to the test was compared in a cross-sectional analysis. There was a slight decline in latency to fall with age, however this did not reach significance (p=0.43).

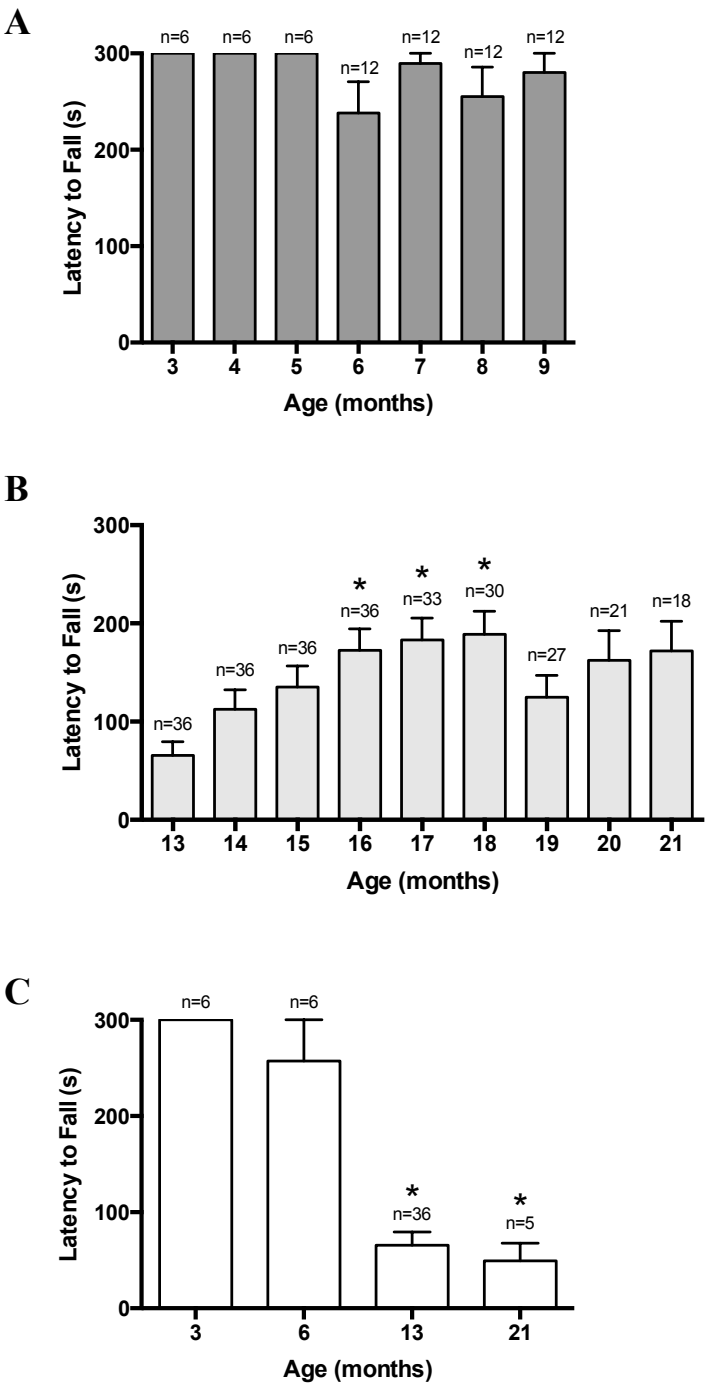

Figure 15. Narrow plank test. A reduction in latency to fall reflected a decline in balance and coordination. (A) Performance was relatively consistent in the young cohort of rats with age ($p=0.40$). (B) There was a significant training effect observed in the older cohort of rats from 13 to 18 months. Performance was relatively plateaued for the remainder of the lifespan ($p<0.05$). (C) First exposure to the test was compared in a cross-sectional analysis. There was a significant reduction in latency to fall with old age ($p<0.05$). *Denotes $p<0.05$ relative to baseline.

1.4 Exploratory Activity

Every two months, animals were placed in an enclosed arena and exploratory activity was recorded for 15 minutes. Using video-tracking software, we then analyzed various movement characteristics by age using both longitudinal and cross-sectional analysis. Similar patterns were observed for all movement characteristics with age. In the young cohort of rats, total distance moved, maximal distance moved, mean velocity, absolute move duration, percentage of time spent moving, and number of rearings all declined between 3 and 9 months. In the older cohort of rats, all movement characteristics declined between 13 and 15 months, and then plateaued for the remainder of the lifespan. Cross-sectional analysis of first exposure to the open field test revealed a significant effect of age on all movement characteristics. A summary of these findings is depicted graphically in figures 16-21.

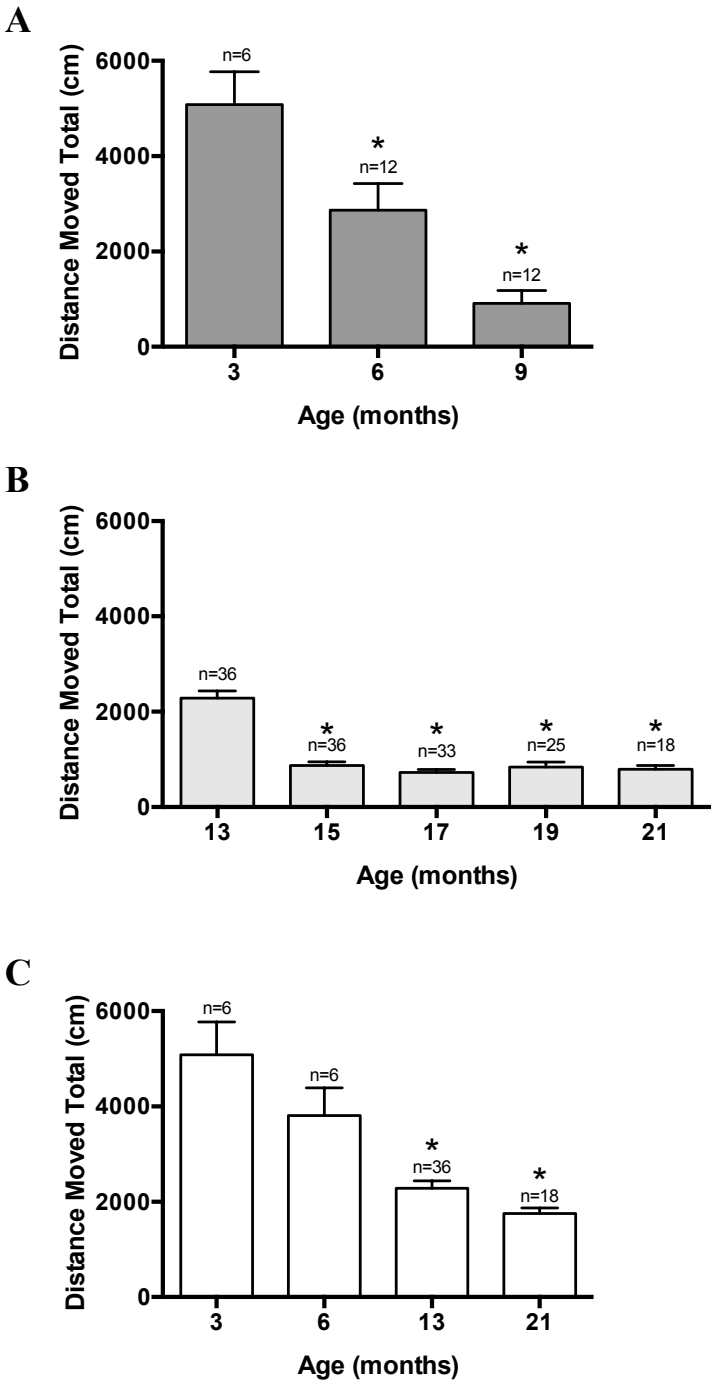

Figure 16. Total distance moved during the recording period. (A) Total distance moved declined with age in the young cohort of rats ($p<0.05$). (B) In the old cohort of rats, performance declined by 15 months and plateaued for the remainder of the lifespan ($p<0.05$). (C) First exposure to the test was compared in a cross-sectional analysis. Total distance moved declined between youth and old age ($p<0.05$). *Denotes $p<0.05$ relative to baseline.

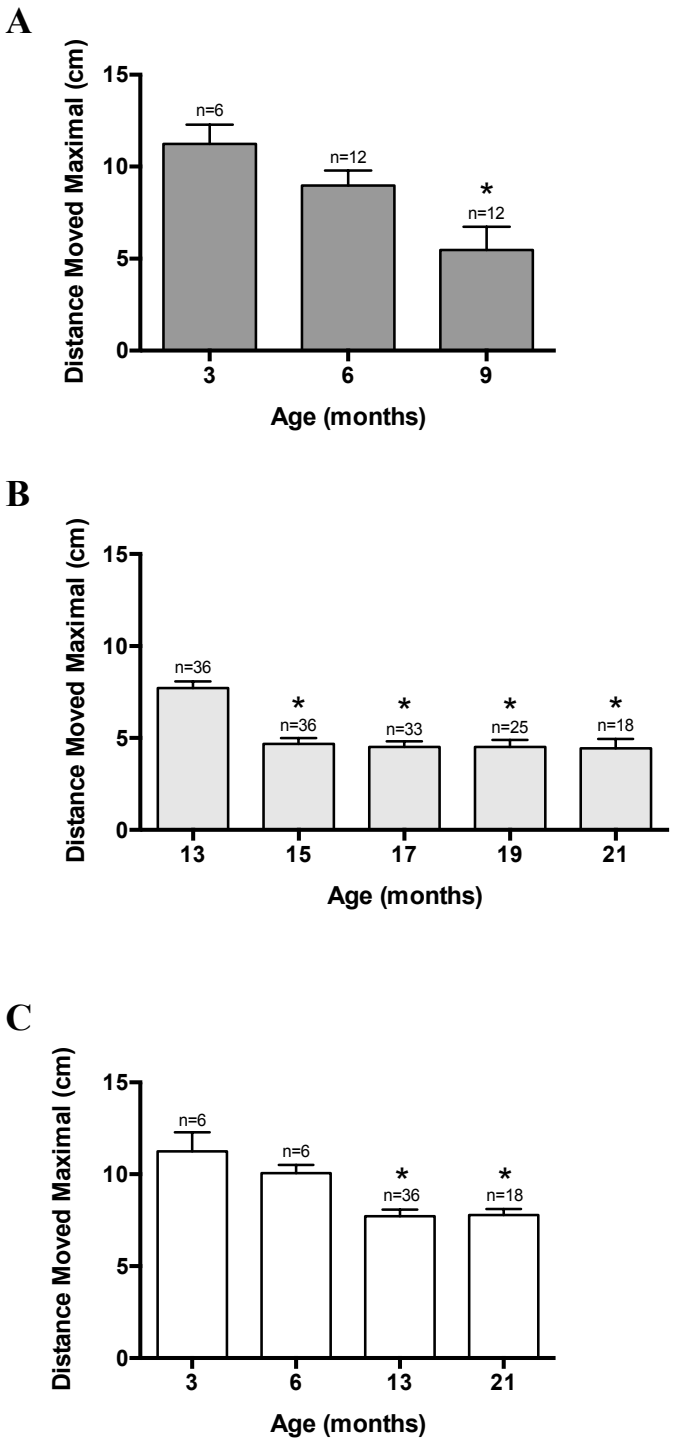

Figure 17. Maximal distance moved during the recording period. (A) Maximal distance declined between 3 and 9 months ($p<0.05$). (B) In the old cohort of rats, performance declined by 15 months and plateaued for the remainder of the lifespan ($p<0.05$). (C) First exposure to the test was compared in a cross-sectional analysis. Maximal distance moved declined between youth and old age ($p<0.05$). *Denotes $p<0.05$ relative to baseline.

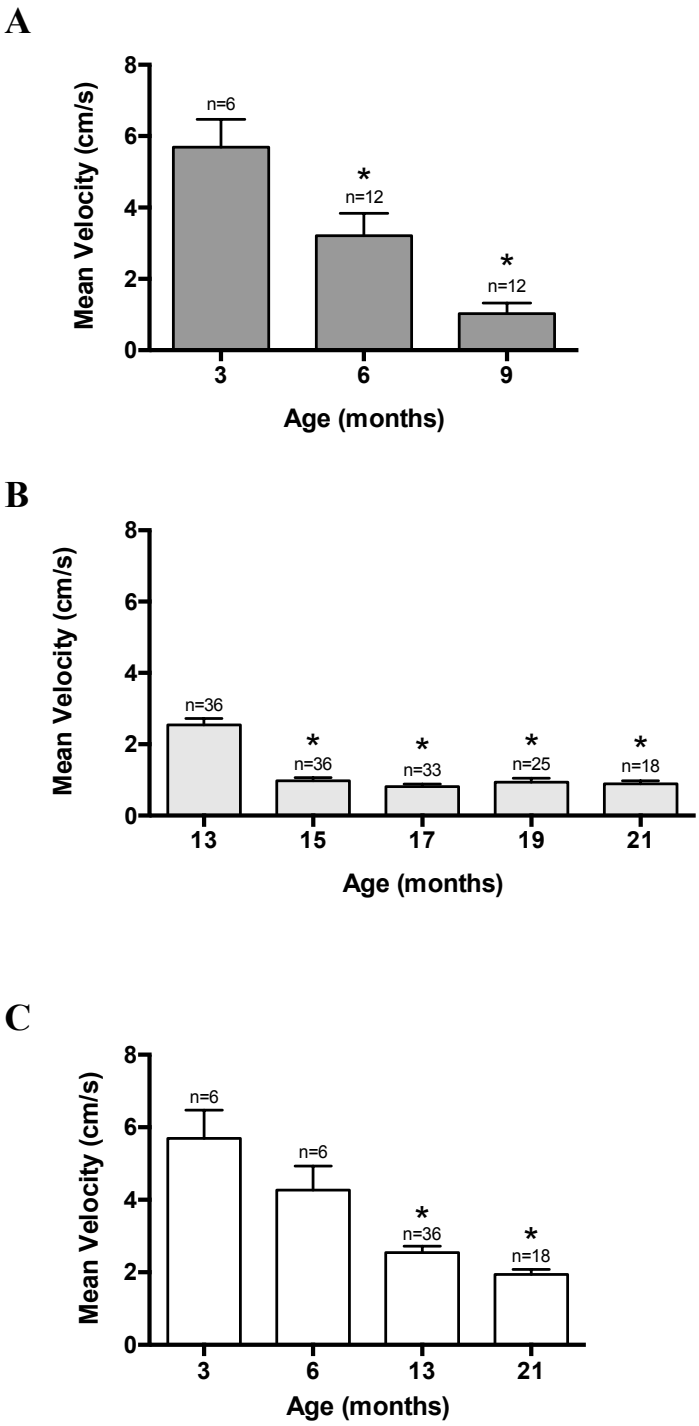

Figure 18. Mean velocity of movement. (A) Mean velocity of movement declined between youth and adulthood in the young cohort of rats ($p<0.05$). (B) In the old cohort of rats, mean velocity declined by 15 months and plateaued for the remainder of the lifespan ($p<0.05$). (C) Mean velocity during the first exposure to the test was compared in a cross-sectional analysis. Mean velocity of movement declined with age ($p<0.05$). *Denotes $p<0.05$ relative to baseline.

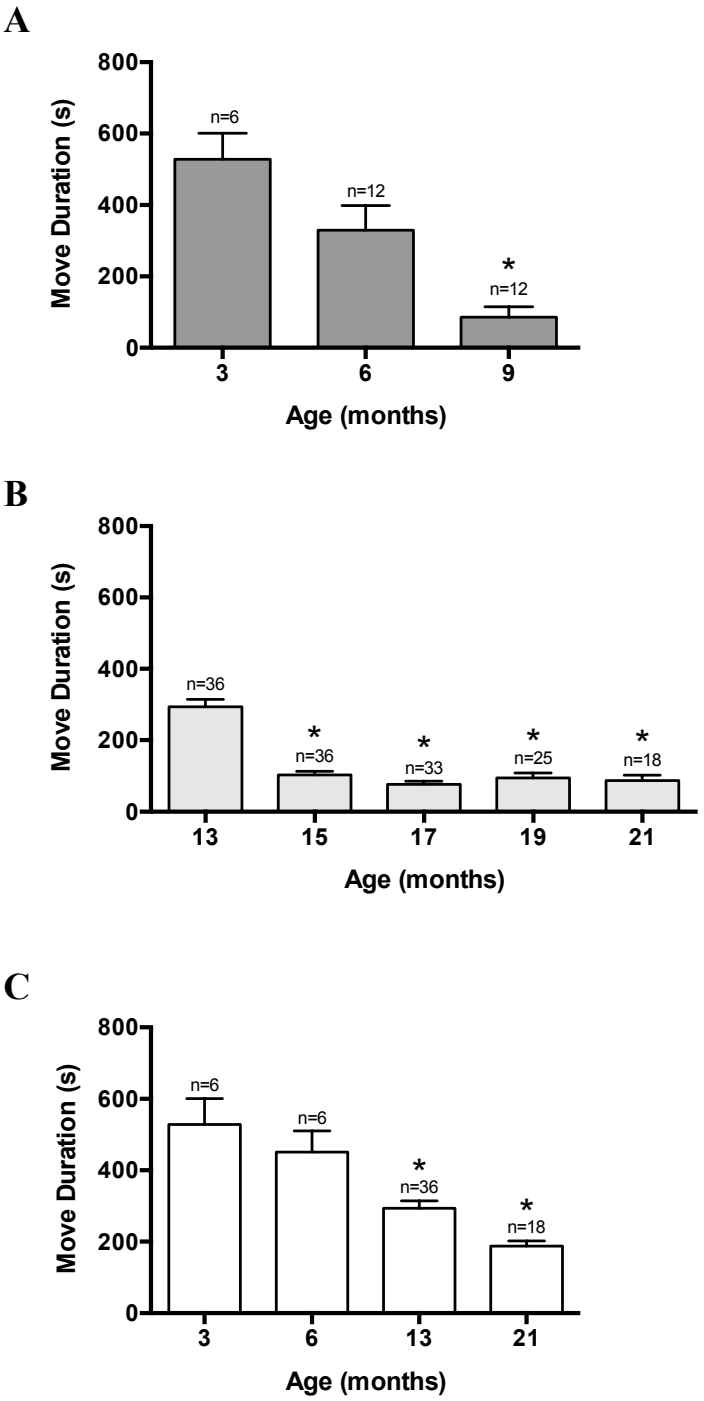

Figure 19. Total duration of movement. (A) Duration of movement declined between 3 and 9 months ($p<0.05$) (B) In the old cohort of rats, movement duration declined by 15 months and plateaued for the remainder of the lifespan ($p<0.05$). (C) Duration of movement during the first exposure to the test was compared in a cross-sectional analysis. Total duration of movement declined between youth and old age ($p<0.05$). *Denotes $p<0.05$ relative to baseline.

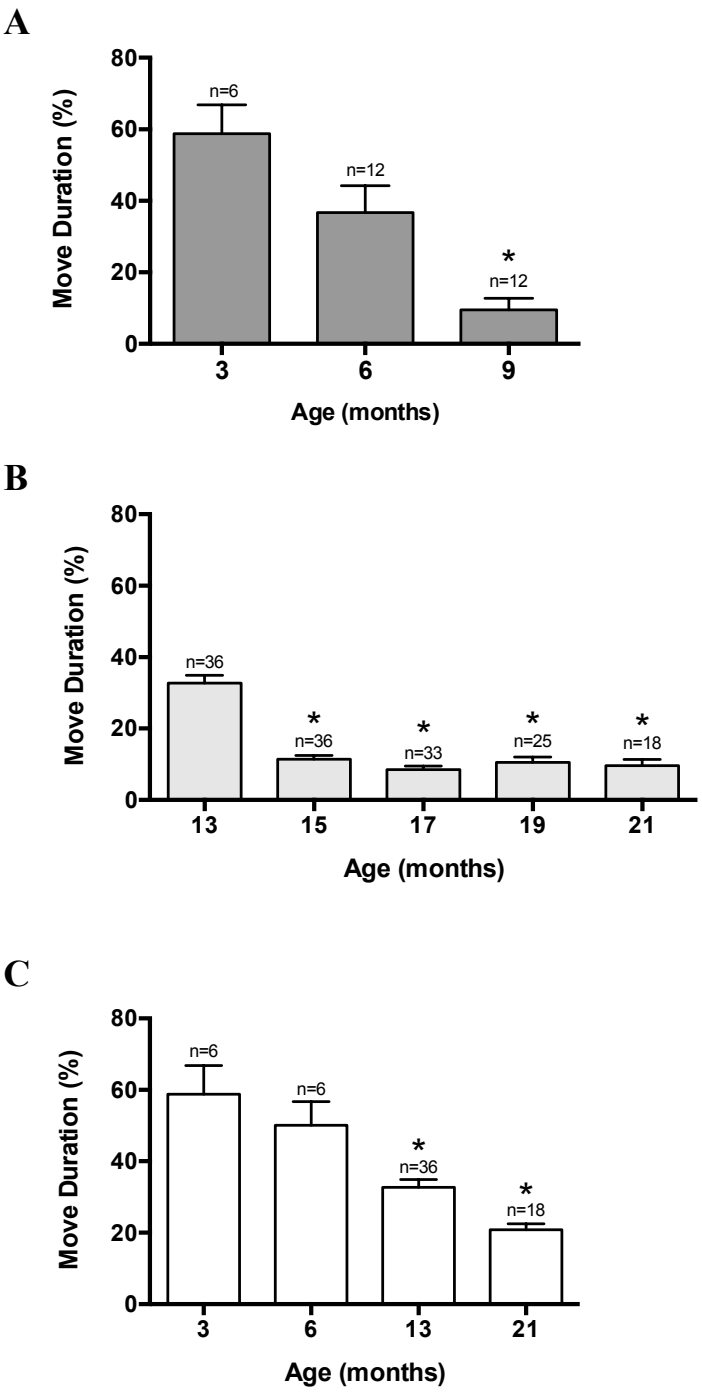

Figure 20. Percentage of time spent moving during the recording period. (A) Duration of movement declined between youth and adulthood in the young cohort of rats ($p<0.05$). (B) In the old cohort of rats, percent time spent moving declined by 15 months and plateaued for the remainder of the lifespan ($p<0.05$). (C) Duration of movement during the first exposure to the test was compared in a cross-sectional analysis. The percentage of time spent moving during the recording period declined with age ($p<0.05$). *Denotes $p<0.05$ relative to baseline.

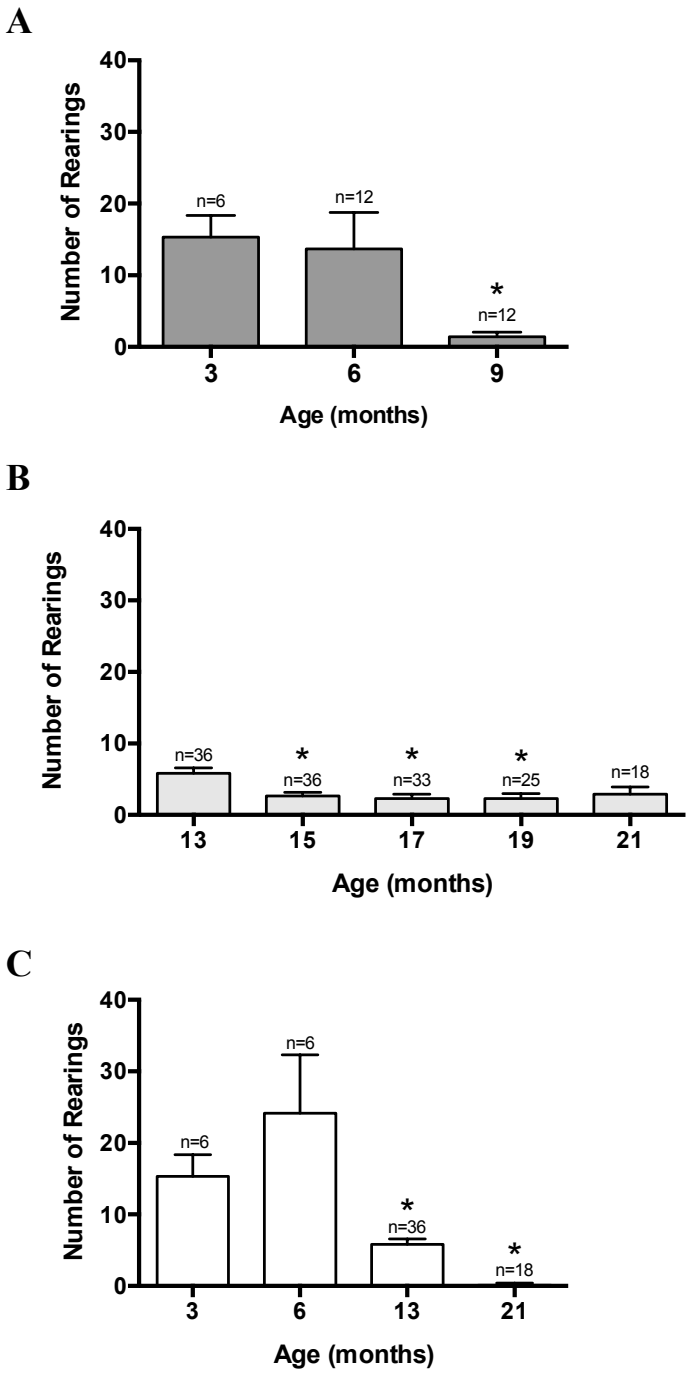

Figure 21. Rearing frequency. (A) There was a sharp decline in rearing frequency by 9 months in the young cohort of rats ($p<0.05$). (B) In the old cohort of rats, rearing frequency declined by 15 months and plateaued for the remainder of the lifespan ($p<0.05$). (C) Rearing frequency during the first exposure to the test was compared in a cross-sectional analysis. There was a dramatic decline in the number of rearings with age ($p<0.05$). *Denotes $p<0.05$ relative to baseline.

1.5 Basic Metabolic Status and Hematology

In the young group of animals, a small volume of blood was collected every 3 months (3, 6 and 9 months) and analyzed. Parameters measured were fairly consistent with increasing age (table 4). In the older group of animals, we collected blood samples for analysis every 4 months (13, 17 and 21 months). There was a significant effect of age on blood urea nitrogen (BUN) and creatinine; other lab parameters were consistent between 13 and 21 months. Interestingly, some parameters (Na^+, total CO_2, hematocrit and hemoglobin) differed between 13 and 17 months (table 5).

Table 4. Basic metabolic status and hematology in young rats.

Parameter	Age (months)		
	3 (n=6)	**6 (n=12)**	**9 (n=12)**
Na^+ (mmol/L)	140±0	142±0*	141±0
K^+ (mmol/L)	5.0±0.2	4.3±0.2*	4.5±0.2
Cl- (mmol/L)	104±1	110±1	91±12
BUN (mg/dL)	18±1	16±1	16±1
Creatinine (mg/dL)	0.3±0.0	0.4±0.0	0.3±0.0
Total CO_2 (mmol/L)	27±1	24±1	25±1
Hematocrit (%)	41±1	36±2	41±2
Hemoglobin (mg/dL)	13.8±0.2	12.4±0.5	13.9±0.5

** Denotes p<0.05 relative to 3 months.*

Table 5. Basic metabolic status and hematology in aged rats.

Parameter	Age (months)		
	13 (n=36)	**17 (n=33)**	**21 (n=18)**
Na^+ (mmol/L)	141±0	142±0*	141±0
K^+ (mmol/L)	4.4±0.1	4.2±0.1	4.9±0.2
Cl- (mmol/L)	110±1	112±1	108±1
BUN (mg/dL)	15±1	14±1	19±1*
Creatinine (mg/dL)	0.3±0.0	0.4±0.0*	0.4±0.0*
Total CO_2 (mmol/L)	26±1	22±1*	25±1
Hematocrit (%)	40±1	35±1*	37±2
Hemoglobin (mg/dL)	13.6±0.4	11.8±0.5*	13.1±0.5

** Denotes p<0.05 relative to 13 months.*

2. Frailty Index

Based on the results of the experiments described in section 1.1 – 1.5, we created a 30-item FI that can be used to assess longitudinal changes in frailty (table 6). Formatting of the FI is modified from that published by Whitehead *et al.* (2014). All variables included in the index satisfied the criteria outlined in methods section 2.7. Subjective variables included in the FI are scored on a graded scale from 0 to 1: 0 = absence of a deficit; 0.5 = a mild deficit; and 1 = a severe deficit. Objective variables, such as changes in grip strength, muscular stamina, nutritional status, metabolic status, hematology and body surface temperature are scored relative to a set of reference values we generated from our data set (table 7).

Reference values for metabolic status, hematology and body surface temperature were generated from the mean and standard deviation of values obtained from 13 month old rats. A value <1 SD away from the mean received a score of 0; 1-2 SD received a score of 0.5; and >2SD received a score of 1. Changes in nutritional status are scored as each animal's percent change from their normal food intake or body weight. We divided the latency to fall at 13 months into tertiles to generate grip strength reference values: animals in the top tertile received a score of 0; those in the middle received a score of 0.5; and animals in the lowest tertile received a score of 1. Inclined plane reference values were generated as follows: animals with a latency to fall >51st percentile received a score of 0; those in the 26th – 50th percentile received a score of 0.5; and animals with a latency to fall <25th percentile received a score of 1.

Table 6. A 30-item index to assess frailty in the male Fischer-344 rat.

	Rating: 0 = absent	0.5 = mild	1 = severe	Comments:	FI Score
Integument					
1. Alopecia	**0**	**0.5**	**1**	_____	
2. Skin lesions	**0**	**0.5**	**1**	_____	
3. Coat condition	**0**	**0.5**	**1**	_____	
Physical/Musculoskeletal					
4. Tumours	**0**	**0.5**	**1**	_____	
5. Hunched posture	**0**	**0.5**	**1**	_____	
6. Body condition score	**0**	**0.5**	**1**	_____	
7. Gait disorder	**0**	**0.5**	**1**	_____	
8. Muscle tone/stamina		_____ **s**		_____	_____
9. Grip strength		_____ **s**		_____	_____
Vestibulocochlear/Auditory					
11. Hearing loss	**0**	**0.5**	**1**	_____	
Ocular/Nasal					
10. Cataracts	**0**	**0.5**	**1**	_____	
11. Chromodacryorrhea	**0**	**0.5**	**1**	_____	
12. Exophthalmos/Microphthalmos	**0**	**0.5**	**1**	_____	
13. Corneal opacity	**0**	**0.5**	**1**	_____	
Neurological					
14. Head tilt	**0**	**0.5**	**1**	_____	
Digestive/Urogenital					
15. Malocclusion	**0**	**0.5**	**1**	_____	
16. Diarrhea	**0**	**0.5**	**1**	_____	
17. Jaundice	**0**	**0.5**	**1**	_____	
Respiratory					
18. Breathing rate/depth	**0**	**0.5**	**1**	_____	
Nutritional					
19. Body weight	_____ **g**			_____	_____
20. Daily food intake	_____ **g**			_____	_____
Metabolic Status					
21. Sodium	_____ **mmol/L**			_____	_____
22. Potassium	_____ **mmol/L**			_____	_____
23. Blood urea nitrogen	_____ **mg/dL**			_____	_____
24. Creatinine	_____ **mg/dL**			_____	_____
25. Carbon dioxide	_____ **mmol/L**			_____	_____
26. Chloride	_____ **mmol/L**			_____	_____
Hematology					
28. Hematocrit	_____ **%PCV**			_____	_____
29. Hemoglobin	_____ **g/dL**			_____	_____
Other					
30. Body surface temperature	_____ **°C**			_____	_____

Table 7. Reference values for scoring objective variables included in the frailty index.

			Scoring		
System	**Variable**	**Mean±SD**	**0**	**0.5**	**1**
Metabolic Status	BUN (mg/dl)	15 ± 3	12–18	9–11 , 19–21	≤8 , ≥22
	Creatinine (mg/dl)	0.3 ± 0.1	0.2–0.4	0.5	≥0.6
	Na^+ (mmol/L)	141 ± 1	140–142	139 , 143	≤138 , ≥144
	K^+ (mmol/L)	4.4 ± 0.7	3.7–5.1	3.0–3.6 , 5.2–5.8	≤2.9 , ≥5.9
	CO_2 (mmol/L)	26 ± 3	23–29	20–22 , 30–32	≤19 , ≥33
	Cl^- (mmol/L)	110 ± 6	104–116	98–103 , 117–122	≤97 , ≥123
	TCO_2 (mmol/L)	25 ± 3	22–28	19–31	≤18 , ≥32
Hematology	Hematocrit (%)	40 ± 7	33–47	26–32 , 48–54	≤25 , ≥55
	Hb (mg/dl)	13.6 ± 2.3	11.3–15.9	9.0–11.2 , 16.0–18.2	≤8.9 , ≥18.3
Nutrition	Food Intake (%)	--	≤15	16–29	≥30
	Weight change (%)	--	≤5	6–9	≥10
Physical	Inclined Plane (s)	--	<142	143–227	≥228
	Grip Strength (s)	--	≥9	5–8	≤4
Other	Body surface temperature (°C)	26.3 ± 0.8	25.5– 27.1	24.7–25.4 , 27.2–27.9	≤24.6 , ≥28.0

3. Frailty Index Outcomes

Using the FI described previously, we generated FI scores for animals at 3, 6, 9, 13, 17 and 21 months. Mean FI scores increased with age. Scores were consistent from youth into adulthood (3 months, 0.032±0.007; 6 months 0.035±0.009; 9 months, 0.051±0.014; p=0.48), and increased from middle age into old age (13 months, 0.071±0.008; 17 months 0.152±0.015; 21 months, 0.241±0.021; p<0.05) (figure 22). To determine the cut-off point at which an animal was frail, we divided the frailty scores generated from the older cohort of rats into tertiles. Animals with FI scores in the upper tertile (>0.250) were deemed frail; those in the lower two tertiles (<0.250) were considered not frail.

We collected survival data on all rats aged to 21 months. Survival in our colony of rats was 52%. Figure 23 illustrates a Kaplan-Meier survival curve for these animals. Mortality occurred when rats died unexpectedly or were euthanized due to illness. A high FI score was associated with an increased risk of mortality ($p<0.05$).

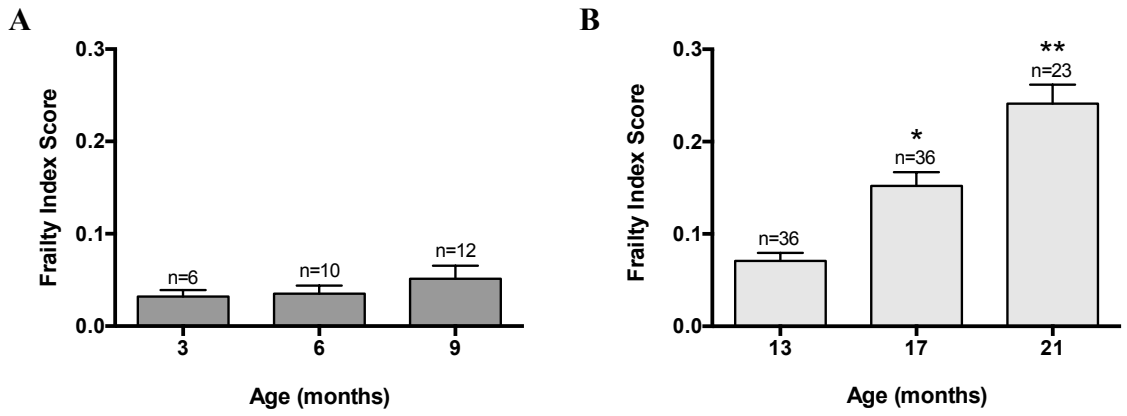

Figure 22. Scores obtained with the 30-item frailty index (FI). (A) There was no significant effect of age on mean FI scores from youth into adulthood ($p=0.48$). (B) Mean FI scores increased from middle age into old age ($p<0.05$). *Denotes $p<0.05$ relative to baseline.

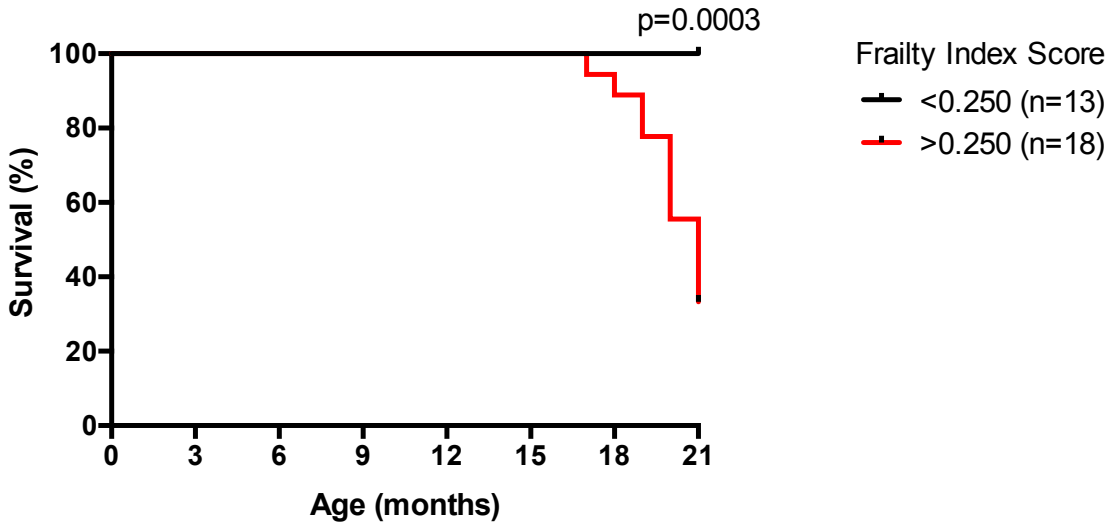

Figure 23. Kaplan-Meier survival curve for mortality. Frailty was associated with an increase in mortality ($p<0.05$).

4. Cardiac structure and function *in vivo*

Following the creation of our FI, we sought to determine if age and frailty had an impact on cardiac structure and function *in vivo.* To investigate this, we aged young rats (3 months, n=6; 6 months, n=6) to adulthood (9 months) and middle-aged rats (13 months, n=36) to old age (21 months). Additional rats underwent echocardiography at 21 months (n=5) to increase the sample size of the 'aged' animals. Serial echocardiography was performed on anesthetized rats at 3, 6, 9 months in the "young" group, and 13, 17 and 21 months in the "old" group. Frailty assessments were performed prior to echocardiography. Animals with a FI score <0.250 were deemed non-frail, while those with a FI score >0.250 were considered frail. Various cardiac structure and function parameters were then compared by age and FI score.

4.1 Impact of age and frailty on cardiac morphology *in vivo*

4.1.1 Left ventricular internal diameter

There was no significant effect of age or FI score on LVID. There was a slight increase in LVIDs (3 months, 0.25±0.03; 6 months, 0.34±0.01; 9 months, 0.3±0.02 cm; $p<0.05$), and LVIDd (3 months, 0.56±0.01; 6 months, 0.64±0.02; 9 months, 0.63±0.01 cm; $p<0.05$) between youth and adulthood. Left ventricular internal diameter plateaued for the remainder of the lifespan: LVIDs (13 months, 0.31±0.01; 17 months, 0.31±0.01; 21 months, 0.32±0.02 cm; $p=0.59$) and LVIDd (13 months, 0.66±0.01; 17 months, 0.68±0.01; 21 months, 0.64±0.02 cm; $p=0.19$). LVID was similar between frail and non-frail animals at systole (FI score >0.250, 0.30±0.02 vs. FI score <0.250, 0.32±0.01 cm; $p=0.35$), and diastole (FI score >0.250, 0.64±0.02 vs. FI score <0.250 0.67±0.01 cm; $p=0.06$) (figures 24-25).

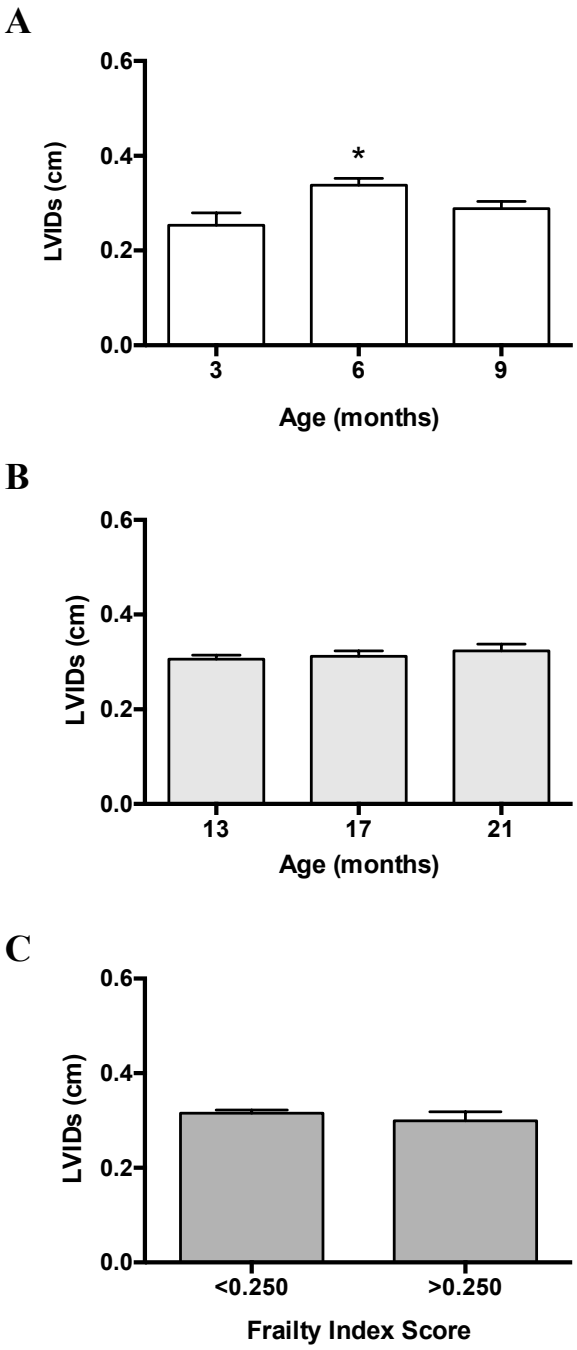

Figure 24. Left ventricular internal diameter during systole (LVIDs) is not affected by age or frailty index score. (A/B) LVIDs increased until 9 months ($p<0.05$), and plateaued for the remainder of the lifespan ($p=0.59$). (C) There was no significant effect of frailty on LVIDs ($p=0.35$). Bars represent mean±SEM. Age: 3m (n=6); 6m (n=10); 9m (n=12); 13m (n=36); 17m (n=33); 21m (n=23). FI score: <0.250 (n=76); >0.250 (n=16). *Denotes $p<0.05$ relative to baseline.

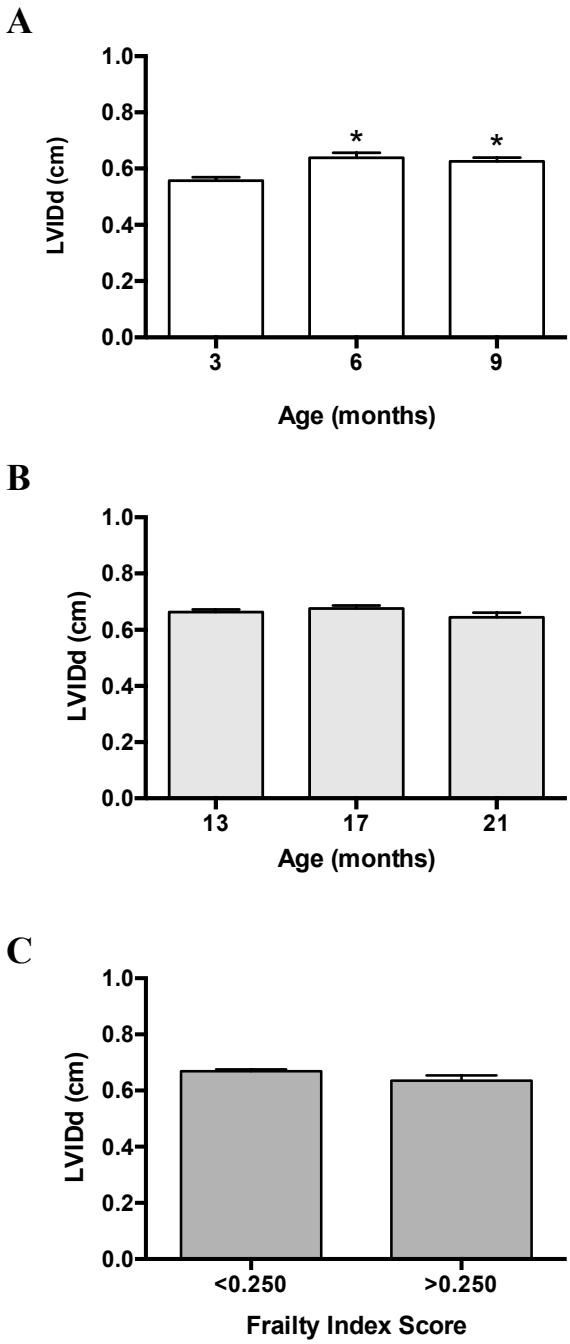

Figure 25. Left ventricular internal diameter at end diastole (LVIDd) is not affected by age or frailty index score. (A/B) LVIDd increased until 9 months ($p<0.05$) and plateaued for the remainder of the lifespan ($p=0.19$). (C) There was no significant effect of frailty on LVIDd ($p=0.06$). Bars represent mean±SEM. Age: 3m (n=6); 6m (n=10); 9m (n=12); 13m (n=36); 17m (n=33); 21m (n=23). FI score: <0.250 (n=76); >0.250 (n=16). *Denotes $p<0.05$ relative to baseline.

4.1.2 Left ventricular posterior wall thickness

LVPW was not affected by age or frailty. Figures 26-27 show that LVPW thickness was similar at systole (3 months, 0.30±0.02; 6 months, 0.32±0.02; 9 months, 0.31±0.01 cm; p=0.76) and diastole (3 months, 0.19±0.02; 6 months, 0.23±0.02; 9 months, 0.19±0.02 cm; p=0.29) in young animals. LVPW plateaued for the remainder of the lifespan at systole (13 months, 0.32±0.01; 17 months, 0.32±0.01; 21 months, 0.31±0.01 cm; p=0.67) and diastole (13 months, 0.19±0.01; 17 months, 0.20±0.01; 21 months, 0.19±0.02 cm; p=0.77). Frailty did not impact LVPWs (FI score <0.250, 0.32±0.01 vs. FI score >0.250, 0.30±0.02 cm; p=0.14) or LVPWd (FI score <0.250, 0.20±0.01 vs. FI score >0.250, 0.18±0.02 cm; p=0.29) (figures 26-27).

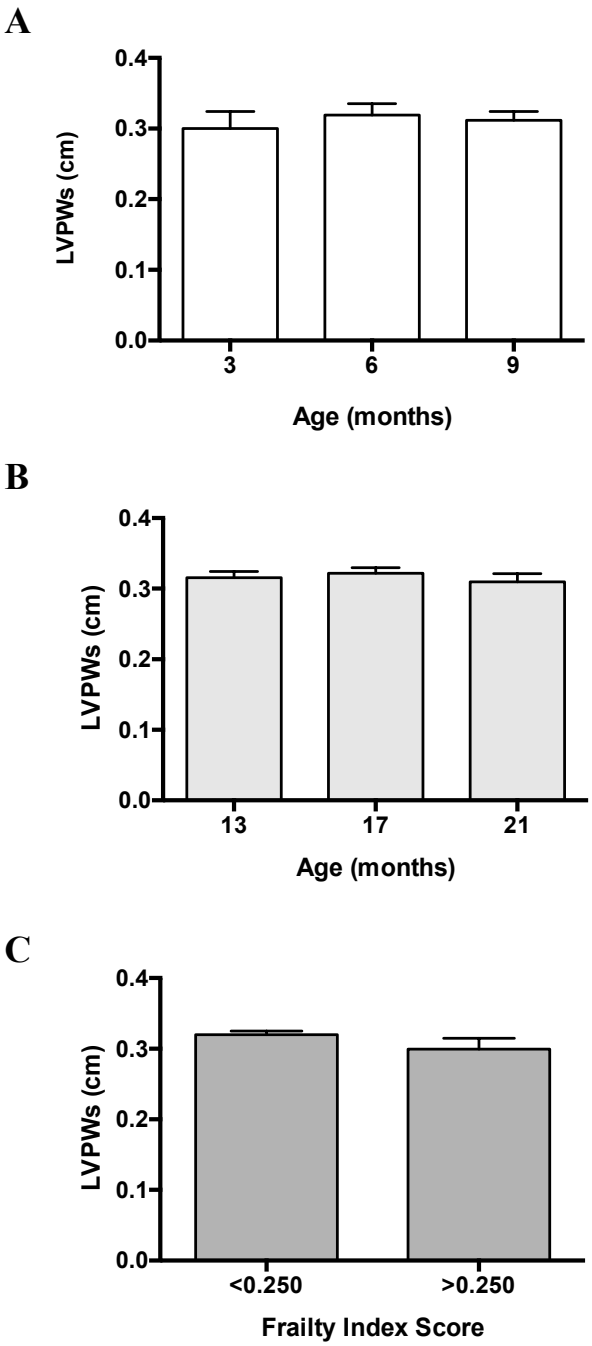

Figure 26. Left ventricular posterior wall thickness during systole (LVPWs) was not affected by age or frailty index score. (A/B) LVPWs was consistent from 3 to 9 months ($p=0.76$), and from 13 to 21 months ($p=0.67$). (C) LVPWs was similar between frail and non-frail animals ($p=0.14$). Bars represent mean±SEM. Age: 3m (n=6); 6m (n=10); 9m (n=12); 13m (n=36); 17m (n=33); 21m (n=23). FI score: <0.250 (n=76); >0.250 (n=16).

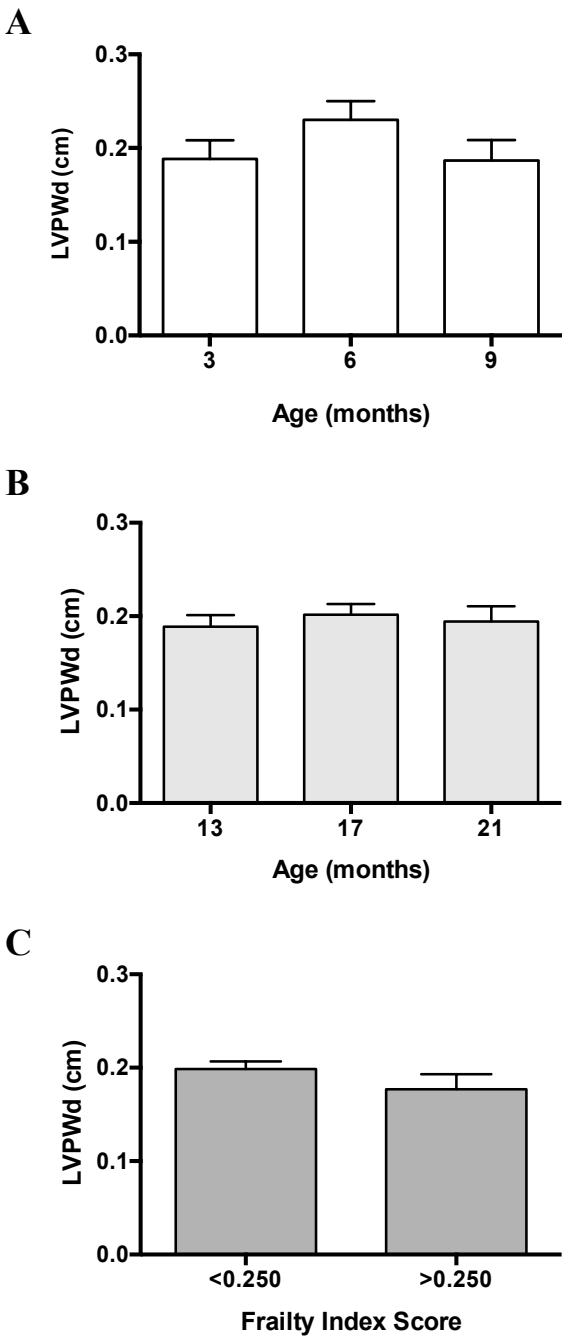

Figure 27. Old age and frailty index score do not affect left ventricular posterior wall thickness at diastole (LVPWd). (A) LVPWd had a parabolic relationship with age from youth into adulthood (p=0.29). (B) LVPWd was similar between middle age and old age (p=0.77). (C) LVPWd tended to be lower in frail animals (p=0.64). Bars represent mean±SEM. Age: 3m (n=6); 6m (n=10); 9m (n=12); 13m (n=36); 17m (n=33); 21m (n=23). FI score: <0.250 (n=76); >0.250 (n=16).

4.2 Impact of age and frailty on cardiac function *in vivo*

4.2.1 Heart rate

HR tended to increase between youth and adulthood (3 months, 340±8; 6 months 324±5; 9 months 361±8 bpm; $p<0.05$). HR declined between 9 and 13 months, but remained fairly consistent throughout the remainder of the lifespan (13 months, 320±4; 17 months 323±4; 21 months 309±7 bpm; $p=0.16$). There was no significant effect of frailty on HR (FI score >0.250, 311±10 vs. FI score <0.250 320±3 bpm; $p=0.26$) (figure 28).

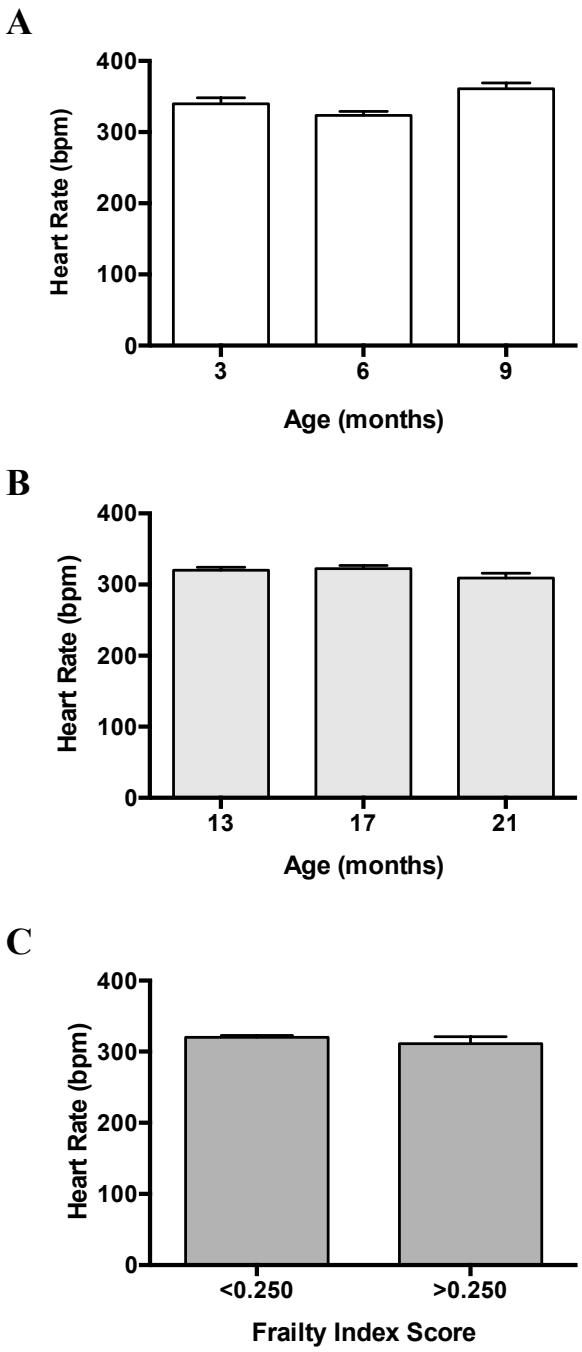

Figure 28. Heart rate (HR) is not affected by old age or frailty index score *in vivo*. (A) HR was variable between youth and adulthood ($p<0.05$). (B) HR was similar between middle age and old age ($p=0.16$). (C) There was no significant effect of frailty on HR ($p=0.26$). Bars represent mean±SEM. Age: 3m (n=6); 6m (n=10); 9m (n=12); 13m (n=36); 17m (n=33); 21m (n=23). FI score: <0.250 (n=76); >0.250 (n=16).

4.2.2 Systolic function

There was no significant effect of age or frailty on indices of systolic function. EF was consistent throughout the lifespan: it was similar between youth and adulthood (3 months, 88±4; 6 months 83±2; 9 months 88±2%; p=0.13), and between middle age and old age (13 months, 88±1; 17 months 88±1; 21 months 85±1%; p=0.06). There was no significant effect of frailty on EF (FI score <0.250 88±1 vs. FI score >0.250 87±2%; p=0.13) (figure 29). FS was also unaffected by age: it was consistent between 3 and 9 months of age (3 months, 55±5; 6 months, 47±2; 9 months 54±2%; p=0.08), and between 13 and 21 months (13 months, 54±1; 17 months 54±1; 21 months 50±1.6%; p=0.07). FS was not affected by frailty (FI score >0.250, 53±3 vs. FI score <0.250 53±1%; p=0.81) (figure 30).

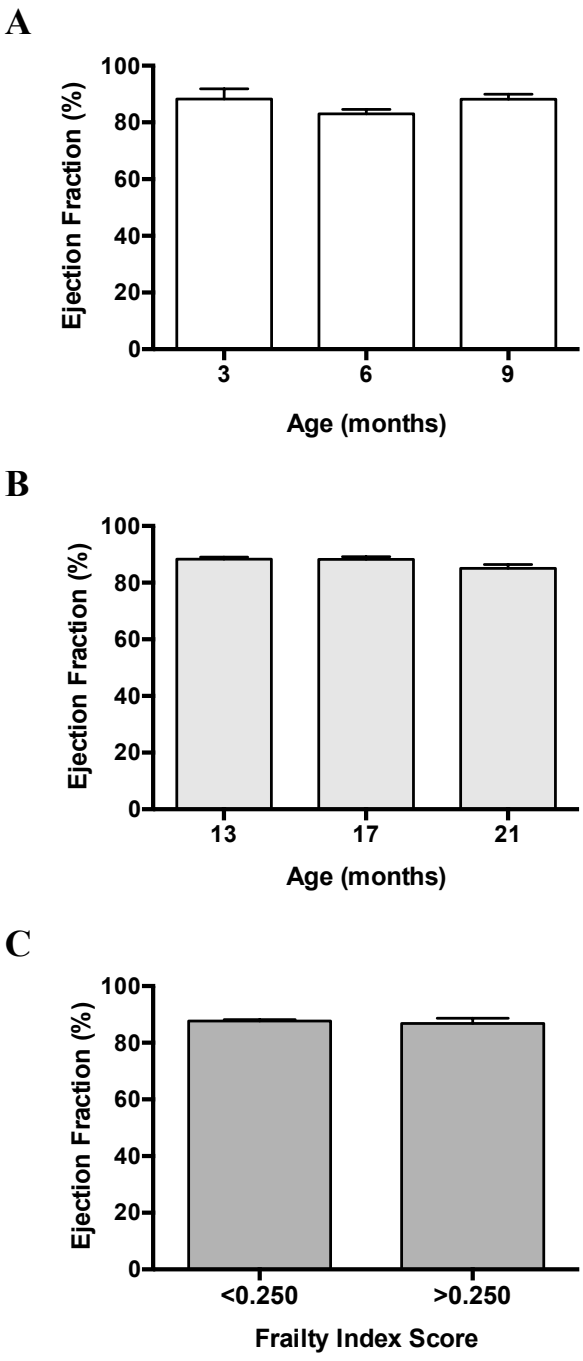

Figure 29. Ejection fraction (EF) is not affected by age or frailty index score *in vivo*. (A/B) There was no significant effect of age on EF from youth into middle age (p=0.13), or from middle age into old age (p=0.06). (C) EF was similar between frail and non-frail rats (p=0.60). Bars represent mean±SEM. Age: 3m (n=6); 6m (n=10); 9m (n=12); 13m (n=36); 17m (n=33); 21m (n=23). FI score: <0.250 (n=76); >0.250 (n=16).

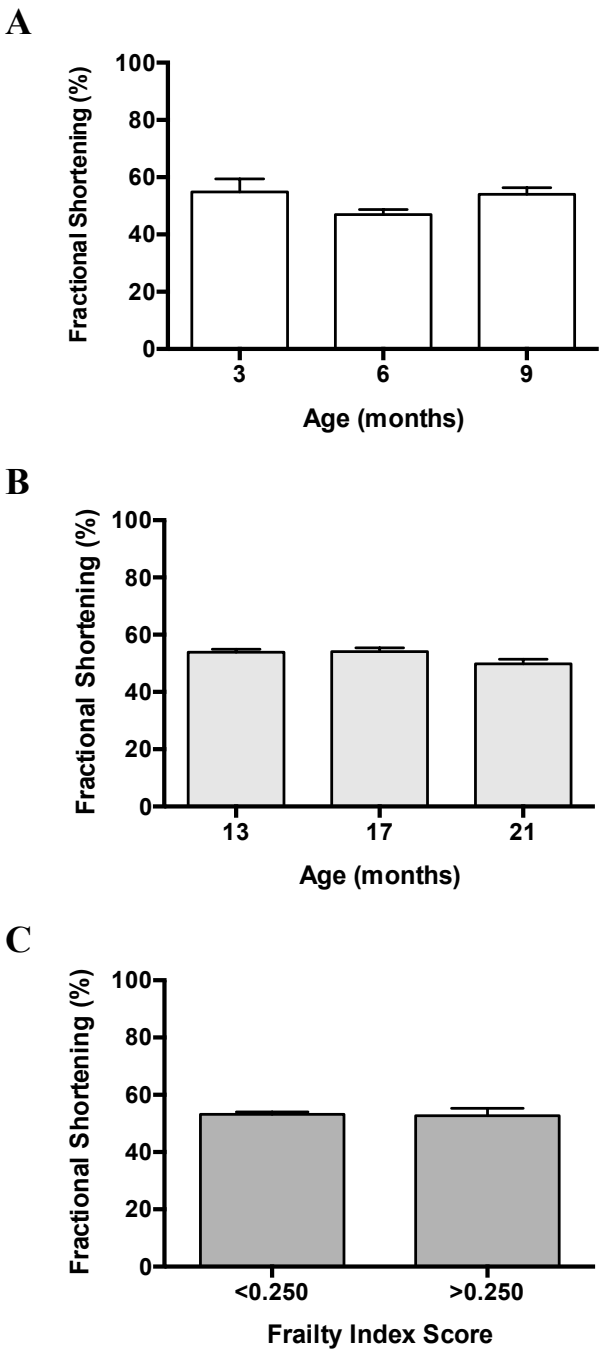

Figure 30. Fractional shortening (FS) was not affected by age or frailty index score. (A/B) FS was consistent from 3 to 9 months (p=0.08), and from 13 to 21 months (p=0.07). (C) FS was similar between frail and non-frail animals (p=0.81). Bars represent mean±SEM. Age: 3m (n=6); 6m (n=10); 9m (n=12); 13m (n=36); 17m (n=33); 21m (n=23). FI score: <0.250 (n=76); >0.250 (n=16).

4.2.3 Diastolic function

Diastolic function was generally unaffected by age *in vivo*. There was a decline in the E/A ratio between 6 and 9 months (6 months, 2.5±0.1 vs. 9 months, 1.8±0.1; $p<0.05$), however mitral DT was similar (6 months, 32.8±2.8 vs. 9 months 29.3±3.1 ms, $p=0.42$). The E/A ratio and mitral DT were fairly consistent between 9 and 17 months. There was no change in the E/A ratio (17 months, 2.0±0.1 vs. 21 months, 2.0±0.1; $p=0.99$), or mitral DT with age (17 months, 29.4± 1.9 vs. 21 months, 27±.0± 3.3 ms; $p=0.50$). E/A ratio was similar between frail and non-frail animals (FI score >0.250, 1.8±0.1 vs. FI score <0.250, 2.1±0.1; $p=0.16$). Mitral DT tended to be lower in frail rats, however this was not statistically significant (FI score>0.250, 24.5±4.0 vs. FI score <0.250, 28.5±1.6 ms; $p=0.42$) (figures 31-32).

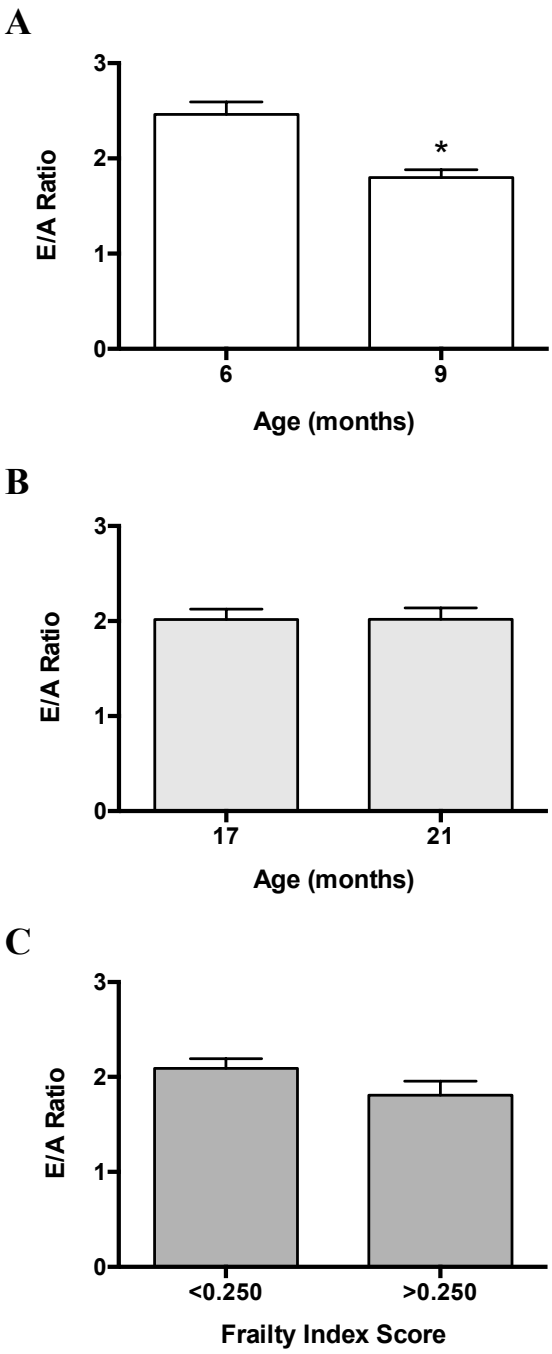

Figure 31. E/A ratio in relation to age and frailty index score. (A) E/A ratio declined between 6 and 9 months ($p<0.05$), and (B) was similar between 17 and 21 months ($p=0.99$). (C) E/A ratio was similar between frail and non-frail animals ($p=0.16$). Bars represent mean±SEM. Age: 6m (n=10); 9m (n=12); 17m (n=32); 21m (n=20). FI score: <0.250 (n=40); >0.250 (n=12). *Denotes $p<0.05$ relative to baseline.

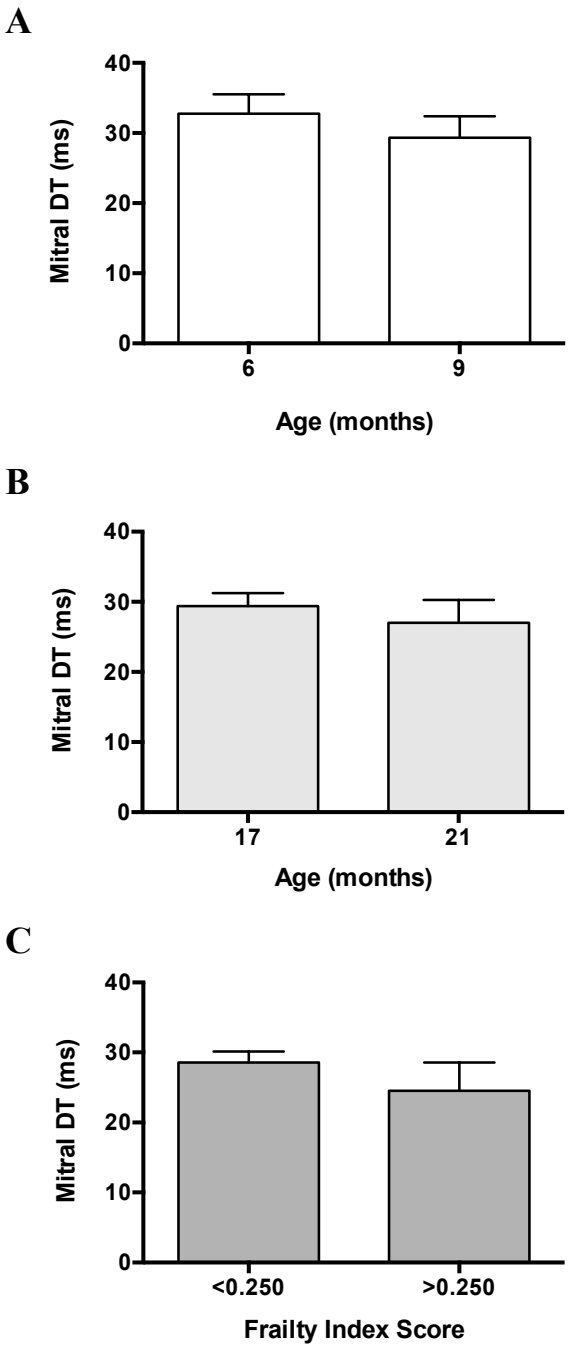

Figure 32. Mitral deceleration time (DT) in relation to age and frailty index score. (A) Mitral DT tended to decrease between 6 and 9 months, however this did not reach statistical significance (p=0.42). (B) Mitral DT was consistent between 17 and 21 months (p=0.50). (C) Mitral DT tended to be lower in frail animals, however this did not attain statistical significance (p=0.27). Bars represent mean±SEM. Age: 6m (n=10); 9m (n=12); 17m (n=32); 21m (n=20). FI score: <0.250 (n=40); >0.250 (n=12).

5. Baseline cardiac function in the isolated working heart

Accurate quantitative measurement of cardiac function by echocardiography is somewhat limited in that poor image quality and resolution can make it difficult to define endocardial borders. Additionally, neurohormonal influences, anesthesia and loading conditions can all influence results in the living animal. Baseline cardiac parameters were therefore further explored in the isolated working heart. Various hemodynamic parameters were collected at baseline in adult (9-months) and aged (21-month) rats. Baseline measurements were recorded at the end of the 5-minute working heart period (prior to arrest with cardioplegia). All hemodynamic parameters were compared by chronological age and FI score. Eleven of 12 adult hearts (92%), and 21 of 23 aged rats (91%) met our baseline inclusion criteria.

5.1 Baseline hemodynamic measurements

5.1.1. Heart Rate

There was no significant effect of age on HR at baseline (9 months, 241±10 vs. 21 months, 234±7 bpm; p=0.56). Frail animals tended to have a slightly higher HR relative to non-frail animals, however this did not attain statistical significance (FI>0.250, 246±10 vs. FI<0.250, 233±6.3 bpm; p=0.33) (figure 33).

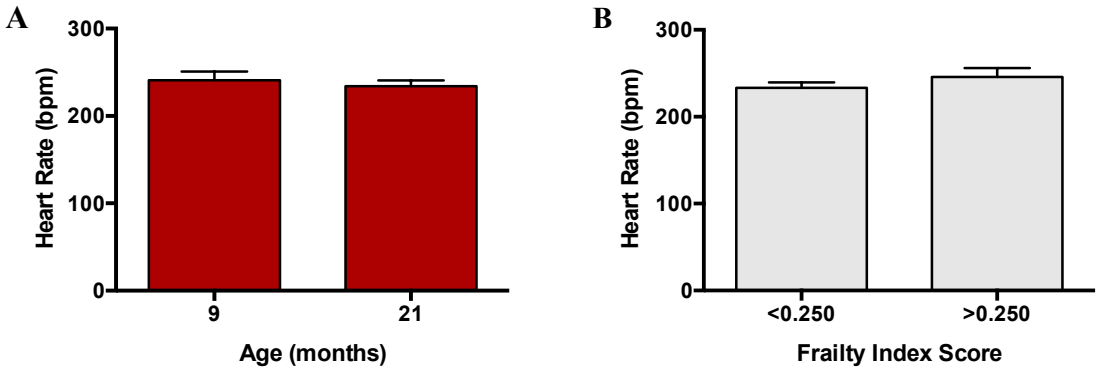

Figure 33. Baseline heart rate (HR) in the isolated working heart. (A) Baseline HR was similar between adult and aged animals (p=0.56). (B) There was no significant effect of frailty index score on HR (p=0.33). Bars represent mean±SEM. Age: 9m (n=11); 21m (n=21). FI score: <0.250 (n=24); >0.250 (n=8).

5.1.2. Rate pressure product

Baseline RPP was similar between adult and aged rats (31±9 vs. 30±6 x 10^3 mmHg*min^{-1}; p=0.11). There was no significant effect of frailty on RPP (FI<0.250, 30±6 vs. FI<0.250, 31±11 x 10^3 mmHg*min^{-1}; p=0.29) (figure 34).

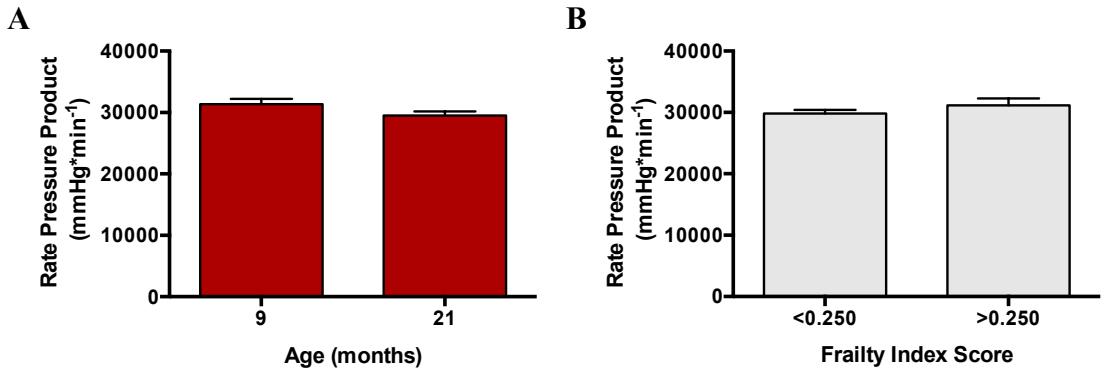

Figure 34. Baseline rate pressure product (RPP) in the isolated working heart. (A) RPP was not affected by age (p=0.11). (B) RPP was similar between frail and non-frail animals at baseline (p=0.29). Bars represent mean±SEM. Age: 9m (n=11); 21m (n=21). FI score: <0.250 (n=24); >0.250 (n=8).

5.1.3 Left ventricular developed pressure

LVDP was similar between adult and aged rats (117±3 vs. 114±2 mmHg; p=0.29). LVDP was not affected by FI score (114±3 vs. 115±2 mmHg; p=0.82) (figure 35).

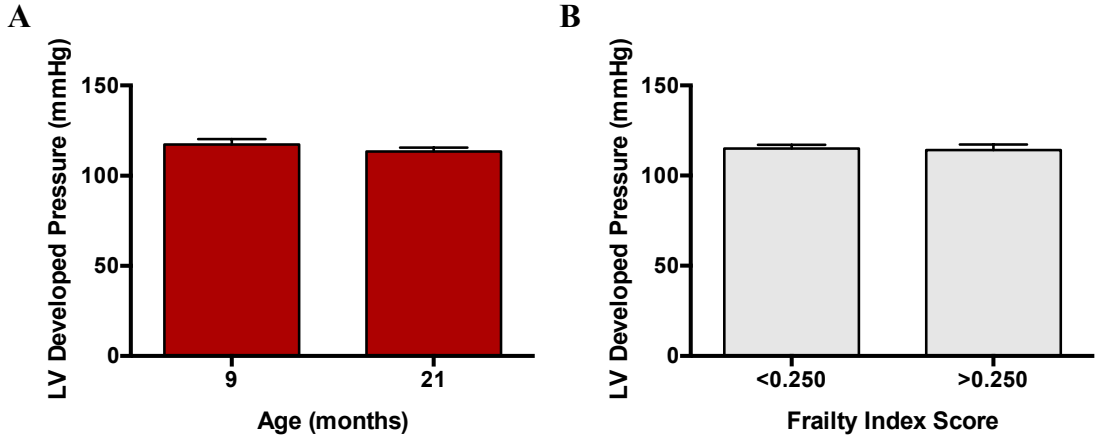

Figure 35. Baseline left ventricular developed pressure (LVDP) in the isolated working heart. (A) LVDP was similar between aged and adult rats (p=0.29). (B) There was no significant effect of frailty on LVDP (p=0.82). Bars represent mean±SEM. Age: 9m (n=11); 21m (n=21). FI score: <0.250 (n=24); >0.250 (n=8).

5.1.4 Coronary flow

CF tended to be lower in aged animals, however this did not attain statistical significance (4.0±0.1 vs. 4.3±0.3 ml*min^{-1}*cm^{-1}; p=0.20). CF was similar between frail and non-frail animals (4.0±0.1 vs. 4.0±0.1 ml*min^{-1}*cm^{-1}; p=0.92) (figure 36).

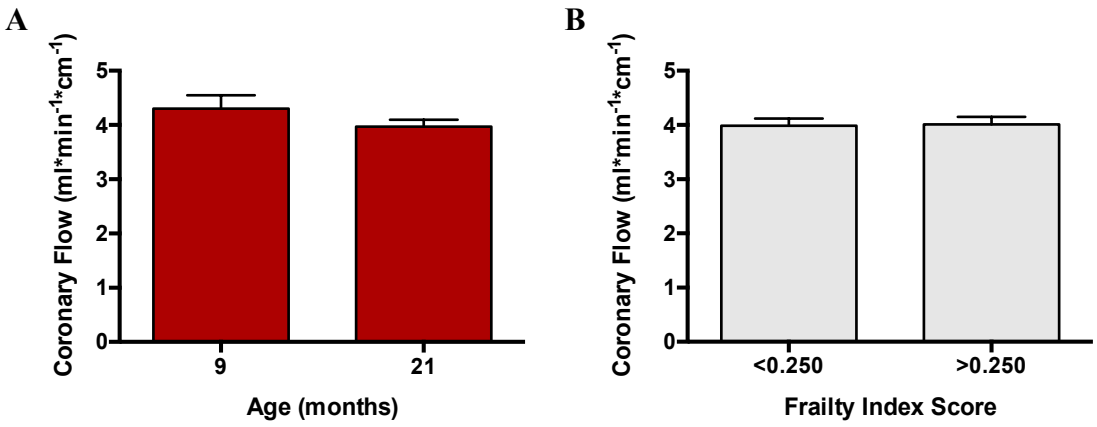

Figure 36. Baseline coronary flow (CF) in the isolated working heart. (A) CF tended to be lower in aged animals, however this was not statistically significant (p=0.20). (B) There was no significant effect of frailty index score on CF at baseline (p=0.92). Bars represent mean±SEM. Age: 9m (n=11); 21m (n=21). FI score: <0.250 (n=24); >0.250 (n=8).

5.1.5 Cardiac output

Aged animals tended to have a lower CO relative to adult animals; however this was not statistically significant (9.9±0.4 vs. 10.9±0.5 ml*min^{-1}*cm^{-1}; p=0.18). There was no significant effect of FI score on CO (FI<0.250, 10.4±0.4 vs. FI>0.250, 10.0±0.7 ml*min^{-1}*cm^{-1}; p=0.63) (figure 37).

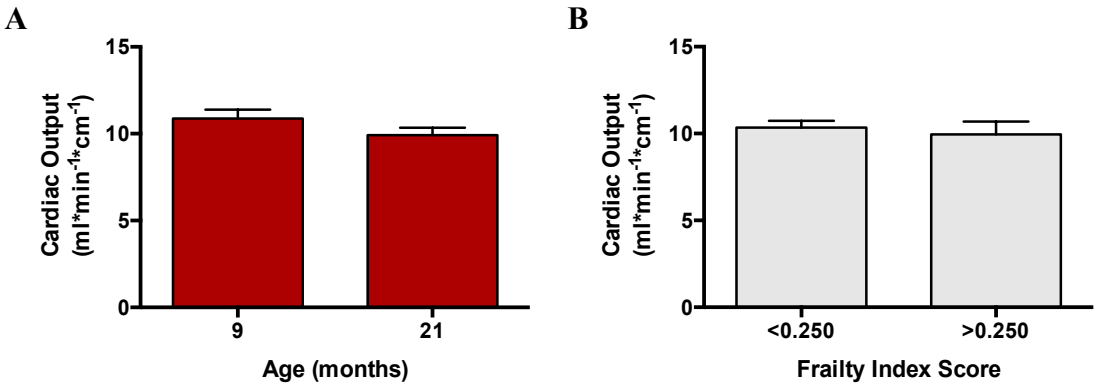

Figure 37. Baseline cardiac output (CO) in the isolated working heart. (A) CO tended to be lower in aged hearts, however this did not attain statistical significance (p=0.18). (B) CO was similar between frail and non-frail animals (p=0.63). Bars represent mean±SEM. Age: 9m (n=11); 21m (n=21). FI score: <0.250 (n=24); >0.250 (n=8).

5.1.6 Stroke volume

SV tended to be lower in aged animals (9 months, 46±2 vs. 21 months, 42±1 x 10^{-3} ml*cm^{-1}; p=0.16), and frail animals (FI score >0.250, 41±2 vs. FI score <0.250, 44±1 x 10^{-3} ml*cm^{-1}; p=0.16) (figure 38).

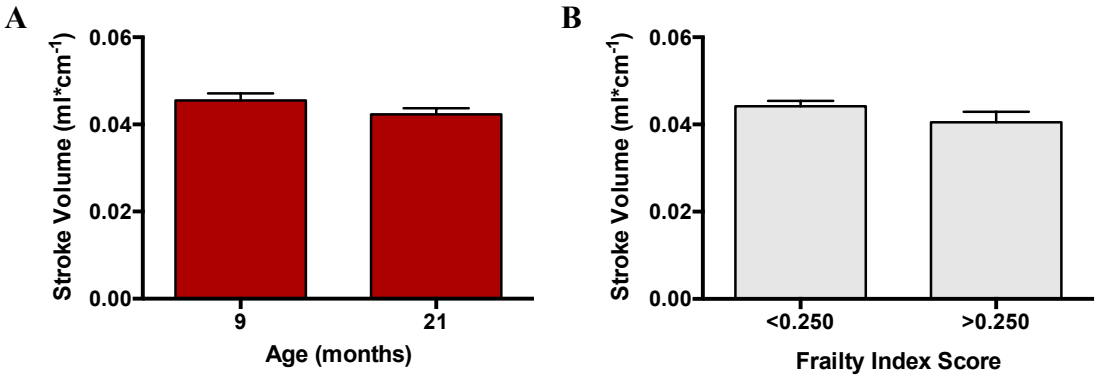

Figure 38. Baseline stroke volume (SV) in the isolated working heart. Baseline SV tended to be lower in both (A) aged (p=0.16), and (B) frail animals (p=0.16), however this did not attain statistical significance. Bars represent mean±SEM. Age: 9m (n=11); 21m (n=21). FI score: <0.250 (n=24); >0.250 (n=8).

5.1.7 Stroke work

Aged animals tended to have a reduced SW relative to adults (5.3±0.2 vs. 6.0±0.3 ml*mmHg*cm^{-1}; p=0.09). SW also tended to be lower in frail animals (FI score >0.250, 5.2±0.3 vs. FI score<0.250, 5.7±0.2 ml*mmHg*cm^{-1}; p=0.24) (figure 39).

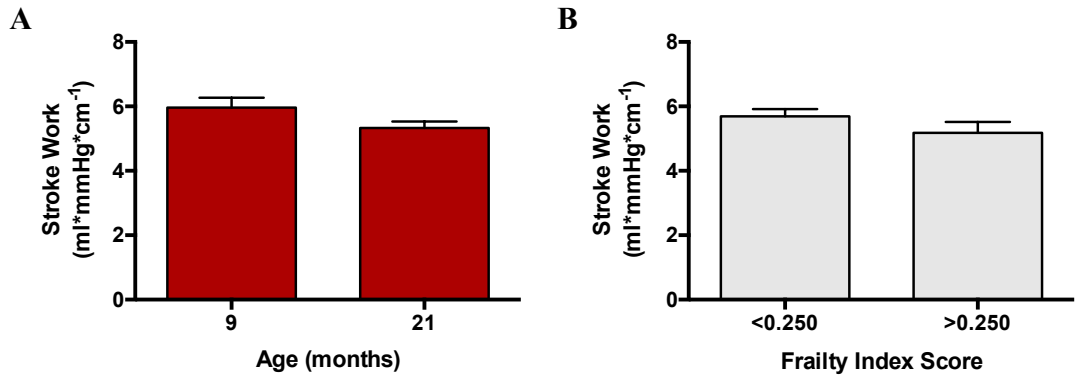

Figure 39. Baseline stroke work (SW) in the isolated working heart. (A) SW tended to be lower in aged (p=0.09), and (B) frail animals (p=0.24), however this did not attain statistical significance. Bars represent mean±SEM. Age: 9m (n=11); 21m (n=21). FI score: <0.250 (n=24); >0.250 (n=8).

5.1.8 Coronary vascular resistance

There was no significant effect of age on CVR (9 months, 1.4±0.1 vs. 21 months, 1.4±0.1 mmHg*ml^{-1}*min*cm^{-1}; p=0.56). CVR was similar between frail and non-frail animals (1.3±0.1 vs. 1.4±0.1 mmHg*ml^{-1}*min*cm^{-1}; p=0.43) (figure 40).

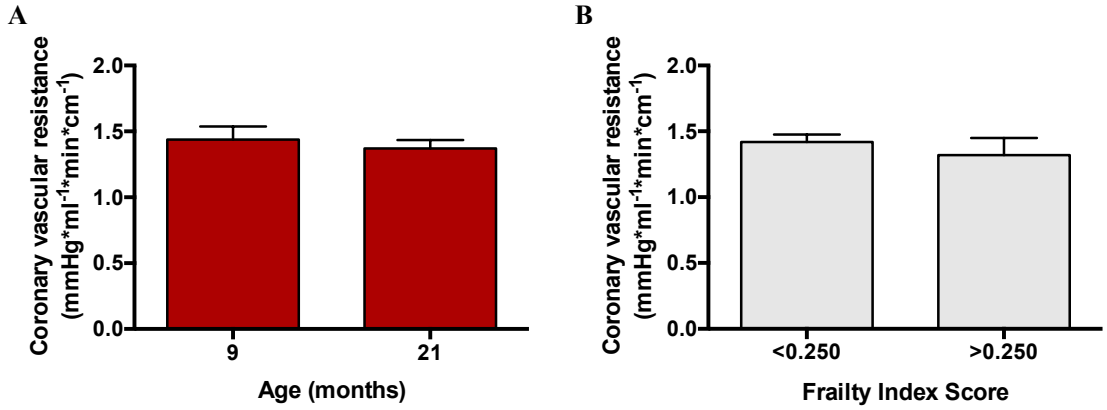

Figure 40. Baseline coronary vascular resistance (CVR) in the isolated working heart. (A) CVR was similar between aged and adult rats at baseline (p=0.56). (B) There was no significant effect of frailty index score on CVR (p=0.43). Bars represent mean±SEM. Age: 9m (n=11); 21m (n=21). FI score: <0.250 (n=24); >0.250 (n=8).

5.2 Arrhythmia

To evaluate arrhythmia, we looked for incidences of VT, VF and PVCs on the ECG or for irregularities in the aortic pressure wave during the baseline working heart period. Aged hearts were more likely to present with arrhythmia relative to adult hearts (9

months, 1/11 hearts arrhythmic vs. 21 months, 8/21 hearts arrhythmic; $p<0.05$). Frail hearts had a greater incidence of arrhythmia at baseline relative to less frail hearts (FI score >0.250, 4/8 hearts arrhythmic vs. FI score <0.250, 4/21 hearts arrhythmic; $p<0.05$) (figure 41).

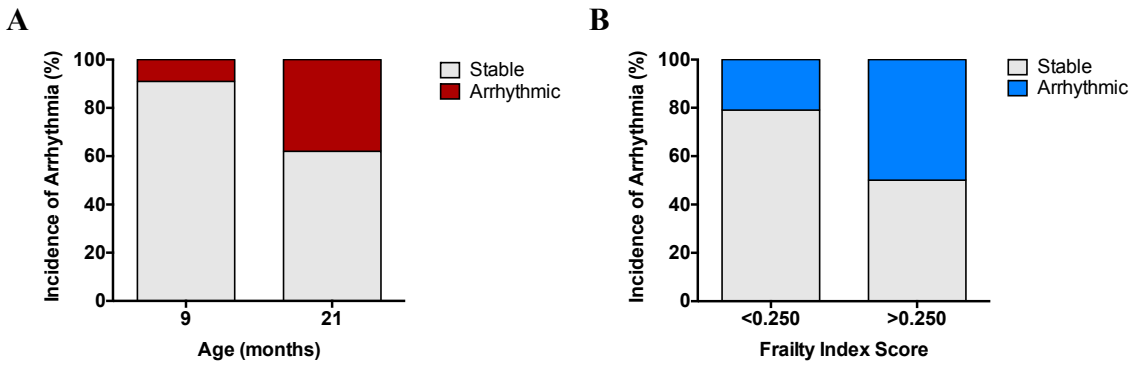

Figure 41. Baseline arrhythmia incidence in the isolated working heart. (A) Aged hearts were more likely to present with arrhythmia at baseline relative to adult hearts ($p<0.05$). (B) Frail hearts were more arrhythmic at baseline relative to less frail hearts ($p<0.05$). Bars represent mean±SEM. Age: 9m (n=11); 21m (n=21). FI score: <0.250 (n=24); >0.250 (n=8).

5.3 Diastolic function

We developed a novel method to assess diastolic function using the isolated working heart preparation in conjunction with echocardiography (as previously described in methods section 4.5.6. Baseline EDV tended to be lower in aged animals (9 months, 0.26±0.01 vs. 21 months, 0.24±0.01 ml; $p=0.29$), however this did not attain statistical significance. There was no significant effect of frailty on baseline EDV (FI score <0.250, 0.25±0.01 vs. FI score >0.250, 0.24±0.02; $p=0.89$) (figure 42).

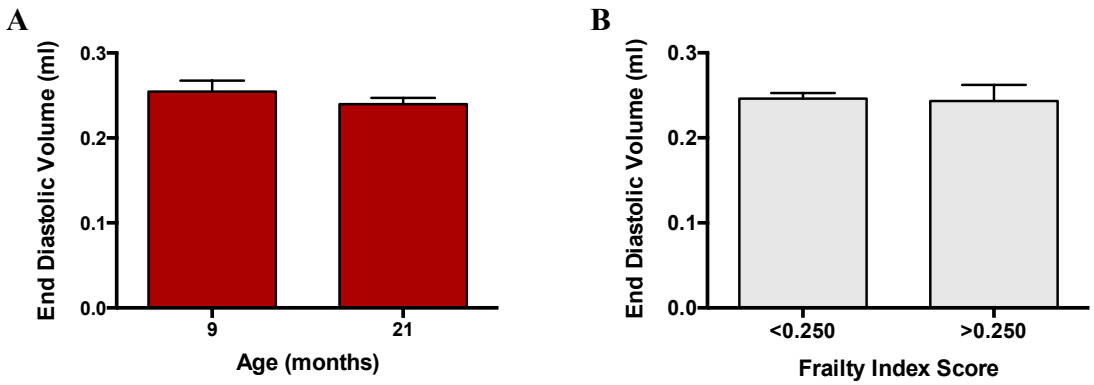

Figure 42. Baseline diastolic function in the isolated working heart. (A) EDV tended to be lower in aged animals, however this did not attain statistical significance (p=0.29). (B) EDV was similar between frail and non-frail animals (p=0.89). Bars represent mean±SEM. Age: 9m (n=11); 21m (n=18). FI score: <0.250 (n=23); >0.250 (n=6).

6. Recovery from cardioplegic arrest in the isolated working heart

6.1 Hemodynamic measurements

Following the characterization of baseline cardiac function in the isolated working heart by age and FI score, we sought to determine if old age or frailty affected recovery from del Nido cardioplegia. Baseline measurements were recorded in working heart mode as previously described. The heart was then arrested with del Nido cardioplegia (60 min). Following a 20-minute reperfusion period, hearts were switched back into working heart mode. Hemodynamic measurements were collected at the following time points during the post-reperfusion period: 0, 5, 10, 15, 30, 45 and 60 minutes. Table 8 summarizes a comparison of the hemodynamic parameters measured in working heart mode between adult (9-month, n=11) and aged rats (21-month, n=21). Table 9 summarizes a comparison of the hemodynamic parameters between frail (FI score >0.250, n=8) and non-frail (FI score <0.250, n=24) animals.

Table 8. Recovery of hemodynamic parameters following arrest with del Nido cardioplegia in relation to age.

		Post-reperfusion (min)						
	Baseline	***0***	***5***	***10***	***15***	***30***	***45***	***60***
Heart rate (bpm)								
Adult	241±10	255±14	250±11	254±11	252±11	257±8	272±14	252±6
Aged	234±7	236±7	242±10	242±8	240±8.5	239±7	241±7	234±6
Rate pressure product ($x10^3$ mmHg*min^{-1})								
Adult	31±9	31±11	30±9	30±9	30±10	30±8	30±11	28±6
Aged	30±6	28±7	29±10	28±6	28±7	27±6	27±5	26±5
Left ventricular developed pressure (mmHg)								
Adult	117±3	110±3	109±3	107±3	107±2	102±2	98±2	98±1
Aged	113±2	108±2	106±2	105±2	104±2	102±2	100±2	98±2
Coronary flow (ml*min^{-1}*cm^{-1})								
Adult	4.3±0.3	4.4±0.2	4.1±0.2	4.1±0.2	4.2±0.2	4.3±0.2	4.2±0.2	4.0±0.2
Aged	4.0±4.3	4.3±0.1	4.0±0.1	4.0±0.1	4.0±0.1	4.0±0.1	3.9±0.1	3.8±0.1
Cardiac output (ml*min^{-1}*cm^{-1})								
Adult	10.9±0.5	9.3±0.5	9.3±0.4	9.3±0.4	9.5±0.4	9.0±0.3	8.5±0.4	7.6±0.3
Aged	9.9±0.4	8.5±0.4	8.6±0.3	8.6±0.3	8.4±0.3	8.4±0.3	8.0±0.3	7.3±0.3
Stroke volume ($x10^{-3}$ ml*$cm^{-1)}$								
Adult	46±2	37±1	38±1	37±1	38±1	35±2	33±2	31±2
Aged	42±1	36±1	36±1	36±1	35±1	35±1	33±1	31±1
Stroke work (ml*mmHg*cm^{-1})								
Adult	6.0±0.3	4.5±0.2	4.6±0.2	4.4±0.2	4.6±0.2	4.1±0.2	3.7±0.2	3.5±0.2
Aged	5.3±0.2	4.3±0.2	4.2±0.2	4.2±0.2	4.1±0.2	4.0±0.2	3.8±0.2	3.5±0.2

Table 9. Recovery of hemodynamic parameters following arrest with del Nido cardioplegia in relation to frailty.

		Post-reperfusion (min)						
	Baseline	***0***	***5***	***10***	***15***	***30***	***45***	***60***
Heart rate (bpm)								
Frail	233±6	243±8	242±7	245±7	241±7	243±6	251±8	240±5
NF	246±10	241±11	255±20	250±16	254±17	252±13	254±14	241±10
Rate pressure product (x10^3 mmHg*min^{-1})								
Frail	30±6	29±6	29±6	28±7	28±6	28±8	27±5	29±6
NF	31±11	30±11	30±8	30±9	29±9	29±7	27±6	30±11
Left ventricular developed pressure (mmHg)								
Frail	115±2	108±2	107±2	105±2	105±2	101±1	98±1	98±1
NF	114±3	109±4	107±5	107±5	106±4	103±3	101±4	100±3
Coronary flow (ml*min^{-1}*cm^{-1})								
Frail	4.0±0.1	4.2±0.1	3.9±0.1	4.0±0.1	4.0±0.1	4.0±0.1	3.9±0.1	3.8±0.1
NF	4.0±0.1	4.4±0.2	3.9±0.1	4.0±0.1	3.9±0.1	4.1±0.3	4.1±0.3	3.9±0.3
Cardiac output (ml*min^{-1}*cm^{-1})								
Frail	10.3±0.4	8.8±0.3	8.9±0.3	8.8±0.3	8.8±0.3	8.5±0.3	8.1±0.3	7.4±0.2
NF	10.0±0.7	8.2±0.8	8.2±0.6	8.2±0.7	8.2±0.7	8.2±0.8	7.6±0.7	7.0±0.7
Stroke volume (x10^{-3} ml*cm$^{-1)}$								
Frail	44±1	37±1	37±1	37±1	35±1	33±1	31±1	37±1
NF	41±2	33±3	33±3	33±3	33±3	31±3	30±3	33±3
Stroke work (ml*mmHg*cm^{-1})								
Frail	5.7±0.2	4.5±0.2	4.5±0.1	4.3±0.1	4.4±0.2	4.1±0.2	3.8±0.2	3.5±0.2
NF	5.2±0.3	4.1±0.4	4.0±0.4	4.1±0.4	4.0±0.4	3.9±0.4	3.5±0.4	3.3±0.3

Abbreviations: NF = non-frail.

6.1.1 Heart rate

HR tended to be higher in adult rats throughout the post-reperfusion period relative to aged animals; however this was not statistically significant (p=0.15). There was no significant effect of age on functional recovery from arrest: adult rats recovered to 106% of values attained at baseline; aged animals had a 100% recovery. HR was consistent from beginning to end of the reperfusion period in both adult (0 min=255±14 vs. 252±6 bpm; p=0.99) and aged (0 min=236±7 vs. 234±6 bpm; p=0.99) animals (figure 43).

HR was similar between frail and non-frail rats during the post-reperfusion period (p=0.58). FI score had no effect on recovery from arrest: frail animals recovered to 104% of values attained at baseline while non-frail rats had a 98% recovery. HR was consistent throughout the post-reperfusion period in both frail (0 min = 243±8 vs. 60 min 240±5 bpm; p=0.99) and non-frail (0 min = 241±11 vs. 60 min = 241±10 bpm; p=0.99) animals (figure 43).

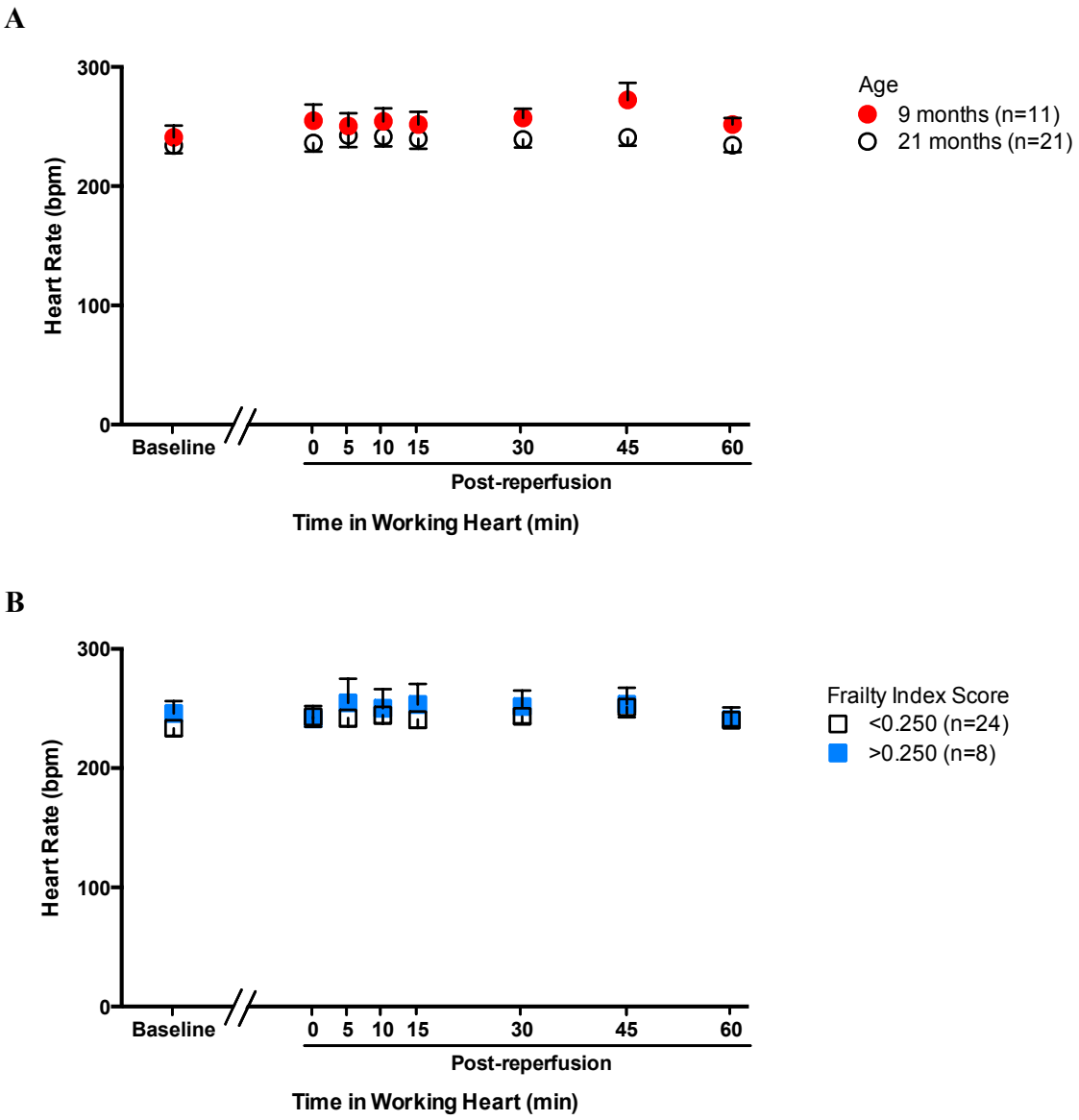

Figure 43. Recovery of heart rate (HR) following arrest with cardioplegia is similar by age and FI score. (A) HR tended to be higher in adult rats throughout the post-reperfusion period; however percent recovery was not affected by age (p=0.15). (B) Recovery of HR was similar between frail and non-frail animals (p=0.58). Data points represent mean±SEM. Age: 9m (n=11); 21m (n=21). FI score: <0.250 (n=24); >0.250 (n=8).

6.1.2 Rate pressure product

RPP was consistently higher in adult rats throughout the post-reperfusion period relative to aged animals ($p<0.05$). Adult rats recovered to 100% of values attained at baseline; aged animals recovered to 93% of baseline values. RPP declined similarly throughout the post-reperfusion period in adult and aged animals: it declined by 10% in adult animals (0 min = 31±11 vs. 60 min = 28±6 x 10^3 mmHg*min^{-1}; $p<0.05$), and by 7% in aged animals (0 min = 28±7 vs. 26±5 x 10^3 mmHg*min^{-1}; $p<0.05$) (figure 44).

There was no effect of FI score on RPP in the post-reperfusion period ($p=0.39$). Both groups recovered to 97% of values attained at baseline. There was no significant decline in RPP throughout the post-reperfusion period in frail rats (0 min = 29±6 vs. 60 min = 29±6 x 10^3 mmHg*min^{-1}; $p<0.05$); however RPP significantly declined in non-frail animals (0 min = 30±11 vs. 60 min = 30±11 x 10^3 mmHg*min^{-1}; $p<0.05$) (figure 44).

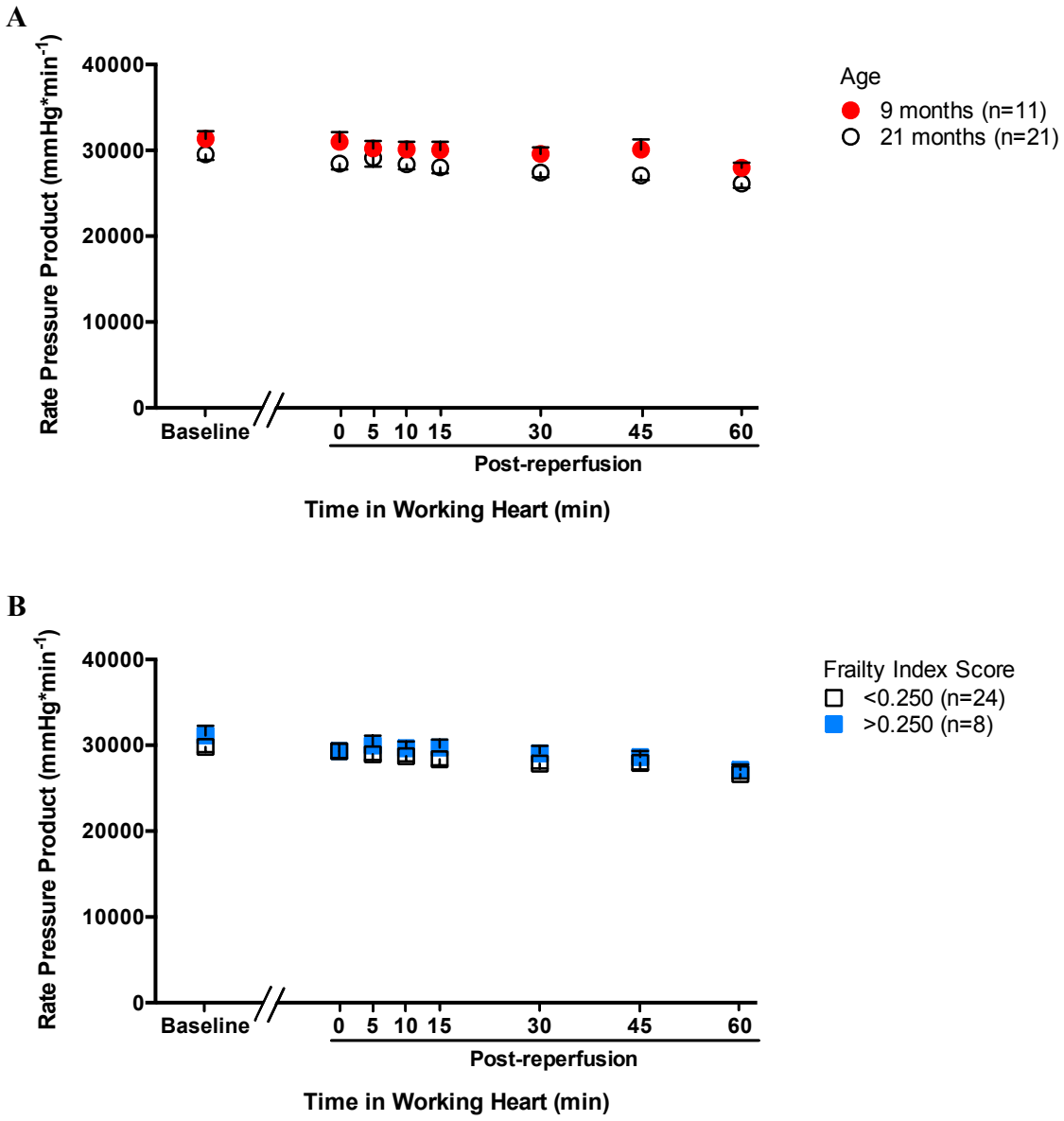

Figure 44. Recovery of rate pressure product (RPP) following arrest with cardioplegia is similar by age and FI score. (A) RPP tended to be higher in adult animals in the post-reperfusion period ($p<0.05$). (B) Recovery of RPP was analogous between frail and non-frail animals ($p=0.39$). Data points represent mean±SEM. Age: 9m (n=11); 21m (n=21). FI score: <0.250 (n=24); >0.250 (n=8).

6.1.3 Left ventricular developed pressure

There was no effect of age on recovery of LVDP following arrest with cardioplegia ($p=0.64$). Adult animals recovered to 94% of baseline values while aged animals recovered to 96%. LVDP declined similarly throughout the remainder of the post-reperfusion period: LVDP declined by 11% in adult animals (0 min = 110±3 mmHg; $p<0.05$) and by 10% in aged animals (0 min = 108±2 vs. 60 min = 98±2 mmHg; $p<0.05$) (figure 45).

LVDP was similar between frail and non-frail rats throughout the post-reperfusion period ($p=0.75$). Frail rats recovered to 94% of baseline values while non-frail rats experienced a 96% recovery. There was a similar decline from beginning to end of the post-reperfusion period between groups: LVDP declined by 10% in frail animals (0 min = 98±1 vs. 108±2 mmHg; $p<0.05$) and by 8% in non-frail animals (0 min = 109±4 vs. 60 min = 100±3 mmHg; $p<0.05$) (figure 45).

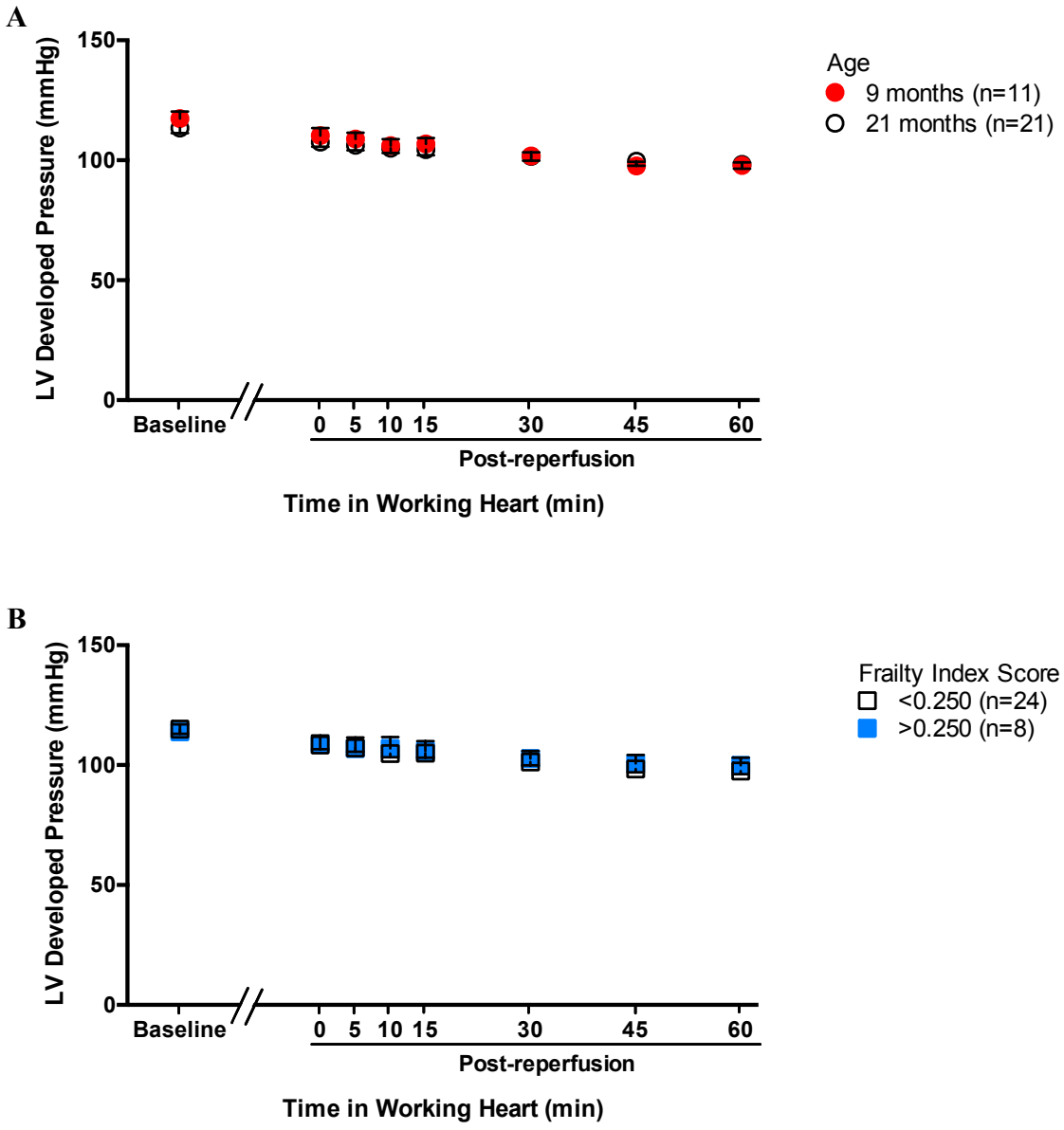

Figure 45. Recovery of left ventricular developed pressure (LVDP) is similar when compared by age and frailty index score. (A) Recovery of LVDP from arrest was not affected by age (p=0.64). (B) There was no significant effect of frailty index score on LVDP in the post-reperfusion period (p=0.75). Data points represent mean±SEM. Age: 9m (n=11); 21m (n=21). FI score: <0.250 (n=24); >0.250 (n=8).

6.1.4 Coronary flow

CF tended to be higher in adult rats relative to aged rats during the post-reperfusion period; however this was not statistically significant (p=0.31). There was no significant effect of age on recovery of CF from cardioplegic arrest: adult rats recovered to 102% of baseline values while aged animals recovered to 108%. CF declined by 9% throughout the post-reperfusion period in adult rats (0 min = 4.4±0.2 vs. 60 min 4.0±0.2 $ml*min^{-1}*cm^{-1}$; p=0.09), and by 12% in aged animals (0 min = 4.3±0.1 vs. 60 min = 3.8±0.1 $ml*min^{-1}*cm^{-1}$; p=0.35) (figure 46).

CF was similar between frail and non-frail animals from beginning to end of the post-reperfusion period (p=0.76). Frail animals recovered to 105% of baseline values while non-frail rats recovered to 110%. There was a similar decline in CF throughout the post-reperfusion period between groups: it declined by 10% in frail animals (0 min = 4.2±0.1 vs. 60 min 3.8±0.1 $ml*min^{-1}*cm^{-1}$; p=0.99), and by 11% in non-frail animals (0 min = 4.4±0.2 vs. 60 min = 3.9±0.3 $ml*min^{-1}*cm^{-1}$; p=0.38) (figure 46).

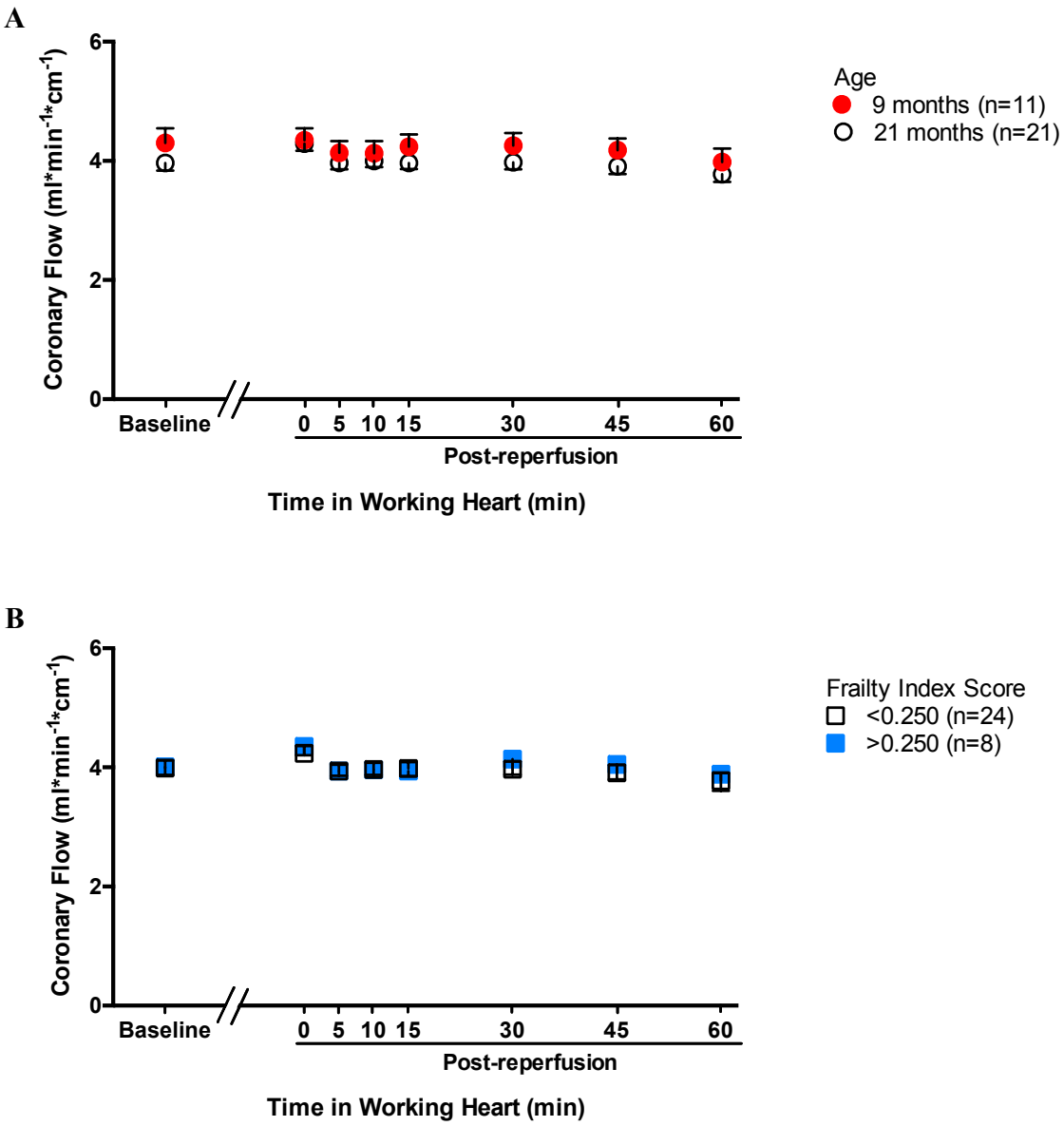

Figure 46. Recovery of coronary flow (CF) is similar when compared by age and frailty index score. (A) CF tended to be higher in adult animals in the post-reperfusion period; however percent recovery was similar (p=0.31). (B) Recovery of CF was analogous between frail and non-frail animals (p=0.76). Data points represent mean±SEM. Age: 9m (n=11); 21m (n=21). FI score: <0.250 (n=24); >0.250 (n=8).

6.1.5 Cardiac output

CO tended to be higher in adult animals throughout the post-reperfusion working heart period; however this was not statistically significant (p=0.14). Recovery of CO from cardioplegic arrest was similar in both groups: adults recovered to 85% of baseline values while aged animals recovered to 86%. There was a similar decline in CO from beginning to end of the post-reperfusion period: CO declined by 18% in adult animals (0 min = 9.3±0.5 vs. 60 min = 7.6±0.4 $ml*min^{-1}*cm^{-1}$; $p<0.05$), and by 14% in the aged animals (0 min = 8.5±0.4 vs. 60 min = 7.3±0.3 $ml*min^{-1}*cm^{-1}$; $p<0.05$) (figure 47).

CO tended to be lower in the frail group of animals throughout the entire post-reperfusion period; however this was not statistically significant (p=0.37). Frail animals recovered to 85% of baseline CO values while less frail animals recovered to 82%. CO declined similarly throughout the post-reperfusion period between groups: it declined by 16% in frail animals (0 min = 8.8±0.3 vs. 60 min = 7.4±0.2 $ml*min^{-1}*cm^{-1}$; $p<0.05$), and by 15% in non-frail animals (0 min = 8.2±0.8 vs. 60 min = 7.0±0.7 $ml*min^{-1}*cm^{-1}$; $p<0.05$) (figure 47).

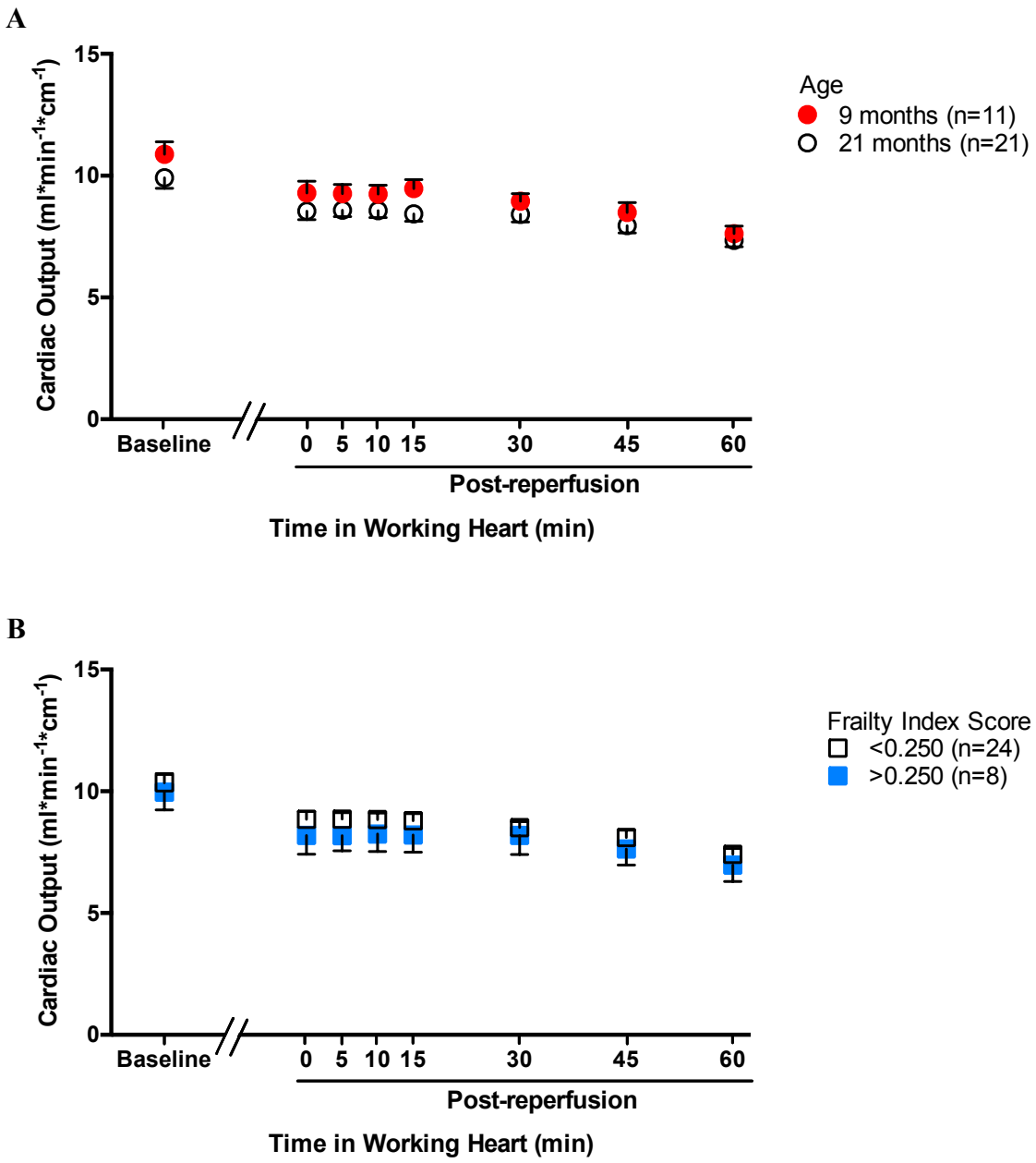

Figure 47. Recovery of cardiac output (CO) is similar when compared by age and frailty index score. (A) Aged rats tended to have a reduced CO throughout the post-reperfusion period; percent recovery from arrest was similar (p=0.14). (B) CO tended to be lower in frail animals in the post-reperfusion period. Recovery was similar between groups (p=0.37). Data points represent mean±SEM. Age: 9m (n=11); 21m (n=21). FI score: <0.250 (n=24); >0.250 (n=8).

6.1.6 Stroke volume

There was no significant effect of age on recovery of SV in the post-reperfusion working heart period ($p=0.50$). Following arrest, SV recovered to 80% of baseline values in adult rats, and 86% in aged animals. There was similar decline in SV between the two groups during the post-reperfusion period: it declined by 16% in adults (0 min = 37±1 vs. 60 min = 31±2 x 10^3 ml*cm^{-1}; $p<0.05$), and by 14% in aged animals (0 min = 36±1 vs. 60 min = 31±1 x 10^{-3} ml*cm^{-1}; $p<0.05$) (figure 48).

Frail animals tended to have a lower SV throughout the post-reperfusion working heart period; however this was not statistically significant ($p=0.17$). Following arrest, SV recovered to 84% of baseline values in frail animals, and 81% in non-frail animals. There was no decline in SV from beginning to end of the post-reperfusion period in frail (0 min = 37±1 vs. 60 min = 37±1 x 10^{-3} ml*cm^{-1}; $p<0.05$) or non-frail animals (0 min = 33±3 vs. 60 min = 33±3 x 10^{-3} ml*cm^{-1}; $p<0.05$) (figure 48).

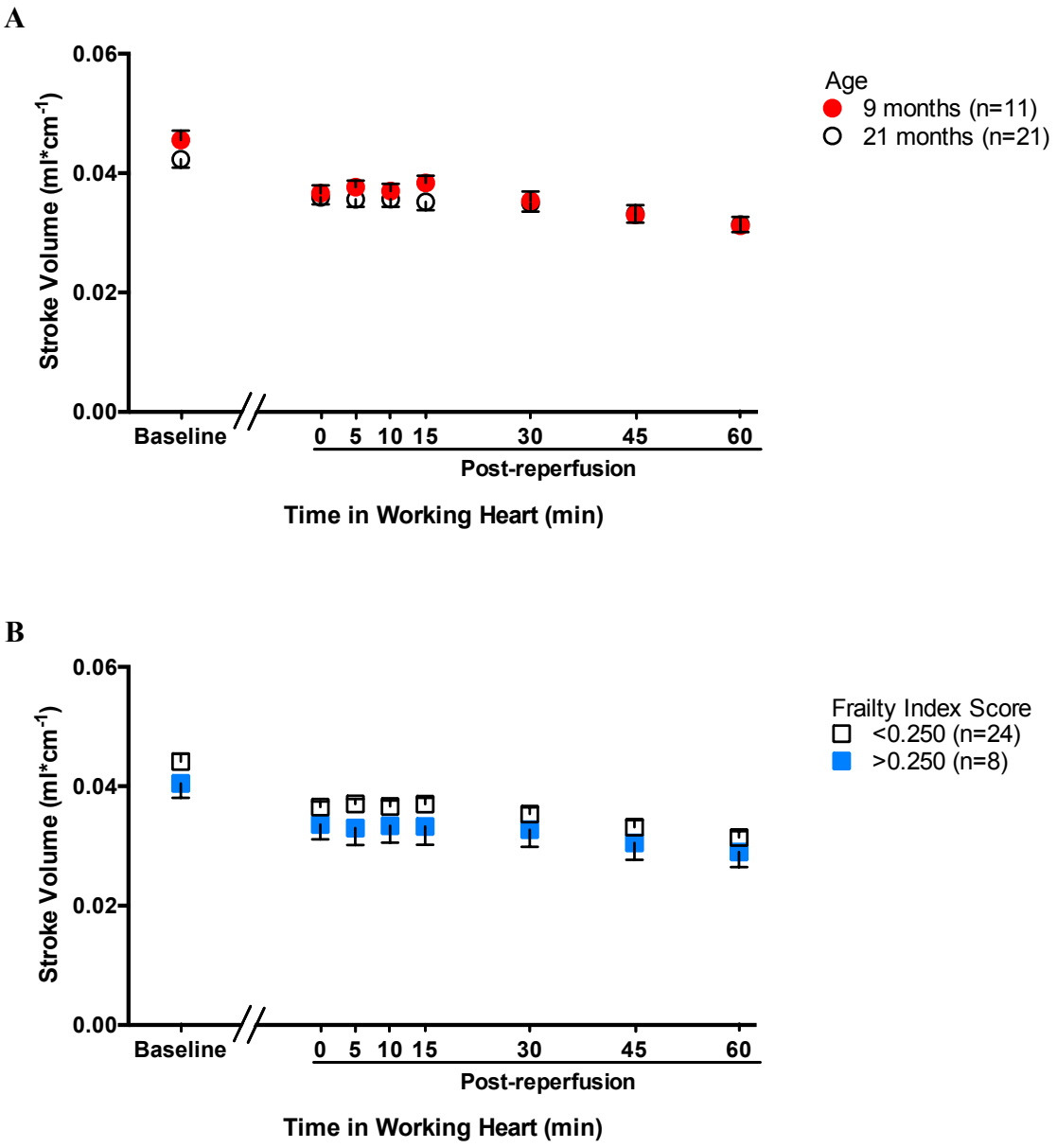

Figure 48. Recovery of stroke volume (SV) is not affected by age or frailty index score. (A) SV declined following arrest with cardioplegia. Recovery was similar between aged and adult animals (p=0.50). (B) SV tended to be lower in frail animals throughout the post-reperfusion period. Recovery was not affected by FI score (p=0.17). Data points represent mean±SEM. Age: 9m (n=11); 21m (n=21). FI score: <0.250 (n=24); >0.250 (n=8).

6.1.7 Stroke work

There was no significant effect of age on SW in the post-reperfusion working heart period (p=0.41). Following arrest, SW recovered to 75% of baseline values in adults and to 81% in aged rats. SW declined throughout the post-reperfusion period: it declined by 23% in adult rats (0 min = 4.5±0.2 vs. 3.5±0.2 ml*mmHg*cm^{-1}; $p<0.05$), and by 19% in aged rats (0 min = 4.3±0.2 vs. 60 min = 3.5±0.2 ml*mmHg*cm^{-1}; $p<0.05$) (figure 49).

SW tended to be lower in the post-reperfusion period in frail animals; however this was not statistically significant (p=0.32). Both groups recovered to 79% of values attained at baseline. SW declined from beginning to end of the post-reperfusion period: it declined by 23% in frail rats (0 min = 4.5±0.2 vs. 60 min = 3.5±0.2 ml*mmHg*cm^{-1}; $p<0.05$), and by 19% in non-frail rats (0 min = 4.1±0.4 vs. 60 min = 3.3±0.3 ml*mmHg*cm^{-1}; $p<0.05$) (figure 49).

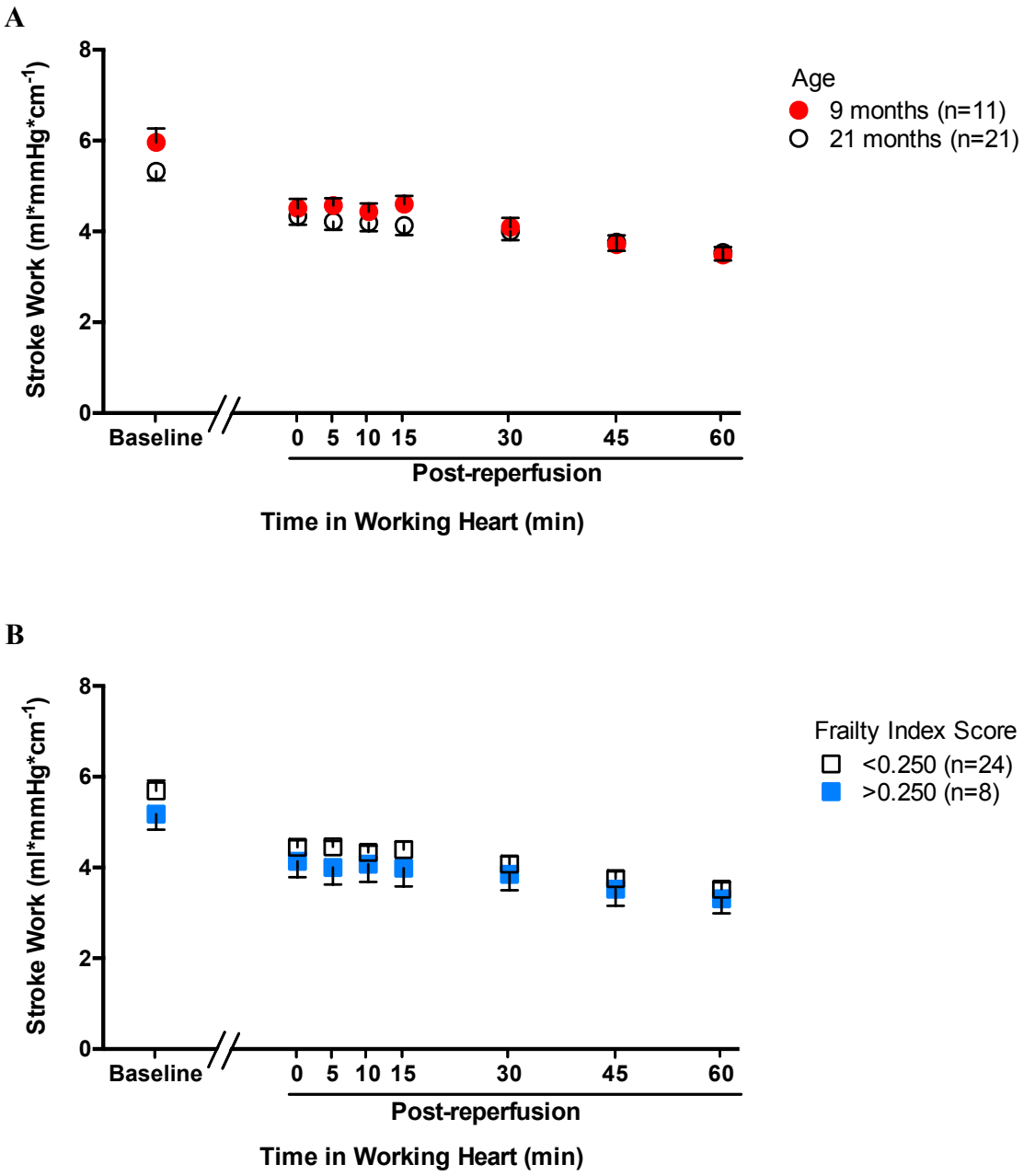

Figure 49. Recovery of stroke work (SW) was similar when compared by age and frailty index score. (A) There was a sharp decline in SW following arrest with cardioplegia ($p<0.05$). Recovery was similar between aged and adult animals ($p=0.41$). (B) SW declined following arrest ($p<0.05$). Frail animals had a reduced SW throughout the post-reperfusion period. Recovery was similar between groups ($p=0.32$). Data points represent mean±SEM. Age: 9m (n=11); 21m (n=21). FI score: <0.250 (n=24); >0.250 (n=8).

6.1.8 Coronary vascular resistance

There was no significant effect of age on CVR during the reperfusion period ($p=0.36$). CVR declined by 17% following arrest with cardioplegia in adult hearts (baseline = 1.44±0.10 vs. reperfusion = 1.19±0.10 $mmHg*ml^{-1}*min^{-1}*cm^{-1}$; $p<0.05$), and by 20% in aged hearts (baseline = 1.37±0.06 vs. reperfusion = 1.10±0.02 $mmHg*ml^{-1}*min^{-1}*cm^{-1}$; $p<0.05$) (figure 50).

There was no significant effect of FI score on CVR during the reperfusion period ($p=0.49$). CVR declined by 16% following arrest in the frail hearts (baseline = 1.32±0.13 vs. reperfusion = 1.10±0.04 $mmHg*ml^{-1}*min^{-1}*cm^{-1}$; $p<0.05$), and by 20% in non-frail hearts (baseline = 1.42±0.06 vs. reperfusion = 1.14±0.05 $mmHg*ml^{-1}*min^{-1}*cm^{-1}$; $p<0.05$) (figure 50).

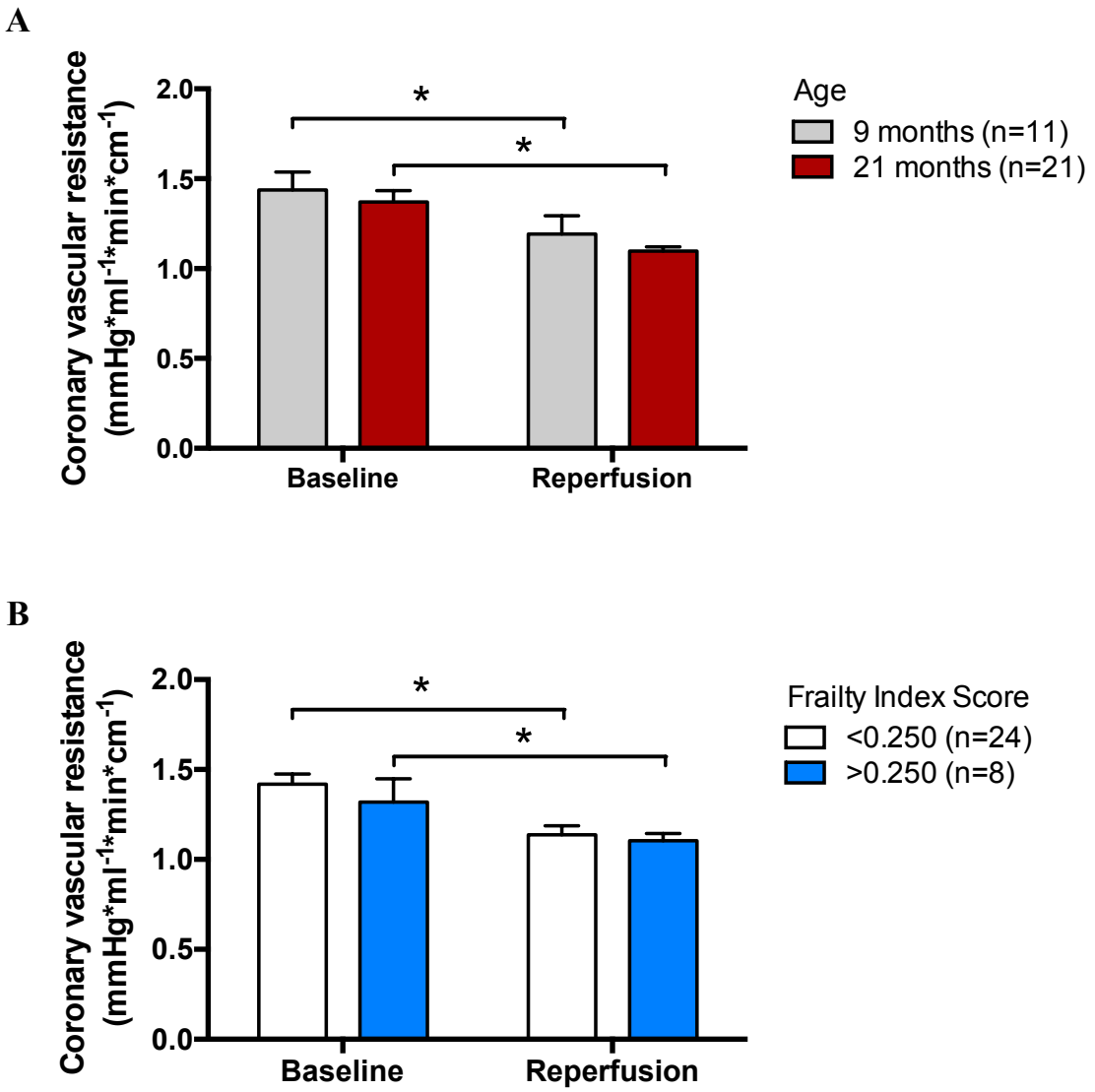

Figure 50. Recovery of coronary vascular resistance (CVR) is not affected by age or frailty index score. (A) CVR declined following arrest in both adult and aged animals ($p<0.05$). Recovery of CVR was similar between groups ($p=0.36$). (B) CVR declined following arrest in both frail and non-frail rats ($p<0.05$). Recovery was similar between groups ($p=0.49$). Bars represent mean±SEM. Age: 9m (n=11); 21m (n=21). FI score: <0.250 (n=24); >0.250 (n=8). *Denotes $p<0.05$ relative to baseline.

6.2 Arrhythmia

We looked for incidences of VT, VF and PVCs on the ECG and irregularities in the aortic pressure waveform throughout the reperfusion period. Aged hearts were just as likely to present with arrhythmia during reperfusion as adult hearts (9 months, 6/11 hearts arrhythmic vs. 21 months, 13/21 hearts arrhythmic; p=0.39). There was no significant effect of frailty on arrhythmia incidence during reperfusion (FI score >0.250, 4/8 hearts arrhythmic vs. FI score <0.250, 13/21 hearts arrhythmic; p=0.12) (figure 51).

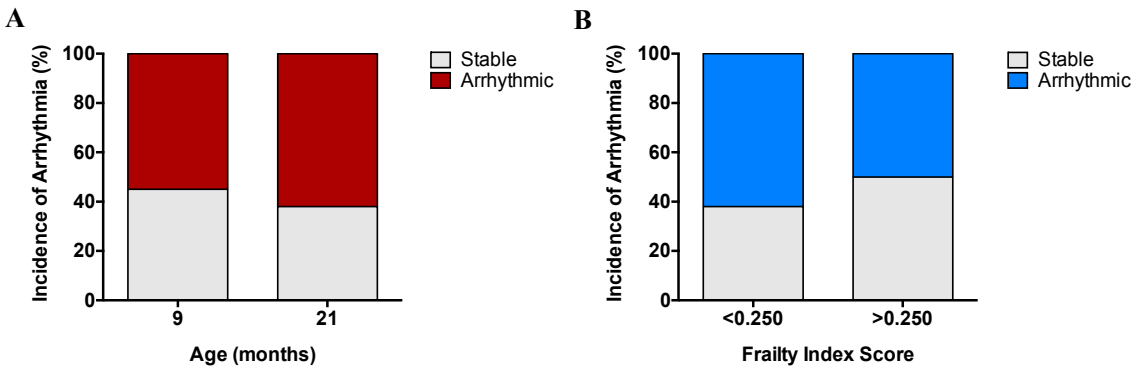

Figure 51. Arrhythmia incidence during reperfusion. (A) There was no significant effect of age on arrhythmia incidence during reperfusion (p=0.39). (B) Frail hearts were just as likely to present with arrhythmia during reperfusion as less frail hearts (p=0.12). Bars represent mean±SEM. Age: 9m (n=11); 21m (n=21). FI score: <0.250 (n=24); >0.250 (n=8).

6.3 Diastolic function

We developed a novel method to assess diastolic function using the isolated working heart in conjunction with echocardiography (described in methods section 4.5.6). Aged hearts tended to have a lower EDV following arrest with del Nido cardioplegia; however this was not statistically significant (p=0.25). EDV declined by 15% following arrest in both adult (baseline = 0.26±0.01 vs. reperfusion = 0.22±0.01 ml; p<0.05), and aged animals (baseline = 0.24±0.01 vs. reperfusion = 0.21±0.01 ml; p<0.05). Non-frail animals tended to recover better than frail animals: EDV declined by 13% following arrest in non-frail animals (baseline = 0.25±0.01 vs. reperfusion = 0.21±0.01 ml; p<0.05), and by 19% in frail animals (baseline = 0.24±0.02 vs. reperfusion = 0.19±0.02; p<0.05). Recovery between groups was not statistically significant (p=0.50) (figure 52).

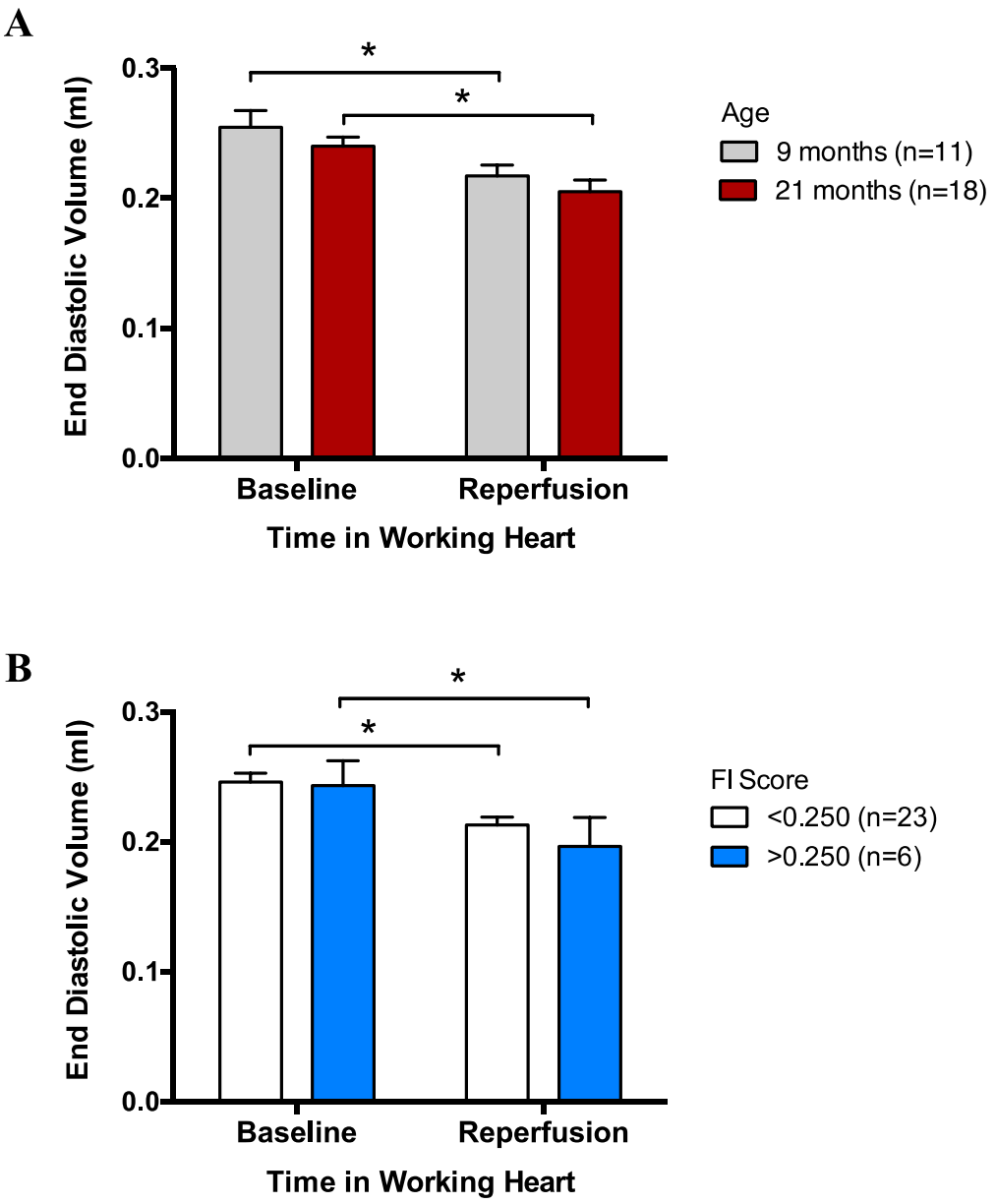

Figure 52. Recovery of diastolic function by age and frailty index score. EDV declined between baseline and reperfusion with both age ($p<0.05$) and frailty ($p<0.05$). (A) Percent recovery of EDV following arrest with del Nido cardioplegia was similar between aged and adult animals ($p=0.25$). (B) Recovery of EDV tended to be lower in frail animals, however this was not statistically significant ($p=0.50$). Bars represent mean±SEM. Age: 9m (n=11); 21m (n=18). FI score: <0.250 (n=23); >0.250 (n=6). *Denotes $p<0.05$ relative to baseline.

6.4 Troponin-I release into coronary effluent

To determine the extent of myocardial injury following arrest with del Nido cardioplegia, we measured levels of cTnI in the coronary effluent during reperfusion. cTnI levels were similar between adults and aged rats (0.07±0.06 vs. 0.10±0.08 ng/ml; p=0.85). Non-frail animals tended to have higher levels of cTnI relative to frail animals; however this was not statistically significant (0.11±0.01 vs. 0.02±0.02 ng/ml; p=0.47) (figure 53).

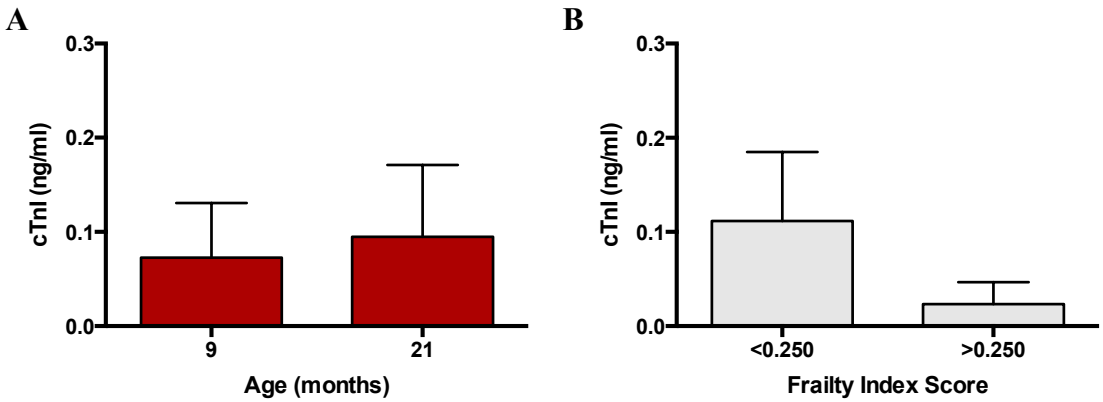

Figure 53. Cardiac troponin-I (cTnI) levels in coronary effluent following arrest with del Nido cardioplegia. (A) There was no significant effect of age on cTnI levels found in the coronary effluent (p=0.85). (B) Non-frail animals had higher levels of cTnI relative to frail animals (p=0.47). Bars represent mean±SEM. Age: 9m (n=11); 21m (n=21). FI score: <0.250 (n=24); >0.250 (n=8).

6.5 Myocardial edema

To evaluate the impact of age and frailty on myocardial edema, water content of the ventricular myocardium was measured at the end of each study. Indexed myocardial water was similar between adult and aged animals (0.175±0.004 vs. 0.171±0.004 $g*cm^{-1}$; p=0.51). There was no significant effect of FI score on indexed myocardial water (FI score >0.250, 0.178±0.006 vs. FI score <0.250, 0.171±0.003 $g*cm^{-1}$; p=0.24) (figure 54).

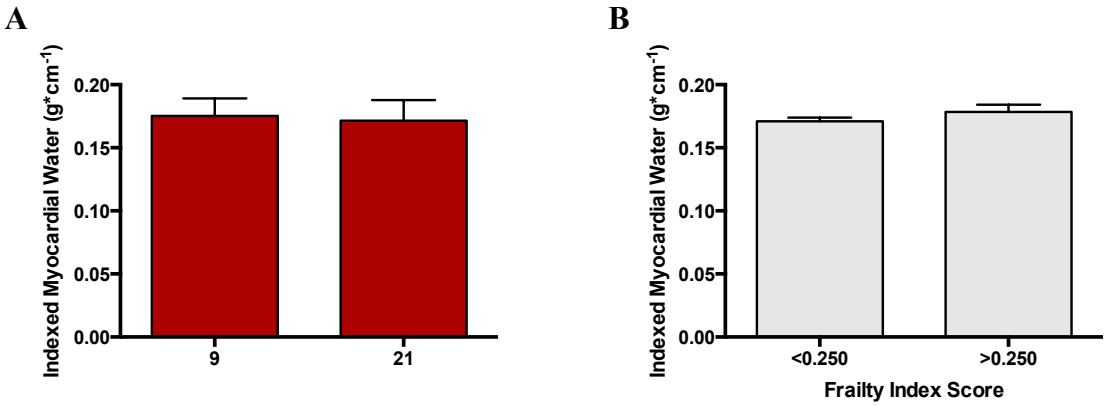

Figure 54. Myocardial edema is not affected by age or frailty index score. (A) Indexed myocardial water is similar between adult and aged rats (p=0.51), and (B) between frail and non-frail rats (p=0.24). Bars represent mean±SEM. Age: 9m (n=11); 21m (n=21). FI score: <0.250 (n=24); >0.250 (n=8).

6.6 Cardiac hypertrophy

To determine if age and frailty affected cardiac hypertrophy, we examined the heart weight to tibia length ratio (HW/TL). Aged rats had a reduced HW/TL relative to adult rats (4.4±0.1 vs. 4.1±0.1 mg*mm^{-1}; p<0.05). There was no significant effect of frailty on HW/TL (FI score <0.250, 4.2±0.1 vs. FI score >0.250, 4.3±0.1 mg*mm^{-1}; p=0.67) (figure 55).

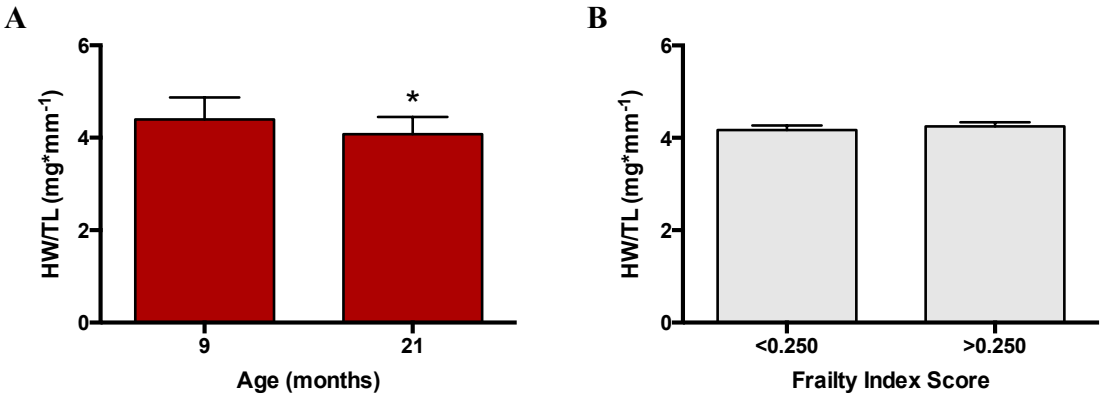

Figure 55. Heart weight to tibia length (HW/TL) ratio is affected by age, but not frailty index score. (A) HW/TL was greater in adult animals (p<0.05). (B) HW/TL was similar between frail and non-frail animals (p=0.67). Bars represent mean±SEM. Age: 9m (n=11); 21m (n=21). FI score: <0.250 (n=24); >0.250 (n=8). *Denotes p<0.05 relative to baseline.

CHAPTER 4: DISCUSSION

1. Key findings

The first objective of this study was to develop a method to quantify frailty in a novel animal model, the F344 rat. We hypothesized that we could develop an FI using the principle of deficit accumulation where mean FI scores would increase with age, and a high FI score would be associated with adverse health outcomes. To test this hypothesis, we serially collected data on survival and 45 variables in health systems known to change with age in a longitudinal study of F344 rats. From this study, we developed a 30-item FI. There was an exponential-like relationship between age and frailty: mean FI scores were similar between youth and adulthood, and significantly increased from middle age into old age. A high FI score was associated with an increased risk of mortality. These findings demonstrate that we were able to develop a 30-item FI for the F344 rat where frailty increases with age and is associated with an increased risk of an adverse health outcome.

The second objective of this study was to determine the effects of chronological age and frailty on baseline cardiac structure and function *in vivo*. We hypothesized that both age and frailty would be associated with changes in cardiac structure and function. Results were similar between groups: left ventricular internal diameter and posterior wall thickness were similar when compared by both age and FI score. HR and indices of systolic function were also similar when compared by age and FI score. Indices of diastolic function tended to decline with frailty; however the differences did not reach statistical significance. Taken together, these data suggest that age and frailty do not significantly impact cardiac morphology and function *in vivo.*

To further examine the effects of age- and frailty-related changes on intrinsic cardiac function, we conducted experiments in the isolated working heart. This allowed us to study the heart independent of loading conditions, anesthesia, and neurohormonal influences that complicate comparisons in the living animal. Old age and a high FI score were associated with arrhythmic activity at baseline. While no statistically significant differences were found, age and frailty might have an impact on certain hemodynamic

variables: SV and SW both tended to be lower in aged and frail animals. Using the isolated working heart in conjunction with echocardiography, we developed a novel method to assess diastolic function by measuring changes in EDV. There was no significant effect of age or frailty on baseline EDV.

Lastly, we sought to determine if old age and frailty would have an impact on recovery from arrest with del Nido cardioplegia. We hypothesized that frailty would be associated with impaired recovery following arrest, as frailty is associated with a loss of resilience in homeostatic regulation with exposure to acute stress (Mitnitski et al., 2013). Our lab has previously demonstrated that del Nido cardioplegia is protective in aged hearts; therefore we hypothesized that age would not impact functional recovery from arrest (Govindapillai et al., 2016; O'Blenes et al., 2011). Recovery of EDV tended to be lower in frail animals, however this was not statistically significant. Recovery of hemodynamic function and heart rhythm, release of cTnI into the coronary effluent, and myocardial edema were similar when compared by age and FI score. These data suggest that neither age nor FI score are significant predictors of recovery from arrest with del Nido cardioplegia.

2. Interpretation of results

2.1 Quantification of frailty in the Fischer-344 rat

Frailty is a concept increasingly explored in the geriatric literature to account for the heterogeneity of health outcomes among elderly people (Rockwood & Mitnitski, 2007). It has been well established that frailty can be quantified in humans; however how to best measure frailty remains controversial (Koller & Rockwood, 2013). Currently, the two most common approaches for assessing frailty are the frailty phenotype and FI (Fried et al., 2001; Mitnitski et al., 2001). Regardless of the measurement tool used, it has been well established that frailty is associated with increased vulnerability to adverse health outcomes, including increased risk of mortality, institutionalization and further deficit accumulation (Rockwood & Mitnitski, 2007). What remains unclear however, is how the biology of frailty renders individuals more susceptible to these adverse health outcomes.

Quantifying frailty in animal models of aging is an important step towards answering this question (Howlett, 2015; Kirkland & Peterson, 2009).

Recent studies by Parks *et al.* (2012) and Whitehead *et al.* (2014) have taken a bedside to bench side approach to quantify frailty in a natural animal model of aging. In both studies, frailty was quantified using the same theory of deficit accumulation as that used to develop the FI in humans (Mitnitski et al., 2001). Briefly, both studies created an FI for C57BL/6 mice by measuring health related variables in different systems known to change with age in this rodent model. Parks *et al.* (2012) developed a 31-item invasive FI appropriate for the cross-sectional study of frailty. Whitehead *et al.* (2014) developed a 31-item non-invasive clinical FI appropriate for longitudinal studies of frailty. In the present study, we sought to build on this body of work to determine if an FI could be created in a larger rodent model of aging, the F344 rat.

To develop our FI, we compiled a list of 45 health-related variables in systems known to change with age. Each of these potential deficits was associated with an adverse health outcome. Many of the potential deficits examined were similar to those used in the frailty indices developed by Parks *et al.* (2012) and Whitehead *et al.* (2014). Novel variables explored as potential deficits for our FI included: daily food intake, chromodacryorrhea, contextual processing, balance and coordination, prehensile strength and muscular stamina. All potential deficits were serially measured in a longitudinal study of rats aged from youth into adulthood, and middle age into old age. The results of these tests are discussed in the following subsections.

2.1.1 Nutritional status

To assess nutritional status, food intake and body weight were evaluated. Mean body weight scores increased with age from 3 to 13 months, plateaued between 13 and 20 months, and decreased by 21 months of age. Mean daily food intake was relatively consistent from 3 to 13 months, but gradually declined between 13 and 21 months. The observed changes in mean body weight agreed with the literature on aging F344 rats fed *ad libitum* (Baskin et al., 1979; Turturro et al., 1999). Mean daily food intake values however were slightly lower than that observed with old age by the NIH (Turturro et al., 1999). A rapid loss of body weight associated with a decline in food intake was observed

near the end of life, regardless of age at the time of death. This terminal decline in body weight and food intake has been previously described (Blanton et al., 1998). Because body weight and food intake both declined with age, and were associated with an increased risk of mortality, we included these variables in our FI.

2.1.2 Clinical signs of deterioration

Twenty-three clinical signs of deterioration were observed weekly. We selected these potential deficits through consultation with a veterinarian and a review of the literature. Many of the clinical deficits explored were similar to those used by Whitehead *et al.* (2014) in their clinical FI for C57BL/6 mice. Eighteen of the 23 deficits measured were included in the final FI. The rationale for omitting 3 of 5 excluded deficits (change in fur colour, unusual sounds and tremor) was that their presence was highly variable, and could change from week to week. We excluded distended abdomen from the final FI because the rats were averse to abdominal palpation, thus it was difficult to reliably examine this deficit unless animals were subjected to anesthesia. Finally, we excluded rectal prolapse as a potential deficit in our final FI because it was not observed in our colony of rats.

2.1.3 Sensorimotor function

Measuring changes in cognition has been identified as a key factor for comprehensive frailty assessment (de Vries et al., 2011). Previous animal models of frailty and frailty assessment tools have somewhat neglected the psychological domain of frailty (Ko et al., 2012; Parks et al., 2012; Whitehead et al., 2014). It is increasingly recognized that changes in sensorimotor and cognitive function are interrelated (Li & Lindenberger, 2002). As such, sensorimotor decline may be associated with cognitive decline. We attempted to measure the psychological domain of frailty by measuring changes in sensorimotor function. Potential sensorimotor deficits were assessed using a battery of tests: contextual processing (blind alley test); prehensile strength (grip strength test); motor coordination and vestibulomotor function (plank tests); and motor coordination and muscle strength (inclined plane test).

Our results demonstrated that only some aspects of sensorimotor function become impaired with age. Prehensile strength declined with age in both cross-sectional and longitudinal analyses. Motor coordination and muscular strength (as tested by the inclined plane) declined with age in the longitudinal analysis, but not with cross-sectional analysis. It is evident from our findings that some impairments in sensorimotor function are maintained until old age (inclined plane); while others decline earlier with age (prehensile strength). These findings agree with the literature (Ingram et al., 1994; Joseph et al., 1983; Markowska et al., 1998).

In contrast, results from the blind alley and plank tests did not agree with previous findings. Cross-sectional analysis of the blind alley and narrow plank tests revealed no significant effect of age, while longitudinal analysis indicated a significant learning effect with repeated test exposure. There was no significant effect of age observed with the medium and wide plank width test. This contrasts with the findings of Markowska *et al.* (1998) and Joseph *et al.* (1983), which observed a significant decline in these parameters with age. The majority of research on the effects of sensorimotor performance in aged rats use a cross-sectional study design because some tests are not suitable for repeated measures analysis (Altun et al., 2007). Thus, when a longitudinal learning effect was observed, we performed a cross-sectional analysis of each animal's performance on first exposure to the test. It is possible that we still did not observe significant differences with this analysis because of the relatively small sample size in the aged group. Thus, further evaluation of sensorimotor performance with first test exposure with a larger sample of aged rats is recommended.

Of the six sensorimotor function tests explored as potential deficits, we included only the inclined plane and prehensile strength test, as results of these tests suggested an age-related decline in performance and no longitudinal learning effect. We proposed that changes in these sensorimotor function tests could be used as a means of measuring the psychological domain of frailty. It is important to recognize however that changes in behaviour on these tests provide information with regards to multiple systems (sensory transduction, central integration/processing, motor unit recruitment, muscle strength, etc.) (Altun et al, 2007). Thus, deficits in sensorimotor function provide information regarding both the physical and cognitive domains of frailty.

2.1.4 Exploratory activity

A common characteristic of aging is a decline in physical activity and explorative behaviour (Fried et al., 2001). We sought to evaluate changes in explorative behaviour by evaluating different characteristics of movement during the open field test. Our results suggested a significant learning effect with repeated exposure to the open field test in both the young and old cohort of rats. In a cross-sectional analysis of first exposure to the open field test, there was a gradual decline in explorative behaviour and locomotion with age. This agrees with the findings of Smith *et al.* (2007), which demonstrated that rats are more active in a novel environment relative to a familiar environment. The age-related decline in novel open field explorative behaviour has been previously described in the literature (Altun et al., 2007; Markowska et al., 1998). Changes in explorative behaviour with age have been attributed to a loss of hind limb muscle, cardiovascular function, motor coordination and anxiety (Altun et al., 2007).

Because of the longitudinal learning effect, the open field test did not satisfy our inclusion criteria for our FI. Age-related differences were observed with first exposure to the test, therefore changes in explorative behaviour could potentially be incorporated as deficits in a cross-sectional frailty assessment.

2.1.5 Basic metabolic status

A blood chemistry panel was utilized to evaluate basic organ function. Many of the measured parameters were similar to those explored by Parks *et al.* (2012), including Na^+, K^+, Cl^-, BUN, hematocrit and hemoglobin. Serum total CO_2 was measured as a surrogate for serum bicarbonate. Creatinine was included as an additional measure of renal health. The majority of parameters measured were generally consistent throughout the lifespan with a few exceptions: mean creatinine and BUN levels increased with age. This is not surprising considering that male F344 rats are known to develop nephropathy with age (Razzaque et al., 1998). While most of these variables did not have an association with age, we included them in our FI because they provide a good measure of overall system health. Furthermore, blood chemistry has been previously incorporated into frailty indices for both mice and humans with success (Howlett et al., 2014; Parks et al., 2012; Rockwood et al., 2015). In future, it would be interesting to consider adding

serum bilirubin levels to the blood chemistry panel given the high incidence of jaundice in our colony of rats.

2.2 Frailty index

We demonstrated for the first time that frailty could be quantified in a rat using the principle of deficit accumulation. The deficits in this index primarily reflect the physical domain of frailty, including changes in nutritional status, mobility, energy and strength (de Vries et al., 2011). Unfortunately, many of the novel deficits we hoped to include with regards to the psychological domain were inappropriate for longitudinal frailty assessments (e.g., blind alley and plank tests). As such, the cognitive component of frailty is poorly reflected by this index. Novel deficits that satisfied our criteria for inclusion include chromodacryorrhea, jaundice, muscular stamina, prehensile strength, and head tilt.

The majority of clinical deficits comprising our FI are similar to those used by Whitehead *et al.* (2014). This is not surprising considering that many of the assessment tools used by veterinarians to assess clinical deterioration in rodents are similar between mice and rats. That being said there are some key species-specific differences. For example, stiffening of the mouse-tail tendon occurs in C57BL/6 mice but there is little evidence to suggest this occurs in F344 rats (Eriksen et al., 2014). While vision loss can be used as a deficit in C57BL/6 mice, light-induced retinal degeneration is universal among albino rats exposed to fluorescent or incandescent light (Williams, 2002). Thus, it is expected that our FI would closely resemble those previously developed in mice with some species-specific differences.

It was important to include 30 or more deficits in our FI because previous studies have suggested that graded levels of frailty are difficult to distinguish when fewer than 30 deficits are measured (Peña et al., 2014). For example, Whitehead *et al.* (2014) demonstrated that a 31-item clinical FI was able to distinguish between different levels of frailty in aged mice where an 8-item FI could not. A second reason why it was important to include a large number of potential deficits is because it allows for multiple phenotypic expressions of frailty. Deficits accumulate with age, however we accumulate different

deficits at different rates. Thus, it is important to incorporate a large number of deficits to allow for multiple frailty phenotypes (Rockwood & Mitnitski, 2011).

Of the 30 deficits included in our FI, 16 deficits are subjective measures while the remaining variables are objective. Incorporation of objective research measures into evaluation tools is important for minimizing observer bias, such as the observer-expectancy or halo effect (Morrow et al., 2016). While not explored in this study, the inclusion of objective variables in our index may also help to improve inter-rater reliability (Morrow et al., 2016). While deficits measured subjectively are more prone to bias, when raters receive appropriate training they can perform rapid, simplified deficit assessment with good inter-rater reliability (Feridooni et al., 2015). There is also a history of subjectivity with regards to frailty assessment: prior to the development of today's frailty assessment tools, frailty was assessed using the end-of-the-bed, or "eyeball" test (Afilalo et al., 2014). Thus there is evidence to support the inclusion of both subjective and objective measures in an FI.

2.3 Frailty index outcomes

A major goal of this study was to characterize the relationship between age and frailty in the F344 rat. Results from our longitudinal study demonstrated that frailty had an exponential-like relationship with age. While this is the first time that this relationship has been demonstrated in rats, these findings agree with what is known in mice and humans. Parks *et al.* (2012) were the first to demonstrate in mice that frailty increases with age. Two years later, Whitehead *et al.* (2014) replicated this finding with their 31-item clinical FI. Interestingly, Whitehead *et al.* (2014) also compared FI data from the Survey of Health, Ageing and Retirement in Europe with FI data from the invasive and clinical FI for C57BL/6 mice by normalizing FI scores to the age of 90% mortality in both groups. Results suggested that the relationship between FI scores and age was almost identical between mice and humans. Furthermore, the rate of deficit accumulation and submaximal limit of frailty were also similar between groups (Whitehead et al., 2014). To further validate our FI, the relationship between FI score and age in rats should be compared with that in C57BL/6 mice and humans.

In both humans and mice, frailty has been proposed as a correlate for physiological age that predicts vulnerability to increased health outcomes (Mitnitski et al., 2013). An FI should therefore be able to measure individual variations in health and a high FI score should be associated with a greater likelihood of adverse health outcomes. In humans, a high FI score has been associated with mortality, institutionalization, and further deficit accumulation (Rockwood & Mitnitski, 2007). In mice, frailty has been associated with impairments in cardiovascular function (Parks et al., 2012; Sun, 2014). A second goal of this study was therefore to determine if frailty in rats was associated with a greater likelihood of adverse health outcomes. Results suggested that a high FI score was associated with an increased risk of all-cause mortality.

It is generally accepted that the increased vulnerability to adverse health outcomes among frail individuals arises from a systemic loss of functional reserves (Mitnitski et al., 2013). This leads to deterioration in the maintenance of homeostatic processes in key physiological systems, rendering frail individuals more susceptible to stress (Fulop et al., 2010; Rockwood & Mitnitski, 2007). Aging, in the absence of significant comorbidity, is also associated with a loss of functional reserves. For example, the loss of ventricular myocytes in the heart, decreased osteogenesis, and impaired glucose handling all represent the loss of functional reserves with age (Fulop et al., 2015). In this model of aging, death occurs when the functional reserves are considered empty (Fulop et al., 2015). Frailty has been previously described as accelerated aging (Kuh et al., 2007). The exhaustion of functional reserves is accelerated in frailty, which could account for the increased mortality observed in frail rats in this study.

The chronic, low-grade inflammation that occurs with aging has been proposed as a possible mechanism leading to the decline in functional reserve (Fulop et al., 2010). This so-called ‘inflammaging’ has been implicated in frailty syndrome as operationalized by the Fried phenotype. For example, higher baseline levels of total white blood cells have been associated with an increased risk of frailty and mortality (Baylis et al., 2013). Frail individuals have also been shown to have higher levels of IL-6 and neopterin relative to less frail age-matched controls (Leng et al., 2002; 2011). It would be interesting to investigate levels of pro-inflammatory biomarkers and frailty in a natural

animal model of aging, as this may provide insight into the biological basis of frailty with the potential of pursuing inflammation as a therapeutic target.

Insights into this relationship have been previously described in the literature using the IL-$10^{tm/tm}$ mice as an animal model of frailty(Walston et al., 2008). These mice exhibited an increase in systemic inflammation coupled with a loss of skeletal muscle strength (Ko et al., 2012; Walston et al., 2008). The translatability of these findings is limited however because the extent to which this knockout mouse mimics natural aging is unclear. Furthermore, while this mouse mimics characteristics of the frailty phenotype, frailty has yet to be quantified in this model (Howlett, 2015). Therefore there is still a need to further explore the relationship between aging, frailty and inflammation.

In summary, we were able to demonstrate that our 31-item FI for F344 rats exhibits many characteristics similar to those previously described in the literature for mice and humans: the FI could differentiate between individual variations in health, FI scores increased with age, and a high FI score was associated with an increased risk of an adverse health outcome (mortality). To further validate this index, survival curves, the rate of deficit accumulation, and maximal FI scores should be compared with data obtained in mice and humans. A larger study of differences in frailty in the F344 as well as an inter-rater reliability study is also recommended.

3. Changes in *in vivo* cardiac structure and function in relation to age and frailty

This study was the second to evaluate changes in *in vivo* cardiac morphology and function as a function of frailty in an animal model, but the first to do so in rats. Thus, little is known about the effects of frailty on cardiac morphology and function in this animal model. Sun *et al.* (2014) previously investigated the relationship between frailty and changes in *in vivo* cardiac morphology and function in C57BL/6 mice. Results suggested that frailty was associated with a decline in indices of systolic function and reduced LV internal diameter at both systole and diastole (Sun, 2014). This contrasts with our findings in the F344 rat, which indicated that LV wall thickness, internal diameter, EF, and FS were similar between frail and less frail rats. Interestingly, our results suggest that frailty might be associated with impaired diastolic function, given frail animals had a normal E/A ratio and reduced mitral deceleration time (not statistically significant).

Additionally, methods for assessing diastolic function in the rat heart have yet to be clearly defined because little is known about changes in the E/A ratio and mitral DT in rats.

Interpreting diastolic function in rats has been done with conflicting results. Some authors purport an increase in the E/A ratio indicates diastolic dysfunction, while others suggest a decrease in the E/A ratio is associated with impaired diastolic function (Brenner et al., 2001; Walker et al., 2006). In humans, the progression of diastolic dysfunction has been well-documented using pulsed-wave Doppler echocardiography. With normal diastolic function, the E wave is greater than the A wave, resulting in a normal E/A ratio. Mitral deceleration time is approximately 140 ms (Khouri et al., 2004). In mild diastolic function, abnormalities in active relaxation lead to a reduction in early filling. There is a compensatory increase in late filling velocity, leading to a reversal of the E/A ratio (E<A). Mitral deceleration time is prolonged (Khouri et al., 2004; Mossahebi & Kovács, 2014). Interpreting the E/A ratio becomes problematic with moderate diastolic dysfunction because a reduction in LV compliance yields a compensatory increase in LA pressure to maintain CO. This results in a normal E/A ratio (E>A) and a decline in mitral DT (Khouri et al., 2004; Mossahebi & Kovács, 2014). Assuming the compensatory reflexes to impairments in active relaxation and reduced LV compliance are similar between rats and humans, a normal E/A ratio coupled with a decline in mitral DT could suggest that frailty is associated with impaired diastolic function in the rat.

In addition to frailty, changes in cardiac morphology and function were also analyzed by age. We did not observe a significant effect of age on any of the parameters measured with echocardiography. Age-related changes in cardiac structure and function have not been well characterized in the F344 rat, as the majority of echocardiography studies have been conducted in other strains, most notably the F344/Brown Norway F1 hybrid rat. Previous studies examining the effects of age on echocardiographic parameters in rats agree that aging is associated with LV wall thickening, but yield conflicting results with regards to age-related changes in systolic and diastolic function. For example, the findings of Walker *et al.* (2006) suggest an increase in LV wall thickness, a decrease in diastolic function, and preservation of systolic function with age. In contrast, Hacker *et al.* (2005) reported an increase in wall thickness and a decline in both diastolic and

systolic function with age. Fannin *et al.* (2014) reported that aging is associated with LV wall thickening, LV chamber dilatation, preserved systolic function, and a preserved E/A ratio. It is important to note that many of the different results observed in these studies can be attributed to differences in age, strain, anesthesia protocol, body weight, or sex (Watson et al., 2004).

It is also possible that we did not observe differences in baseline cardiac function because there are limitations to using echocardiography in small animals. Suboptimal ultrasound windows and limited resolution/image quality can make it difficult to define endocardial borders. Deviation from proper angle alignment during pulsed wave Doppler will underestimate transmitral flow velocity (Huang & McLean, 2012). Furthermore, echocardiography has poor inter-rater reliability, however we controlled this in our study by using a single blinded echocardiographer.

In summary, this is the first study to characterize changes in cardiac morphology and function by frailty and chronological age in a longitudinal study of F344 rats. We did not observe any significant differences by age or frailty; however it may be useful to further explore the relationship between diastolic dysfunction and frailty. A replication of this study with a larger sample size is recommended to explore this relationship. Specialized small animal echocardiography equipment may also improve the sensitivity of the assessment.

4. Baseline cardiac function in the isolated working heart

Age-related changes in cardiac structure and function lower the threshold for cardiovascular disease manifestation by impairing cardiac performance (Strait & Lakatta, 2012). To further examine the impact of age and frailty on baseline cardiovascular performance, we utilized an isolated working heart preparation. While data exists on baseline cardiac function in the isolated working heart in both young and aged rats, few studies have directly compared these data by age. For example, our lab has previously examined baseline cardiac function using the isolated working heart in both aged and young F344 rats; however these data were not directly compared by age (Govindapillai et al., 2016). There have been no studies investigating the effects of frailty on cardiac

function with this experimental model. Thus, the effects of age and frailty on baseline cardiac function are not well characterized in the isolated working rat heart.

4.1 Heart rhythm

We observed an increase in the incidence of ventricular arrhythmia with both age and frailty in F344 rats. We did not examine mechanisms underlying age- and frailty-related alterations in the electrophysiological properties of the heart; therefore we can only postulate how changes in the ventricular myocardium or conduction system may contribute to a higher incidence of ventricular arrhythmia in senescent rats. Previous work has demonstrated that age-related collagen deposition provides a substrate for arrhythmia. When coupled with cardiovascular stress, this can promote triggered activity that can result in VT or VF in senescent rats (Morita et al., 2014). While we did not examine fibrosis in our colony of rats, increased collagen deposition is well documented in the aged F344 rat heart (Anversa & Capasso, 1991; Eghbali et al., 1989). If fibrosis was greater in aged and frail hearts relative to the young and non-frail hearts, it is possible that the stress of isolating the heart could have triggered the development of PVCs, VT or VF. Our results suggest that there may be difference in the cardiac electrophysiological properties of aged versus young rats, and frail versus non-frail rats. Further electrophysiological study is recommended to explore the mechanisms underlying these changes.

4.2 Hemodynamic function

Baseline hemodynamic values are similar to those previously observed by our lab in aged F344 rats. Few differences in hemodynamic function were observed between groups. SV and SW tended to be lower in aged and frail rats, however this did not reach statistical significance. That being said, it has been previously demonstrated that there is a decline in SV between 20 and 29 months in the F344 rat (Anversa & Capasso, 1991). Aging is associated with a loss of cardiac myocytes. Compensatory hypertrophy occurs in remaining cardiac myocytes to maintain cardiac function (Anversa et al., 1986). While we did not directly explore this in our study, Sun *et al.* (2014) demonstrated that both age and

frailty were associated with cardiac myocyte hypertrophy in mice. Thus it is possible that cellular hypertrophy may have helped maintain cardiac function in both aged and frail hearts.

Observed deficits in cardiovascular performance with age and frailty are attributed to a decline in functional reserve and are often observed in response to stress. For example, resting HR and SV are not affected by age; but there is an age-related decline in peak HR and SV in response to exercise (Strait & Lakatta, 2012; Vella & Robergs, 2005). It is therefore possible that we did not observe changes in baseline function with age or FI score because changes may only be seen when homeostatic mechanisms are challenged.

4.3 Coronary vascular resistance

In our study, baseline CVR was similar with age and frailty. CVR is determined by the diameter of the coronary arterioles. *In vivo*, smooth muscle tone is maintained by the sympathetic nervous system, in addition to the myogenic mechanism and local metabolic autocrine regulators (Wang et al., 1995). In the isolated working heart, only the latter two mechanisms influence CVR. It has been previously demonstrated *in vivo* that coronary blood flow to the left ventricle at rest does not differ as a function of age (Hachamovitch et al., 1989). Maximal coronary blood flow (coronary reserve) however is decreased with age. Again, this suggests that the aged heart is able to maintain normal function under baseline conditions, but has an impaired reserve capacity that may only be observed with exposure to stress (Hachamovitch et al., 1989).

4.4 Diastolic function

We developed a novel method to quantify diastolic function using the isolated working heart in conjunction with echocardiography to measure changes in EDV at a fixed filling pressure. At baseline, EDV was reduced in aged hearts (not statistically significant), suggesting that diastolic function may be impaired with age in the F344 rat. Previous work in the F344 rat has shown that there is an increase in the slope of the end diastolic pressure volume relationship with age (Anversa & Capasso, 1991; Pacher et al., 2004). There was no effect of FI score on EDV. It is possible that a difference was not

observed between frail and non-frail rats because of the small sample size in the frail group of animals.

Cardiac myocyte hypertrophy and alterations in the myocardial collagen network, including increased collagen deposition and collagen cross-linking, reduce left ventricular compliance with age (Anversa et al., 1986). When coupled with impairments in active relaxation, there is a diminished capacity to fill at low left-atrial pressures. As a result, the left ventricular pressure-volume loop is displaced up and to the left. For a given filling pressure, there will be a reduction in left ventricular volume (Aurigemma & Gaasch, 2004; Zile & Brutsaert, 2002). Our aged hearts did not have a greater myocardial mass relative to adult hearts; therefore the reduction in EDV observed at baseline may be due to age-related alterations in the myocardial collagen network.

5. Functional recovery following arrest with del Nido cardioplegia

5.1 Rhythm during reperfusion

During ischemia and reperfusion, there is a disruption in intracellular Na^+ and Ca^{2+} homeostasis. Hyperkalemic arrest depolarizes the membrane, opening a proportion of the voltage-gated Na^+ channels. While many of these channels inactivate, a small proportion remain open during ischemia creating a window current, otherwise known as the late Na^+ current (I_{Na}) (Dobson & Jones, 2004). Intracellular Na^+ accumulation through the late I_{Na} leads to an increased exchange of intracellular Na^+ for extracellular Ca^{2+} through reverse mode NCX during reperfusion. Ca^{2+} accumulates in the cell, which can cause spontaneous release of Ca^{2+} from the sarcoplasmic reticulum through calcium induced calcium release. This can result in ventricular repolarization abnormalities, such as beat-to-beat variability in action potential duration and after-depolarizations (Belardinelli et al., 2006). Reperfusion arrhythmias are therefore indicative of ischemia-reperfusion injury. We observed a similar incidence of reperfusion arrhythmias between adult versus aged animals, and frail versus non-frail animals.

5.2 Recovery of hemodynamic function

Impaired ventricular function following ischemia has been associated with accelerated accumulation of intracellular Ca^{2+} (Belardinelli et al., 2006). As such, strategies to limit Ca^{2+} overload in reperfusion are not just recommended to inhibit arrhythmia, but also to improve functional recovery following arrest. As described earlier, del Nido cardioplegia contains lidocaine, a compound that inhibits Ca^{2+} overload by inhibiting intracellular Na^{+} accumulation during ischemia. Previous research in our lab has demonstrated that the use of del Nido cardioplegia is an effective strategy to reduce myocardial damage and improve functional recovery in the aged heart (Govindapillai et al., 2016; O'Blenes et al., 2011). A comparison of hemodynamic parameters in these studies revealed that percent recovery of RPP, SP, LVDP, CF, CO, SV, and SW was improved following arrest with del Nido relative to standard cardioplegia in aged hearts. In our study, results suggested that functional recovery from arrest with del Nido cardioplegia was similar between aged versus adult hearts, and frail versus non-frail hearts. del Nido cardioplegia may therefore be an effective myocardial protection strategy in the aged and frail heart.

5.3 Coronary vascular resistance

There was a similar decline in CVR between baseline and reperfusion when compared by age and FI score. This decline may be attributable to the composition of del Nido cardioplegia (Matte & del Nido, 2012). Del Nido cardioplegia is a hyperkalemic solution that contains lidocaine. Previous studies (albeit in pigs) have demonstrated that hyperkalemic cardioplegic arrest is associated with a decrease in myogenic reactivity and intrinsic vascular tone (Wang et al., 1995). In rats, lidocaine has been shown to promote vasodilation in coronary arterioles and inhibit the accumulation of neutrophils in the coronary arteries following ischemia-reperfusion injury (Homeister et al., 1990; Johns et al., 1985). Previous research by our lab has suggested that arrest with del Nido cardioplegia reduces CVR during reperfusion relative to standard cardioplegia (Govindapillai et al., 2016). Because the decline in CVR was similar between adult and aged rats, and frail and non-frail rats, it is possible that del Nido cardioplegia is effectively protecting aged and frail myocardium against increases in CVR.

5.4 Recovery of diastolic function

There was a significant decline in EDV following reperfusion after arrest with del Nido cardioplegia in all groups, suggesting that diastolic function was impaired. It has been previously demonstrated that recovery from cardioplegia is associated with reduced left ventricular compliance in the rat (Hwang et al., 2009; Rach et al., 1999). For example, Rach *et al.* (1999) and Hwang *et al.* (2009) have both demonstrated that the slope of the end diastolic pressure volume relationship is elevated following arrest with cardioplegia. Ca^{2+} overload resulting from ischemia-reperfusion injury can result in relaxation abnormalities, such as increased LV end diastolic pressure and tension (Belardinelli et al., 2006). It has been previously demonstrated that inhibiting late I_{Na} improves left ventricular compliance following reperfusion. For example, there is a reduction in the LVEDPVR when ranolazine, a late I_{Na} inhibitor, is added to hyperkalemic cardioplegia (Hwang et al., 2009). Del Nido cardioplegia contains lidocaine, an I_{Na} inhibitor. It should therefore protect against diastolic dysfunction following reperfusion. Our results suggested a similar recovery of EDV for a given filling pressure when compared by age and FI score. Thus, del Nido cardioplegia may be an efficient cardioplegia strategy to protect diastolic function in both aged and frail hearts.

5.5 Cardiac troponin-I release into coronary effluent

cTnI is a protein found on the thin myofilament that regulates the Ca^{2+}-mediated interaction between actin and myosin (Sharma et al., 2004). Following myocardial damage, there is an efflux of cTnI from the myocyte into the coronary effluent. It can be easily and reliably detected with immunoassays; thus it is an ideal biomarker of myocardial damage (Korff et al., 2006). Levels of cTnI were low in all groups (<0.2 ng/mL). Interestingly, frail rats had the lowest levels of cTnI (not significant), suggesting that del Nido cardioplegia may be protective in the frail heart.

5.6 Cardiac hypertrophy

Previous studies in rats have demonstrated that mild hypertrophy is a common characteristic of aging (Walker et al., 2006; Yin et al., 1982). For example, Walker *et al.* (2006) reported an increase in heart weight to tibia length ratio in F344/Brown Norway F1 hybrid rats by 30-months. Yin *et al.* (1982) reported that the left ventricle is hypertrophied by 28-months in Wistar rats. In F344 rats however, it has been shown that there is a decline in cardiac mass at 19-21 months of age (Anversa et al., 1986). Thus, there are strain-related differences in cardiac mass with age. Our results suggested an age-related decline in the HW/TL ratio. It has been previously documented in male F344 rats the aged left ventricle is composed of a smaller number of hypertrophied cells (Anversa et al., 1986). Hypertrophy occurs to maintain normal cardiac function despite a loss of ventricular myocytes. It is possible that the age-related decline in cardiac mass observed in our study reflects the loss of myocytes at an age where compensatory hypertrophy has yet to occur.

5.7 Myocardial edema

Myocardial edema occurs when there is abnormal fluid accumulation in the myocardium. It has been hypothesized that capillary integrity is compromised during ischemia or with reperfusion (Bragadeesh et al., 2008). Fluid accumulates in the interstitial space (becoming hypotonic), which can result in myocyte swelling and myofibrillar edema (Bragadeesh et al., 2008). With edema, there is suboptimal overlap of the actin-myosin filaments, which impairs force generation during cross-bridge cycling (Bragadeesh et al., 2008). Indeed, myocardial edema has been associated with myocardial dysfunction following ischemia (Powell et al., 1976). In our study, myocardial edema was similar between groups, which may account for the similar recovery of cardiac function between groups. Del Nido cardioplegia contains mannitol, a hyperosmotic compound that has been shown to reduce myocardial edema (Powell et al., 1976). These results suggest that del Nido cardioplegia may be protective against myocardial edema in aged and frail hearts.

6. Limitations

It is important to address some of the limitations of our study. With regards to the deficits comprising the FI, we were unable to measure deficits in the psychological domain. While we attempt to infer cognitive impairments through a decline in sensorimotor function, the inclusion of more pointed learning/memory assessments such as the object recognition or water maze test should be considered. While our FI exhibited characteristics similar to those described in the literature for mice and humans, direct comparisons with these data are still required. Furthermore, we did not evaluate the influence of observer bias in this study, therefore the need for a study of inter- and intra-rater reliability is recognized.

In any longitudinal study of aging, survival bias must be considered. Our results suggested that a high FI score was associated with an increased risk of mortality. Therefore a number of frail animals did not undergo cardiac structure and function tests. As such, animals that lived to the terminal end point of the study may represent a relatively healthier subset of animals, which could account for the similarities observed between cardiac parameters. It is also important to address how we selected at what age animals were considered old. Using survival data, animals are generally classified as senescent at the age where 50% colony mortality occurs (Lakatta & Sollott, 2002). In our colony, this occurred at 21 months. This is slightly younger than that reported in the literature, where 24-month old, cage-paired rats commonly represent senescence (Lakatta & Sollot, 2002). Isolated rats (like those used in our study) typically have 50% mortality between 20-22 months (Yu et al., 1982). Few studies characterizing age-related changes in cardiac structure and function have reported data in isolated F344 rats. It is therefore possible that we did not observe changes in cardiac parameters measured because the myocardium was not sufficiently aged.

Many hemodynamic parameters are rate-dependent and load-dependent. Because our hearts were not paced, it is possible that differences in HR may have affected the results of our isolated working heart experiments. During these experiments, hearts were filled at a fixed preload and ejected against a fixed afterload, which may have also influenced hemodynamic outcomes. It is also possible that we did not observe differences following reperfusion because healthy hearts are typically not exposed to cardioplegia. It

may therefore be interesting to pursue age- and frailty-related differences in an animal model of CVD.

This study characterized age-related changes in structure and function in male rats. We therefore cannot comment on sex differences with relation to age, frailty and cardiac structure and function. Age-related diastolic dysfunction and frailty tend to be more prevalent in females, therefore it would be interesting to explore if these sex differences exist in the F344 rat (Rockwood et al., 2005; Gutierrez & Blanchard, 2012).

7. Summary

In the present study, we developed a 30-item FI for the F344 rat where frailty increased with age and was associated with an increased risk of mortality. Aged and frail hearts were more likely to be arrhythmic at baseline, however neither age nor frailty were associated with significant changes in cardiac structure, inotropic, hemodynamic, or diastolic function *in vivo* or in the isolated working heart. Post-reperfusion functional recovery and cTnI release into the coronary effluent was similar between groups, suggesting that del Nido cardioplegia may be an effective strategy for protecting the aged and frail heart. In our model, any differences in cardiac function and recovery from cardiologic arrest are subtle. Del Nido cardioplegia is effective in protecting the heart regardless of age and frailty. It is possible that a longer, more clinically relevant period of ischemia may bring out differences in recovery from cardioplegia.

REFERENCES

1. Afilalo, J. (2011). Frailty in Patients with Cardiovascular Disease: Why, When, and How to Measure. *Current Cardiovascular Risk Reports*, *5*(5), 467–472. doi:10.1007/s12170-011-0186-0
2. Afilalo, J. (2014). Androgen deficiency as a biological determinant of frailty: hope or hype? *Journal of the American Geriatrics Society*, *62*(6), 1174–1178. doi:10.1111/jgs.12835
3. Afilalo, J., Alexander, K. P., Mack, M. J., Maurer, M. S., Green, P., Allen, L. A., et al. (2014). Frailty assessment in the cardiovascular care of older adults. *Journal of the American College of Cardiology*, *63*(8), 747–762. doi:10.1016/j.jacc.2013.09.070
4. Afilalo, J., Karunananthan, S., Eisenberg, M. J., Alexander, K. P., & Bergman, H. (2009). Role of frailty in patients with cardiovascular disease. *The American Journal of Cardiology*, *103*(11), 1616–1621. doi:10.1016/j.amjcard.2009.01.375
5. Alexander, K. P., Anstrom, K. J., Muhlbaier, L. H., Grosswald, R. D., Smith, P. K., Jones, R. H., & Peterson, E. D. (2000). Outcomes of cardiac surgery in patients > or = 80 years: results from the National Cardiovascular Network. *Journal of the American College of Cardiology*, *35*(3), 731–738.
6. Altun, M., Bergman, E., Edström, E., Johnson, H., & Ulfhake, B. (2007). Behavioral impairments of the aging rat. *Physiology & Behavior*, *92*(5), 911–923. doi:10.1016/j.physbeh.2007.06.017
7. Anversa, P., & Capasso, J. M. (1991). Cellular basis of aging in the mammalian heart. *Scanning Microscopy*, *5*(4), 1065–1074.
8. Anversa, P., Hiler, B., Ricci, R., Guideri, G., & Olivetti, G. (1986). Myocyte cell loss and myocyte hypertrophy in the aging rat heart. *Journal of the American College of Cardiology*, *8*(6), 1441–1448.
9. Aurigemma, G. P., & Gaasch, W. H. (2004). Clinical practice. Diastolic heart failure. *The New England Journal of Medicine*, *351*(11), 1097–1105. doi:10.1056/NEJMcp022709
10. Barzilay, J. I., Blaum, C., Moore, T., Xue, Q.-L., Hirsch, C. H., Walston, J. D., & Fried, L. P. (2007). Insulin resistance and inflammation as precursors of frailty: the Cardiovascular Health Study. *Archives of Internal Medicine*, *167*(7), 635–641. doi:10.1001/archinte.167.7.635
11. Baskin, S. I., Roberts, J., & Kendrick, Z. V. (1979). Effect of age on body weight, heart rate and blood pressure in pair-caged, male, Fischer 344 rats. *Age*, *2*(2), 47–50. doi:10.1007/BF02432256
12. Baylis, D., Bartlett, D. B., Syddall, H. E., Ntani, G., Gale, C. R., Cooper, C., et al. (2013). Immune-endocrine biomarkers as predictors of frailty and mortality: a 10-year longitudinal study in community-dwelling older people. *Age (Dordrecht, Netherlands)*, *35*(3), 963–971. doi:10.1007/s11357-012-9396-8
13. Belardinelli, L., Shryock, J. C., & Fraser, H. (2006). Inhibition of the late sodium current as a potential cardioprotective principle: effects of the late sodium current inhibitor ranolazine. *Heart (British Cardiac Society)*, *92 Suppl 4*(suppl_4), iv6–iv14. doi:10.1136/hrt.2005.078790

14. Benjamin, E. J., Levy, D., Anderson, K. M., Wolf, P. A., Plehn, J. F., Evans, J. C., et al. (1992). Determinants of Doppler indexes of left ventricular diastolic function in normal subjects (the Framingham Heart Study). *The American Journal of Cardiology*, *70*(4), 508–515.
15. Blanton, C. A., Horwitz, B. A., Murtagh-Mark, C., Gietzen, D. W., Griffey, S. M., & McDonald, R. B. (1998). Meal patterns associated with the age-related decline in food intake in the Fischer 344 rat. *The American Journal of Physiology*, *275*(5 Pt 2), R1494–1502.
16. Bouillon, K., Kivimaki, M., Hamer, M., Sabia, S., Fransson, E. I., Singh-Manoux, A., et al. (2013). Measures of frailty in population-based studies: an overview. *BMC Geriatrics*, *13*(1), 64. doi:10.1186/1471-2318-13-64
17. Bragadeesh, T., Jayaweera, A. R., Pascotto, M., Micari, A., Le, D. E., Kramer, C. M., et al. (2008). Post-ischaemic myocardial dysfunction (stunning) results from myofibrillar oedema. *Heart (British Cardiac Society)*, *94*(2), 166–171. doi:10.1136/hrt.2006.102434
18. Brenner, D. A., Apstein, C. S., & Saupe, K. W. (2001). Exercise training attenuates age-associated diastolic dysfunction in rats. *Circulation*, *104*(2), 221–226.
19. Cheng, S., Fernandes, V. R. S., Bluemke, D. A., McClelland, R. L., Kronmal, R. A., & Lima, J. A. C. (2009). Age-related left ventricular remodeling and associated risk for cardiovascular outcomes: the Multi-Ethnic Study of Atherosclerosis. *Circulation. Cardiovascular Imaging*, *2*(3), 191–198. doi:10.1161/CIRCIMAGING.108.819938
20. Claessens T.E., Rietzschel E.R., De Buyzere, M.L. et al. (2007). Noninvasive assessment of left ventricular and myocardial contractility in middle-aged men and women: disparate evolution above the age of 50? *Am J Physiol Heart Circ Physiol, 292,* H856-65.
20. Cook, C.S. (1991). *Eye and Ear.* (T.C. Jones, U. Mohr, & R.D. Hunt). Springer Berlin Heidlberg.
21. de Souza, R. R. (2002). Aging of myocardial collagen. *Biogerontology*, *3*(6), 325–335.
22. de Vries, N. M., Staal, J. B., van Ravensberg, C. D., Hobbelen, J. S. M., Olde Rikkert, M. G. M., & Nijhuis-van der Sanden, M. W. G. (2011). Outcome instruments to measure frailty: a systematic review. *Ageing Research Reviews*, *10*(1), 104–114. doi:10.1016/j.arr.2010.09.001
23. Demetrius, L. (2006). Aging in mouse and human systems: a comparative study. *Annals of the New York Academy of Sciences*, *1067*(1), 66–82. doi:10.1196/annals.1354.010
24. Dobson, G. P., & Jones, M. W. (2004). Adenosine and lidocaine: a new concept in nondepolarizing surgical myocardial arrest, protection, and preservation. *The Journal of Thoracic and Cardiovascular Surgery*, *127*(3), 794–805. doi:10.1016/S0022
25. Eghbali, M., Eghbali, M., Robinson, T. F., Seifter, S., & Blumenfeld, O. O. (1989). Collagen accumulation in heart ventricles as a function of growth and aging. *Cardiovascular Research*, *23*(8), 723–729.
26. Eriksen, C., Svensson, R. B., Scheijen, J., Hag, A. M. F., Schalkwijk, C., Praet, S. F. E., et al. (2014). Systemic stiffening of mouse tail tendon is related to dietary advanced glycation end products but not high-fat diet or cholesterol. *Journal of Applied Physiology (Bethesda, Md. : 1985)*, *117*(8), 840–847. doi:10.1152/japplphysiol.00584.2014

27. Fannin, J., Rice, K. M., Thulluri, S., Dornon, L., Arvapalli, R. K., Wehner, P., & Blough, E. R. (2014). Age-associated alterations of cardiac structure and function in the female F344xBN rat heart. *Age (Dordrecht, Netherlands)*, *36*(4), 9684–9712. doi:10.1007/s11357-014-9684-6
28. Feridooni, H. A., Sun, M. H., Rockwood, K., & Howlett, S. E. (2015). Reliability of a Frailty Index Based on the Clinical Assessment of Health Deficits in Male C57BL/6J Mice. *The Journals of Gerontology. Series a, Biological Sciences and Medical Sciences*. doi:10.1093/gerona/glu161
29. Fleg, J. L., Das, D. N., Wright, J., & Lakatta, E. G. (1990). Age-associated changes in the components of atrioventricular conduction in apparently healthy volunteers. *Journal of Gerontology*, *45*(3), M95–100.
30. Forman, D. E., Rich, M. W., Alexander, K. P., Zieman, S., Maurer, M. S., Najjar, S. S., et al. (2011). Cardiac care for older adults. Time for a new paradigm. *Journal of the American College of Cardiology*, *57*(18), 1801–1810. doi:10.1016/j.jacc.2011.02.014
31. Franceschi, C., & Campisi, J. (2014). Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. *The Journals of Gerontology. Series a, Biological Sciences and Medical Sciences*, *69 Suppl 1*(Suppl 1), S4–9. doi:10.1093/gerona/glu057
32. Fried, L. P., Tangen, C. M., Walston, J., Newman, A. B., Hirsch, C., Gottdiener, J., et al. (2001). Frailty in older adults: evidence for a phenotype. *The Journals of Gerontology. Series a, Biological Sciences and Medical Sciences*, *56*(3), M146–156.
33. Fruitman, D. S., MacDougall, C. E., & Ross, D. B. (1999). Cardiac surgery in octogenarians: can elderly patients benefit? Quality of life after cardiac surgery. *The Annals of Thoracic Surgery*, *68*(6), 2129–2135.
34. Fulop, T., Larbi, A., Witkowski, J. M., McElhaney, J., Loeb, M., Mitnitski, A., & Pawelec, G. (2010). Aging, frailty and age-related diseases. *Biogerontology*, *11*(5), 547–563. doi:10.1007/s10522-010-9287-2
35. Fulop, T., McElhaney, J., Pawelec, G., Cohen, A. A., Morais, J. A., Dupuis, G., et al. (2015). Frailty, Inflammation and Immunosenescence. *Interdisciplinary Topics in Gerontology and Geriatrics*, *41*, 26–40. doi:10.1159/000381134
36. Gharacholou, S., Tashiro, T., Cha, S., Scott, C., Takahashi, P., & Pellikka, P. (2015). Echocardiographic Indices Associated With Frailty in Adults ≥65 Years. *Am J Cardiol , 116* (10), 1591-1595.
37. Govindapillai, A., Hancock Friesen, C., & O'Blenes, S. B. (2016). Protecting the aged heart during cardiac surgery: single-dose del Nido cardioplegia is superior to multi-dose del Nido cardioplegia in isolated rat hearts. *Perfusion*, *31*(2), 135–142. doi:10.1177/0267659115588633
38. Gutierrez, C., & Blanchard, D. (2004). stolic heart failure: challenges of diagnosis and treatment. *Am Fam Physician , 69* (11), 2609-2616.
39. Hachamovitch, R., Wicker, P., Capasso, J. M., & Anversa, P. (1989). Alterations of coronary blood flow and reserve with aging in Fischer 344 rats. *The American Journal of Physiology*, *256*(1 Pt 2), H66–73.
40. Hacker, T. A., McKiernan, S. H., Douglas, P. S., Wanagat, J., & Aiken, J. M. (2006). Age-related changes in cardiac structure and function in Fischer 344 x Brown Norway hybrid rats. *American Journal of Physiology - Heart and Circulatory Physiology*, *290*(1), H304–311. doi:10.1152/ajpheart.00290.2005

41. Harman, S. M., Metter, E. J., Tobin, J. D., Pearson, J., Blackman, M. R., Baltimore Longitudinal Study of Aging. (2001). Longitudinal effects of aging on serum total and free testosterone levels in healthy men. Baltimore Longitudinal Study of Aging. *The Journal of Clinical Endocrinology and Metabolism*, *86*(2), 724–731. doi:10.1210/jcem.86.2.7219
42. Homeister, J. W., Hoff, P. T., Fletcher, D. D., & Lucchesi, B. R. (1990). Combined adenosine and lidocaine administration limits myocardial reperfusion injury. *Circulation*, *82*(2), 595–608.
43. Howlett, S. E. (2015). Assessment of Frailty in Animal Models. *Interdisciplinary Topics in Gerontology and Geriatrics*, *41*, 15–25. doi:10.1159/000381131
44. Howlett, S. E., Rockwood, M. R. H., Mitnitski, A., & Rockwood, K. (2014). Standard laboratory tests to identify older adults at increased risk of death. *BMC Medicine*, *12*(1), 171. doi:10.1186/s12916-014-0171-9
45. Huang, S. J., & McLean, A. S. (2012). Appreciating the strengths and weaknesses of transthoracic echocardiography in hemodynamic assessments. *Cardiology Research and Practice*, *2012*(3). doi:10.1155/2012/894308
46. Hwang, H., Arcidi, J. M., Hale, S. L., Simkhovich, B. Z., Belardinelli, L., Dhalla, A. K., et al. (2009). Ranolazine as a cardioplegia additive improves recovery of diastolic function in isolated rat hearts. *Circulation*, *120*(11 Suppl), S16–21. doi:10.1161/CIRCULATIONAHA.108.844167
47. Ingram, D. K., Joseph, J. A., Spangler, E. L., Roberts, D., Hengemihle, J., & Fanelli, R. J. (1994). Chronic nimodipine treatment in aged rats: analysis of motor and cognitive effects and muscarinic-induced striatal dopamine release. *Neurobiology of Aging*, *15*(1), 55–61.
48. Johns, R. A., DiFazio, C. A., & Longnecker, D. E. (1985). Lidocaine constricts or dilates rat arterioles in a dose-dependent manner. *Anesthesiology*, *62*(2), 141–144.
49. Joseph, J. A., Bartus, R. T., Clody, D., Morgan, D., Finch, C., Beer, B., & Sesack, S. (1983). Psychomotor performance in the senescent rodent: reduction of deficits via striatal dopamine receptor up-regulation. *Neurobiology of Aging*, *4*(4), 313–319.
50. Kahn, C.M., Line, S., Merck & Co. (2010). *The Merck Veterinary Manual* (10 ed.). Whitehouse Station, N.J.:Merck.
51. Khouri, S. J., Maly, G. T., Suh, D. D., & Walsh, T. E. (2004). A practical approach to the echocardiographic evaluation of diastolic function. *Journal of the American Society of Echocardiography : Official Publication of the American Society of Echocardiography*, *17*(3), 290–297. doi:10.1016/j.echo.2003.08.012
52. Kirkland, J. L., & Peterson, C. (2009). Healthspan, translation, and new outcomes for animal studies of aging. *The Journals of Gerontology. Series a, Biological Sciences and Medical Sciences*, *64*(2), 209–212. doi:10.1093/gerona/gln063
53. Ko, F., Yu, Q., Xue, Q.-L., Yao, W., Brayton, C., Yang, H., et al. (2012). Inflammation and mortality in a frail mouse model. *Age (Dordrecht, Netherlands)*, *34*(3), 705–715. doi:10.1007/s11357-011-9269-6
54. Koller, K., & Rockwood, K. (2013). Frailty in older adults: implications for end-of-life care. *Cleveland Clinic Journal of Medicine*, *80*(3), 168–174. doi:10.3949/ccjm.80a.12100
55. Korff, S., Katus, H. A., & Giannitsis, E. (2006). Differential diagnosis of elevated troponins. *Heart (British Cardiac Society)*, *92*(7), 987–993. doi:10.1136/hrt.2005.071282

56. Kuh, D., New Dynamics of Ageing (NDA) Preparatory Network. (2007). A life course approach to healthy aging, frailty, and capability. *The Journals of Gerontology. Series a, Biological Sciences and Medical Sciences*, *62*(7), 717–721.
57. Lakatta, E. G., & Levy, D. (2003). Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: Part II: the aging heart in health: links to heart disease. *Circulation*, *107*(2), 346–354.
58. Lakatta, E. G., & Sollott, S. J. (2002). Perspectives on mammalian cardiovascular aging: humans to molecules. *Comparative Biochemistry and Physiology. Part a, Molecular & Integrative Physiology*, *132*(4), 699–721.
59. Lee, D. H., Buth, K. J., Martin, B.-J., Yip, A. M., & Hirsch, G. M. (2010). Frail patients are at increased risk for mortality and prolonged institutional care after cardiac surgery. *Circulation*, *121*(8), 973–978. doi:10.1161/CIRCULATIONAHA.108.841437
60. Lee, L., Heckman, G., & Molnar, F. J. (2015). Frailty: Identifying elderly patients at high risk of poor outcomes. *Canadian Family Physician Médecin De Famille Canadien*, *61*(3), 227–231.
61. Leng, S. X., Tian, X., Matteini, A., Li, H., Hughes, J., Jain, A., et al. (2011). IL-6-independent association of elevated serum neopterin levels with prevalent frailty in community-dwelling older adults. *Age and Ageing*, *40*(4), 475–481. doi:10.1093/ageing/afr047
62. Leng, S., Chaves, P., Koenig, K., & Walston, J. (2002). Serum interleukin-6 and hemoglobin as physiological correlates in the geriatric syndrome of frailty: a pilot study. *Journal of the American Geriatrics Society*, *50*(7), 1268–1271.
63. Li, K. Z. H., & Lindenberger, U. (2002). Relations between aging sensory/sensorimotor and cognitive functions. *Neuroscience and Biobehavioral Reviews*, *26*(7), 777–783.
64. Markowska, A. L., Mooney, M., & Sonntag, W. E. (1998). Insulin-like growth factor-1 ameliorates age-related behavioral deficits. *Neuroscience*, *87*(3), 559–569.
65. Matte, G., & del Nido, P. (2012). History and use of del Nido cardioplegia solution at Boston Children's Hospital. *J Extra Corpor Technol , 44* (3), 98-103.
66. Mecklenburg, L., Kusewitt, D., Kolly, C., Treumann, S., Adams, E.T., Diegel, K., et al. (2013). Proliferative and non-proliferative lesions of the rat and mouse integument. *Journal of Toxicologic Pathology, 26*(3 Suppl), 27S-57S. doi:10.1293/tox.26.27S
67. Mitnitski, A. B., Mogilner, A. J., & Rockwood, K. (2001). Accumulation of deficits as a proxy measure of aging. *TheScientificWorldJournal*, *1*, 323–336. doi:10.1100/tsw.2001.58
68. Mitnitski, A., Song, X., & Rockwood, K. (2013). Assessing biological aging: the origin of deficit accumulation. *Biogerontology*, *14*(6), 709–717. doi:10.1007/s10522-013-9446-3
69. Morita, N., Mandel, W. J., Kobayashi, Y., & Karagueuzian, H. S. (2014). Cardiac fibrosis as a determinant of ventricular tachyarrhythmias. *Journal of Arrhythmia*, *30*(6), 389–394. doi:10.1016/j.joa.2013.12.008
70. Morrow, J., Mood, D., Disch, J., & Kang, M. (2016). ***Measurement and Evaluation in Human Performance*** (5 ed.). Windsor, ON: Human Kinetics.

71. Mossahebi, S., & Kovács, S. J. (2014). The isovolumic relaxation to early rapid filling relation: kinematic model based prediction with in vivo validation. *Physiological Reports*, *2*(3). doi:10.1002/phy2.258
72. Nadon, N. L. (2007). Animal models in gerontology research. *International Review of Neurobiology*, *81*, 15–27. doi:10.1016/S0074-7742(06)81002-0
73. Newman, A. B., Gottdiener, J. S., McBurnie, M. A., Hirsch, C. H., Kop, W. J., Tracy, R., et al. (2001). Associations of subclinical cardiovascular disease with frailty. *The Journals of Gerontology. Series a, Biological Sciences and Medical Sciences*, *56*(3), M158–66.
74. Nicolini, F., Agostinelli, A., Vezzani, A., Manca, T., Benassi, F., Molardi, A., & Gherli, T. (2014). The evolution of cardiovascular surgery in elderly patient: a review of current options and outcomes. *BioMed Research International*, *2014*(5). doi:10.1155/2014/736298
75. O'Blenes, S. B., Friesen, C. H., Ali, A., & Howlett, S. (2011). Protecting the aged heart during cardiac surgery: the potential benefits of del Nido cardioplegia. *The Journal of Thoracic and Cardiovascular Surgery*, *141*(3), 762–770. doi:10.1016/j.jtcvs.2010.06.004
76. Olivetti, G., Melissari, M., Capasso, J. M., & Anversa, P. (1991). Cardiomyopathy of the aging human heart. Myocyte loss and reactive cellular hypertrophy. *Circulation Research*, *68*(6), 1560–1568.
77. Pacher, P., Mabley, J. G., Liaudet, L., Evgenov, O. V., Marton, A., Haskó, G., et al. (2004). Left ventricular pressure-volume relationship in a rat model of advanced aging-associated heart failure. *American Journal of Physiology - Heart and Circulatory Physiology*, *287*(5), H2132–2137. doi:10.1152/ajpheart.00405.2004
78. Parks, R. J., Fares, E., Macdonald, J. K., Ernst, M. C., Sinal, C. J., Rockwood, K., & Howlett, S. E. (2012). A procedure for creating a frailty index based on deficit accumulation in aging mice. *The Journals of Gerontology. Series a, Biological Sciences and Medical Sciences*, *67*(3), 217–227. doi:10.1093/gerona/glr193
79. Peña, F. G., Theou, O., Wallace, L., Brothers, T. D., Gill, T. M., Gahbauer, E. A., et al. (2014). Comparison of alternate scoring of variables on the performance of the frailty index. *BMC Geriatrics*, *14*(1), 25. doi:10.1186/1471-2318-14-25
80. Powell, W. J., DiBona, D. R., Flores, J., & Leaf, A. (1976). The protective effect of hyperosmotic mannitol in myocardial ischemia and necrosis. *Circulation*, *54*(4), 603–615.
81. Public Health Agency of Canada. (2009-2011). [Analyses were performed using Health Canada's DAIS edition of anonymized microdata from the *Canadian Community Health Survey 2009: Healthy Aging,* prepared by Statistics Canada].
82. Puts, M. T. E., Visser, M., Twisk, J. W. R., Deeg, D. J. H., & Lips, P. (2005). Endocrine and inflammatory markers as predictors of frailty. *Clinical Endocrinology*, *63*(4), 403–411. doi:10.1111/j.1365-2265.2005.02355.x
83. Rach, C., Gandhi, M., Docherty, J., Finegan, B. A., & Clanachan, A. S. (1999). Deficiency in myocardial NO biosignalling after cardioplegic arrest: mechanisms and contribution to post-storage mechanical dysfunction. *British Journal of Pharmacology*, *128*(4), 891–902. doi:10.1038/sj.bjp.0702863
84. Radnoti Working Heart Rat System 120101BEZ Instruction Manual. www.radnoti.com

85. Razzaque, M. S., Shimokawa, I., Nazneen, A., Higami, Y., & Taguchi, T. (1998). Age-related nephropathy in the Fischer 344 rat is associated with overexpression of collagens and collagen-binding heat shock protein 47. *Cell and Tissue Research*, *293*(3), 471–478.
86. Rieu, I., Magne, H., Savary-Auzeloux, I., Averous, J., Bos, C., Peyron, M. A., et al. (2009). Reduction of low grade inflammation restores blunting of postprandial muscle anabolism and limits sarcopenia in old rats. *The Journal of Physiology*, *587*(Pt 22), 5483–5492. doi:10.1113/jphysiol.2009.178319
87. Rockwood, K., & Mitnitski, A. (2007). Frailty in relation to the accumulation of deficits. *The Journals of Gerontology. Series a, Biological Sciences and Medical Sciences*, *62*(7), 722–727.
88. Rockwood, K., & Mitnitski, A. (2011). Frailty defined by deficit accumulation and geriatric medicine defined by frailty. *Clinics in Geriatric Medicine*, *27*(1), 17–26. doi:10.1016/j.cger.2010.08.008
89. Rockwood, K., Fox, R. A., Stolee, P., Robertson, D., & Beattie, B. L. (1994). Frailty in elderly people: an evolving concept. *CMAJ : Canadian Medical Association Journal = Journal De l'Association Medicale Canadienne*, *150*(4), 489–495.
90. Rockwood, K., McMillan, M., Mitnitski, A., & Howlett, S. E. (2015). A Frailty Index Based on Common Laboratory Tests in Comparison With a Clinical Frailty Index for Older Adults in Long-Term Care Facilities. *Journal of the American Medical Directors Association*, *16*(10), 842–847. doi:10.1016/j.jamda.2015.03.027
91. Rockwood, K., Song, X., MacKnight, C., Bergman, H., Hogan, D. B., McDowell, I., & Mitnitski, A. (2005). A global clinical measure of fitness and frailty in elderly people. *CMAJ : Canadian Medical Association Journal = Journal De l'Association Medicale Canadienne*, *173*(5), 489–495. doi:10.1503/cmaj.050051
92. Rodriguez-Mañas, L., Féart, C., Mann, G., Viña, J., Chatterji, S., Chodzko-Zajko, W., et al. (2013). Searching for an operational definition of frailty: a Delphi method based consensus statement: the frailty operative definition-consensus conference project. (Vol. 68, pp. 62–67). Presented at the The journals of gerontology. Series A, Biological sciences and medical sciences, Oxford University Press. doi:10.1093/gerona/gls119
93. Rothwell, T.L., & Everitt, A.V. (1986). Exopthalmos in ageing rats with Harderian gland disease. *Laboratory Animals, 20*(2), 97-100.
94. Rowe, R., Iqbal, J., Murali-Krishnan, R., Sultan, A., Orme, R., Briffa, N., et al. (2014). Role of frailty assessment in patients undergoing cardiac interventions. *Open Heart*, *1*(1). doi:10.1136/openhrt-2013-000033
95. Schoenenberger, A. W., Stortecky, S., Neumann, S., Moser, A., Jüni, P., Carrel, T., et al. (2013). Predictors of functional decline in elderly patients undergoing transcatheter aortic valve implantation (TAVI). *European Heart Journal*, *34*(9), 684–692. doi:10.1093/eurheartj/ehs304
96. Searle, S. D., Mitnitski, A., Gahbauer, E. A., Gill, T. M., & Rockwood, K. (2008). A standard procedure for creating a frailty index. *BMC Geriatrics*, *8*(1), 24. doi:10.1186/1471-2318-8-24
97. Sepehri, A., Beggs, T., Hassan, A., Rigatto, C., Shaw-Daigle, C., Tangri, N., & Arora, R. C. (2014). The impact of frailty on outcomes after cardiac surgery: a systematic review. *The Journal of Thoracic and Cardiovascular Surgery*, *148*(6), 3110–3117. doi:10.1016/j.jtcvs.2014.07.087

98. Sharma, S., Jackson, P. G., & Makan, J. (2004). Cardiac troponins. *Journal of Clinical Pathology*, *57*(10), 1025–1026. doi:10.1136/jcp.2003.015420
99. Sharp, P., & Villiano, J.S. (2012). *The Laboratory Rat, Second Edition.* CRC Press.
100. Sikka, G., Miller, K. L., Steppan, J., Pandey, D., Jung, S. M., Fraser, C. D., et al. (2013). Interleukin 10 knockout frail mice develop cardiac and vascular dysfunction with increased age. *Experimental Gerontology*, *48*(2), 128–135. doi:10.1016/j.exger.2012.11.001
101. Statistics Canada. (2014, September 26). Canada's population estimates: Age and sex, 2014. *Statistics Canada*. Retrieved November 2, 2015, from http://www.statcan.gc.ca/daily-quotidien/140926/dq140926b-eng.htm
102. Strait, J. B., & Lakatta, E. G. (2012). Aging-associated cardiovascular changes and their relationship to heart failure. *Heart Failure Clinics*, *8*(1), 143–164. doi:10.1016/j.hfc.2011.08.011
103. Stratton, J., Levy, W., Cerqueira, M., Schwartz, R., & Abrass, I. (1994). Cardiovascular responses to exercise: effects of age and exercise training in healthy men. *Circulation , 89* (4), 1648-1655.
104. Sun, M. H. (2014, August 11). *Impact of Frailty on Cardiac Contractile Function in an Aging Mouse Model* (Unpublished master's thesis). Dalhousie University, Halifax.
105. Sündermann, S. H., Dademasch, A., Seifert, B., Rodriguez Cetina Biefer, H., Emmert, M. Y., Walther, T., et al. (2014). Frailty is a predictor of short- and mid-term mortality after elective cardiac surgery independently of age. *Interactive Cardiovascular and Thoracic Surgery*, *18*(5), 580–585. doi:10.1093/icvts/ivu006
106. Turturro, A., Witt, W. W., Lewis, S., Hass, B. S., Lipman, R. D., & Hart, R. W. (1999). Growth curves and survival characteristics of the animals used in the Biomarkers of Aging Program. *The Journals of Gerontology. Series a, Biological Sciences and Medical Sciences*, *54*(11), B492–501.
107. University of Pennsylvania. (2011). *IACUC Guideline: Humane Intervention and Endpoints for Laboratory Animal Species.* (pp. 1-7).
108. Vella, C. A., & Robergs, R. A. (2005). A review of the stroke volume response to upright exercise in healthy subjects. *British Journal of Sports Medicine*, *39*(4), 190–195. doi:10.1136/bjsm.2004.013037
109. Walker, E. M., Nillas, M. S., Mangiarua, E. I., Cansino, S., Morrison, R. G., Perdue, R. R., et al. (2006). Age-associated changes in hearts of male Fischer 344/Brown Norway F1 rats. *Annals of Clinical and Laboratory Science*, *36*(4), 427–438.
110. Walston, J., Fedarko, N., Yang, H., Leng, S., Beamer, B., Espinoza, S., et al. (2008). The physical and biological characterization of a frail mouse model. *The Journals of Gerontology. Series a, Biological Sciences and Medical Sciences*, *63*(4), 391–398.
111. Walston, J., McBurnie, M. A., Newman, A., Tracy, R. P., Kop, W. J., Hirsch, C. H., et al. (2002). Frailty and activation of the inflammation and coagulation systems with and without clinical comorbidities: results from the Cardiovascular Health Study. *Archives of Internal Medicine*, *162*(20), 2333–2341.
112. Wang, S. Y., Friedman, M., Franklin, A., & Sellke, F. W. (1995). Myogenic reactivity of coronary resistance arteries after cardiopulmonary bypass and hyperkalemic cardioplegia. *Circulation*, *92*(6), 1590–1596.
113. Watanabe, S., Suzuki, N., Kudo, A., Suzuki, T., Abe, S., Suzuki, M., et al. (2005). Influence of aging on cardiac function examined by echocardiography. *The Tohoku Journal of Experimental Medicine*, *207*(1), 13–19.

114. Watson, L. E., Sheth, M., Denyer, R. F., & Dostal, D. E. (2004). Baseline echocardiographic values for adult male rats. *Journal of the American Society of Echocardiography : Official Publication of the American Society of Echocardiography*, *17*(2), 161–167. doi:10.1016/j.echo.2003.10.010
115. Whitehead, J. C., Hildebrand, B. A., Sun, M., Rockwood, M. R., Rose, R. A., Rockwood, K., & Howlett, S. E. (2014). A clinical frailty index in aging mice: comparisons with frailty index data in humans. *The Journals of Gerontology. Series a, Biological Sciences and Medical Sciences*, *69*(6), 621–632. doi:10.1093/gerona/glt136
116. Wiedemann, D., Bernhard, D., Laufer, G., & Kocher, A. (2010). The elderly patient and cardiac surgery - a mini-review. *Gerontology*, *56*(3), 241–249. doi:10.1159/000248761
117. Williams, D. L. (2002). Ocular disease in rats: a review. *Veterinary Ophthalmology*, *5*(3), 183–191.
118. Yazdanyar, A., & Newman, A. B. (2009). The burden of cardiovascular disease in the elderly: morbidity, mortality, and costs. *Clinics in Geriatric Medicine*, *25*(4), 563–77– vii. doi:10.1016/j.cger.2009.07.007
119. Yin, F. C., Spurgeon, H. A., Rakusan, K., Weisfeldt, M. L., & Lakatta, E. G. (1982). Use of tibial length to quantify cardiac hypertrophy: application in the aging rat. *The American Journal of Physiology*, *243*(6), H941–947.
120. Yu, B. M., Murata, I., Bertrand, H., & Lynd, F. (1982). Life span study of SPF Fischer 344 male rats fed ad libitum or restricted diets: longevity, growth, lean body mass and disease. *J Gerontol , 37* (2), 130-141.
121. Zile, M. R., & Brutsaert, D. L. (2002). New concepts in diastolic dysfunction and diastolic heart failure: Part I: diagnosis, prognosis, and measurements of diastolic function. *Circulation*, *105*(11), 1387–1393.
122. Zingone, B., Gatti, G., Rauber, E., Tiziani, P., Dreas, L., Pappalardo, A., et al. (2009). Early and late outcomes of cardiac surgery in octogenarians. *The Annals of Thoracic Surgery*, *87*(1), 71–78. doi:10.1016/j.athoracsur.2008.10.011

APPENDIX A: SCORING OF CLINICAL DEFICITS

System/ Deficit	*Assessment of Deficit*	*Scoring Guidelines*
Integument		
Alopecia	Restrain and inspect the animal's body for signs of fur loss.	0 = normal fur density 0.5 = focal fur loss 1= widespread fur loss
Change in fur colour	Observe the animal for changes in fur colour from white to yellow.	0 = normal colour 0.5 = focal yellow patches of fur 1 = yellow fur throughout body
Skin lesions	Using gentle restraint, inspect the animal's body for skin lesions.	0 = absent 0.5 = focal lesions 1 = widespread or multifocal lesions
Coat condition	Observe the animal's fur for signs of poor grooming.	0 = smooth/sleek coat 0.5 = slightly ruffled coat 1 = matted fur, unkempt/ungroomed coat
Physical/Musculoskeletal		
Tumours	Observe/palpate the animal for subcutaneous masses.	0 = absent 0.5 = suspicious palpable mass (<1 cm) 1 = obvious tumour; multiple small masses
Hunched posture	Observe the animal for hunched posture (head down, feet together).	0 = absent 0.5 = slightly hunched; normal movement 1 = obvious hunched posture; animal does not move willingly
Gait disorders	Observe freely moving animal to detect limping, unsteadiness and falling.	0 = absent 0.5 = abnormal gait; animal moves easily 1 = abnormal gait; impaired movement
Tremor	Observe the animal for involuntary tremor.	0 = no tremor 0.5 = slight tremor 1 = marked shaking/tremor
Body condition	On a flat surface, gently restrain the rat and palpate the flesh/fat on the sacroiliac region for excess or insufficient flesh/fat.	0 = bones palpable with slight pressure, not prominent 0.5 = bones easily palpable, or only with firm pressure 1 = bones extremely prominent/visible or disappear under subcutaneous fat
Distended abdomen	Observe rat for symmetry. Palpate abdomen for rigidity and fluid accumulation.	0 = no fluid, belly soft 0.5 = some fluid accumulation/asymmetry 1 = noticeable fluid accumulation, belly quite firm, or asymmetric
Neurological		
Head tilt	Observe freely moving animal for abnormal, asymmetrical head or neck position.	0 = normal head/neck position 0.5 = modest change in head tilt 1 = marked change in head tilt

Auditory		
Hearing loss	Acoustic startle reflex: hold a clicker ~10 cm from rat, sound it 3 times and record responses. Wait 30 seconds between each click stimulus.	0 = always reacts (3/3 times) 0.5 = reacts 1/3 or 2/3 times 1 = unresponsive (0/3 times)
Cataracts	Observe for signs of opacity in the centre of the eye.	0 = absent 0.5 = minimal changes, some opacity 1 = opaque lens
Chromodacryorrhea	Observe for signs of ocular or nasal porphyrin staining.	0 = absent 0.5 = slight staining/accumulation around nose/eyes 1 = obvious staining/accumulation around nose/eyes
Exophthalmos	Observe for swelling or bulging in the eyes.	0 = normal size 0.5 = one or both eyes slightly larger 1 = one or both eyes very large
Microphthalmos	Observe for small or sunken eye(s).	0 = normal size 0.5 = one or both eyes slightly small/sunken 1 = one or both eyes noticeably small/sunken
Corneal opacity	Observe for clouding of the cornea, or superficial white spots.	0 = absent 0.5 = slight clouding/spotting of cornea 1 = marked clouding/spotting of cornea
Digestive/Urogenital		
Malocclusion	Grasp the rat by the neck scruff, invert and expose teeth. Observe for overgrown/misaligned incisors. May have to be done under light anesthesia.	0 = mandibular longer than maxillary incisors 0.5 = incisors slightly uneven, or misaligned 1 = incisors overgrown and misaligned
Rectal prolapse	Grasp rat by the base of the tail and invert to detect signs of tissue protruding from the rectum. Tissue appears red and may bleed.	0 = absent 0.5 = small amount of prolapsed tissue 1 = prolapsed tissue clearly visible
Diarrhea	Observe for fecal smearing in home cage or on fur.	0 = normal feces 0.5 = some fecal smearing on fur or in home cage 1 = noticeable fecal smearing on fur or in home cage, stools soft and watery

Jaundice	Inspect the animal for yellowing of the feet, nose, ears and tail. Urine will also be quite concentrated and yellow.	0 = no signs of jaundice 0.5 = slight yellowing, some signs of jaundice 1 = intense yellowing; urine quite concentrated/yellow.
Respiratory		
Breathing rate/depth	Observe the animal at rest. Note rate and depth of breathing.	0 = normal 0.5 = some change in breathing rate and/or depth 1 = dramatic changes in rate/depth, laboured breathing
Pain/Discomfort		
Unusual sounds	Observe the animal. Listen for snuffling, or acute vocalizations when touched. The rat may display increased aggressiveness when handled.	0 = normal 0.5 = modest wheezing, congestion or mild vocalization 1 = obvious wheezing, congestion or marked vocalizations
Other		
Body surface temperature	Using infrared thermometer, measure abdominal body surface temperature. Record the average of 3 measures.	0 = differs by <1 SD from mean reference value 0.5 = differs by 1 – 2 SD from mean reference value 1 = differs by >2 SD from mean reference value